



## Alfalfa breeding for intercropping and grazing tolerance

Bernadette Julier, Paolo Annicchiarico, Philippe Barre, Gaëtan Louarn,  
Luciano Pecetti

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# PROCEEDINGS OF THE SECOND WORLD ALFALFA CONGRESS

## GLOBAL INTERACTION FOR ALFALFA INNOVATION

NOVEMBER 11-14, 2018

CORDOBA, ARGENTINA

Organized by Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina

Co-sponsored by US National Alfalfa & Forage Alliance (NAFA), China Grassland Association (CGA) and Commission Intersyndicale de Deshydrateurs Européens (CIDE)

*Edited by*

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Córdoba, Argentina

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## PREFACE

Alfalfa (*Medicago sativa* L.) is the most valuable forage crop worldwide because of its high quality as an animal feed. In 2017, international trade of alfalfa hay reached 8.3 million metric tons for a total value of 2.3 billion US Dollars. Based on the growing demand from Asian and Middle East countries, it is predicted that alfalfa hay demand will continuously increase in the near future. To meet this need, alfalfa producing countries must be very effective in providing larger amounts of high quality hay while using natural resources, especially irrigation water, in a highly efficient way.

Global climate change is producing environmental modifications that impact not only on alfalfa productivity but also on insect and disease behavior. In this regard, alfalfa breeding programs around the world are emphasizing abiotic and biotic stress resistance as breeding objectives. Water shortages in several countries, as well as the increasing need of fresh water for human consumption, are exerting pressure on using medium to low quality irrigation waters. The permanent demand for high quality alfalfa, particularly for dairy production, is challenging breeding programs to improve alfalfa nutritional value by both conventional and biotechnological approaches. De-regulation of GE alfalfas in several countries created the need for developing co-existence models in order to produce alfalfa for any market, i.e. sensitive (non-GE) and non-sensitive (GE). The many services provided by alfalfa -such as N<sub>2</sub> fixation, water table lowering, bird nesting, beneficial insect refuges, among others- are revitalizing the consideration of its remarkable environmental role, especially on the sustainability of the production systems. The latter is confirmed by the increasing interest on alfalfa grazing or its use in intercropping systems to widen biotic diversity. On the other hand, new uses of alfalfa, either for human feed or cosmetic and pharmaceutical industries, are enriching the scope of alfalfa production in different countries.

In this context, the Alfalfa Group of INTA Manfredi organized the Second World Alfalfa Congress (2WAC). This second edition –as the previous World Alfalfa Congress held in Bengbu, China in 2015- received the institutional support of NAFA (National Alfalfa and Forage Alliance, USA), CIDE (Commission Intersyndicale de Deshydrateurs Européens) and CGA (China Grassland Association). The 2018 event had two main scientific objectives: I) to promote updated discussions on the most important issues that impact the crop; and II) to emphasize international cooperation on research and industrial developments that improve alfalfa production and use under sustainable premises.

Scientific structure of the 2WAC included 44 oral presentations by 38 speakers from 11 countries of South & North America, Europe, Asia and Oceania. There were also 47 poster presentations from 9 countries. Oral presentations were divided into 8 sessions, designed to cover most of the important topics pointed out above. These sessions were: I- World Alfalfa Production and Demand; II- Increasing Water Use Efficiency in Alfalfa Production; III- Abiotic Stresses (drought, salinity and cold): Breeding and Field Management; IV- Biotic Stresses: Insects and Diseases; V- GE Alfalfas; VI- Breeding for Alfalfa Quality; VII- Alfalfa Processing, New Uses, New Areas and Export Market; and VIII- Alfalfa-based Production Systems to Meet Economic and Environmental Challenges.

This publication includes the abstracts of both oral and poster presentations. We expect the reader will find here the core concepts that were discussed along the conference with the hope that they could be useful for enriching their work.

**The editors**

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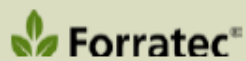
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## SESSION CHAIRS AND INVITED SPEAKERS

### **SESSION I - World Alfalfa Production and Demand**

#### ***Chairs:***

**Daniel Basigalup** (Argentina): Alfalfa breeder at INTA Manfredi – President Organizing Committee Second World Alfalfa Congress.

**Lu Xinshi** (China): Professor at Beijing Forestry University and President of the China Grassland Association

#### ***Speakers:***

**Lu Xinshi** (China) is a Grassland-Agronomist, Professor at the Beijing Forestry University, President of the National Grassland Industry Innovation Alliance, President of China Grassland Association and Task Force Leader of “Alfalfa Rejuvenation Campaign”. He has published many scientific papers.

**Alan Humphries** (Australia) is an alfalfa breeder at the (SARDI). His interests include selection for improved tolerance to drought, grazing and acidic soils. He has authored/co-authored more than 30 scientific papers and released seven alfalfa cultivars.

**Daniel Basigalup** (Argentina) is Senior Scientist at INTA Manfredi and responsible for INTA's National Alfalfa Breeding Program. He has released nine alfalfa cultivars, edited/co-edited 3 alfalfa books and authored/co-authored several book chapters and scientific papers.

**Eiko Jan Duursema** (The Netherlands) is the President of CIDE (European Dehydrators Association) since 2006, President of the Dutch Dehydrators Association (1996-present) and member of the Board of Directors of Agrifirm Feed Norht West Europe (2017-present).

**Dan Gardner** (USA) is the Chief Marketing and Technology Officer at S&W Company since 2012. He is responsible for Breeding and Sales Teams in Alfalfa, Sorghum, Sunflower and Stevia. He worked for 18 years at Dairyland Seed Co. (Dow AgroSciences group), and as alfalfa breeder (1994-2012).

**Gabriel Osatinsky** (Argentina) is a businessman with a vast experience in international trade. Since April 2016 is the President of the Argentine-Emirates Chamber of Commerce and since February 2018 is the President of the Argentine-Emirates Business Platform.

**Qian Guixia** (China) is a Professor at the Inner Mongolia University and a researcher at the Grassland Research Institute, Chinese Academy of Agricultural Science. At present, she is studying the sustainable development of Chinese forage industry and pastoral animal husbandry.

### **SESSION II - Increasing Water Use Efficiency in Alfalfa Production**

#### ***Chair:***

**Daniel Putnam** (USA): Faculty member at the UC-Davis and chair of the California Alfalfa Workgroup, the California and Western Alfalfa Symposia and founding member of the California Alfalfa & Forage Association.

#### ***Speakers:***

**Rodolfo Bongiovanni** (Argentina) is a researcher at INTA's Manfredi Experimental Station and Professor of Economics at the Córdoba Catholic University (UCC). He leads the INTA's Project on Life-Cycle Assessment of Industrial Crops, which deals with environmental footprints.

**Haijun Yan** (China) is Professor and Dean of the College of Water Resources & Civil Engineering at China Agricultural University (CAU). He published over 100 papers and 4 books and is conducting research on sprinkler and micro irrigation technology and fertigation in alfalfa and other crops.

**Sharon Benes** (USA) is a Plant Pathologist/Physiologist at the Dept. of Pl. Science, CA State University, Fresno. Her research deals with salinity and drainage management, irrigation with saline water, and use of wastewaters for irrigation. She has produced many scientific publications.

**Dan Putnam** (USA) is an alfalfa specialist at the UC-Davis conducting research on alfalfa agronomic practices, varieties, harvest schedules, irrigation techniques and soil salinity practices, weed and pest management, forage quality, crop rotation effects, biofuels and alternative forage crops.

**Steven Evett** (USA) is Soil Scientist, USDA-ARS and President of the ASA. He published 26 books/chapters and over 300 papers. Currently, is studying energy and water balances of irrigated crops through modeling and conducting irrigation projects in Egypt, Israel, Jordan and Uzbekistan.

### **SESSION III - Abiotic stresses (drought, salinity and cold): Breeding and Field Management**

#### ***Chairs***

**Paolo Annicchiarico** (Italy) is a legume breeder and Director of Research at the Centre for Animal Production and Aquaculture (CREA), Lodi, Italy.

**E. Charles Brummer** (USA) is the Director of the Plant Breeding Center, Professor at the Department of Pl. Sci.-UC Davis and President of the Crop Science Society of America.

#### ***Speakers:***

**Bernadette Julier** (France) is Senior Scientist at INRA Lusignan. Her research is related to the improvement of alfalfa forage quality and yield, adaptation of alfalfa-grass mixtures for increasing sustainability and use of molecular markers in alfalfa breeding. She published many scientific papers.

**Twain Butler** (USA) is a Professor and Forage Agronomist at the Noble Foundation in Ardmore, OK, USA. His latest research is related to the improvement of grazing management of mixed and pure alfalfa pastures. He has published many scientific papers and released several forage cultivars.

**Annick Bertrand** (Canada) is Research Scientist at Agriculture and Agri-Food Canada in Quebec. She works on physiological and molecular aspects of winter survival and stress tolerance in alfalfa and developed selection techniques for higher freezing tolerance and more cell wall digestibility.

**Alan Humphries** (Australia) is an alfalfa breeder at the (SARDI). His interests include selection for improved tolerance to drought, grazing and acidic soils. He has authored/co-authored more than 30 scientific papers and released seven alfalfa cultivars.

**Charlie Brummer** (USA) is at UC-Davis. His lab is investigating the genetic control of autumn dormancy in alfalfa, developing methods to improve biomass yield, and creating alfalfa breeding pools from the USDA germplasm collection. He published many scientific papers and book chapters.

**Paolo Annicchiarico** (Italy) is at CREA, Lodi and has coordinated several research projects on legume breeding and genetic resources in Europe. Currently is working on alfalfa tolerance to abiotic stresses and genomic selection. He published 250 scientific papers and released several cultivars.

## **SESSION IV - Biotic stresses: insects, diseases and weeds**

### ***Chair:***

**Deborah Samac** (USA) is a Research Geneticist and Plant Pathologist with USDA-Agricultural Research Service at the University of Minnesota-St. Paul.

### ***Speakers:***

**Deborah Samac** (USA) works for the USDA-ARS in UMN and conducts research on resistance to alfalfa diseases and on diversity of alfalfa pathogen populations. Her lab also uses biotechnological approaches for alfalfa improvement.

**Verónica Trucco** (Argentina) is a Plant Virologist at the Institute of Plant Pathology of the Agricultural and Cattle Research Center (CIAP), INTA, Córdoba. At present, she and her group are studying the Alfalfa Viral Complex Disease using molecular tools.

**Fengling Shi** (China) is Professor at College of Grassland and Resource Environment Science, Inner Mongolia Agricultural University. As an alfalfa breeder, he is conducting research on male sterility, heterosis application, and alfalfa seed production. He has released eight forage varieties.

**Scott Johnson** (Australia) is Associate Professor at the Australian Research Council Future Fellow. He leads a group that addresses how environmental factors shape plant chemistry and how this in turn affects herbivorous pests. Much of this research focuses on legumes, but also pasture grasses.

**Jorge Frana** (Argentina) is an entomology, biological control and integrated pest management specialist at INTA Rafaela, Santa Fe, Argentina. Much of his researchs focuses on integrated management of harmful organisms in various crops.

## **SESION V - GE Alfalfas**

### ***Chairs:***

**Mark McCaslin** (USA): Alfalfa breeder and Vice President Research of Forage Genetics International.

**Federico Trucco** (Argentina): is the Chief Executive Officer of Bioceres and in 2005-11 he was Director of Product Development and General Manager at INDEAR.

### ***Speakers:***

**Zengyu Wang** (China/USA) is currently the Director of Core Research Facilities of The Samuel Roberts Noble Foundation, USA. His lab focuses on translational research for the improvement of forage grasses and legumes.

**Stephen Temple** (England/USA) is the Director of Biotechnology for Forage Genetics International. He was biotechnology leader for the development and commercialization of Roundup Ready and HarvXtra alfalfas and has been involved on several alfalfa biotechnology and biochemistry aspects.

**Gabriela Soto** (Argentina) is the head of Alfalfa Genetic Engineering group and CONICET researcher at Institute of Genetics (IGEAF) INTA-Castelar. She has worked on several alfalfa genetic topics and has led the developing of a transgenic ammonia glufosinate tolerant alfalfa.

**Daniel Putnam** (USA) is an alfalfa specialist at UC-Davis. He has been involved on several extension and research activities related to alfalfa and forage crops, particularly on variety evaluation, forage quality, harvest schedules, irrigation techniques and soil salinity practices.

**Martín Lema** (Argentina) is Director of Biotechnology at the Ministry of Agroindustry of Argentina. Martín has a vast experience in biotechnology and biosafety aspects.

## **SESION VI - Breeding for alfalfa quality**

### ***Chairs:***

**Don Miller** (USA) is Director of Product Development for Alforex Seeds, Nampa ID and has been involved in plant breeding for over 35 years, developing alfalfa, teff (*Eragrostis tef*) and red clover varieties.

**Mike Peterson** (USA) is currently the Lead for Global Traits at Forage Genetics International, a breeder and worldwide marketer of alfalfa seed in USA. Mike was Senior Breeder, Research Director, and Brand Manager for W-L Research.

### ***Speakers:***

**Vincent Beguier** (France) is the research head of the Jouffray-Drillaud company, France. Vincent is a forage grass and legume breeder, Director of GIE GRASS, President of the French Forage Plant Breeder Association and member of the French Forage Variety Review Board.

**Jonathan Reich** (USA) currently serves as the Global Alfalfa Breeding Leader for CORTEVA Agriscience, the Agriculture Division of DowDupont. Jon has been involved in plant breeding professionally for over 34 years during which time the programs under his direction have commercialized over 200 improved alfalfa varieties, 30 improved clover and grass varieties, and 9 improved oilseed varieties.

**Ariel Odorizzi** (Argentina) is the Head of the Animal Production Department and Senior Scientist at INTA Manfredi, where he has worked on several alfalfa breeding aspects for over 15 years. He also published several scientific papers and co-authored book chapters.

**Mark McCaslin** (USA) currently serves as Vice President Research of Forage Genetics International. Mark has worked on alfalfa breeding for over 30 years and has been responsible for developing more than 250 alfalfa cultivars.

**Jinhui Shao** (China) is currently the General Manager of Beijing Rtyway Seed Co. Ltd. and Vice President of China Grassland Association.

## **SESION VII**

### ***Chair:***

**Valeria Arolfo** (Argentina) is a Senior Scientist at INTA Manfredi, where is also the Head of the Forage Resources Group. She works on alfalfa and *Melilotus albus* improvement and coordinates the National Alfalfa Cultivar Evaluation Network.

### ***Speakers:***

**Chengzhang Wang** (China) is Professor in College of Animal Science and Veterinary Medicine at Henan Agricultural University, Zhengzhou. He is also Director of Chinese Grassland Society and Vice Chairman of both Feed Production Committee and Grassland Education Committee.



**Daniel Putnam** (USA) is an alfalfa specialist at UC-Davis. He has been involved on several extension and research activities related to alfalfa and forage crops, particularly on variety evaluation, forage quality, harvest schedules, irrigation techniques and soil salinity practices.

**Duarte Vilela** (Brazil) is Senior Scientist at the Animal Production Department of EMBRAPA, where was Head Dairy Cattle Division as well. He was also Supporting Coordinator in Sectorial Chambers of the Ministry of Agriculture, Livestock & Food Supply, and FAO Advisor for Food Safety in Africa.

**Qamar Shakil** (Pakistan) is Assistant Botanist at Fodder Research Section of the Ayub Agricultural Research Institute (AARI), Faisalabad. Currently, he is also Supervisor of Research Activities and Drawing & Disbursing Officer at Fodder Research Sub-Station, AARI.

**Abid Mahmood** (Pakistan) is Director General of Agriculture Research at the Ayub Agricultural Research Institute (AARI) in Faisalabad. He has a vast experience in many agricultural topics for the Punjab region and has published nearly 100 scientific papers.

**Francisco Tabuenca** (Spain) is President of AEFA (Dehydrated Industry Association of Spain), as well as Managing Director of Raigones Agrícola S.L., CEO of Forrajes San Agustín, and Managing Director & CEO of Amaetón, Huesca.

## **SESION VIII**

### ***Chair:***

**Christian Huyghe** (France) is a Senior Scientist and Deputy Scientific Director for Agriculture Sector of INRA, Paris. He is also ACTA's President of the Scientific Orientation Council & GEVES' President of the Administrative Board. He has many scientific publications

### ***Speakers:***

**Guillaume Jégo** (Canada) is a Scientist at the Quebec Research & Development Centre, Agriculture and AgriFood Canada. He works on agro-ecosystems modeling for biomass and yield predictions and evaluation of environmental impacts of agricultural activities and water and Nitrogen cycles.

**Pablo Tiftonelli** (Argentina) is coordinator of INTA's National Program for Natural Resources, Environmental Management and Ecoregions. He is also Associate Professor at the Universities of Wageningen, Montpellier (Sibaghe Graduate School) and Lomas de Zamora.

**Nicolás Ayub** (Argentina) is an Independent Researcher from CONICET working at Ewald A. Favret Genetic Institute, INTA-Castelar. His research focuses on analyzing various microorganism-plant interaction processes, particularly with alfalfa.

**Christian Huyghe** (France) is a Senior Scientist and Deputy Scientific Director for Agriculture Sector of INRA, Paris. He is also ACTA's President of the Scientific Orientation Council, GEVES' President of the Administrative Board and Member of the Bio-Based Industries Joint Undertaking Committee.

**Eduardo Comerón** (Argentina) is a Senior Scientist at INTA Rafaela with 30 years of experience on dairy production. He was INTA's Dairy Research Coordinator between 1994 and 2018 and now represents INTA at the Dairy International Federation. Since 1985, he is Professor at UTN Rafaela.

**Xiangyang Hou** (China) is the General Director of the Institute of Grassland Research of the Chinese Academy of Agricultural Sciences (CAAS), Hohhot. He is also Head of Key Lab of Grassland Ecosystem and Protection from the Ministry of Agriculture, China



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## ORAL PRESENTATIONS

## Alfalfa improvement in China\*

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China is one of the world's largest alfalfa countries, with a planting area of 4.7 million hectares and where alfalfa is used mainly in three aspects: a) environment vegetation restoration; b) commercial hay/fodder market supply; and c) self-consumption in family farms. Alfalfa production in China has a long history of planting, dating back to the Han dynasty about 2,300 years ago.

China is part of the center of origin of alfalfa. Native germplasm is mainly distributed in northern of Xinjiang area, which is a part of the center of origin in Central Asia. The main germplasm sources are fall-dormant and very fall-dormant ecotypes. By 2018, China has developed 94 new alfalfa varieties, which clearly demonstrates the importance China devotes to research on alfalfa germplasm resources. Three forage gene banks have been established so far, and more than 3,000 alfalfa germplasm accessions have been collected and conserved and more than one-third of them have been already genetically evaluated. Alfalfa is a very important focus of scientific research, and 70% of published academic papers are referred to alfalfa research areas.

China's alfalfa commodity production has had a tremendous development since 2008, with the production increased from 150,000 tons in 2008 to 1.4 million tons in 2017. Alfalfa hay and silage are the main commodity forms, coming from multiple production areas and receiving support from national policies. At present, China's 7.5 million dairy cows consume an average of 3 million tons of alfalfa per year. By 2020, alfalfa consumption will reach 4 million tons, and by 2030, it will reach 6 million tons. The current alfalfa industrial policy in China will encourage farmers and enterprises to continually increase the planting area and the production of alfalfa products in China. In addition, it will expand the door opening for alfalfa imports from the world. China's alfalfa production, consumption and market will become an integral part of alfalfa production and marketing in the world.

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## Overview of the economic utilization and traits of interest of lucerne in Australia\*

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**KEYWORDS:** alfalfa, drought, acidity, adaptation

**INTRODUCTION:** Lucerne (alfalfa, *Medicago sativa* L.) is grown on an estimated 3 – 3.5 Million hectares of Australia, concentrated in the south-eastern, medium rainfall agricultural zones. Over 90% of the total area is grown under rainfed conditions, where it is predominantly used as a specialist pasture that is cut opportunistically for hay and grazed by sheep and cattle for meat, dairy, and in rotations with cereal crops in low-medium rainfall environments. In this mixed cropping and livestock farming system a 3 – 6 year pasture phase provides nitrogen and a disease break for the following sequence of grain crops. Lucerne also has a minor use as a water efficient, heat tolerant summer grazing option in the dairy industry (usually under irrigation) where it is also cut for hay or silage. There is also approximately 100,000 hectares of lucerne managed for intensive lucerne hay production in NSW, concentrated along river valleys.

The south-east of South Australia is home to one of the world's largest areas of lucerne seed production. The region produces 4-8,000t of seed per year from 20-25,000 ha of rainfed and irrigated production (Lucerne Australia Pers. Comm. 2018). Irrigated seed yields range between 600 and 1500 kg/ha, dependent on seasonal climate variability.

In southern Australia the deep rooted nature of this perennial legume is valued for extending the growing season of winter-based pasture into summer, where it is used for finishing lambs, growing out vealer calves, or improving the fertility of maternal stock. In northern Australia rainfall is dominant in the summer months, and in these environments the quality of lucerne is valued for improving feed quality and the utilisation of C4 grasses.

### Hay production for domestic and export markets

The domestic market for lucerne hay is a considerable size, but the volume is difficult to estimate because it is dominated by cash sales for the equine industries and local livestock markets (transported <100km). Hay yields in northern NSW from well managed commercial irrigated stands are in the order of 15-25t/ha across six-seven cuts per year. This is comparable to experiments that have reported 25.7 t DM/ha in northern Victoria (Greenwood et al. 2006) and 24.2 t DM/ha in south-eastern Queensland (Lowe et al. 2010). A recent change from winter active (fall dormancy) class 5 to 7 varieties has resulted in an increase in the number of cuts per year that can be achieved from 5 to 6 or 7 (with an average of 2.5 t/ha per cut), plus the possibility of an additional winter silage cut. Producers target 15-18% moisture quality, with around 55% being delivered to premium horse markets and the remaining 45%, lower quality hay, sold to cattle producers. The quality of lucerne hay is still largely purchased on appearance by horse enthusiasts, with colour, stem thickness and purity used to estimate nutritive value.

Under rainfed conditions only a single hay cut is possible in most areas, with the rest of the season used for grazing. Lucerne is increasingly conserved as silage where on-farm use for beef or dairy production is intended. The dairy industry is no longer a large buyer of lucerne or clover (*Trifolium* spp.) hay, having largely transferred to using cereal hay.

Australia does not have a significant export hay market for lucerne. Australia exports around 1.1 Million tonnes of hay and cereal straw a year to China, Japan and Korea, but it doesn't have the right environmental conditions to produce a consistent supply of quality lucerne hay for export. The large oaten hay exporters (Gilmac,

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Johnsons, Balco) are positioned in the cereal zones of WA, SA and Victoria, far away from the hay producing regions for lucerne. In Australia irrigation water is a scarce resource, and in the last two decades has transitioned to being used for either higher value crops or applied more efficiently to supplement the growth of annual crops growing during cooler months. In the last five years, the only considerable lucerne hay exports occurred during a US port strike that ran for four months, and this was < 1000t in total. There are some lucerne chaff exports, with one NSW company exporting around 1000t per year in addition to their domestic production of 6000 t/year.

### **Lucerne as a pasture for livestock systems**

Lucerne is estimated to be adapted to over 30 million ha in Australia (Hill and Donald 1998), but its current area of cultivation of 3.2 million ha is approximately half of the area suggested as a realistic upper limit for adoption (Robertson 2006). In southern Australia lucerne is valued for its capacity to extend the growing season of winter-based pasture. The high nutritive value feed can be used to switch autumn calving or lambing times into spring. In cattle, spring calving can substantially reduce large costs associated with supplementing the lactating cow during winter (McKiernan et al. 2005). The weaners are younger, but the system allows for higher stocking rates (more calves per ha) that can be finished with lucerne on farm or supplied to feedlots. Producers are also using lucerne to hold onto weaner stock for longer into summer, adding weight and attracting a premium price for selling later in the season.

One of the greatest current drivers for promoting the expansion of lucerne in southern Australia is related to the aim to provide a consistent supply of preferred specification meat production to local meat processors. The capacity for livestock producers to supply this product is constrained by production systems that have a highly variable feed supply. Lucerne is one of the few options that can underpin a consistent feedbase, and has been linked to higher carcass compliance and eating quality beef in comparison to grain and ryegrass finished pasture (Frank et al. 2014). The opportunity to finish livestock at a younger age with lucerne also presents opportunities for reducing methane emissions intensity.

The limitation for further expansion of lucerne in Australia is that it is considered to be a specialist pasture, due to perceived difficulties with integration into the farm system. Also, for many environments, lucerne is adapted to only some areas of the farm, with seasonal waterlogging and acidic soils restricting its adaptation. Grazing systems in most areas are extensive, with a lack of rotational grazing over the farm contributing to challenges with adoption and integration. A single on-farm field of lucerne provides challenges for rumen function when taking livestock from dry senesced pastures and putting them onto highly digestible lucerne. Sudden changes to diet can increase the risk of bloat in cattle and red-gut in sheep, which is particularly relevant where lucerne is grown as a monoculture. New research is required to demonstrate the production of lucerne in mixtures with perennial grasses and chicory, including the development of new varieties that are adapted to growing in pasture mixtures.

### **Traits of importance for lucerne in Australia**

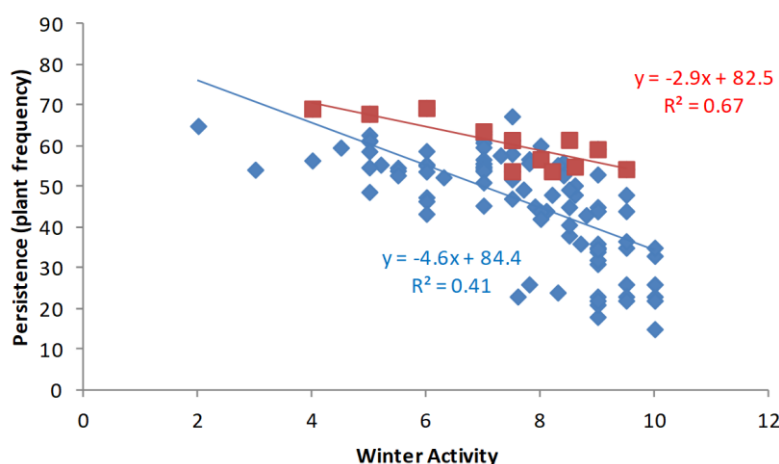
Broad adaptation to diverse environmental conditions and farming systems is critical in Australia, as the market is not large enough to segregate varieties into rainfall, soil types or farming systems. Lucerne varieties with winter activity (fall dormancy) classes of 3 to 10 are sold, with persistent, multipurpose class 7 varieties being the most popular by volume.

#### **1. Persistence under grazing**

New grazing tolerant varieties released by SARDI and PGG Wrightsons (SARDI Grazer and Stamina GT5, FD5-6) have persisted extremely well in field evaluation trials under farmer management. An analysis of persistence versus winter activity from nine farmer managed sites after four years (Humphries, unpublished) shows (Figure 1) that persistence was improved for entries previously selected for tolerance to continuous grazing across a range of winter activity.

## 2. Drought tolerance

Lucerne forage production in south-eastern Australia is dependent on a variable rainfall supply. In much of southern Australia, deterioration of pasture quality and availability over summer and autumn (McKiernan et al. 2005) places lucerne under pressure from overgrazing when other feed sources are scarce. Lucerne is often set-stocked during drought conditions, and whilst this can be important to keeping livestock alive, the drought x grazing stress is a major cause of lucerne population decline.



**Figure 1.** The relationship between persistence and winter activity for conventionally bred (diamonds) and grazing tolerant lucerne lines (squares) at nine commercially managed sites in Australia (Coolac, Cowra, Culcairn, Grenfell, Mingbool, Bendigo, Timboon, Rochester, Tintinara).

Climate change predictions indicate further warming of 0.8-2.8 °C and decreases of rainfall of 0-20% by 2050 (Ghahramani et al. 2013). This is likely to place greater pressure on a production system that is already constrained by a winter dominant rainfall pattern. The climate models suggest that later breaks to the winter growing season are likely, potentially coupled with warmer winters and less spring rainfall (Ghahramani et al. 2013). This may have a large impact on the ability to establish new lucerne pastures in low rainfall environments. This could see establishment being pushed from autumn and spring into a narrower zone of opportunity in winter. Seedling vigour and high winter activity (low fall dormancy) are likely to increase as important traits, in order to maximise seedling growth in cooler conditions. Despite these concerns, a higher incidence of summer rainfall has resulted in the prediction that an increased use lucerne is likely to have the greatest effect in reducing the negative impact of climate change (Ghahramani & Moore 2013).

The deep rootedness and drought tolerance of lucerne also has benefits for improved production efficiency using scarce irrigation water. In the Riverina region (Murray River irrigation), Kelly et al. (2005) showed that lucerne is very tolerant to deficit irrigation, with no loss of yield under an irrigation strategy that reduced the yield of white clover by 70%. Current recommendations for dairy farmers in hot climates with water restrictions are to incorporate winter-active lucerne on up to 20% of farm area, with a focus on supplementing natural rainfall with irrigation to produce feed from winter-growing annual species during the cooler growing seasons.

## 3. Aphid and disease tolerance

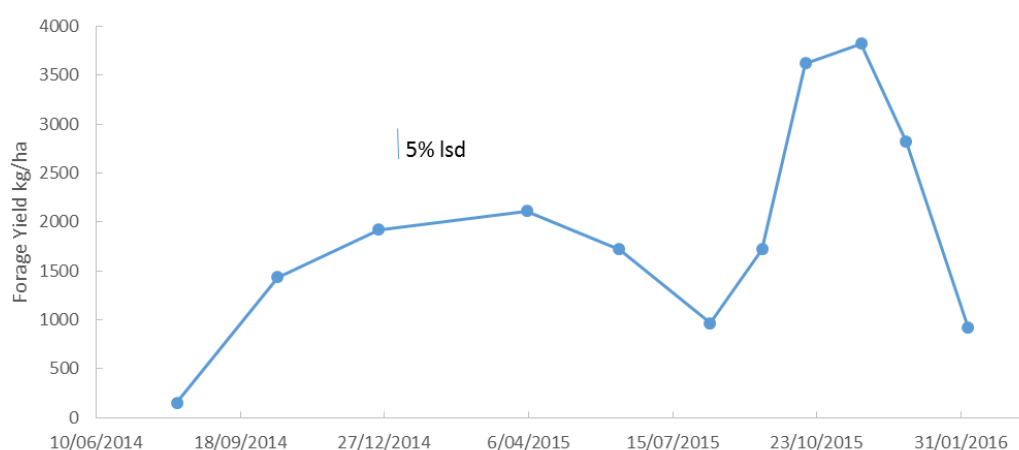
The four major pests and diseases of lucerne in Australia are bluegreen (*Acyrtosiphon kondoi*) and spotted alfalfa (*Therioaphis trifolii*) aphids, *Phytophthora* root rot (*P. medicaginis*) and Anthracnose (*Colletotrichum trifolii*). Plant-based resistance to bluegreen aphid (BGA, *Acyrtosiphon kondoi* Shinji) has broken down in south-eastern Australia (Humphries et al. 2012), with virulent biotypes now requiring chemical control when growing conditions are ideal for aphid development. Lucerne fields are also managed for lucerne flea (*Sminthurus viridis*) and red-legged earth mite (*Halotydeus destructor*) during seedling development, which has reduced the awareness of the new bluegreen aphid being a serious pest. An increase in reliance on chemicals

for control of insects has already led to the development of chemical resistance in red-legged earth mite, and there are concerns that this could also develop in aphids. There are opportunities to breed for resistance using existing genetic variability to the new bluegreen aphid biotype, as well as maintain resistance to other pests and diseases. This aim needs to be realised if plant-based resistance is to have a future role in maintaining lucerne stability.

#### 4. Tolerance to acidic soils

Acidic soils are strongly prevalent in the medium-high rainfall zones of southern Australia, with 11.2 M ha of agricultural land with surface pH<sub>Ca</sub> 5.0-5.5 and a further 6.2 M ha with pH<sub>Ca</sub> 4.5-5. An acid tolerant lucerne variety would be expected to have the greatest impact in areas where lucerne is currently grown but often at sub-optimum production levels, including the Riverine Plains, Central West and Northern Tablelands of NSW and Victoria and the south-east of South Australia.

The performance of new lucerne varieties and rhizobia strains selected for improved tolerance to soil acidity have recently been evaluated across a range of environments in south eastern Australia (Humphries et al. 2017). The results of this project have demonstrated that lucerne is more tolerant to highly acidic soils than previously described in literature. Forage production in the first calendar year after sowing ranged from 11 to 14 t/ha under rainfed conditions at three of the sites with soil pH<sub>Ca</sub> 4.1-4.3 (Figure 2). However, the addition of 1.2 t/ha lime partially ameliorated the surface soil to pH<sub>Ca</sub> to 4.6 and increased forage yield by 33%, nitrogen fixation by 26%, and plant persistence by 28%. The research showed that lucerne can be productive on acidic soils, but the long term production in these environments is associated with greater risk when this stress is combined with drought, grazing or seasonal waterlogging. The research has culminated in the release of a new acid tolerant rhizobia strain, SRDI736, to be used as a specialised strain for lucerne on acid soils (<pH<sub>Ca</sub> 5.0).



**Figure 2.** Average distribution of lucerne forage production on highly acidic soils (pH<sub>Ca</sub> 4.1 to 4.3) at Tooperang, Pewsey Vale (SA) and Holbrook (NSW). Lucerne produced 11 – 14 t/ha with 52% of production over summer (Humphries et al. 2017).

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## An overview of alfalfa (*Medicago sativa* L.) situation in Argentina\*

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**KEYWORDS:** Lucerne, hay production, hay market Argentina,

**INTRODUCTION** - From the approximately 3.2 million ha of alfalfa grown in Argentina in 2018, about 60% is planted as pure stands and 40% in mixtures with temperate forage grasses. Pure stands are primarily used for dairy and hay production, while mixtures with grasses are usually devoted to beef production. More than 426 varieties have been registered between 1980 and March 2018, and about 120 varieties are currently in the market. Among these varieties, 65% belong to fall dormancy (FD) rates 8-10 and 35% to FD 6-7. The majority of those cultivars come from private companies, either domestic or international. Most of the new releases are provided by USA and, to a lesser extent, Australian breeding programs; however, INTA's varieties currently have about 15% market share. The National Alfalfa Cultivar Evaluation Network is being conducted by INTA Manfredi since 1992 in an attempt to help farmers in the process of choosing the most adapted varieties. This network has 17 locations across the country which represents the wide range of environmental conditions and production systems (rain fed or irrigated). Forage yield ( $\text{t DM ha}^{-1} \text{ year}^{-1}$ ) and stand density (persistence) are statistically analyzed and published annually (Arolfo and Odorizzi, 2017). Results from the last 10 years show that average forage production for FD 5-7 cultivars ranges from 5 t (metric tons)  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Anguil (Semiarid Pampa Region-5 cuts) to 24 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Marcos Juárez (Sub-humid Pampa Region-6 cuts) under rain fed conditions; and 17.2 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Hilario Ascasubi (Southern Pampa Region-6 cuts) to 20 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Viedma (Northern Patagonia-6 cuts) under irrigation. For the non-dormant cultivars (FD 8-10), forage production ranges from 5.3 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Anguil (5 cuts) to 25.2 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Marcos Juárez (8 cuts) under rain fed conditions; and 17.3 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Catamarca (Northwestern Region-8 cuts) to 23 t  $\text{DM ha}^{-1} \text{ year}^{-1}$  in Santiago del Estero (North-Western Region-8 cuts) under irrigation. In bovine production, rotational grazing is still very important in Argentina. Compared to other production systems, grazing alfalfa offers lower production costs and better utilization of alfalfa forage quality; on the other hand, it increases the risk of bloat and requires longer cattle finishing periods. Nonetheless, the use of feed-lot type operations has dramatically increased in the last 15 years. Utilization of corn or sorghum silage and alfalfa hay has become increasingly important for most dairy and beef production systems that use varied degrees of cattle confinement. Despite this, nearly 55% of milk is still produced in grazing-based systems in which alfalfa represents 50% (winter) to 80% (spring and summer) of the total diet.

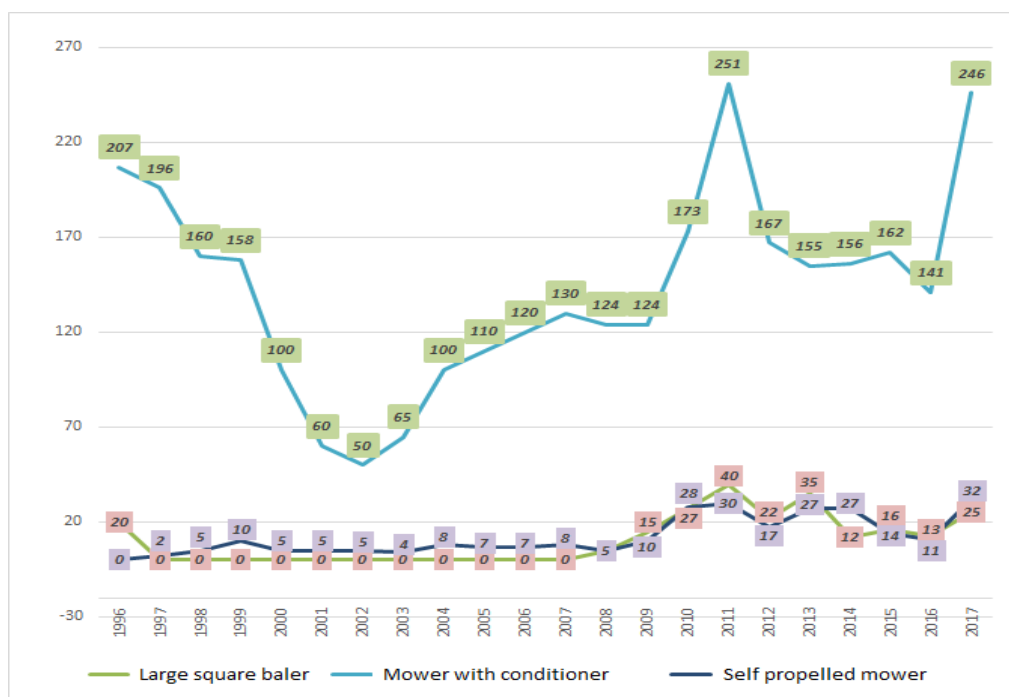
**Hay production** - Nearly 850,000 ha of alfalfa were cut for hay and another 150,000 ha were cut for silage (haylage) in 2017. The vast majority of hay -either as small bales (22 kg), round bales (350 to 450 kg) or prismatic big bales (400 or 750 kg)- is used within the farm or sold in the domestic market. In the latter, quality evaluation is based on subjective (color, presence of leaves, smell, etc.) rather than on objective criteria, i.e. crude protein (CP), relative feed value (RFV), fiber (ADF and NDF) content, *in vitro* dry matter digestibility (IVDMD) and/or moisture. Therefore, the overall hay quality in the internal market is low to medium. In addition, buyers usually pay by weight rather than by quality classes. This has resulted in sub-standard practices in hay making (i.e., improper cutting machinery, delayed cutting, inadequate bale storage, etc.) that are quite common. Conversely, and co-existing with the situation described above, the number of farmers and companies aimed at produce high quality hay has significantly increased in the last decade. The country has excellent environmental conditions for growing high quality alfalfa and the capacity to easily increase the planting area within a few years. Updated technology, including sophisticated hay machinery (Figure 1) and better weed and insect control practices, are also available. Practically all hay is sun cured; however, since the rainfall is mostly concentrated from spring to fall, some cuts along the growing season do not reach the necessary quality to meet international

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\* **CITATION:** Basigalup, D., Giletta, M., Odorizzi, A., Arolfo, V., Sánchez, F. and Urrets Zavalía, G. 2018. An overview of alfalfa (*Medicago sativa* L.) situation in Argentina. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



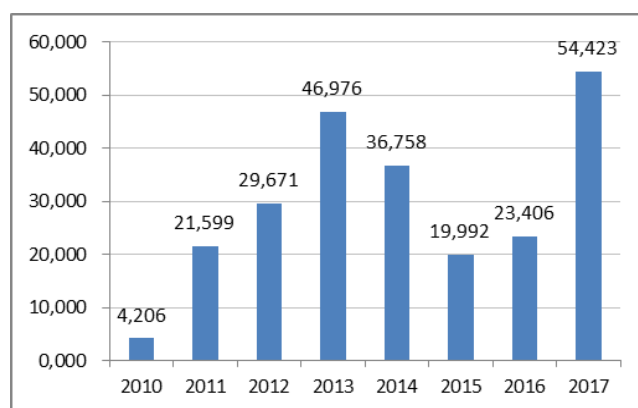
standards. In an attempt to solve this problem, there is an increasing interest in establishing dehydration plants for increasing the production of uniform high-quality hay. Although there is at present only one dehydration plant functioning in Argentina, there are other projects on the way for building other plants.



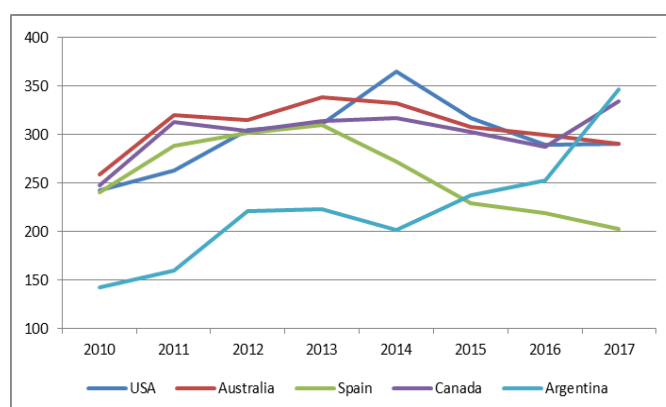
**Figure 1.** Number of technically-advanced hay machinery sold in Argentina during the last 20 years.

Growing alfalfa for hay can be done under rain fed or irrigated conditions. Within the Pampa Region, production is totally rain fed. On the contrary, in the Northwestern, Cuyo and Patagonia regions, irrigation from either rivers or wells (underground water) is used. The most common system is flood irrigation, but the utilization of pressurized systems (like central pivot) is increasing due to its higher water use efficiency. At present, there are about 60,000 ha under rain fed conditions and approximately 80,000 ha under irrigation devoted to high quality –and so potentially exportable- hay production across the country. However, the potential for significantly increasing those areas in the near future is very high. Overall, on-farm actual average alfalfa yields vary greatly, according to soil and climatic conditions, management practices and moisture availability (rainfall or irrigation). For rain fed operations, production can go from 4-6 t DM ha<sup>-1</sup> year<sup>-1</sup> under 3-5 cut systems in La Pampa to 18 t DM ha<sup>-1</sup> year<sup>-1</sup> under 7-8 cut systems in Southeastern Córdoba. Under irrigation, yields can range from 10-12 t DM ha<sup>-1</sup> year<sup>-1</sup> in 4-5 cut systems in Southern La Pampa to 16-18 t DM ha<sup>-1</sup> year<sup>-1</sup> in 8-9 cut systems in Santiago del Estero.

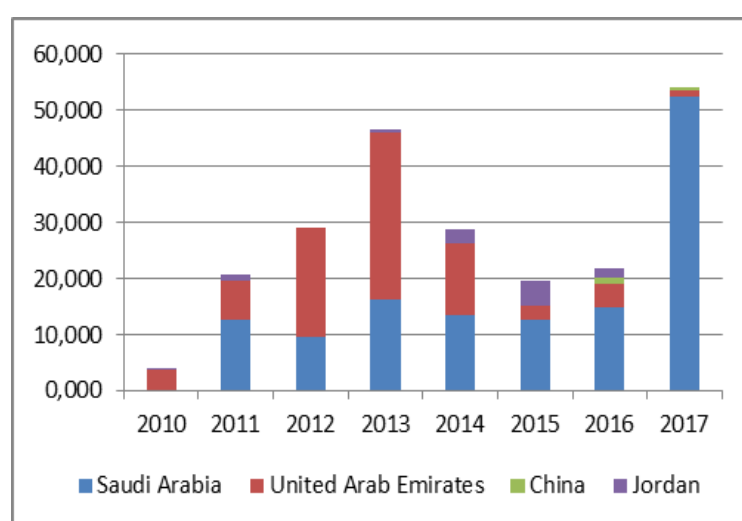
**Hay exports** – During the last decade, alfalfa hay world trade has grown 70% in volume and >95% in value, reflecting the latter the intensity of the demand. In 2017, the global market reached 8.3 million metric tons (ITC, 2018); to this amount, Argentina only contributed 0.7% (54,423 metric tons) (Figure 2). Even though a marginal contributor to the global market, Argentina is gradually improving the average price of its exports since 2014/15, and so considerably diminishing the gap with the main exporters (Figure 3).



**Figure 2.** Argentine alfalfa exports (metric tons) in 2010/2017 period (ITC, 2018).



**Figure 3.** Average international alfalfa hay prices (US\$/ton) in 2010/2017 period (ITC, 2018). The most important export destinations are by far United Arab Emirates (UAE) and Saudi Arabia (SA), and to a much lesser extent, Jordan (Figure 4). In the last two years, there were some exports to China, a destination that likely will be increasingly important in the near future.

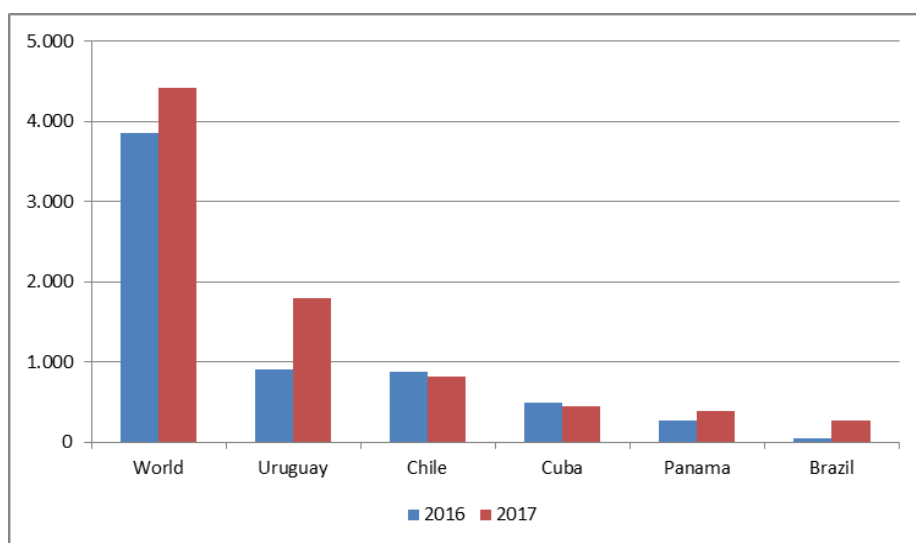


**Figure 4.** Destinations of Argentine alfalfa hay exports (metric tons) in 2010/2017 period (SENASA, 2018).



From Figure 2 it can be noted that there is a general trend for Argentine hay exports to slowly grow, with the exception of 2015 and 2016, when excessive rains prevented to meet the minimum international quality requirements for most of the cuts. Presently, hay exports in the first trimester of 2018 grew 58% compared to the first trimester in 2017: 28,815 vs. 14,447 metric tons, respectively (SENASA, 2018). In the first semester of 2018, hay exports reached 28,206 metric tons with a mean price of USD 342 per t.

In the case of pellets and alfalfa meal, world exports reached 1.2 million t and a global value of USD 305 million in 2017 (ITC, 2017). Differently from hay, pellets are used primarily for horses, rabbits, chickens, pets, and even pigs. Alfalfa meal is becoming increasingly important for different animal ration preparations and other new uses of alfalfa. Argentina exports pellets to mostly Latin American countries, like Uruguay, Brazil, Chile, Colombia, Venezuela and Panama. In 2017, the exported volume was 4,400 t (Figure 5).



**Figure 5.** Exports of alfalfa pellets (metric tons) from Argentina in 2016 and 2017 (SENASA, 2017).

A significant event for strengthening Argentine hay production in 2017 was the creation of the *Cámara Argentina de la Alfalfa* (Argentine Alfalfa Chamber) or CAA for its name in Spanish. This is a private institution that nucleates most of the important hay, pellet and cube companies in the country. CAA's main objectives are to promote the crop; to define best management practices for increasing high quality production; to establish a traceability system and a uniform quality classification for both domestic and international markets; to define a uniform sampling protocol and quality analysis methodology with a qualified lab network; to contribute to solve logistic problems; and to serve as a bridge between hay offer and demand in both international and internal markets.

Another important fact that occurred in Argentina during 2018 was the deregulation of the GE alfalfa containing two stacked events: reduced lignin (HarvXtra™) and Roundup Ready (RR) traits. These varieties will be commercialized in Argentina in 2019. Independently of the advantages of this technology, its advent raises the need for defining a series of measures that allows the coexistence of conventional and GE alfalfas in order to satisfy different market requirements. In doing this, it will be critical to prevent the adventitious presence of GE in hay, seeds and/or honey for sensitive markets.

**CONCLUDING REMARKS** – Based on a solid demand from Asia and Middle East, Argentina has the opportunity to implement an ambitious strategic plan for organizing the alfalfa value chain. The goal should be to consistently increase the production of high quality hay and other value-added products. International quality standards should also promote the search for higher quality in the domestic market, with the subsequent improvement of Argentine animal production chains. To prevent overproduction in the domestic market, a strategy based on product diversification should be implemented. In doing so, geographical production capabilities, high quality crop management protocols, innovative industrial processes and efficient logistics must be established. The latter is extremely important because logistic deficiencies derive in unnecessary higher

costs that impact on Argentine hay export competitiveness. For the domestic market, it is expected a steady increase in meat (beef, pork, sheep and poultry) production; since all of these meat production systems are based on high efficiency and increasing production scale, it is expected that this situation will therefore emphasize alfalfa demand. In addition, milk production is going towards higher scale production models where grazing is being progressively replaced by totally confined systems in which alfalfa hay is provided by specialized third parties from outside the dairy farms. This will also promote the need for higher quality alfalfa feeds.

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## Alfalfa situation in the USA and Canada\*

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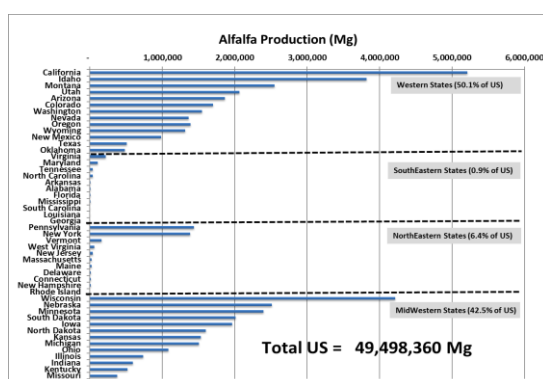
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United States has historically been the largest producer of alfalfa (*Medicago sativa* L.) in the world, followed by Argentina. In 2017, alfalfa production in the form of hay, haylage and greenchop was the 3<sup>rd</sup> largest economic crop in the United States, following corn and soybean (Table 1). All hay (grasses and alfalfa) is the #3 economic crop in the US. Alfalfa, considered alone, has competed from year to year with wheat for the #3 or #4 spot (Table 1). It is important to note that the largest value of forages is realized through animal products, not hay itself. In 2017, cattle and milk production, both highly dependent upon alfalfa and forage crops, totaled well over \$100 billion in economic value in the US (Table 1). Taken as a whole, these are the most important agricultural products in the US.

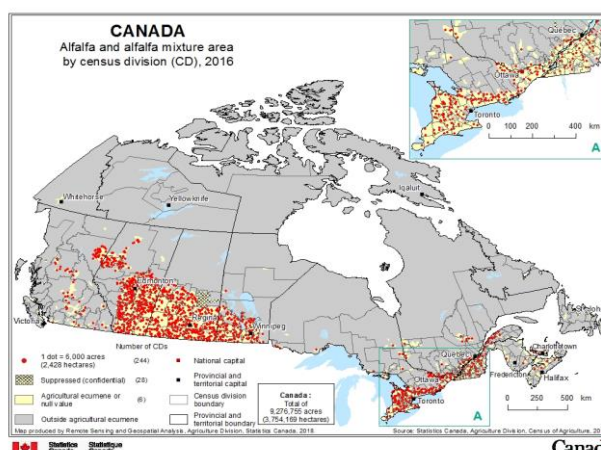
Alfalfa in the United States is concentrated in the West and upper Midwest states (Figure 1), with about 50% in western regions, 42% in the upper Midwest, and about 6% in the Northeast. Very little alfalfa is grown in the southeastern or southern US states, although grass hays are significant in those regions.

Crop/Product	2014	2015	2016	2017	RANK (\$)
(US\$ Billion Dollars)					
Corn Grain	52.9	49.3	51.7	48.5	1
Cattle and Calves	71.0	78.7	65.9	69.4	
Milk and Cream	49.6	35.9	34.7	38.1	
Soybean	39.5	35.2	40.9	40.0	2
Hay/Forage (all)	19.0	16.5	15.6	16.1	3
Hay (alfalfa)	10.5	8.4	7.5	9.3	(3)
Wheat (all)	11.9	10.0	9.1	8.1	4
Cotton (all)	5.1	4.0	5.6	7.2	5
Potatoes	3.9	3.9	3.9	4.6	6
Rice	3.1	2.4	2.4	2.2	7
Peanuts	1.1	1.2	1.1	1.6	8
Sorghum	1.7	2.1	1.3	1.2	9
Sugarbeet	1.4	1.0	1.3	1.2	10
All Field Crops	149.9	136.1	142.6	141.4	
All Fruit and Nuts	31.9	27.1	28.4	30.0	

Source: USDA-NASS (NASS.USDA.GOV)



**Figure 1.** US Production of alfalfa hay, haylage and greenchop (2012 Census).

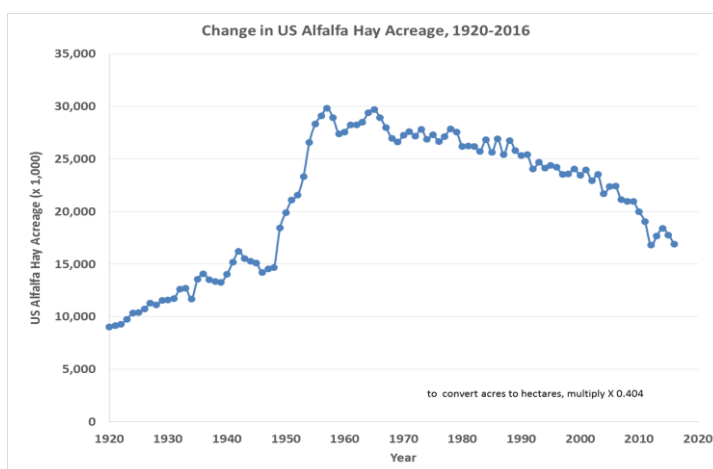


**Figure 2.** Acreage of alfalfa in Canada

Alfalfa production in Canada, at about 3.7 million hectares in 2016, is concentrated in the western prairies, southern Ontario and the eastern provinces (Figure 2).

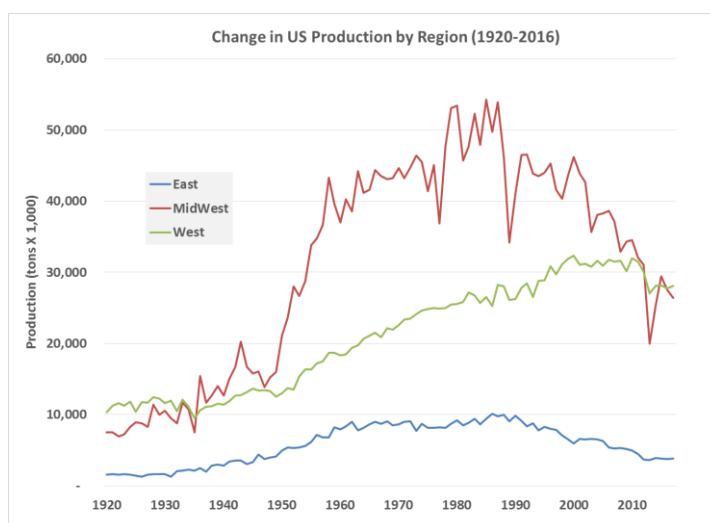
\* **CITATION:** Gardner, D.; Putnam, D. H. 2018. ALFALFA SITUATION IN THE USA AND CANADA. In: Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

**Area has declined.** Acreage of alfalfa in the US and Canada has declined significantly during the past 40 years (Figure 3), accelerating in the past 10 years (Figure 3). (Note that this number should be about 5-8% higher than shown here, adding the haylage, grazing and greenchop numbers). The US alfalfa acreage reached a maximum acreage and production in the 1960s through the 1990s of 25-30 million acres (12 million ha) and declined to less than 17 million acres (6.9 million ha) in 2016 (Figure 1). Production was less than 60 million tons (54 million Metric Tons) in 2016, approximately a 33% decline since the maximum of 90 million tons in the early 1980s (Figure 3). Similarly, Canada has seen a decline in acreage from 2011 to 2016, falling 17% in 5 years from 4,544,662 ha to 3,754,169 ha (Canada Ag. Statistics).



**Figure 3.** Changes in US alfalfa hay acreage, 1920-2016 (USDA-NASS).

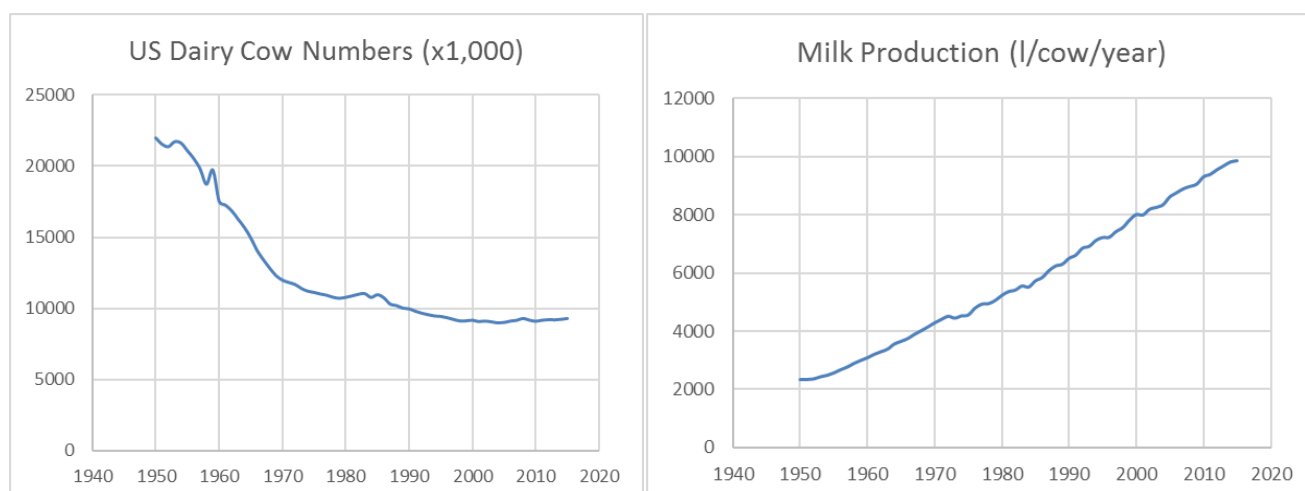
**Regional Differences.** While western and midwestern alfalfa production regions were about equal in the early 20<sup>th</sup> Century, after 1950, midwestern production (mostly rainfed) exploded and increased to high levels from 1950 through the early 1990s, dominating US production (Figure 4). This increase was made possible by improved alfalfa varieties and techniques and the expansion of midwestern dairying from 1950-1980. Western alfalfa hay production (>95% under irrigation) steadily increased 140% from 1950 through 2012, driven largely by the movement of dairy production to the West. Currently, nearly 50% of US milk production occurs in the 11 western states. Eastern states alfalfa production has always been lower than other regions, but has also declined from the late 1980s. The most dramatic decline has been in the Midwestern corn-belt states (Figure 4).



**Figure 4.** Regional changes in alfalfa hay production, 1920-2016 (USDA-NASS).

**Why these trends?** The reasons for these changes are not always 100% clear, but a few causes can be described. Here are some of the important factors:

- Alfalfa must compete with subsidized crops (corn, wheat, cotton,) for acreage.
- Alfalfa does not receive government subsidies nor crop insurance programs.
- All forage has declined as a percentage of dairy rations, including alfalfa.
- Concentrates of various types have increased as a % of rations for dairy & beef.
- Corn, sorghum and small grain silage are low-cost, high energy forages.
- Stagnant yield improvements in alfalfa compared with corn.
- Profitability of corn during the 2000s in the Midwest due to ethanol expansion.
- Increase in high value crops, orchards and vineyards in irrigated regions.
- Higher equipment and labor requirement for than alfalfa compared with grains.
- Aging farm population and land tenure favors simpler cropping patterns (grains).



**Figure 5.** US dairy cow numbers and production per cow, 1950-2015 (USDA-NASS).

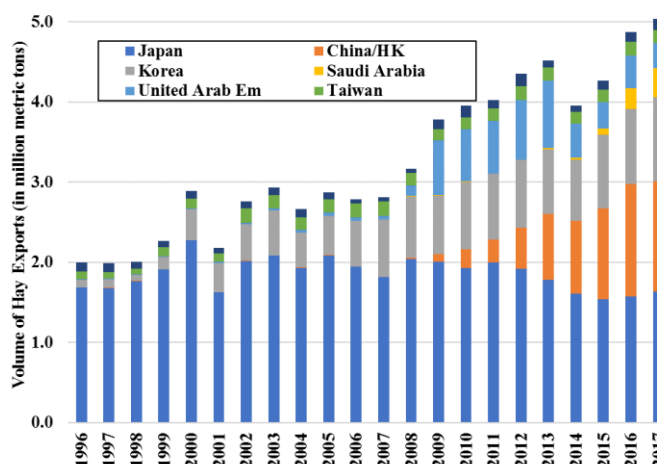
**Dynamic Dairy Trends Drive Alfalfa.** It's important to look at trends in US dairy production dynamics to understand alfalfa trends. While dairy cow numbers in the US have declined since the 1950s, the numbers have been relatively stable over the past 20 years (Figure 5). However, production efficiency has increased dramatically – increases in milk production per cow has ranged from 2-2.5% per year over many years, so that an average cow in 2015 produces roughly 5 times as much milk as in 1950 (Figure 5)! These increases continue today. *How does this relate to alfalfa?* Dairy rations have changed dramatically in the past 30 years from high forage to lower forage components, adapting high levels of concentrates (e.g. canola meal, cottonseed, soybean meal, corn grain, almond hulls or citrus pulp). Corn silage has increased significantly in the major dairy regions of Wisconsin, California, Idaho, New Mexico and other states, often replacing alfalfa as a percentage of the ration. It's been estimated that the average ration of alfalfa fell from about 11.5 lbs/cow/day (5.2 kg/day) in 2009 to 7.5 lbs/cow/day (3.4 kg/day) in 2017 in California (CDFA data), a decline of 34% in 8 years. Much of this is driven by the relative high price of alfalfa hay compared with other feeds, including corn silage, grain and concentrates.

*What are alfalfa's unique properties?* In spite of these trends, alfalfa still has a key role in dairy rations. There are properties that are unique to this crop and not strictly subject to least-cost ration balancing (Robinson, 2014). In terms of quality, alfalfa has a unique combination of digestible energy, intake properties, palatability and protein that is still the mainstay for dairy rations and hard to duplicate with other feedstocks. When alfalfa is priced favorably, it is the highly-preferred forage crop, in combination with corn silage and other feedstuffs. The role of 'functional fiber' should not be ignored – which is one of the key values for alfalfa hay. We should note that some feeds may be cheaper sources of energy, or cheaper protein sources, but few have the combination of 'functional fiber' along with protein, intake potential and digestible energy.

**Yield Gap.** Although competing crops and changes in ration have been important, one cannot ignore the relatively stagnant improvements in yield for alfalfa. Michael Russelle discussed this issue in detail (Russelle, 1917) and analyzed by Brummer & Putnam (2017). Russelle (2017) estimated that the top producers harvested 1.5 to 2.7 times as much dry hay per acre vs. middle producers, equivalent to a net profit of \$285 to \$690/acre. There is clearly a scope to dramatically improve yields of alfalfa.

**Exports.** One important trend in the USA is the development of export markets. Western hay exports (alfalfa and grasses) have increased rapidly in recent years, and was about 5.0 million Metric Tons (MT) in 2017 (Figure 6), valued in excess of \$1.5 billion. The major growth areas have been China and Saudi Arabia. Approximately 4.7% of US production of alfalfa is exported (see Putnam et al., 2018).

**Adoption of GE Alfalfa.** Genetically Engineered (GE) traits, have been in production in alfalfa in the US since 2005. There are currently two GE traits that are commercialized in alfalfa: **Roundup Ready Alfalfa (RRA, 2005)**. – This trait confers tolerance to glyphosate in alfalfa, enabling the use of glyphosate (Roundup) herbicide for alfalfa forage and seed production. **Reduced Lignin (HarvXtra, 2014)** – This GE trait down-regulates enzymes which produce lignin in alfalfa, resulting in a reduced lignin phenotype. Both RRA and HarvXtra have been of strong interest to alfalfa growers in the US, but have not been adapted by the majority of growers, as has been the case with GE corn, soybean, cotton, and sugarbeets. GE traits remain controversial in the US and some markets (organic and export) do not accept GMO alfalfa.



**Figure 6.** Export trends for all hay shipped from western US ports. Approximately 55% is alfalfa.

## SUMMARY

Although North America leads in world alfalfa production, there have been dramatic reductions in US alfalfa acreage in the past 30 years. There are a series of factors which contribute to these changes in acreage, including changes in dairy ration formulation, competing crops, and lack of scientific progress in alfalfa yields. The reduction in acreage should be a cause concern for the alfalfa industry leading to improvements in production technology, yields, genetic improvements, and innovation to improve existing markets and develop new markets. Two meaningful trends in the past 10 years have been the increase in exports and adaptation of GE traits in alfalfa. Importantly, alfalfa has a key role to play in sustainability of agriculture due to its many environmental benefits.

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## Supply and demand of alfalfa in China.\*

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**KEYWORDS :** Alfalfa, Supply, Demand, Price

In recent years, alfalfa industry in China has made rapid progress. Chinese government launched “The Action Plan for developing alfalfa production to revitalize the dairy industry” in 2012, in which the alfalfa industry was paid more attention. The central government provided some support policies including subsidies for alfalfa enterprises and farmers. Alfalfa planting area has increased a lot in China, and commodity rate is also increasing. It is currently the golden period for alfalfa in China.

The supply of alfalfa mainly includes both planting output and import. The alfalfa planting is distributed in 23 provinces of China. The planting area of the commercial alfalfa in Inner Mongolia and Gansu Province has reached 2/3 of the total area of alfalfa in China by now. From 2004 to 2007, affected by policies on subsidies and protection of grain, farmers and herdsmen decreased alfalfa planting areas. In 2005, China's alfalfa planting area decreased by 466,700 hm<sup>2</sup>, and remained at around 3,733,300 hm<sup>2</sup>. After the outbreak of the “melamine incident” in 2008, dairy enterprises and farmers were more fully aware of the important role of alfalfa in the safety of dairy production. The increasing demand has stimulated the enthusiasm of farmers and enterprises to plant alfalfa. China's alfalfa planting area increased steadily, reaching 3.408 million hm<sup>2</sup> in 2016. But the sales fluctuated. In 2009, the sales were only 1.83 million tons, lowest in the past 10 years. In 2017, affected by the policy, the sales soared to 3.41 million tons.

Before 2008, China's imports of alfalfa hay accounted for less than 0.1% of the total supply of alfalfa. From 2006 to 2010, the import of alfalfa hay increased from 0.03 million tons to 218,000 tons, an increase of more than 700 times; in 2017, it increased further to 1.4 million tons, and the import price increased from US\$270.8/t in 2010 to US\$303/t. The alfalfa hay is mainly imported from USA, Canada, Spain and other countries.

In recent years, planting area and yield of Chinese alfalfa seeds have declined to a certain degree, but the sales has increased. In 2015, the sales of alfalfa seeds reached 8478.9 tons, accounting for 31.71% of the total seed sales in China, up 10% year-on-year. The proportion increased by 5.5% compared with 2001, and the output increased by 3.55 times. The increase in seed sales has improved the supply in the market. However, domestic alfalfa seeds do not have an advantage in terms of the stability of quality and price, and the supply of alfalfa seeds is still insufficient.

The demand is from dairy farming, other animal husbandry, and export. The development of dairy industry and animal husbandry has led to an increase in the consumption of alfalfa. The overall domestic demand of alfalfa in China increased from 1,725,200 tons in 2008 to 5.38 million tons in 2015, an increase of nearly 3.7 million tons. The average annual growth rate of alfalfa demand was 17% from 2008-2015. In China, the current export of alfalfa products is very small. Alfalfa is mainly exported to countries such as East Asia and Southeast Asia. Although the demand for alfalfa in Japan and South Korea has been strong in recent years, Japan and South Korea often imposed trade restrictions and technical barriers on China's alfalfa due to geographical relations and economic friction, which affects the sustainability and stability of China's alfalfa export. After 2012, China's alfalfa supply cannot meet the domestic demand. China has become an alfalfa net import country. Import price of alfalfa is significantly higher than export price. The import dependence of alfalfa is high in China.

Despite the rapid development of the alfalfa industry, alfalfa supply cannot meet the increasing demand. Especially high-quality alfalfa needs to be imported from other countries. The gap between alfalfa supply and demand is increasing. In order to balance the alfalfa supply and demand, the factors affecting alfalfa were

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analyzed by using panel data of China's main planting alfalfa provinces in 2001-2015. The result shows that alfalfa supply is mainly influenced by last-year purchase price, the last-year planting area, and the last-year related commodity prices. The demand of alfalfa is mainly influenced by the purchase price, the price of raw milk, cow stock, and the processing ability of the enterprises. By using the Grey Markov model and secondary exponential smoothing model to forecast the supply and demand of alfalfa, the result shows that supply and demand gap of alfalfa will increase.



## Water footprint of alfalfa hay production in Córdoba, Argentina \*

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**KEYWORDS:** Water use, irrigation, environment, weather, Cropwat

**INTRODUCTION:** In 2017, Argentina exported 58,848 t of alfalfa feed, for a value of nearly 19.8 million USD (UNcomtrade, 2018). Even though it only represents 0.7% of the world trade, it involves large amounts of virtual water in the product, which deserves the attention of the stakeholders of the alfalfa value chain, for a sustainable, efficient and equitable use of natural resources. In this sense, the water footprint is one of the family of environmental footprints that quantifies the amount of water consumed, evaporated and polluted. Worldwide, various activities consume or pollute water, but most of the water use occurs in agriculture (Hoekstra, Chapagain, Aldaya, & Mekonnen, 2011). Water consumption and pollution can be associated with specific activities, but until recently, there was little awareness to relate it to the structure of the global economy that supplies the various consumer goods and services. Hoekstra and Chapagain (2008) quantified the effects of consumption and trade on water resources use, by visualizing the hidden water in the products. Fresh water is a global resource, but due to the world trade, there is a spatial disconnection between the use of water resources and its consumers. For instance, this is the case of alfalfa hay exported from Argentina to the Middle East, i.e., production and final consumption are located in different places. Therefore, in order to study the impacts of consumption of alfalfa on the globe's water resources, it is necessary to model the supply chain in order to trace the origins of the product. Visualizing the hidden link between consumption and water use forms the basis for the formulation of new strategies of water governance; in which producers, traders and consumers have a role, not only as direct water users, but also as indirect water users. Therefore, the importance of a water footprint study relies on various reasons: a national government that imports alfalfa hay may be interested in knowing its dependency on foreign water resources, or a local government may be interested to know the sustainability of water use in the areas where import products originate.

In essence, the water footprint of a product is the total volume of fresh water that is used directly or indirectly to produce the product, considering water consumption and pollution in all steps of the production chain. The accounting procedure is similar to all sorts of products, including the agricultural, industrial or service sectors. The water footprint of all products is formed by a green, blue and grey component, and in the case of agricultural products, it is expressed in m<sup>3</sup> t<sup>-1</sup> or liters kg<sup>-1</sup> (Hoekstra, Chapagain, Aldaya, & Mekonnen, 2011).

In the scientific literature there are two parallel developments: the methodology of the Water Footprint Network (WFN), and on the other hand, the water footprint estimated as part of the Life Cycle Assessment (LCA), which developed comprehensive methodologies to include environmental impacts related to water in LCA studies and framed the international standard 14046 on water footprint (ISO, 2014). These approaches have been in conflict in the past few years. Both methods have the goal to preserve water resources, but in different ways. The LCA estimates the potential environmental impacts of human activities on climate change, human respiratory impacts, land use, etc., including water use. LCA calculates quantitative impact indicators related to global warming, eutrophication, acidification and toxicity to human and ecosystems. The LCA method focuses on the sustainability of products, with a comprehensive approach, whereby water (LCAwater) is just one area of attention among others (e.g., carbon footprint, land use). On the other hand, the WFN method addresses freshwater resources appropriation, including the quantification and mapping of the three distinct types of water use: the blue, grey and green water footprints. WFN focuses on analyzing the sustainable, efficient and equitable allocation and use of freshwater in both local and global context with either a product, consumption pattern or geographic focus (Boulay, Hoekstra, & Vionnet, 2013).

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Mekonnen and Hoekstra (2010) quantified the green, blue and grey water footprint of 126 crops in the world, for the period 1996-2005. They used a gridbased dynamic water balance model to calculate crop water use over time, taking into account the daily soil water balance and climatic conditions for each grid cell, including the water pollution associated with the use of nitrogen fertilizer in crop production. They also calculated the water footprint of more than two hundred derived crop products, including various flours, beverages, fibres and biofuels, using the WFN method. Nevertheless, alfalfa was not included in this study.

There is little research on the water footprint of alfalfa. Fulton, Cooley & Gleick (2012) reported that alfalfa has the second greatest water requirements in the state of California, one of the crops that provide the primary inputs to California's meat and dairy industry. It also supplies the demand for alfalfa as animal feed to the expanding global dairy industry, particularly in China, Japan, and the United Arab Emirates (WFN, 2015). Other authors study the water footprint from the demand side in dry areas (Mojtabavi, Shokoohi, Etedali, & Singh, 2018).

**Objectives:** The objective of this work was to assess the green, blue and grey water footprints of alfalfa hay, produced in both rainfed and irrigated systems, in Córdoba, central Argentina, in dry, wet and neutral periods.

**MATERIALS AND METHODS:** The research followed the method of Hoekstra et al. (2011), which assesses the amount of water used in production. It calculates the quantity of surface water and groundwater required to produce a good (Blue Water Footprint), the volume of rainwater necessary for the crop (Green Water Footprint) and the amount of freshwater needed to dilute the wastewater generated, in order to maintain water quality, as determined by local regulations (Grey Water Footprint).

Blue Water Footprint (**Blue**) is *“the volume of surface and groundwater consumed as a result of the production of a good or service. Consumption refers to the volume of freshwater used and then evaporated or incorporated into a product. It also includes water abstracted from surface or groundwater in a catchment and returned to another catchment or the sea. It is the amount of water abstracted from groundwater or surface water that does not return to the catchment from which it was withdrawn”* Green Water Footprint (**Green**) is *“the precipitation on land that does not run off or recharge the groundwater but is stored in the soil or temporarily stays on top of the soil or vegetation. Eventually, this part of precipitation evaporates or transpires through plants. Green water can be made productive for crop growth (although not all green water can be taken up by crops, because there will always be evaporation from the soil and because not all periods of the year or areas are suitable for crop growth)”*. The Grey Water Footprint (**Grey**) of a product is *“an indicator of freshwater pollution that can be associated with the production of a product over its full supply chain. It is defined as the volume of freshwater that is required to assimilate the load of pollutants based on natural background concentrations and existing ambient water quality standards. It is calculated as the volume of water that is required to dilute pollutants to such an extent that the quality of the water remains above agreed water quality standards”* (Hoekstra, Chapagain, Aldaya, & Mekonnen, 2011).

The area under study included 62,516 ha for pure alfalfa and 6,111 ha of mixed pasture, concentrated in the Departments of Río Segundo, Tercero Arriba and Río Primero (Córdoba, Argentina). The average temperature is 16.5 ° C, with a frost-free period of 255 to 270 days and average rainfall of 650 mm, seasonal distribution monsoon type. 67.2% of the soils are class III, suitable for agriculture. The class VI and VII soil types for grazing occupy 28.1%, while the rest corresponds to land not suitable for agricultural use. In this area there are two milk basins: Centro, and Villa María, with 18.9% and 14.7% of dairy farms, respectively. The production of alfalfa in this area has a relevant participation within the distribution of implanted crops. For the production of alfalfa, a soil analysis is performed before sowing, to determine the fertilization needs. It usually requires the application of phosphorous fertilizer. Another soil analysis is conducted every year to help maintaining the fertility. The seeding density is 12 kg per hectare of inspected, inoculated and pelleted seed (with resistance to pests and diseases). Direct sowing is done in March, in deep, well-drained soil, with a pH of 6.5 to 7.5, low amount of stubble on the surface, with special care in achieving a uniform sowing depth (0.5 to 1.5 cm). Before seeding, a chemical control of weeds is done, as well as the use of post-emergent herbicides and aphicides. The useful life of the alfalfa crop assumed in this study is three years. Most of this area was devoted to the production of both prismatic and round bales. The crop management is described in Barberis et al. (2015). The useful crop life considered for this model was three years, with a dry matter yield of 12, 15 and 13 t ha<sup>-1</sup> year<sup>-1</sup> of for the first, second and third year respectively. The small prismatic bales had an average weight of 22 kg, while round and

large prismatic bales weighted 500 kg. For the production of alfalfa under irrigation, the work of Barrenecha et al. (1999) was used as a reference, which reported average yield differences of 48.21% above the rainfed crop. In order for this model to be representative and to reflect the variability of weather, the production of alfalfa was studied during a dry period (2003, 2004 and 2005), a neutral period (2006, 2007 and 2008); and a wet one (2014, 2015 and 2016).

The software Cropwat 8.0 (FAO, 2009) was used to estimate the water requirements, based on weather, soil and ecophysiological variables. The climatic data was obtained from the nearest and most representative meteorological stations of Manfredi, Pilar and Córdoba. The edaphic variables were defined according to the INTA's Soil Atlas (Cruzate et al., 2018), while the ecophysiological variables contained in the Cropwat / FAO database were reviewed with current data. The first step was to calculate the reference evapotranspiration (ET<sub>0</sub>) and crop water requirements. Although several methods exist to determine ET<sub>0</sub>, the Penman-Monteith Method has been recommended as the appropriate combination method to determine ET<sub>0</sub> from climatic data on temperature, humidity, sunshine and windspeed. Specifically, Cropwat required the input of climatic, crop and soil data, as indicated in Table 1.

**Table 1:** Input data required by the software Cropwat

CLIMATIC DATA	CROP DATA	SOIL DATA
Maximum temperature (°C)	Crop coefficient (Kc)	Soil type
Minimum temperature (°C)	Phenological stages (days)	Moisture content (%)
Average temperature (°C)	Seeding date	Maximum infiltration (mm day <sup>-1</sup> )
Precipitation (mm/ha)	Root depth (cm)	Maximum root depth (cm)
Relative humidity (%)	Crop height at harvest (cm)	Initial soil moisture content (%)
Sunshine hours (h)	Permanent wilting point (%)	
Average daily windspeed (m sec <sup>-1</sup> )	Crop yield (t ha <sup>-1</sup> )	

With this information, the software calculates the specific crop evapotranspiration (ET<sub>c</sub>), as  $ET_c = ET_0 * K_c$  (in mm day<sup>-1</sup>), where K<sub>c</sub> = Crop coefficient. The following step is to calculate the crop water requirement (CWR) or Green Water Footprint (**Green**), in m<sup>3</sup> ha<sup>-1</sup>, as  $CWR = \sum ET_c$  (accumulated over the entire growth period). The last step in Cropwat is to calculate the water need, the irrigation requirement, or Blue Water Footprint (**Blue**), as the crop water requirement (CWR) minus the effective precipitation:  $BWF = CWR - \text{effective Ppt}$ .

Finally, in order to determine the Grey Water Footprint (**Grey**), the application of phosphorus fertilizer was considered, establishing a leaching coefficient of 3% and a maximum allowed concentration of 4 µg L<sup>-1</sup> (Franke et al., 2013).

**RESULTS:** Table 2 shows the results obtained for the Green, Blue, Grey and Average Water Footprints in the production of alfalfa in rainfed and irrigated regimes respectively, for dry, neutral and wet years.

**Table 2.** Water footprint ( $\text{m}^3$  of water per t of hay).

		RAINFED				IRRIGATED				
		Green	Grey	Total	Avg	Green	Blue y	Gre	Total	Avg
Dry	2003	758	91	849	859	140	382	37	560	774
	2004	902	49	951		473	341	30	844	
	2005	728	48	776		619	264	35	918	
Neutral	2006	728	84	812	881	247	317	37	601	728
	2007	826	47	874		440	293	30	763	
	2008	908	48	956		482	303	35	819	
Wet	2014	562	75	638	819	355	221	37	614	743
	2015	812	38	850		488	227	30	745	
	2016	927	42	969		683	151	35	869	

Even though there is not much scientific information about the water footprint for alfalfa, the estimates from the California Agricultural Statistics Office (2012) show that the average water footprint of alfalfa hay in California is  $950 \text{ m}^3 \text{ t}^{-1}$ . Therefore, the average value of for  $800 \text{ m}^3 \text{ t}^{-1}$  for Argentina is 15% lower than California, which might be a competitive advantage in markets willing to pay a premium for the reduced water footprint.

Regarding virtual water, a quick estimation indicates that if all the exports from Argentina for the year 2017 were from a rainfed production, the exported virtual water would have been 50 million  $\text{m}^3$ , whereas it would have been 44 million  $\text{m}^3$  if irrigated (Table 3).

**Table 3:** Estimation of the virtual water contained in alfalfa hay exports

	Tons of hay	VIRTUAL WATER ( $\text{m}^3 \text{ YEAR}^{-1}$ )	
		Rainfed	Irrigated
2013	46,976	40,058,158	35,137,474
2014	36,758	31,344,894	27,494,535
2015	19,194	16,367,428	14,356,877
2016	23,406	19,959,154	17,507,402
2017	58,848	50,181,847	44,017,585

**CONCLUSIONS:** The average water footprint of alfalfa hay in Argentina ranged between 819 and  $881 \text{ m}^3 \text{ t}^{-1}$ . This variability is due to changes in rainfall between years. On average, the total water footprint increased 5% in dry years and 8% in neutral years. When the crop was irrigated, the average footprint decreased to a range between 728 and  $774 \text{ m}^3 \text{ t}^{-1}$ . On average, the total water footprint increased 4% in dry years. The irrigation

decreased the total average water footprint an 11% in dry years, 21% in average years, and 10% in wet years. This study successfully characterized the water footprint of alfalfa hay production in Córdoba, Argentina and opened the way for further research. Simultaneously, it draws the attention to the amount of virtual water exported annually.

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## Irrigation research on yield and quality in China\*

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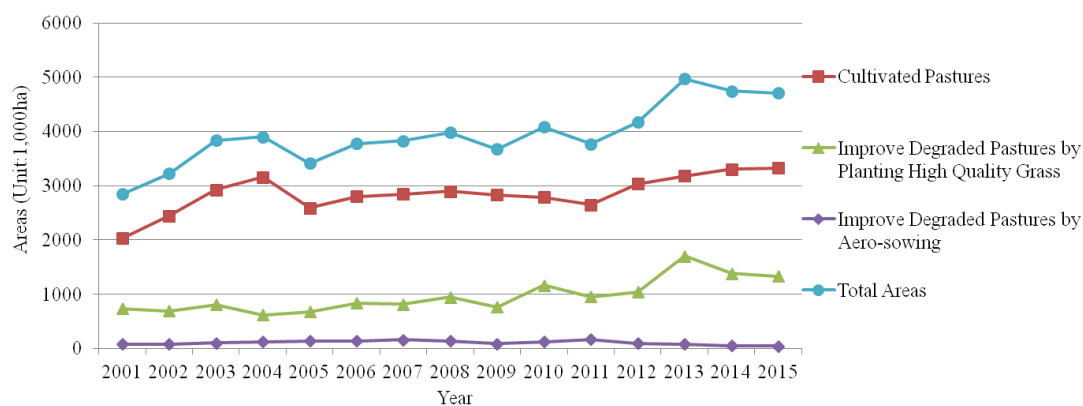
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**KEYWORDS:** Irrigation, alfalfa, yield, quality, China

### Distributions of Main Alfalfa in China

The areas of alfalfa in China was 4.71 million hectares by 2015 (China, 2016), including cultivated pastures and improved degraded pastures. These degraded pastures, accounting 30% of the total areas, improved the productivity by aero-seeding and artificially planting high quality grass (Li et al., 2015). Since 2009, China has introduced a series of policies to encourage the development of alfalfa industry, which greatly inspired farmers' passion (Fig.1). The areas of alfalfa were generally increasing from 2001 to 2015, although the areas decreased slightly in some years (Tab.1). However, the production of commercial alfalfa is far from meeting the requirements of animal husbandry due to small areas (0.43 million hectares) and low commodity rate, with 3.67 million tons by 2015. The alfalfa is often considered an extravagant use of water compared with other crops due to its greater evapotranspiration rates. Many studies showed that alfalfa production had high related to the irrigation. Usually, the water requirement of alfalfa is mainly affected by climate region, harvest times, irrigation conditions and other factors. The annual water requirement of alfalfa differs geographically in China. Specially, the annual water requirement of alfalfa is about 500 to 700 mm in Northeast region, 600 to 750 mm in North China Plain, 700 to 900 mm in Loess Plateau and Hetao-irrigated region, and 600 to 1300 mm in the Northwest (Sun et al., 2005). Over 70% of alfalfa areas distributes in the arid and semiarid regions, such as Xinjing, Gansu, Inner Mongolia, Ningxia, etc. (Fig. 2 (a)). The productivity of alfalfa in these areas where the annual rainfall is about 200 to 400 mm (Fig.2 (b)) are extremely limited by the shortage of water resources, with average yield of 6068kg ha<sup>-1</sup> (China, 2016). The total alfalfa irrigated areas reached 695,000 ha in China, including underground water irrigated areas of 163,000 ha (China, 2016).



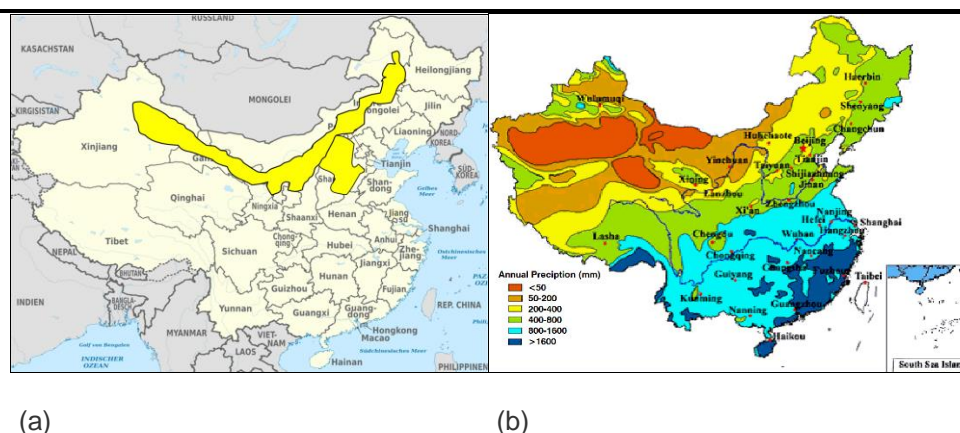
**Figure 1:** Alfalfa areas from 2001 to 2015 in China

\* **CITATION:** Yan, H.\*, Li, M., Wang, Y. 2018. Irrigation research on yield and quality in China. :IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



**Table 1:** Alfalfa hay yield and total production in China

Year	Hay yield (kg ha <sup>-1</sup> )	Total production (1,000 tons)
2001	7500	21360
2002	7530	24040
2003	7785	29890
2004	8535	33300
2005	8595	29310
2006	8850	33430
2007	8370	32050
2008	8025	31910
2009	7725	28450
2010	7350	30000
2011	7425	28020
2012	6915	28780
2013	6690	33250

**Figure 2:** Distributions of commercial alfalfa (a) and precipitation (b) in China

### Alfalfa Water Use Characteristics

Alfalfa is a high-water use crop because it has a long growing season, a deep root system, and a dense mass of vegetation. The amount of water needed is governed by temperature, wind, humidity, and the amount and intensity of light. However, the irrigation requirement for alfalfa is governed by rainfall and the water holding capacity of the soils growing the crop. Commonly cited ranges in water requirements for alfalfa are 400 to 2250 mm of water per season (Sun et al., 2005). It is crucial to yield and quality of alfalfa from branch to bud stage in which should be ensured supply of water (Dong et al., 2006; Yang et al., 2008). Alfalfa is quite tolerant of drought or extended periods without highly available water. As much as 50 to 65% of the available soil moisture can be removed between irrigations early and late in the season, but only 35 to 50% removal will give better results during high water-use periods (Wang et al., 2004; Kou et al., 2014). Maintaining higher levels, especially for slow-draining soils, may cause loss of stand and competition from grass invasion.

### Irrigation in Alfalfa

Alfalfa yield and quality are both related to irrigation amount. Irrigation has become one of the major limiting factors for alfalfa production in the main alfalfa areas in China because of severe water shortages. Deficit irrigation has been an effective way to provide irrigation management for water shortage areas (Li et al. 2017; Lindenmayer et al., 2011), although many studies have shown that alfalfa yield benefits from irrigation (Pembleton et al., 2009). Reducing the irrigation amount caused a reduction in yield (Lindenmayer et al., 2011) but improved water use efficiency (WUE) and alfalfa quality. Water stress was generally highly favorable for alfalfa quality because drought delayed alfalfa maturation (Halim and Buxton, 1989). The crude protein (CP) concentration declined when the alfalfa biomass yield significantly increased, especially during the growth period from the branching stage to harvest (Zhang, 2007).

The irrigation methods has high related to growth and WUE of alfalfa by distribution of water and root in soil. The main irrigation methods in China included surface irrigation (pipe irrigation and border irrigation), sprinkler irrigation (center-pivot irrigation, hose-fed traveler irrigation, solid-set irrigation) and drip irrigation (surface and subsurface drip irrigation). The WUE of alfalfa under subsurface drip irrigation, sprinkler irrigation and surface irrigation were 2.03 to 2.47, 1.90 to 2.6 and 1.37 to 2.26 kg m<sup>-3</sup>, respectively (Guo et al., 2014; Tao et al., 2015; Sun et al., 2005; Li et al., 2017). Surface irrigation is the most widely used irrigation method in alfalfa irrigation area, although surface irrigation has lower WUE than others. Drip irrigation is considered as one of the efficient water-saving irrigation methods by reducing the loss of water (runoff and invalid evaporation of soil) during the irrigation process. Compared to surface drip irrigation, subsurface drip irrigation is more suitable for alfalfa due to its deep rooted perennial crop. However, there are still a lot of problems in the application of surface and subsurface drip irrigation for alfalfa, such as the clogging of dripper, the damage of drip lines caused by machinery during the harvest and the insufficient water supply at seedling stage (Camp, 1998). Therefore, center-pivot irrigation system has been widely used method of water-saving, especially in the developed countries of alfalfa industry in the world. In recent years, the use of center-pivot irrigation systems has gradually increased in China (Yan et al., 2009) because of advantages in irrigation efficiency, coverage of irrigated area, automation, and labor costs. It was estimated that over 70,000 ha of irrigated alfalfa were equipped with center-pivot systems in the semiarid region of Inner Mongolia, which is one of the largest commercial grass zones of China.

### **A case of center-pivot irrigated alfalfa in China**

This research was conducted in 2014 and 2015 at Saiwusu, Inner Mongolian Plateau, Northwest China (38°56'N, 106°49'E). The climate was a typical temperate continental semiarid monsoon with a summer precipitation pattern. Over 50% of the precipitation occurs from July to September, and long-term average annual precipitation was approximately 250 mm. The soil type was sandy loam with bulk density of 1.3 g cm<sup>-3</sup> and the pH of 8.5. In the study, three irrigation levels (100%, 80%, 60% ET) were used to evaluate the effects of irrigation amounts on alfalfa yield and quality. For assessing the effects of water distribution variation of center pivot system on alfalfa yield and quality, water application depths, alfalfa yield and quality between the first span, second span, overhang, and end gun were also compared. The results showed there was no significant difference in annual yield between 100% and 80% ET irrigation levels. Compared to the irrigation at 100% and 80% ET level, the irrigation at 60% ET level caused a significant reduction of yield by 10% and 11%, respectively. As the irrigation amounts decreased, total crop water use significantly declined from 617 to 405 mm, and WUE increased from 21.8 to 29.8 kg ha<sup>-1</sup> mm<sup>-1</sup>. The water production functions of alfalfa were parabolic in each harvest. The proportions of seasonal total actual water applied in each cutting were approximately 25%, 32% and 43%, with contributions to annual yield accounting for 54%, 30% and 16%, respectively, indicating that the third harvest of alfalfa had a great potential to improve WUE and save more water. Irrigation levels had noticeable effect on the relative feed values (RFV), but no effect on crude protein (CP) concentrations. The 60% ET irrigation level was conducive to increase CP concentration and RFV of alfalfa but was of no help to improve its grade. The spatial distributions of annual yield and quality were highly related to the water spatial distribution of the center pivot irrigation system. The coefficient of variations (CVs) in annual yield, RFV and CP of the whole system were 5% to 12%, 2% to 8% and 1% to 8% respectively, while the CVs in actual irrigation amounts ranged from 11% to 13%. Over-irrigation caused by end gun slightly increased alfalfa annual yield, but it reduced the quality and WUE. Therefore, an end gun in the center pivot irrigation system should be carefully selected for improving uniformity of the water application. Irrigation level of 80% ET in the first and second cuttings and 60% ET irrigation level in the third cutting were recommended for alfalfa production in semi-arid region such as western Inner Mongolia in China.

### **Future of alfalfa irrigation in China**

Although the China government has established many alfalfa demonstration bases of high production, there is still an important lack of knowledge about the quantification of the effects of management practices on yield and quality. Main alfalfa areas concentrated in arid and semiarid regions of China where the environmental impacts may be more important than yields. It is important to study and take into account the effects of alfalfa management practices in the environment, specially the use of water. Future research should focus on the optimization the decisions on irrigation with limited water resources. The new irrigation technology, like variable irrigation, offers the ability to very precisely apply water, nutrients, and other chemicals in the alfalfa root zone at



the timing and frequency needed. However, the further study on the adaptation and adoption of new irrigation technology is needed. The water-saving irrigation technology, an effective way to solve the problem of water shortage, has been promoted in the alfalfa irrigated regions in China. Many application problems in irrigation engineering, such as poor uniformity, inadequate irrigation facilities, should be attracted attention in the future.

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# Alfalfa (*Medicago sativa* L.) is more tolerant to salinity than guidelines indicate: Implications of field and greenhouse studies\*

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**Keywords:** salt tolerance, saline irrigation, alfalfa, sodium, abiotic stress

## Abstract

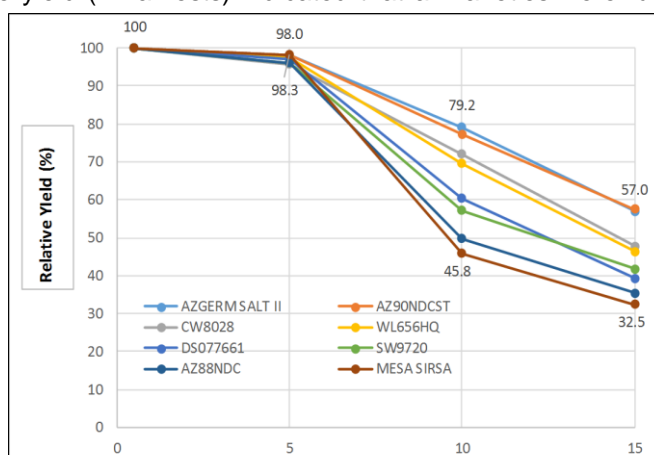
Alfalfa has historically been classified as moderately sensitive to salinity with yield declines predicted at >2 dS/m EC<sub>e</sub> (electrical conductivity of the saturated soil paste extract). However, greenhouse and field studies over the past 5 years have confirmed that alfalfa can be grown with limited negative effects at much greater salinity levels. A broad collection of alfalfa varieties have shown a range of resistance at irrigation water salinities >5 dS/m EC<sub>w</sub> in greenhouse trials, with significant variation due to variety. A three-year field study on clay loam soil with applications of 5-7 dS/m EC<sub>w</sub> irrigation water indicated normal yields and excellent stand survivability. A second field study in the same soil type with irrigation water salinities of 8-10 dS/m and resulting soil salinities of 10-16 dS/m EC<sub>e</sub> (0-90 cm depth) had yield reductions of 9-13% when averaged across varieties, and only two of the 21 varieties lost more than 20% yield under these highly saline-sodic soil conditions. Soil boron concentrations under high saline (HS) irrigation reached 10 mg/kg total boron, suggesting a very high level of boron tolerance in the varieties tested. Field evaluation of variety performance was subject to greater variation due to secondary sodicity effects (poor water infiltration and crusting) than to salinity, per-se. Providing adequate irrigation water availability to the crop may be as important as salinity in impacting yields under basin irrigation in these saline-sodic soils. Utilization of saline waters for alfalfa irrigation will likely increase in many irrigated regions due to drought and water scarcity; however, long term impacts on soil quality and the volume of water required for leaching should be taken into consideration.

## Greenhouse study (established plants- 7 month duration)

Nineteen alfalfa varieties, including new materials selected for salt tolerance, were grown in tall pots in a greenhouse to determine the relative salt tolerance of established plants to saline irrigation with four treatments (0.5, 5, 10 and 15 dS/m EC<sub>w</sub>). The cumulative relative yield (7 harvests) indicated that all varieties were fully tolerant to saline irrigation at EC<sub>w</sub> of 5 dS/m, and differentiation of the varieties was apparent only at 10 and 15 dS/m (Figure 1).

The relative yield (RY) for shoots and for root + crown dry matter decreased significantly when the EC<sub>w</sub> was > 5 dS/m. Tolerant varieties had a RY for shoot dry matter of 48 to 58%, even at an irrigation water salinity of 15 dS/m EC<sub>w</sub>; whereas for sensitive varieties, shoot RY was only 33% to 41% at 15 dS/m (Table 1).

On an absolute basis (cumulative yield for seven cuts), a consistent pattern was observed whereby the varieties which yielded the highest under nonsaline irrigation also yielded the highest at all levels of saline irrigation; e.g. varieties CUF101, AZNDCST,



**Fig 1.** Relative shoot biomass for eight alfalfa varieties exhibiting a range of salt tolerance in a greenhouse experiment. Shoot biomass is based on the cumulative yield for seven cuts over the seven-month experiment. Values are means with n=3

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AZGERMSALTII, FG96T707 and Hybriforce 800 were the top five yielding regardless of salinity level (data not shown). Notable was the high absolute biomass production under saline irrigation of CUF-101, a variety grown in the San Joaquin Valley for many years that was not necessarily bred for salt tolerance. On a relative basis, it was ranked as moderately sensitive with large yield reductions (on a percentage basis) for the 10 and 15 dS/m EC<sub>w</sub> treatments; however, it still yielded higher than most other varieties at these salinity levels. The other top-yielding varieties under saline irrigation were selected for salt tolerance.

**Table 1. Relative shoot biomass (% of non-saline control<sup>†</sup>) of 19 greenhouse-grown alfalfa varieties irrigated with different levels of salinity (0.5, 5, 10, 15 dS m<sup>-1</sup>). Tolerance levels (left column) is based primarily on yields at 10 dS m<sup>-1</sup> EC<sub>w</sub>.**

Tolerance	Var.#	Variety name <sup>††</sup>	Relative Yield (%)		
			----- EC <sub>w</sub> (dS/m) -----		
			5	10	15
T	18	AZGERM SALT II	98.0	79.2	57
"	17	AZ90NDCST	98.0	77.3	57.6
"	7	HYBRIFORCE800	95.5	76.2	56.3
"	9	FG96T707	97.9	74.7	53.6
"	8	DS067092	95.6	75.2	48.0
"	2	SW8421S	97.6	70.3	52.6
MT	13	CW8028	95.8	72.1	47.8
"	5	WL656HQ	97.5	69.6	46.4
"	3	6906N	97.2	68.3	46.4
"	12	CW58S	96.2	65.7	42.7
"	15	SW9215	96.9	64.5	43.2
MS	11	CW48S	96.2	62.2	40.8
"	14	DS077661	97.2	60.5	39.3
"	1	SW9720	96.2	57.2	41.9
"	6	AMERISTAND901SQ	97.0	57.9	40.8
"	20	CUF101(a)	94.7	54.9	44.3
"	4	CUF101(b)	94.6	54.7	43.9
S	10	CW9S	97.0	44.6	40.6
"	16	AZ88NDC	96.1	49.8	35.4
"	19	MESA SIRSA	98.3	45.8	32.5

<sup>†</sup> calculated from cumulative biomass for seven cuts taken during the seven-month experiment.

<sup>††</sup>AZ = Arizona, FG= Forage Genetics, CW = CalWest (now Alforex), WL=W-L Alfalfa, SW= S&W Seed.

For most varieties, Na<sup>+</sup> and Cl<sup>-</sup> concentrations in shoots (and roots + crowns) steadily increased (and K<sup>+</sup> decreased) as salinity increased: e.g. shoot Na<sup>+</sup> was 1.7 to 2.8 times greater than the non-saline control at 10 dS/m EC<sub>w</sub> and 3.0 to 4.8 times greater at 15 dS/m EC<sub>w</sub>, with the more sensitive varieties (based on shoot RY) having the greatest increases. Differences in sodium accumulation amongst the varieties were greatest at the 7<sup>th</sup> (last cut) in which case, the most sensitive varieties accumulated 2 to 3% sodium (= 870 – 1305 mmol/kg) in the shoot tissue at the highest salinity level. The most tolerant varieties showed less increase in Na<sup>+</sup> and Cl<sup>-</sup> (and decrease in K<sup>+</sup>) in shoot tissue. Toxic ion exclusion and K<sup>+</sup> discrimination (over Na<sup>+</sup>) may be important tolerance mechanisms in these alfalfa varieties.

#### Field trial 1 (2009–2012; basin irrigation, 5.5–6.5 dS/m EC<sub>w</sub>)

This trial was conducted in a clay loam soil at the University of California Westside Research and Extension Center (WSREC) in western Fresno County, California, testing the forage yield response of a broad range of alfalfa varieties to irrigation waters of 5.5-6.5 dS/m EC<sub>w</sub> which resulted in soil salinities of about 9.0 dS/m EC<sub>e</sub> by the end of the trial. In this trial, yields were in the normal range for this site (e.g. 12.3 tons/acre average) over the 3 years of saline irrigation (see <http://alfalfa.ucdavis.edu/+producing/variety/data/2012/09WSFS10-12.htm> for data). In fact, in this trial, varieties gained in their yield potential from year 1 to year 3, with yields increasing from 22.0 to 33.0 t/ha, in spite of an increase in salinity over the period of the trial. This trial was conducted only with saline water (no non-saline controls) with 6 replications and the varieties were significantly different over 3 years, ranging from an average low of 24.9 to a high of 29.8 t/ha.

Table 2. Alfalfa variety performance in a three year field trial, Five Points, CA.

Variety	Yield (MT/h)									
	2015		2016		2017		3 Year Avg.		Cum. Yield	
	LS	HS	LS	HS	LS	HS	LS	HS	LS	HS
SW9812	14.65	16.51	18.73	24.4	21.15	26.91	18.18	22.61	54.54	67.82
SW 9813	17.79	16.16	20.77	22.76	20.73	28.63	19.76	22.52	59.28	67.55
FG R914W259S	18.64	17.05	22.33	21.01	23.46	23.79	21.48	20.62	64.43	61.86
AmeriStand 915TS RR	17.03	15.75	19.56	20.79	20.14	24.05	18.91	20.19	56.72	60.58
SW 8421- RRS	17.42	16.38	23.3	19.13	27.16	22.81	22.63	19.44	67.88	58.32
Saltana	16.47	15.34	21.62	19.57	25.83	23.33	21.31	19.41	63.92	58.24
AmeriStand 901TS	16.44	14.22	21.73	19.41	25.83	23.21	21.33	18.95	64	56.85
FG R814W257S	17.23	14.32	19.7	21.6	20.8	20.37	19.24	18.76	57.73	56.28
CUF101	17.57	15.64	22.22	20.21	23.75	19.67	21.18	18.51	63.54	55.52
9R100	17.44	15.66	21.68	18.48	23.27	21.05	20.8	18.4	62.39	55.19
SW9215	15.57	15.46	17.96	18.25	24.37	21.02	19.3	18.24	57.9	54.73
Sun Quest	16.55	14.86	21.91	17.08	27.8	22.11	22.09	18.02	66.27	54.05
AZ- 90NDC-ST	17.71	13.77	22.18	18.77	23.73	21.11	21.21	17.88	63.62	53.65
CW050085	17.51	14.15	22.14	17.88	23.33	20.8	20.99	17.61	62.98	52.83
FG R814W258S	15.71	14.6	20.83	18.6	24.8	19.01	20.45	17.4	61.34	52.21
AZ-88NDC	16.62	14.09	20.81	17.37	21.35	18.95	19.59	16.8	58.78	50.41
SW 9215-RRS	16.98	13.95	23.04	16.55	23.66	18.83	21.23	16.44	63.68	49.33
CW058071	17.92	13.81	20.2	16.68	21.28	18.54	19.8	16.34	59.4	49.03
Desert Sun 8.10RR	16.78	14.4	19.16	16.55	21.98	17.84	19.31	16.27	57.92	48.8
SW 9106	18.69	13.16	25.07	16.59	28.2	17.77	23.99	15.84	71.97	47.52
SW 8421-S	17.23	13.58	20.29	14.25	20.81	18.17	19.45	15.33	58.34	46
<b>Mean</b>	<b>17.05</b>	<b>14.9</b>	<b>21.2</b>	<b>18.85</b>	<b>23.5</b>	<b>21.33</b>	<b>20.58</b>	<b>18.36</b>	<b>61.74</b>	<b>55.08</b>
<b>CV%</b>	<b>10.91</b>	<b>23.54</b>	<b>19.59</b>	<b>28.2</b>	<b>30.96</b>	<b>30.3</b>	<b>21.82</b>	<b>25.8</b>	<b>27.13</b>	<b>30.69</b>
<b>LSD (p=0.05)</b>	<b>1.17</b>	<b>2.21</b>	<b>2.62</b>	<b>3.36</b>	<b>4.59</b>	<b>4.08</b>	<b>0.26</b>	<b>0.27</b>	<b>2.02</b>	<b>2.04</b>
<b>Yield loss due to salinity</b>	<b>13%</b>		<b>11%</b>		<b>9%</b>		<b>11%</b>		<b>11%</b>	

## Field trial 2 (2015-2017; basin irrigation, 8-10 dS/m EC<sub>w</sub>)

In this trial, the alfalfa was planted into pre-salinized, clay loam soil (~4.0 dS/m EC<sub>e</sub>) and the 24 varieties were challenged with higher salinity water (8-10 dS/m EC<sub>w</sub>) which resulted in soil salinities of 10 – 16.5 dS/m EC<sub>e</sub> (0-90 cm depth) in the high salinity (HS) basin by the end of the first year and throughout the second and third year. The low salinity (LS) basin received water averaging 1.24 dS/m and soil salinities were 3.2 – 4.6 dS/m EC<sub>e</sub> (0-90 cm depth). Only a 9-13% yield decline was observed over 3 years of HS irrigation (Table 2), as compared to the 50-60 percent yield losses predicted by the Maas Hoffman yield loss coefficients.

Trial 2 provided valuable information on the overall salt tolerance of alfalfa, but we were unable to rank the varieties for salt tolerance due to the lack of uniform salinity (Fig. 2) and uniform soil moisture within the basins. In spite of the replicated plots (4) for each variety and the use of 1 m borders, a significant border effect was observed. Adjustments were made to improve the distribution uniformity of the irrigation water and to increase the depth of water penetration, but care had to be taken to avoid water-logging due to the sensitivity of alfalfa to poorly-aerated soils and irrigation had to cease 10 - 12 days before harvesting. It was expected that the saline-sodic water used for irrigation in the HS basin would lead to clay dispersion and reduced infiltration, but at times infiltration was poor in the LS basin as well, due in part, to the expanding clay loam soil that swells upon wetting and small differences in elevation which created spatial variability in water infiltration. A third field

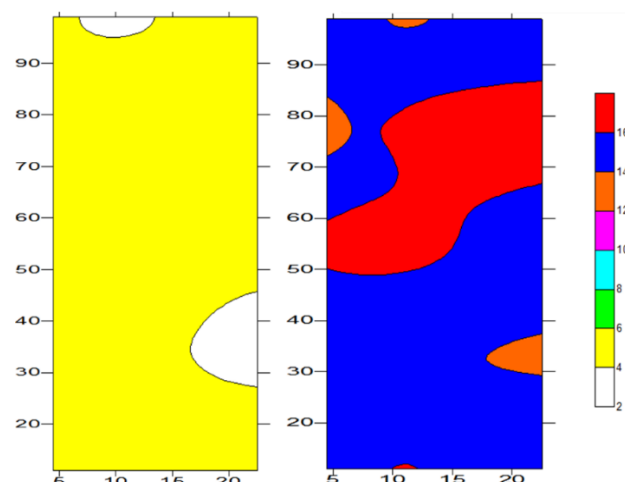


Fig. 2. Soil salinity contour map (EC<sub>e</sub>, dS/m, 0-1.5 m depth) for October 2016 (end of 2<sup>nd</sup> year) of saline irrigation. LS basin (left) and HS basin (right), each 10.7 x 61 m. Variation in soil salinity and soil moisture interfered with alfalfa variety comparisons under saline irrigation.



trial at WSREC was established in spring 2017, utilizing subsurface drip irrigation in combination with surface irrigation (sprinkler systems) to apply saline water in a split plot design.

## Conclusions

What we can conclude from our results is that alfalfa is much more salt tolerant than previously thought, similar to the conclusions of Cornacchione and Suarez (2015). Our results provide strong evidence that irrigation waters of higher salinity (5 – 8 dS/m  $EC_w$ ) which result in soil salinities of 10 dS/m  $E_{ce}$  or higher can be used for alfalfa grown on deep fertile soils, with potential yield losses ranging from negligible to 20% over three years, depending on the variety. In spite of modest yield losses under saline conditions, these are still economically viable yield levels for alfalfa grown under relatively high levels of salinity. Furthermore, the HS irrigation waters used in our study averaged 7.0 ppm (mg/L) of boron. Most water quality standards (e.g. Ayers and Wescot, 1985) consider irrigation waters with B > 2.0 ppm to have a severe restriction and they can only be used to irrigate boron tolerant crops. The high yields observed under our HS irrigation suggest that alfalfa is quite boron tolerant, similar to the results of Maas and Grattan (1999). Furthermore, the deep-rooted characteristics of alfalfa may enable utilization of deeper subsurface moisture, even at moderate to high salinity levels.

However, the long term impact of saline irrigation on soil quality should be considered including the need for excess irrigation water required to return the soil salinity to levels suitable for most other crops— ideally below 5.0 dS/m  $E_{ce}$ . Thus, less saline irrigation should be utilized when future rotations would include more salt-sensitive crops. Soil texture and infiltration characteristics largely determine the extent to which salts can be leached from saline-irrigated fields and many soils are not easily leached due to their saline-sodic condition or subsurface impediments. Soil texture, structure, EC/SAR ratio, pH and other soil chemical properties (Suarez et al. 2006), infiltration properties, volume applied and leaching fraction will determine the true outcome on saline soils. Careful water management during stand establishment, prevention of crusting, and agronomic practices to promote water infiltration and prevent ponding will be particularly important to the successful production of alfalfa under saline and saline-sodic conditions.

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## Deficit irrigation strategies: why alfalfa is the best crop to have in a drought\*

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**KEYWORDS:** *Medicago sativa*, irrigation, water deficit.

Approximately 50% of US alfalfa is produced under western arid conditions and highly dependent upon irrigation (Table 1). Drought and diminished water supplies for irrigation have affected many western states and have been especially acute in California during the record drought of 2012-2017. Strategies are needed to improve economic viability and lessen long-term impacts of drought for alfalfa producers.

### Biological Advantages vis' a vis

**Water.** Although often high in annual water demand, alfalfa has some important advantages when it comes to water issues. It has a high level of water productivity (WP) or water use efficiency, and is well adapted to drought conditions and deficit irrigation strategies. *Medicago* species evolved in the Near East regions (Iraq, Iran) with long dry summers and wet winters. Thus alfalfa exhibits characteristics that make it very useful when a farm is faced water limitations. These include:

**Table 1.** Alfalfa Hay & Alfalfa Forage (hay+silage+greenchop) production, hay equivalent basis (13%DM). (2012 USDA-NASS Agricultural Ag. Census).

Region	Alfalfa Hay	Alfalfa Forage (Hay+Silage+Green)	Alfalfa Forage (% of US)
	tons per year	tons per year	%
MidWest	20,132,158	23,215,460	42.5%
Northeast	2,224,016	3,501,677	6.4%
South	435,991	501,753	0.9%
West	26,677,006	27,348,810	50.1%
<b>Total US</b>	<b>49,469,171</b>	<b>54,567,699</b>	<b>100%</b>

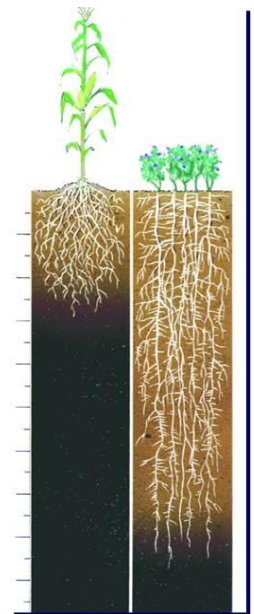
- **Deep-Rootedness**—alfalfa roots can be >1-3 meters in effective rooting depth, obtaining moisture from deep in the soil profile.
- **Perenniality**—Alfalfa rapidly grows with warm spring conditions and does not require annual establishment which uses a large amount of water inefficiently before a full canopy is developed.
- **High Yields**—Alfalfa can yield up to 12 cuttings per year, and under optimum management can obtain yields greater than 35 Mt/ha dry matter.
- **High Harvest Index**—100% of the above-ground plant material is harvested, unlike seed or fruiting crops, where 10-50% of the biomass is harvested.
- **High Water Productivity (WP)**—Alfalfa's high yields and high harvest index generate high water productivities (kg of yield per unit of water applied).
- **Salt Tolerance**—Alfalfa has a high degree of salt tolerance. Increased salinity is common during droughts. The crop can also use degraded saline water supplies.
- **Ability to Survive**—The crown, root structure and storage nutrients of alfalfa enables the crop to recover after months of drought.

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- **Flexibility to Deficit irrigate**—Alfalfa will produce economic yields when partially irrigated, unlike some crops where complete failure is possible under drought.

**What are the Strategies for Low Water Availability on Farm?** There should be a range of strategies for dealing with a water-poor future (Putnam, 2012). The last characteristic (flexibility) is a key attribute of alfalfa, and somewhat unique among crops. The key questions for growers is how to apply deficit irrigation strategies when there is insufficient water supply to satisfy the full irrigation needs of the crop? When faced with a drought, alfalfa growers have the choice of:

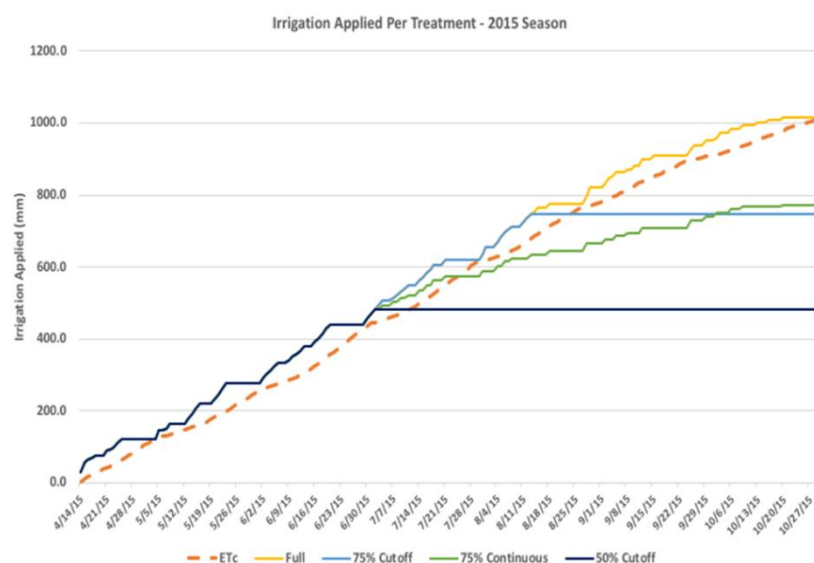
Alfalfa has very deep perennial root structures, conferring water management advantages (Univ. MN illustr.)



- **Triage/Cease Production.** Complete cessation of irrigation on some fields while other fields are fully irrigated.
- **Continual Deficit/Starvation Diet.** Deficit irrigating all fields and applying less water than the alfalfa needs throughout the crop season.
- **Partial Season Irrigation/Sudden Cutoff.** Irrigated fully for a period of time, and then cease irrigations altogether.

Triage (stopping production on some fields) and using water on the best fields makes sense if some fields are in late stages of decay with marginal economic returns. However, deficit strategies, either continual or partial season irrigation strategies may be considered to keep forage production (and animal production) viable during drought periods, or to facilitate economic water transfers for other users.

**Water Transfers and Economics.** In irrigated regions during drought, water can often be transferred for other uses, either between farms, between districts, or from agriculture to cities, or from agriculture to environmental uses. Transfers can be regulatory, by legal fiat, or voluntary transfers. In the Colorado River Basin, overdrafts by all users have caused concern about the long-term sustainability of that water sources and level of the Powell Reservoir (Cabot et al., 2017). Economic transfers of water from agriculture to the massive cities of Los Angeles and San Diego have occurred over the past 15 years. Alfalfa is of particular interest since the hectares are large and it represents a relatively large water footprint. Economists have long argued in favor of a rational approach to water transfers from high availability areas to low-availability areas especially during drought (Howitt et al., 2010). These approaches could (or could not) be favorable to alfalfa growers, depending upon the compensation for yield losses, long-term security of water supplies, and risks involved. Ottman and Putnam (2017) described the yield and economic penalties for yield reductions that would be experienced by farmers. However, in some cases yield penalties can be fully mitigated by payments for water conserved. Such discussions begin by understanding yield penalties and risk of deficit-irrigating alfalfa crops.



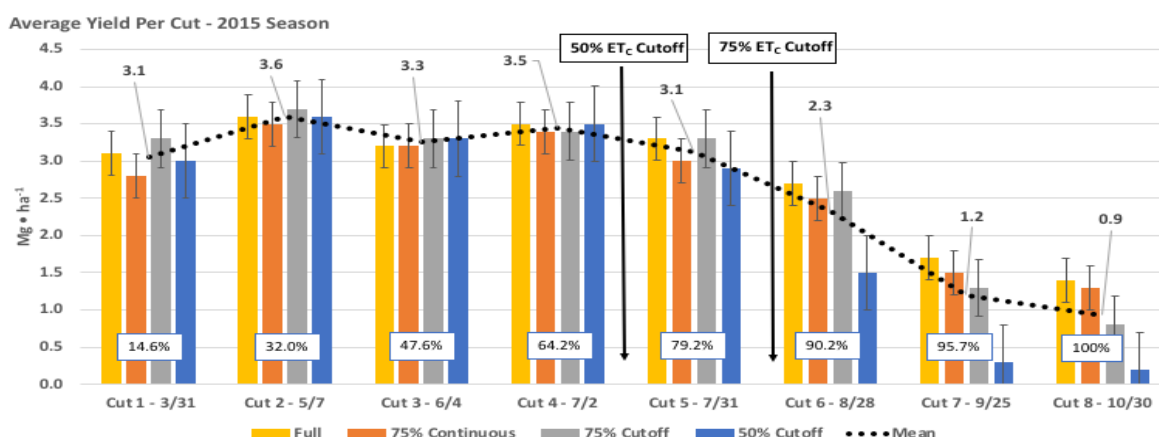
**Figure 1.** Cumulative seasonal ET<sub>c</sub> or crop water requirements (dashed dark orange line) and fully-watered irrigation treatment amounts (yellow line) of applied irrigation water for an alfalfa trial, Davis, CA, 2015. Water applied for the three deficit treatments were 75% of full watering (late cutoff, blue line), 75% of full watering (continuous deficits after mid-season, green line) and 50% (sudden cutoff mid-season – dark blue line).

**Deficit Irrigation- Evidence from Field Trials.** A range of field trials have been conducted over many years on the issue of deficit irrigation. At Davis, California a study was completed in 2017 in a semi-dormant, 6-8 cut system. Fifteen alfalfa cultivars were established in fall 2014 at Davis, CA using a split plot design with four replications with four different irrigation regimes utilizing Subsurface Drip Irrigation (SDI). Irrigation treatments (Figure 1) were: 1) 100% of seasonal ET<sub>c</sub>, applied water, 2) 75% of seasonal ET<sub>c</sub> with an August cutoff, 3) 75% of seasonal ET<sub>c</sub>, -fully irrigated until mid-season, then 1/2 of full ET<sub>c</sub> supplied for the remainder of the season, and 4) 50% of ET<sub>c</sub> –with a complete July cutoff (Figure 1). Over three years of data collection (2015-2017), yields averaged 80% of fully-irrigated plots when receiving 50% water application and averaged of 96% of the fully-watered yields at 75% of water applications to fully match ET<sub>c</sub>. (Figure 2). We found that the cultivars differed significantly in yield potential ( $P < 0.05$ ), but there was no significant interaction between variety and deficit irrigation strategies. There was little consistent excess damage to crop stand resulting from deficits at the end of this 3-year trial.

In a separate research trial in Colorado in high-mountain irrigated environment (3-4 cut system), a total of 6 established alfalfa fields were subjected to irrigation treatments including normal irrigation (control), irrigation stopped later (low-risk), and irrigation stopped early (high-risk) in the growing season for 2 consecutive years (Cabot et al., 2017). All fields then received consistently full irrigation in the third and final year. Not surprisingly, deficit irrigation reduced plant growth and yields relative to the fully irrigated control in the year deficits were imposed. This was more pronounced in the high risk (early season irrigation cutoffs) than in late-season cutoffs. Yield penalty ranged from negligible at some sites to severe (e.g. 75% reduction) at other sites. At some sites, there was no yield penalty for late-season cutoffs or early season cutoffs, presumably due to residual moisture and soil factors (Cabot et al., 2017).

**Quality.** There is considerable evidence that alfalfa under water deficits may exhibit higher quality than fully-watered alfalfa. This may be due to more rapid growth under fully-watered conditions, the trade-off between yield and quality, and greater leaf-stem ratio when crops are grown under stress. In the Colorado study, NDF values were significantly decreased (6 percentage points NDF) when irrigations ceased after 1<sup>st</sup> cutting. Enhanced quality is likely due to delayed maturity resulting in a greater leaf-to-stem ratio and finer stems (Lindenmayer, 2008; Peterson et al., 1992). However, such increases is not likely to fully mitigate yield losses that might occur due to water deficits.

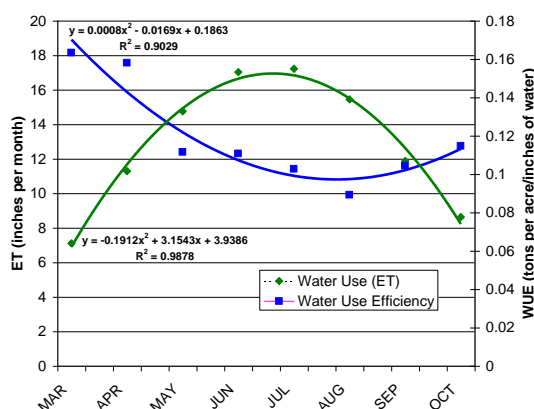




**Figure 2.** Seasonal yield patterns for alfalfa under different deficit strategies, Davis, CA, 2015. The dotted line = average yield of 15 varieties. Boxes below provide % of seasonal yield., Water deficits have a lower impact when implemented later in the year.

**Persistence and Recovery.** In both the California and Colorado studies, alfalfa was generally able to fully recover from water deficits. Multiple studies conducted in California over a 15 year period have shown that alfalfa mostly recovers when deficit irrigated for the later part of the season (Orloff et al., 2005). Actually, deficit irrigated fields may yield more when fully irrigated in the year following deficits than control treatments. In the final year of the Colorado study, the first cut fully irrigated plots yielded 2,279 kg/ha whereas the late and early deficit plots yielded 2,524, and 2,869 kg/ha, respectively, showing over-compensation for previous year deficits. These results are supported by other researchers (Carter and Sheaffer, 1983; Lindenmayer, 2008) and our California study. However, stand persistence after water deficits has not been universal. We have observed stand decline in late-season deficit irrigated fields in the low desert regions of California and Arizona. These are harsh desert conditions high in salinity with clay soils and very high temperatures, and stand loss is common even under full irrigation.

**Partial Season Irrigation vs. Continuous deficits.** In many western US irrigated regions, the best deficit strategy is to fully irrigate the early-season cuttings and then cease irrigation partway through the season. There are several advantages to this tactic. Spring and early summer cuttings are typically the highest yielding (Figure 2), and also the highest quality and highest in Water Productivity (Figure 3). Depending on the production area, approximately 2/3 to 3/4 of the total annual production occurs by mid-summer (Figure 2). In the Davis, CA study, 50% water savings were imposed after about 64% of the seasonal yield was obtained, and the crop yielded 80% of full yields over 3 years, while saving about 50% of the irrigation water.



**Figure 3.** Changes in water demand (ETc) and Water Use Efficiency of alfalfa over the season, Sacramento Valley, CA

**Summary.** Unlike many other crops, alfalfa offers a high degree of flexibility during droughts due to its ability to successfully survive severe deficits and produce some yield—a valuable attribute when deciding how to allocate scarce water. Thus alfalfa may be the best crop to have in a drought. Deficit irrigation strategies could be implemented utilizing either continual deficiencies (starvation diet) or a sudden cutoff after a partial season full irrigation. We recommend ‘sudden cutoff’ strategies, e.g. fully watering the crop early, followed by complete cessation of irrigation. These are effective since 1) most yield is obtained during early harvests, and 2) late harvests of alfalfa can utilize residual moisture from earlier irrigations and 3) the crop normally recovers from this short-term stress. Although yields are reduced under deficit irrigation, harvest costs and pest management costs may also be reduced. Stands have been largely unaffected by short-term deficits, and recovery is excellent, but we have found more severe stand losses in very hot desert environments. Since competition for water resources in irrigated regions is likely to intensify in the future, innovative strategies to cope with reduced water supplies are likely to be important for alfalfa in the future.

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## Technological innovations to improve water management in alfalfa and forage crops.\*

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**KEYWORDS:** variable rate irrigation, subsurface drip irrigation, deficit irrigation, plant feedback, soil water content

In the US, 40% of crop market value is produced by irrigation on only 7.5% of cropped lands, but progress is frustrated by present and likely future water shortages. To sustain and improve agricultural productivity requires increases in irrigation efficiency and crop water productivity (CWP), also known as water use efficiency. Irrigation efficiency is the ratio of water consumed by the crop through evapotranspiration (ET) to the irrigation water applied. Reducing runoff and deep percolation losses are ways in which to increase irrigation efficiency. Water use efficiency (WUE) or crop water productivity (CWP) is defined as the economic yield obtained per unit of water consumed by the crop:  $CWP = Y/(E + T)$  = transpiration efficiency/( $E/T + 1$ ); where  $Y$  is economic yield,  $E$  is evaporation,  $T$  is transpiration, and the transpiration efficiency is  $Y/T$ . Ways to change CWP include choice of crop and variety (affects  $Y/T$ ), irrigation scheduling and amount, irrigation method and management, fertility management, tillage management and soils and climate. Timing of water supply has a large effect on CWP. An otherwise well-watered crop that is short of water during a critical growth period will suffer decreased yield and CWP. Irrigation method can influence WP by affecting the ratio of evaporative loss to crop transpiration. Irrigation methods that reduce soil and crop wetting, and thus evaporative losses, include subsurface drip irrigation and low-energy-precision-application (LEPA) applicators on sprinkler irrigation systems.

Forage yields are as reliant on a stable water supply for increases in yield and CWP as are yields of grain crops. In the western US (largely west of the 95<sup>th</sup> meridian), non-irrigated forage yields are roughly 1 ton/acre less than those in the eastern states. However, in the western states where >31% of forage lands are irrigated, yields are roughly three times greater than those achieved on non-irrigated lands and considerably larger than those achieved in the eastern US where most forage is not irrigated.

### Water savings with microirrigation

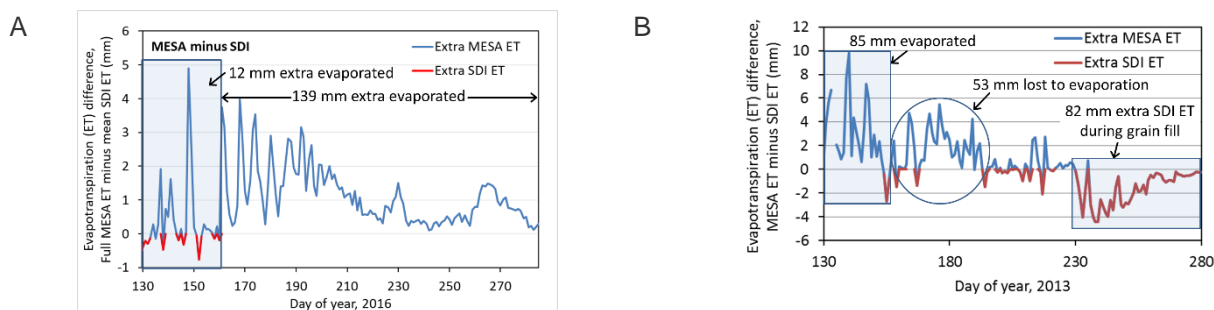
Microirrigation of forage crops, particularly subsurface drip irrigation (SDI), can reduce water consumption for a given yield target by decreasing evaporative losses compared with gravity and sprinkler irrigation methods. Sorghum and corn grown at Bushland, Texas with both SDI and mid-elevation spray application (MESA) sprinklers showed that savings of up to 85 mm of water could be achieved during the period of pre-plant irrigation through 25 days after planting when plant cover became important (Fig. 1). Due to evaporative losses from the plant canopy that occurred with MESA irrigation, another 53 to 139 mm of water were saved with SDI later in the season. Improvements in WUE ranged from 11% for sorghum to 44% for corn. Although WUE was computed for grain yield in these experiments, similar differences in biomass were observed.

SDI has been shown to improve both yield and CWP of alfalfa considerably compared with flood or furrow irrigation at several locations, including Lubbock, TX; NM; Coolidge, AZ; Kansas; Lovelock, NV; the Treasure Valley, ID; and the Imperial Valley and Tulare Lake areas, CA. Other advantages of SDI include elimination of leaf scalding that may occur with sprinkler irrigation; quicker turnaround of harvest operations due to firmer soil, which allows irrigation to be resumed more quickly; and larger yields (Alam et al., 2009). Because irrigation can be continued nearly up to and, depending on soil type, during harvest, irrigation system capacity can be smaller and still achieve adequate irrigation for high yield. Despite improvements in alfalfa yield and CWP, drip tape damage by rodents has been severe in some locations (e.g., the Treasure Valley, Idaho, Neufeld, 2014;

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Coolidge, AZ, Blake, 2009). Longevity of SDI systems is a concern, but SDI installed at both Colby, KS, and Bushland, TX, has lasted >20 years, well beyond the amortization period for the systems and competitive with center pivot irrigation systems in total cost over the life of the system.



**Figure 1.** Differences in evapotranspiration (ET) between sprinkler (mid elevation spray application, MESA) irrigation and subsurface drip irrigation (SDI) at Bushland, Texas. Blue indicates greater ET from MESA irrigation and red indicates greater ET from SDI. (A) Results for corn irrigated in 2016. (B) Results for corn grown in 2013.

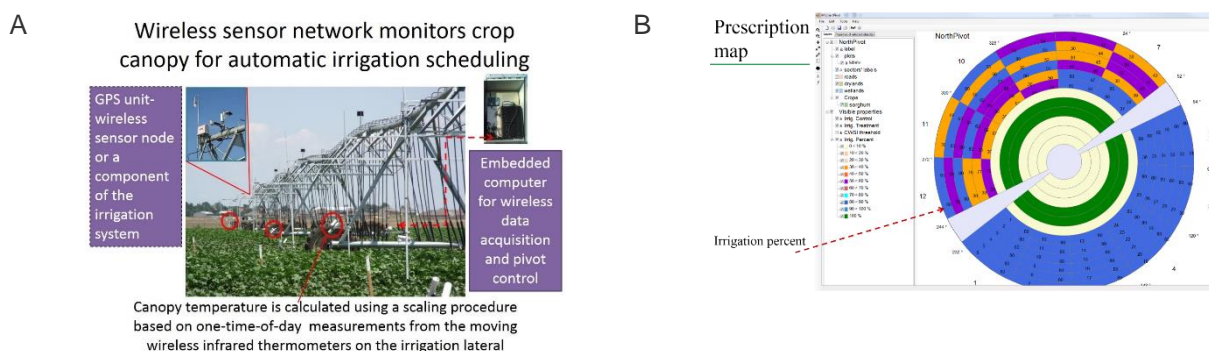
### Yield and crop water productivity increases with advanced center pivot management

In the US, large reductions in annual irrigation amounts coincided with the increased percentage of irrigated land that is sprinkler irrigated (from 19% in 1969 to 56% in 2013). This was achieved because of the greater uniformity of irrigation achieved with these systems when properly set up. Irrigation scheduling is still largely based on producer perceptions of crop water needs. Adoption of measurement based scheduling has lagged due to the difficulties of using soil water sensors of various types, or weather based scheduling programs based on a daily reference ET,  $ET_o$ , and a crop and crop-growth-stage specific crop coefficient,  $K_c$ , where daily crop water use,  $ET_c$ , is calculated as  $ET_c = K_c \times ET_o$ . The  $ET_o$  is calculated from weather station data using, for example, the standardized Penman-Monteith reference ET method (ASCE, 2005). Typically, this procedure gives  $ET_c$  for a well-watered crop managed for high yields. However, greater CWP and often greater profitability can be obtained by irrigating at less than the well-watered rate. When well-managed, this deficit irrigation reduces crop yields only slightly (e.g., 5%) or not at all, but reduces pumping costs and nutrient losses that can occur due to deep percolation and runoff occurring in some parts of fully irrigated fields.

Producers often avoid deficit irrigation for reasons ranging from the risk of considerable yield losses if management is imperfect to uncertainty about irrigation capacity being large enough to keep up with crop water demand during peak water use periods, and to uncertainty regarding the accuracy of  $ET_c$  estimates or of soil water sensors. Another reason for lack of adoption is that, unlike irrigation equipment, scheduling software and equipment is usually not supported by a dealer or manufacturer, leaving the producer to study, choose, acquire, set up and operate an appropriate system. Alternative scheduling methods based on plant water stress sensing are, however, showing the ability to manage deficit irrigation without undue crop yield reductions while increasing CWP and sometimes yield. Earlier versions of this system showed good control of WUE and yield using drip irrigation systems (Evetts et al., 2001, 2006). Most recently, these methods rely on wireless sensor networks of thermal infrared sensors to monitor crop canopy temperature in the field, transferring the data automatically and wirelessly to an embedded computer at the pivot point where the data are processed to produce recommendations for irrigation automatically (Fig. 2A). Recommendations can be for an entire field, or in the case of a variable rate irrigation (VRI) system, a prescription map can be uploaded to the center pivot control panel (Fig. 2B). Irrigation equipment manufacturers are beginning to offer such systems as integral parts of center pivot irrigation systems.

An Irrigation Scheduling Supervisory Control and Data Acquisition (ISSCADA) system was developed and patented by USDA ARS to enable sensor-based irrigation scheduling based on automatic sensing and irrigation needs assessment (Evetts et al., 2014). The system is embodied in a client-server computer program architecture names ARSmartPivot (Andrade et al., 2015), which uses geographical positioning systems (GPS) to allow dynamic spatial mapping of plant water stress and corresponding irrigation scheduling prescriptions

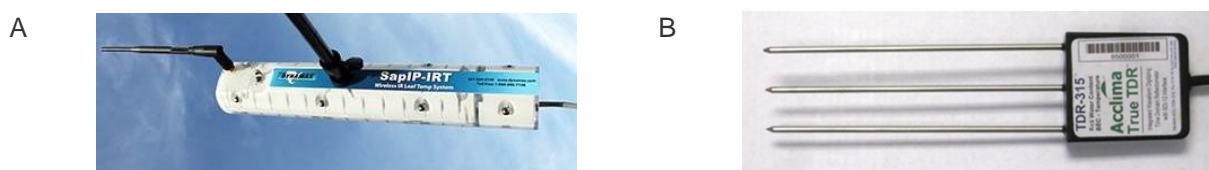
(O'Shaughnessy et al., 2015). ARSmartPivot uses wireless sensor networks and computer algorithms to determine irrigation needs based on a crop water stress index integrated over daylight hours (iCWSI) and soil water sensing. In a series of experiments, O'Shaughnessy et al. (2012a,b) showed that ISSCADA obtained sorghum and cotton yields and WUE values as large as and sometimes larger than those obtained using weekly neutron probe readings for irrigation scheduling (the latter including data from Colaizzi et al., 2004). In particular, sorghum yield and WUE were typically larger for moderate deficit irrigation (55 to 80% of full) compared with full irrigation.



**Figure 2.** (A) A variable rate irrigation (VRI) center pivot system combined with a wireless network of crop canopy temperature sensors (thermal infrared) and soil water sensors used by the ISSCADA system to automatically determine full and deficit irrigation levels and control the VRI system at the USDA, ARS Conservation & Production Research Laboratory, Bushland, Texas. (B) Example of a prescription map produced by the ARSmartPivot software.

### Advanced wireless sensor systems for irrigation management

Numerous plant and soil sensor systems have been developed in the past 20 years, but utilization in producer fields has been hampered by the wiring required to get data from sensors to where it can be used for management. Recently, wireless sensor networks have been developed and adapted to irrigation system management. A recent wireless plant canopy temperature sensor was developed in cooperation with USDA ARS at Bushland, Texas (Fig. 3A). The development of this sensor was based on the work reported by O'Shaughnessy et al. (2012a,b). In 2015, a novel soil water sensor relying on a miniaturized time domain reflectometry (TDR) electronic circuit was patented (Evet et al., 2015) and one of several sensors based on this true TDR circuit was introduced commercially (Fig. 3B). This sensor can be easily field deployed in a wireless sensor network using commercially available wireless dataloggers. Advantages over previous soil water sensors include accuracy sufficient for irrigation management based on management allowed depletion (MAD) concepts, and nearly complete immunity to soil electrical conductivity and temperature problems (low sensitivity at solution conductivities less than 7 dS m<sup>-1</sup>; Schwartz et al., 2015).



**Figure 3.** (A) A wireless thermal infrared thermometer (model SAPIP-IRT<sup>9</sup>, Dynamax, Inc., Houston, TX). (B) A true time domain reflectometry (TDR) sensor (model TDR-315, Acclima, Inc., Meridian, ID).

<sup>9</sup> The mention of trade names of commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotope, etc.) should contact USDA's



## SUMMARY

Strategies to improve agricultural productivity in the face of water limitations range from choice of crop, tillage and agronomic methods that conserve water and utilize it more efficiently to adoption of irrigation application methods that more uniformly apply water, reduce or eliminate conveyance losses and reduce evaporative losses from wetted canopy and soil surfaces. Sprinkler systems equipped with low-elevation-precision-application (LEPA) and low-elevation-spray-application (LESA) devices can reduce evaporative loss from wetted canopies and to some extent from soil surfaces when used in every other crop interrow, but can cause runoff problems. Subsurface drip irrigation (SDI) eliminates losses from canopy wetting and most losses due to evaporation from the soil surface, resulting in increased crop water productivity and allowing high yields with less water pumped. In some cases, yields are larger with SDI than are possible even with full irrigation using gravity methods. Advanced irrigation systems utilizing wireless sensor systems to automatically determine crop water stress have been shown to improve crop water productivity and allow well-regulated deficit irrigation with little user effort. When used with variable rate irrigation systems, these advanced supervisory control and data acquisition (SCADA) systems can allow spatially varying application of irrigation water to avoid flooding low lying areas and to respond to water stress where it occurs in the field. Combined with ever improving wireless plant and soil water sensing systems, SCADA control is poised for rapid commercialization and application in producer fields.

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## Alfalfa breeding for intercropping and grazing tolerance\*

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**KEYWORDS:** *Medicago sativa*, mixture, molecular marker, sustainability

### INTRODUCTION

Current agriculture challenges (energy consumption, pollution, workload and economic return for the farmers) require substantial changes in agronomic practices. Several levers are available. Among them, legumes can play a major role because of their ability to fix atmospheric nitrogen (N<sub>2</sub>). This natural fixation contributes to reduce the energy consumed for chemical nitrogen fertilizers and the nitrogen losses in the environment. In cropping systems, forage legumes can be used as a component of the rotation during which they produce high quality forage with low inputs, and store high N content in the soil for the next crop. They can also be intercropped in mixed stands which include at least one legume species and one grass species. In mixtures, legume and grass compete for light interception and water capture but they interact positively for nitrogen: the legume relies on N<sub>2</sub> fixation and releases a part of the fixed N while the grass N nutrition is based on soil N absorption. Another lever is to reduce harvest costs through animal grazing. No fuel is used for forage harvesting, drying, transportation or storage. Intercropping and grazing are probably two options to increase agriculture sustainability.

Several hundreds of alfalfa varieties are registered over the world. With few exceptions, they have been selected for forage production, quality and stress tolerance in pure stands with a mowing harvest. The new requirements attributed to alfalfa in cropping systems indicate that release of alfalfa varieties adapted to mixed stands with grasses and/or to grazing are expected. Such an objective involves a better knowledge of the genetic variation for these traits, of the correlation between traits and the genetic architecture of the traits. The analysis of progeny is a way to collect all this genetic information. We report studies in which phenotypic traits were measured on progenies obtained from several parental plants. Markers associated to phenotypic traits were identified with a QTL (Quantitative Trait Locus) analysis.

### MATERIAL AND METHODS

#### Intercropping

The progeny of three crosses between three contrasted parental plants (H1, G4 and D3 chosen in the variety 5246 of Pioneer), with 100 to 300 F1 plants each, was studied in mixture with a tall fescue genotype and in monoculture at INRA of Lusignan (France). Plant height and plant biomass were recorded on several cuts over two years. The plants were genotyped with microsatellite and DaRT markers and genetic maps were calculated. QTL for phenotypic traits were identified through analysis of variance for each marker.

#### Grazing

The progeny of four crosses between four contrasted parental plants were obtained. Two parents originated from Camporegio variety (prostrate, dormant, grazing tolerant), and two parents from Mamuntanas variety (erect, non dormant, non grazing tolerant). The 432 F1 plants were submitted to intensive sheep grazing at CREA of Lodi (Italy) and evaluated for growth before and after grazing, cold-season growth and final persistence. Genotyping was performed with GBS markers and genetic maps were obtained. QTL detection was performed by analysis of variance.

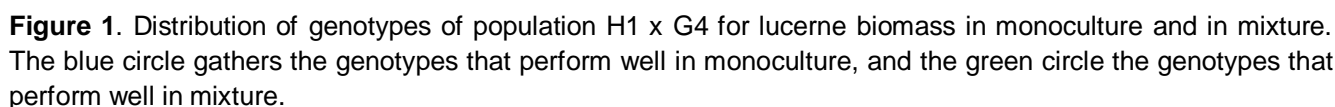
### RESULTS

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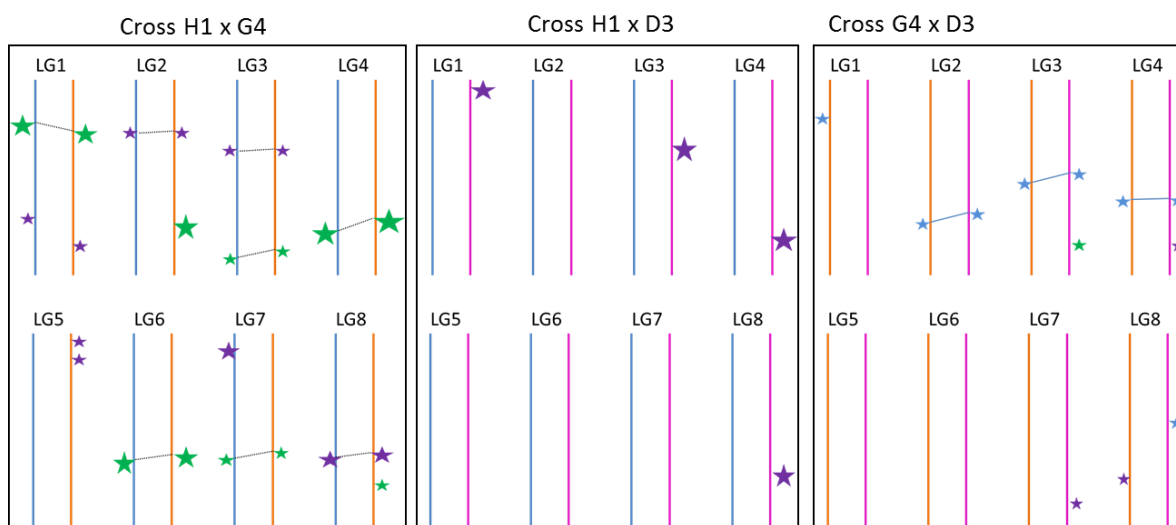
\* **CITATION:** Julier, B., Annicchiarico, P., Barre, P., Louarn, G., Pecetti, L. 2018. Alfalfa breeding for intercropping and grazing tolerance. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



The phenotypic data showed that biomass production of alfalfa genotypes in monoculture and mixture was positively correlated but some genotypes had higher performance in monoculture while other genotypes were more adapted to mixture (Figure 1). Plant height and biomass were highly correlated. Such results have also been observed on a set of genotypes of diverse origins: the correlation between biomass in mixture and biomass in monoculture was positive with the 45 genotypes ( $r = 0.84$ ,  $P < 0.001$ ) but became non-significant when considering the highest yielding genotypes ( $r = 0.53$ ,  $P > 0.05$ ) (Maamouri et al., 2017).



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**Figure 2:** QTL (represented by stars) on the genetic maps of each parent (blue for H1, orange for G4, pink for D3) in each cross. Green stars are for QTL that are common to pure and mixed stands, purple for mixed stands and blue for pure stands.

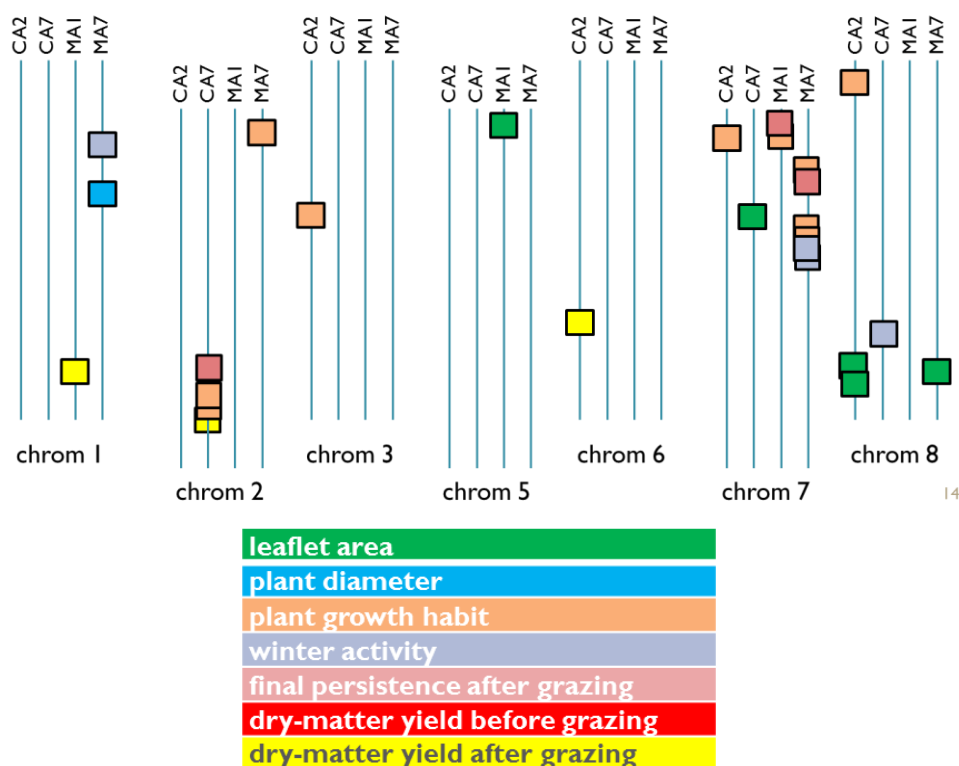
### Grazing

The grazing-tolerance experiment (Figure 3) indicated the presence of inverse genetic correlations of high persistence under intense sheep grazing with erect plant habit ( $r_g$   $-0.42$  to  $-0.87$  depending on the cross) and cold-season growth ( $r_g$   $-0.33$  to  $-0.73$ ) (Pecetti and Annicchiarico 2017).



**Figure 3:** Evaluation of 432 F<sub>1</sub> genotypes issued from four crosses between morphophysiologically-contrasting parents (CA1 and CA2 from Camporegio, MA1 and MA7 from Mamuntanas) for tolerance to severe grazing by sheep and other traits.

The number of QTL for each trait varied from one (for plant diameter) to 10 (for plant growth habit) (Figure 4), and they explained between 12 and 31% of the phenotypic variation. The allelic effect mostly, but not always, followed the expected trend: Camporegio alleles conferred smaller leaflet area, prostrate growth habit, higher winter persistency, and higher yield after grazing.



**Figure 4:** Schematic position of QTL for grazing tolerance on the chromosomes of each parent (CA1 and CA2 for Camporegio, MA1 and MA7 for Mamuntanas).

We observed co-location of QTL explaining several traits (Figure 4), including one QTL on chromosome 2 of CA2 parent related simultaneously to final persistence, biomass yield under grazing and prostrate habit, and another QTL on chromosome 7 of MA7 related to growth habit and winter growth. However, such co-locations did not occur for all traits. This offers a means to select simultaneously for grazing tolerance (biomass yield and final persistence under grazing), low winter dormancy and relatively erect growth habit by MAS, to partly circumvent the negative genetic correlations existing between these traits.

## DISCUSSION

Both studies clarified the genetic control of new traits of interest in alfalfa breeding. For the adaptation to mixture, the positive correlation with monoculture is favourable to breeding. However, there is a possibility to improve specifically the yielding ability in mixtures by selecting the plants that carry the positive alleles for mixture. For the adaptation to grazing, the situation is more complex because of the negative correlation between yield, persistence and winter activity. However, the QTL highlighted a partly different genetic control between these traits, offering a possible way to exploit marker-assisted selection to breed for tolerance to severe grazing.

The F1 plants carrying positive traits and QTL for each growing condition were intercrossed. A few recurrent selection cycles that progressively concentrate the frequencies of positive QTL alleles would be particularly useful in this respect. The evaluation of experimental varieties under contrasted protocols (monoculture vs. mixture, grazing vs. mowing) will definitively attest the opportunity to improve alfalfa for more sustainable conditions.

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## Field management for grazing/drought tolerance\*

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**KEYWORDS:** alfalfa, management, grazing

### INTRODUCTION:

Alfalfa (*Medicago sativa* L) is a perennial legume that can fix its own nitrogen in association with rhizobia bacteria. It has very high nutritional value, and due to the development of grazing type, alfalfa is considered to have tremendous forage potential (Bates et al., 1996; Bouton et al., 1998; Cassida et al., 2006). However, its use as a grazing crop has been limited due to limited persistence under continuous grazing (Smith and Bouton, 1993; Bouton et al., 1998; Kallenbach et al., 2002). The development of grazing tolerant cultivars (Smith et al., 1989; Smith and Bouton, 1993; Bouton et al., 1998; Bouton and Gates, 2003) warrant further investigation of alfalfa utilized under grazing. The objective of this research is to evaluate grazing tolerant alfalfa in various grazing systems. We evaluated various methods to establish alfalfa in perennial grass systems (tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons.] and bermudagrass [*Cynodon dactylon* (L.) Pers.]) prior to evaluating the production and economics of grazing alfalfa in the southern Great Plains of the USA, which is characterized by hot dry summers with bi-modal rainfall distribution in the autumn and spring.

### Establishment

**Alfalfa-Tall Fescue:** A series of experiments evaluated establishment methods and persistence of alfalfa ('Bulldog 505') and both summer-active ('Texoma Max QII') and summer-dormant ('Flecha') tall fescue types (Butler et al., 2011). Successful establishment (based on seedling counts) occurred with all treatments, however mixing seed in the same drill row resulted in one species dominating the stand (usually alfalfa). Planting perpendicular rows also resulted in dominance of alfalfa (although to a lesser extent). Planting in alternating drill rows resulted in successful establishment of both species, however in the second season, cattle preferentially grazed down the row of alfalfa avoiding the tall fescue. Planting in a combination of alternating and perpendicular drill rows ("checkerboard" matrix) resulted in successful establishment and persistence of both species (3 years). Therefore the checkerboard method is the preferred and recommended planting method in our environment.

**Alfalfa-Bermudagrass:** An experiment was conducted at the Red River Farm in the 2013-14 and 2014-15 seasons (unpublished data). Main plot consisted of three planting dates (15 September, 15 October, and 15 February), subplot consisted of three seedbed preparation methods (haying, tillage, and haying plus glyphosate), and sub-subplot consisted of various fungicide and insecticide seed treatments. Establishment (seedling counts 30 days after emergence) was successful with all treatments, however seedlings in the February planting dates and hay only seedbed preparation sub-treatment did not survive or contributed very little to DM yield. Although we did not observe any specific insect damage, the Cruiser® insecticide either alone or in combination consistently provided the greatest number of seedlings and alfalfa DM production. Glyphosate in October was inconsistent: in year one, alfalfa production was equal to tillage, however in year two, early frost negated the effect of the glyphosate and bermudagrass outcompeted the alfalfa. Although both tillage and glyphosate seedbed treatments were similar in September, we recommend glyphosate plus no-till drill due to ease of application compared to tillage.

### Production

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**Alfalfa Monoculture:** In a previous study (3-year average), steers grazing monoculture stands of alfalfa gained  $0.93 \text{ kg day}^{-1}$  and grazed  $504 \text{ days ha}^{-1}$ , which resulted in total gain of  $470 \text{ kg ha}^{-1}$ . Full economic analysis accounted for all input costs (seed, chemical, fertilizer, custom rates for tillage and planting) and output revenue based on total gain and the value of gain (Butler et al., 2012). Alfalfa stands only lasted three years in this experiment due to loss from cotton root rot (*Phymatotrichopsis omnivore*). Amortized on three year stand-life, grazing alfalfa was profitable (US\$314  $\text{ha}^{-1}$ ) and similar to rye (*Secale cereale* L.) /ryegrass (*Lolium multiflorum* L.) graze out system, however net returns were not as great compared to an enterprise budget of alfalfa produced and sold for hay. It is unlikely that producers in the Southern Great Plains of the United States will utilize alfalfa as a grazing crop, since the dry environment allows for successful hay production. However in more humid, high rainfall areas where it is difficult to put up high quality hay, grazing alfalfa could be an option.

**Alfalfa-Tall Fescue:** Mixtures of alfalfa-summer-dormant tall fescue were established using the checkerboard alternating and perpendicular row orientation (described earlier) during the 2013-14 season, which were then compared to a summer-dormant tall fescue only fertilized with  $112 \text{ kg N ha}^{-1}$ .

In 2015 (the second season), Tropical Storm Bill dropped 30.5 cm of rain in 12 hours, which killed the alfalfa; therefore alfalfa was replanted in the autumn of 2015. During the establishment seasons (2013-14 and 2015-16), there was limited production and grazing (April-May). In order to maximize stand life and autumn production, paddocks were not grazed during the summer months (July-August). The economic analysis accounted for two establishment costs during the five year average (Table 1). The first stand was amortized over two years while the second stand was amortized over five years. Due to limited production in both establishment seasons, the five year average was also lower than expected. Total grazing days ( $381 \text{ days ha}^{-1}$ ) were less than the tall fescue monoculture paddocks fertilized with N fertilizer. However, total live weight gains ( $384 \text{ kg ha}^{-1}$ ) were similar to tall fescue fertilized with N fertilizer ( $380 \text{ kg ha}^{-1}$ ) since average daily gains (ADG) were greater with the alfalfa-tall fescue mixtures ( $1.01 \text{ vs } ? \text{ kg day}^{-1}$ ). Consequently, net returns were slightly greater in tall fescue N fertilizer systems when two establishment cost were included during the five year average (US\$279  $\text{ha}^{-1}$ ). The current alfalfa stand is going into its fourth season; however sensitivity analysis indicates the alfalfa must persist five seasons in order for the net returns to equal tall fescue monoculture fertilized with N.

**Wheat-Alfalfa-Crabgrass-Alfalfa Rotation:** A wheat (*Triticum aestivum* L.)-alfalfa-crabgrass [*Digitaria sanguinalis* (L.) Scop.], two paddock rotational system was also evaluated. The growing season was defined as September-August. Alfalfa was seeded at  $13.4 \text{ kg PLS ha}^{-1}$  in one paddock, while the wheat was no-till drilled at  $112 \text{ kg PLS ha}^{-1}$  and fertilized with  $112 \text{ kg N ha}^{-1}$  in the adjacent paddock. The crabgrass was no-tilled drilled into the wheat stubble the first season of the graze-out wheat and allowed to reseed itself in the subsequent seasons. In the establishment season, cattle grazed the wheat pasture from November-April, and then rotated to the alfalfa from April-June, and then rotated to the crabgrass (interseeded into wheat stubble) from July to August. In the subsequent seasons, cattle grazed the alfalfa from September-November, and then rotated to wheat from November-April, back to alfalfa April-June, and then back to crabgrass from late June to August. Total live weight gain (TLWG) was numerically greater in the two paddock system with wheat-alfalfa-crabgrass, compared to the wheat-N only system, tall fescue-alfalfa mixture, and tall fescue-N systems, which were similar. The economic analysis accounted for two establishment costs during the five year average (Table 1). The first stand was amortized over two years while the second stand was amortized over five years. Alfalfa alone had lesser gains and net returns since it was managed as part of the system and grazing was deferred when crabgrass and wheat was being utilized. Alfalfa could complement the wheat-crabgrass system, since it is the only summer forage to provide great ADG, even though net returns were greater with the wheat-crabgrass only system. A producer would need a high quality summer forage during the establishment of the crabgrass in that system in order to be successful. Net returns were similar among alfalfa-tall fescue (US\$279  $\text{ha}^{-1}$ ) and wheat-alfalfa-crabgrass two paddock systems (US\$289  $\text{ha}^{-1}$ ). Therefore alfalfa has potential in an integrated system that aims to provide high quality forage in a year-round grazing system.

**Table 1:** Summary of cool-season grazing system evaluations for the Southern Great Plains averaged over five seasons (2013-18), which were defined as September through August.



<b>5 year avg (2013-18):</b>	Net return	ON date	OFF date	Grazing days	Grazing days ha <sup>-1</sup>	ADG	TLWG
	US\$ ha <sup>-1</sup>			# days	days ha <sup>-1</sup>	kg day <sup>-1</sup>	kg ha <sup>-1</sup>
Wheat ('NF 101')	254	11/26	4/21	118	408	0.98	400
Crabgrass ('Impact')	212	6/28	8/19	47	304	0.71	216
Alfalfa ('Bulldog 505')	114	4/26 and 9/18	6/21 and 11/13	90	321	0.92	295
<b>Wheat-alfalfa-crabgrass</b>	<b>289</b>	11/26	11/13	<b>254</b>	<b>496</b>	<b>0.92</b>	<b>456</b>
'Flecha' SDTF-100N	341	10/30	5/21	144	463	0.82	380
Flecha SDTF-alfalfa	279	9/27	5/18	103	381	1.01	384

**Alfalfa-Bermudagrass:** Interseeding alfalfa into established bermudagrass pastures can increase nutritive value and seasonal forage distribution as well as contribute to the nitrogen needs of bermudagrass. The objective of this study is to evaluate stocking rate, forage allowance, grazing days ha<sup>-1</sup>, and animal performance of bermudagrass grazing systems in Ardmore, OK. Forage treatments are 1) monoculture bermudagrass with 0 N, 2) monoculture bermudagrass with 0 N and protein supplement (0.5% BW), 3) monoculture bermudagrass with 112 kg N ha<sup>-1</sup>, 4) monoculture bermudagrass with 112 kg N ha<sup>-1</sup> and protein supplement (0.5% BW), and 5) bermudagrass interseeded with '800RR' alfalfa in September following hay removal and application of 1.12 kg ai ha<sup>-1</sup> glyphosate. All treatments have a continuous (4.9 ha) and rotationally (9.9 ha) stocked (with 21 day rest period) component with three replications in a completely randomized design.

**Table 2.** Bermudagrass treatment and stocking method effect on grazing days, stocking rate, and animal performance averaged across 2016 and 2017 growing seasons (April-November).

Bermudagrass treatment	Stocking method	Grazing days	Stocking rate	ADG	Grazing days	Total gain
		# days	AU ha <sup>-1</sup>	kg day <sup>-1</sup>	days ha <sup>-1</sup>	kg ha <sup>-1</sup>
800RR alfalfa	Continuous	182	2.7	0.40	616	247
800RR alfalfa	Rotational	189	3.3	0.40	754	301
N fertilizer	Continuous	151	5.6	0.16	822	131
N Fertilizer	Rotational	154	6.0	0.16	916	145
N fertilizer + 0.5% suppl.	Continuous	151	5.7	0.47	842	393
N fertilizer + 0.5% suppl.	Rotational	154	5.7	0.43	844	366
0 N	Continuous	140	4.7	0.18	617	112
0 N	Rotational	140	4.0	0.21	565	118
0 N + 0.5% suppl.	Continuous	140	4.6	0.38	648	244
0 N + 0.5% suppl.	Rotational	140	4.9	0.42	685	286

Continuously and rotationally stocked paddocks generally did not differ in grazing days, ADG, and TLWG per hectare within bermudagrass treatment, therefore data is pooled across years (random) and stocking method. Greatest ADG occurred with both supplementation treatments and alfalfa interseeded into bermudagrass.

However, greatest TLWG occurred with the bermudagrass + N fertilizer plus 0.5% BW supplementation treatment. A full economic analysis will be conducted after the third season is completed, however preliminary results based on two years of data suggest the bermudagrass with protein/energy supplement at 0.5% BW provided the lowest value of gain when compared to the control of no N fertilizer and no feed supplement.

## SUMMARY AND CONCLUSION

Alfalfa does have potential to be utilized as a grazing forage crop, however due to the limited persistence under grazing (3 year stand life), it has not compared favorably to other alternatives. In order for it to be successful, the seed price must be reduced or the length of stand life must be increased to five years. Grazing management (i.e. rotational stocking method or deferred summer grazing when grown in a tall fescue mixture) can improve persistence. We will continue to monitor persistence to determine final economic outcome. Current efforts in alfalfa breeding need to address the persistence issues, with the hope of developing a cultivar that will persist for five years, or greater, under grazing.

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## Breeding alfalfa for cold tolerance in Canada\*

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**KEYWORDS:** Alfalfa, recurrent selection, cold tolerance, high-throughput genotyping

### INTRODUCTION

Lack of winter of alfalfa is largely attributable to insufficient freezing tolerance which greatly reduces the persistence of this forage legume in northern climates. The development of winter hardy cultivars has been historically hardiness based on selection of genotypes that survived winters within field nurseries. Cultivars (Apica, AC Caribou, AC Viva, AC Brador, AC Mélodie, Manitou, Totem, Maska, Calypso and AAC Nikon) released by our group during the last 30 years were developed using traditional breeding approaches (Michaud and Richard. 1992; Michaud et al. 1983). However, this is a long, costly and highly unpredictable process that often requires the assessment of plants at multiple sites over many years in order to identify genotypes with superior potential (Limin and Fowler, 1991). Furthermore, improvement of freezing tolerance using conventional breeding approaches is limited by its quantitative inheritance and large G x E interactions (Miklas et al. 2006). Thus, faster and more repeatable approaches are required to incorporate freezing tolerance into cultivars with high agronomic value.

### Recurrent selection performed under controlled conditions

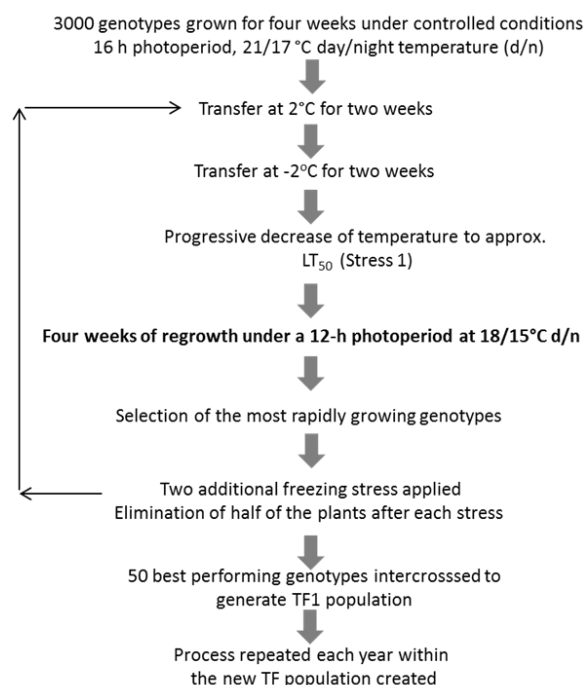
To address this issue, we developed and applied a recurrent selection protocol entirely performed under controlled conditions to produce alfalfa populations selectively improved for their tolerance to freezing (Castonguay et al. 2009 and Fig. 1). To avoid selecting highly-dormant alfalfa genotypes with low yield potential, a 4-wk regrowth phase under a 12h-photoperiod allowing identification and selection of low-dormant genotypes was included after the freezing test (Bertrand et al. 2018). Several cycles of recurrent phenotypic selection have been performed in various genetic backgrounds and new synthetic populations have been produced by intercrossing elite genotypes (Castonguay et al. 2006). We obtained significant increases in freezing tolerance in advanced cycles of selection (Fig. 2) which translated into superior winter survival and spring regrowth in the field (Castonguay et al. 2009).

Plant responses to recurrent selection for superior freezing tolerance were characterized. We observed differences in concentration of cold-induced metabolites, including increase in the concentration of cryoprotective sugars, and changes in amino acids concentrations (proline, asparagine, arginine), and of transcript levels of cold-regulated genes (Bertrand et al. 2017, Castonguay et al. 2011a) between initial cultivars and advanced cycles of selection. Extensive proteomic changes associated with cold acclimation and/or freezing tolerance were also observed in recurrently selected red clover populations (Bertrand et al. 2016).

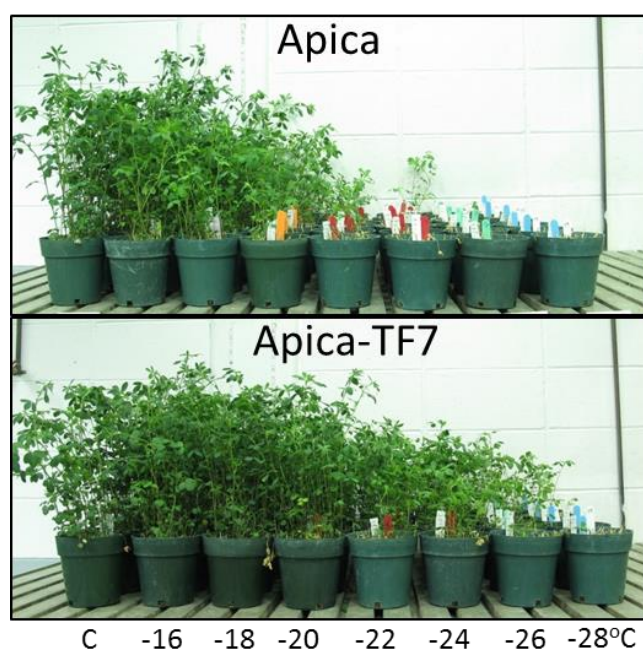
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## SELECTION FOR FREEZING TOLERANCE (TF) AND REDUCED DORMANCY



**Figure 1.** Recurrent procedure used for the selection of freezing-tolerant and low-dormant populations.



**Figure 2.** Plants of alfalfa cultivar Apica and of population Apica-TF7 issued from seven cycles of recurrent selection for freezing tolerance within Apica after exposure to subfreezing temperatures followed by three-week of regrowth. Control plants (C) were exposed to -2°C. Five (5) pots of 10 plants were tested at each temperature.

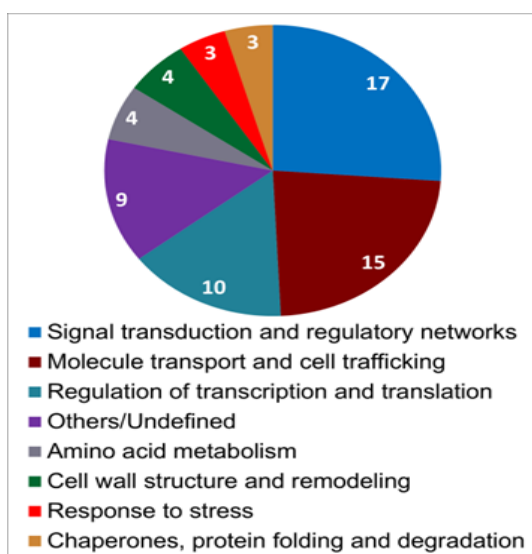
### SNPs under selection pressure

At the genomic level, we observed changes in allele frequency in response to selection pressure when using SRAP (Sequence-Related Amplified Polymorphism) markers (Castonguay et al. 2012). This observation of allele enrichment linked with the observation of large phenotypic differences in freezing tolerance between initial

and selected populations prompted us to combine recurrent selection and polymorphism discovery toward marker-assisted selection to accelerate the selection process.

For this purpose, we undertook genome wide characterisation of DNA variations in recurrently-selected populations of tetraploid alfalfa, using a high throughput genotyping approach (Genotyping By Sequencing, GBS) (Rocher et al. 2015). The analysis of these GBS data was performed with UNEAK (Glaubitz et al. 2014), a pipeline specifically designed for species lacking reference genome like alfalfa. Although several SNP under selection pressure were identified (>100 unlinked SNP), this study was not designed to detect QTL associated with freezing tolerance. First, the phenotypic test used to evaluate freezing tolerance is destructive and based on population response to stress. As such, it does not allow phenotypic evaluation of individual genotypes, which prevents the identification of markers associated with freezing tolerance using conventional quantitative genetic approaches. Also the lack of tetraploid reference genome of *M. sativa* is a major limitation, as UNEAK pipeline delivers only bi-allelic SNP markers with insufficient coverage to infer allele dosage, which prevents to fully exploit the extensive allelic variation that exists in heterozygous tetraploid populations. Solutions to face these issues are currently being addressed by our team through the development of single-genotype based phenotyping methods to complete freezing tolerance evaluations and the utilisation of analysis methods based on population genetics approaches to identify candidate genes linked with superior freezing tolerance.

On the other hand, SNPs loci under strong selection pressure identified between an initial alfalfa population (Apica) and recurrently selected populations with superior freezing tolerance (Apica-TF6) were mostly located in genes involved in a limited number of functional categories, including signal transduction, regulatory network and molecule transport (Fig. 3).



**Figure 3.** Number of candidate genes containing SNP loci under selection pressure in alfalfa populations under recurrent selection for superior freezing tolerance distributed within functional categories.

## CONCLUSION

Taken together, our results suggest that recurrent selection for superior freezing tolerance in alfalfa populations has a major impact on alfalfa biology, from genome organisation to physiological pathways. Not only will the use of molecular genetic tools help us unravel molecular determinism of this complex trait but it will also be added to the tools available to breed cold-tolerant alfalfa. These resources might be used to accelerate the development of enhanced alfalfa cultivars and to implement alfalfa breeding strategies.

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## Introgression of alfalfa crop wild relatives for climate change adaptation\*

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**ABSTRACT:** Crop wild relatives (CWR) have evolved to survive in extreme environments, and have developed many different strategies for drought tolerance. This adaptation provides the potential to contribute novel alleles for the breeding of more resilient forages in the face of global climate change. Through our partnership with the Crop Trust, we aim to identify, conserve and introgress crop wild relatives of alfalfa into modern plant varieties. The new pre-bred lines have overcome issues with inter-specific hybridisation, allowing plant breeders to more easily introduce new diversity into their breeding programs. The ultimate aim is to breed new resilient varieties for developing countries that benefit subsistence and smallholder farmers, who are most vulnerable because they live in marginal food production environments and are least equipped to deal with change.

Extreme examples of drought tolerant alfalfa accessions were identified and acquired from the USDA-ARS (USA), Vavilov Institute (Russia), Kazakhstan and the Australian Pastures Genebank (APG). In addition, the most drought tolerant Russian ecotypes (or species using Russian taxonomy) described by Sinskaya (1950) were targeted to maximise diversity from this primary centre of evolution. Recent collection trips by Kew (UK), SARDI (Australia) and KSRIAPG (Kazakhstan) to Georgia, Italy, Azerbaijan and Kazakhstan have also provided access to new diversity.

This paper reports on progress with the development of new hybrid and pre-bred lines developed from crosses between alfalfa (*Medicago sativa* subsp. *sativa*) and *M. sativa* subsp. *falcata*, *M. sativa* subsp. *caerulea*, *M. arborea*, *M. strasseri*, *M. sativa* subsp. *glomerata* and *M. truncatula*, and highlights the free availability of all accessions and pre-bred lines for further research and development under the SMTA from the Australian Pastures Genebank.

### 1. Identification, acquisition and conservation of new CWR accessions with a focus on drought tolerance

The book 'Alfalfa and wild relatives' (Small 2011) was used to identify germplasm at a species level with the potential to contribute to drought tolerance and previous success at intra/inter species crosses with *M. s. sativa*. From this review, *M. prostrata*, *M. arborea*, *M. strasseri*, *M. s. falcata*, *M. s. caerulea* and *M. ruthenica* were identified as major sources of drought tolerance diversity.

Accessions from the APG and USDA genebanks with putative drought tolerance were selected based on their passport data, including average annual rainfall combined with location and collection notes. Accessions were also selected if other harsh environmental conditions such as salt tolerance (increasing osmotic stress), grazing tolerance or evolution with competitive rhizomatous grasses could be identified. KSRIAPG have also recently donated 62 accessions to the APG from arid environments in Western Kazakhstan (Table 1).

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As part of the CWR program, Kew has conducted targeted collection missions to conserve crop wild relatives in environments that are underrepresented in genebanks and most at risk of local extinction in the face of climate change. Recent plant collections in collaboration with the Università degli Studi di Pavia (Italy) and the National Botanical Garden of Georgia (NBGG, Republic of Georgia) have resulted in the acquisition and conservation of new alfalfa genetic resources (Table 1).

The Russian 'Flora of cultivated plants of the USSR' (Sinskaya 1950) book was used to identify ecotypes (or species using the Russian taxonomy) with extreme sources of drought tolerance in *M. s. falcata* and *M. s. caerulea* (Table 2). Accessions representing these ecotypes were acquired by APG from the N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) in 2017, with seed now available under a SMTA (Table 2).

**Table 2.** *Medicago sativa* ecotypes specifically mentioned with high levels of drought tolerance in 'Flora of cultivated plants of the USSR' (Sinskaya 1950)

Ecotype description	sub species	APG Accession
<b>South Russian Steppe Regional ecotype – Ukraine, central</b>	<i>falcata</i>	84259, 84260, 84261, 84262
<b>Caucasian foothill steppe ecotype</b>	<i>falcata</i>	84270, 84271
<b>South Russian floodland ecotype – Kubin</b>	<i>falcata</i>	842645, 84266
<b>Siberian Steppe regional ecotype – variety Omskaya 2251</b>	<i>falcata</i>	84267
<b>Western Siberian forest steppe ecotype, Leningrad Oblast deep rooted</b>	<i>falcata</i>	84268, 84269
<b><i>Medicago trautvetteri</i> eastern ecotype</b>	<i>varia</i>	84272, 84273
<b><i>Medicago trautvetteri</i> western ecotype</b>	<i>varia</i>	84274, 84275

Two additional ecotypes, 'Altyn Emel' and 'Ural root suckering' (from Western Siberia) were not sourced.

## 2. Introgression of CWR into *M. sativa* subsp. *sativa* plant varieties

Crop wild relatives of alfalfa targeted in this project are being introgressed into alfalfa varieties, with the aim of developing a cohort of pre-bred lines for use in alfalfa breeding programs around the world. A list of the new lines developed to date are shown in Table 3.

New lines developed from crosses between *M. s. caerulea* and alfalfa were made using both diploid (relying on the production of 2n pollen) and tetraploid versions of accession APG 42382. This accession was targeted because it was collected from the most arid site in Azerbaijan (the Abseron peninsula) where this species was represented (Auricht et al. 2009). Drought tolerance may be associated with ploidy in this diploid subspecies of *sativa*, representing a challenge for plant breeders working in tetraploid *M. s. sativa*.

A total of 55 new lines have been made between *M. s. falcata* and *M. s. varia* (*M. trautvetteri*) and alfalfa varieties from Australia, China and Kazakhstan. The accessions in these crosses were obtained from VIR, identified by N. Dzyubenko (Pers. Comm 2017), representing drought tolerant ecotypes (in Table 1) described by Sinskaya (1950). A pre-bred line developed earlier (CTA011, Table 3) has shown promise for high yield production at Almaty in Kazakhstan, indicating the potential success of introgressing CWR into modern varieties. This CWR accession in this cross co-existed in a dense sward with *Agropyron*. We are interested in the potential of the *M. s. falcata* subspecies to improve the compatibility of alfalfa in mixtures with perennial grasses, and thus extend the production of alfalfa into permanent pastures.

The development of *M. sativa* x *M. arborea* hybrids (called Alborea) has been pioneered by Edwin Bingham (2009). The potential of Alborea in restructuring a number of traits in alfalfa has also been reported by Irwin et al. (2016). Bingham has kindly donated the population Alborea-101, which we started to seed increase with a 500 plant population, employing a single seed descent method over five generations to promote gene mixing and the development of new phenotypes following tetrasomic inheritance. Seed of earlier generations will also be available from the APG under the SMTA. In addition, we have also attempted our own crosses between 22 accessions of *M. arborea* plants (15 year old plants growing at the Waite Institute) and alfalfa in 2017/18. We did not use a male sterile mid-parent as described by Bingham (2009), hoping to maximise low fall dormancy



(maximum winter or fall growth rates). Our crosses concentrated on using high seed yielding, non-fall dormant alfalfa (class 10) germplasm from Australia and the USA.

**Table 1.** Summary of accessions identified and acquired with new diversity for drought tolerance.

<b>Taxon</b>	<b>No. accessions</b>	<b>Key APG* accessions</b>	<b>Country / region</b>	<b>Donators</b>	<b>Collection / breeding notes of key accessions</b>
<i>M. arborea</i>	24	-	Greece	1	15 year old plants at the Waite Institute
<i>M. ruthenica</i>	1	43029	China, Inner Mongolia	2	Selected for erect habit
<i>M. sativa</i> subsp. <i>sativa</i>	1	Patagonian alfalfa	Chile	10	Seed collected from 80 year old plants growing in Patagonia Chile
	1	16453	Iran	1	Low rainfall, shallow soil (MJM 7318)
	1	6567	Spain	1	YI-1, selection made at Aula Del, Zaragoza for arid zones
<i>M. s. caerulea</i>	1	42382	Azerbaijan, Nardaran	1	Very dry, hot and overgrazed by sheep.
	10	84831, 84834	West Kazakhstan, Aktobe	3	Arid sand to sandy loam sites
<i>M. s. falcata</i>	17	38808, 38116, 38690	Kazakhstan Ayagoz	1, 4	Co-existence with <i>Agropyron</i> spp., saline, soil pH 10
	9	854, 856	Italy	5,6	High relative yield under rainfed / irrigation.
	19	84767, 84775	West Kazakhstan, Aktobe	7, 3	Mugalzhar mountains, Khromtau district, in association with <i>Agropyron</i> and <i>Astragalus</i> .
<i>M. s. glomerata</i>	10	84245, 84247, 84248	Georgia	8	CWR 867308. Good biomass, large flowers
<i>M. s. varia</i>	43	84779, 84781, 84805	West Kazakhstan, Aktobe	7, 3	Feathergrass ( <i>Stipa</i> ) and <i>Agropyron</i> .
<i>M. strasseri</i>	1	41909	Spain (ex Greece)	9	Improved leaf holding capacity relative to <i>M. arborea</i> .

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**Table 3.** New pre-bred lines and hybrids developed

APG or project identifier*	Taxon	Breeder	Fall dormancy	Parents / Description
APG422, APG4293, APG4294	<i>M. sativa</i> subsp. <i>varia</i>	Humphries	2-4	Field selections for persistence in South Australia. Diverse background
CTA007-CTA011	<i>M. sativa</i> subsp. <i>varia</i>	Humphries	2-4	Five lines FD7 lucerne x Kazakhstan (38690, 38815-18 <i>M. s. falcata</i> from saline areas with <i>Agropyron</i> ) backcrosses back into <i>M. s. sativa</i>
CTA013	<i>M. sativa</i> subsp. <i>varia</i>	Humphries	2-4	32 lines, FD7 lucerne (broad) x VIR <i>falcata</i> APG84261, 84269, 84270 with high yield
CTA014	<i>M. sativa</i> subsp. <i>varia</i>	Humphries	2-4	33 lines, FD7 lucerne (broad) x VIR "trautvetteri" APG84272, 84273, 84274, 84275 with high yield
CTA015	<i>M. sativa</i> x <i>M. s. glomerata</i>	Humphries	2-4	22 lines, FD7 lucerne (broad) x <i>M. s. glutinosa</i> APG84245, 84247, 84248 (large flowers, moderate yield)
CTA016	<i>M. truncatula</i> 4x	Humphries	n/a	28 tetraploid seed lines doubled from <i>M. truncatula</i> cv. Sultan SU
CTA017	<i>M. sativa</i> x <i>M. truncatula</i>	David Peck	?	Tetraploid Tet1-2 <i>M. truncatula</i> x SARDI 7Series 2 lucerne. Three plants.
Alborea-101	<i>M. sativa</i> x <i>M. arborea</i>	Edwin Bingham	3-10	Alborea composite line. 500 plant population
CTA019	<i>M. sativa</i> x <i>M. arborea</i>	Edwin Bingham	9-10	Alborea crosses, FD 9-10. W10 x Genesis
CTA020	<i>M. sativa</i> x <i>M. arborea</i>	Alan Humphries	?	181 seed lines from 22 Arborea accessions x FD7 and FD10 lucerne varieties crossed in 2017
CTA021	<i>M. sativa</i> x <i>M. strasseri</i>	Alan Humphries	?	66 seed lines from APG41909 x FD7 and FD10 lucerne varieties

\*All lines will be given an APG number once seed is available for deposit, APG = Australian Pastures Genebank.

This decision resulted from the impressive growth rates of a M10 x Genesis hybrid (Genesis is a FD7 cultivar), also provided to us by Bingham. The F1 generation will be screened for habit, height, stem thickness and flower colour (yellow flowers are inherited from *M. arborea*) to confirm that true hybrids have been made.

In the same approach we have also attempted to make crosses between alfalfa and *M. strasseri* accession APG41909. *M. strasseri* is closely related to *M. arborea*, and is only found on the island of Crete (Greece). This is likely to be the first time this cross has been attempted, given rareness of this species, with only three accessions housed in genebanks around the world (Small 2011).

Hybrids between alfalfa, *M. truncatula* and *M. intertexta* have also been reported to be successful (Bingham Pers. Comm. 2017). We plan to evaluate and multiply a mixed population of *M. sativa* x *M. truncatula* x *M. intertexta* (referred to as Perennial X Annual Crosses or M.PAC) from Bingham. *M. intertexta* is a high yielding annual species that could be of interest to Australian farmers in low rainfall Mediterranean environments, but is not grown because of large spines on its pods. We are also confident that we have three *M. sativa* x *M. truncatula* (tetraploid following chromosome doubling) hybrid plants. The plants have yellow flowers and an appearance that is close to the tetraploid *M. truncatula* parent (albeit with larger flowers), and look to have impressive forage yield. One of the three putative hybrids has stable pollen. The three plants (and vegetative

clones produced from them) will be crossed back into non-dormant alfalfa and evaluated for perenniality in 2018/19.

**Evaluation and free distribution of new hybrid lines for plant breeders.** The individual APG accessions and hybrid lines described in this paper will be made freely available to researchers through the APG under an SMTA as consistent with the International Treaty on Plant Genetic Resources for Food and Agriculture. A cohort containing the main outputs of the project will be available under the name 'CT\_alfalfa cohort' from June 2020. Seed can be ordered online from the APG public web site at <https://apg.pir.sa.gov.au/gringlobal>. The cohort is being evaluated by the project partners in South Australia, central and southern Chile, south and north Kazakhstan and in Inner Mongolia, China (some early results presented in posters at this conference). Full details of pre-bred lines and associated data can soon be found on Germinate 3 at <https://ics.hutton.ac.uk/cwr/alfalfa/#home>.

The aim of the Crop Trust CWR alfalfa project is to promote the availability of these new hybrids and pre-bred lines in breeding programs around the world, with the understanding that will take a collaborative effort to develop new resilient varieties adapted to a changing climate.

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# Genome-enabled and phenotypic selection of alfalfa for widely-diversified cropping environments\*

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## INTRODUCTION

The decrease of rainfall amount due to climate change, and the reduction of irrigation water caused by growing water demand for non-agricultural uses, emphasize the importance of breeding novel alfalfa varieties that are more tolerant to drought under rain-fed cropping or modest supplemental irrigation, and more tolerant to salt to exploit low-quality, saline irrigation water. A sharp need for such improved germplasm is emerging in the Mediterranean basin, where alfalfa plays a key role for forage production. Drought-tolerant cultivars are needed also elsewhere, for example in Argentina, where they could allow to expand westward the alfalfa rain-fed cropping. Alfalfa typically features very low rates of genetic yield gain (Annicchiarico et al. 2015a), urging the development of cost-efficient marker-based selection.

The multi-site yield testing of alfalfa varieties and landraces across countries of the western Mediterranean basin revealed outstanding genotype  $\times$  environment (GE) interaction of cross-over type associated with three major types of target environments: i) rain-fed or irrigated environments featuring limited spring-summer water available and low salinity, ii) salt-stress environments, and iii) moisture-favorable environments (Annicchiarico et al. 2011). Eco-physiological research identified various mechanisms that contribute to specific-adaptation responses (Annicchiarico et al. 2013). Moderately wide cultivar adaptation may be desirable, given the wide year-to-year climatic variation of sites in this region.

A Mediterranean reference population of alfalfa was developed from elite germplasm within the ERA-Net project REFORMA. A genotype training set was sorted out from it, to verify the ability of genomic selection (Heffner et al. 2009) to predict breeding values for biomass yield across a range of widely-diversified cropping environments. Genotype breeding values were estimated according to responses of their half-sib progenies, as required by the crop outbred system (Annicchiarico et al. 2015a). The genotyping of parent genotypes was carried out by Genotyping-by-Sequencing (Elshire et al. 2011) after optimizing for alfalfa some elements of its protocol (Annicchiarico et al. 2017). Concurrently, the project verified the ability of managed-stress environments of Italy to predict genotype yield responses in distant agricultural environments, following earlier work showing good ability to predict cultivar responses across agricultural environments of Italy (Annicchiarico and Piano 2005). This study aimed to provide an initial assessment of the ability of genomic selection to predict breeding values in each cropping environment and across stress environments, on the basis of preliminary yield data and GBS-generated data.

## MATERIALS AND METHODS

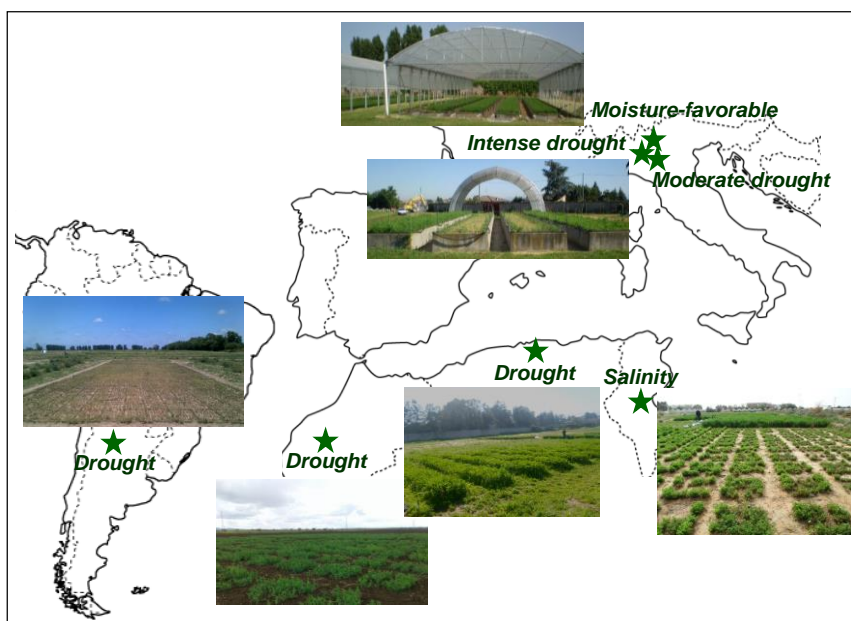
The Mediterranean reference population was developed by repeated intercrossing of genotypes from the drought-tolerant Sardinian landrace Mamuntanas, the salt-tolerant Moroccan landrace Erfoud 1 and the Australian variety SARDI 10 [which is widely adapted across moisture-favorable and drought-prone sites: Annicchiarico et al. 2011], as described in Annicchiarico et al. (2015b). Some 128 half-sib progenies were simultaneously phenotyped in three managed-stress (MS) environments of northern Italy that featured contrasting drought-stress level, two drought-prone rain-fed sites of Algeria (Alger) and western Argentina

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(Santiago del Estero), one drought-prone Moroccan site managed with limited supplemental irrigation (Marrakech), and a Tunisian site (Médénine) irrigated with moderately saline (9.37 dS/m) water (Figure 1). Dry-matter yield was recorded over five months in the moisture-favorable MS environment, and over a period ranging from nine months (Santiago del Estero) to two years (intense-drought MS) in the other environments. Data from one more year of evaluation will be available in the future for most sites.

Annicchiarico et al. (2015b) described the GBS analysis of the parent genotypes (which used the *ApeKI* restriction enzyme and the KAPA *Taq* polymerase) and the genotype SNP calling, which envisaged two homozygote classes AAAA and aaaa and one heterozygote class pooling AAAa, AAaa and Aaaa variants (given the inability to produce sufficient SNP data to estimate reliably the allele dosage for heterozygous loci). We retained for analyses 9,269 polymorphic SNP markers with less than 20% missing data across genotypes, imputing missing data by the KNNI method (K = 4). Genome-enabled predictions were assessed by two models, i.e., Ridge Regression BLUP and Support Vector Regression with linear kernel, which stood forward in the comparison of models reported in Annicchiarico et al. (2015b).



**Figure 1.** Test environments for biomass yield phenotyping of 128 alfalfa half-sib progenies

## RESULTS

Estimates of genetic correlation for yield of half-sib progenies between pairs of environments were mostly low and occasionally negative, indicating the large size of GE interaction even across drought-prone environments. The ability of MS environments of Italy to predict yield responses in distant agricultural environments was generally modest, probably because of differences for other environmental factors (e.g., temperature pattern). High genetic correlation occurred between MS environments with moderate drought and moisture-favorable conditions. The large size of GE interactions was confirmed by cross-over interactions occurring among top-yielding half-sib progenies as revealed by AMMI analysis.

The two genomic selection models exhibited similar predictive ability (data not shown). Environment-specific genomic predictions, which are reported in Table 1 for the best-predicting model, were fairly high for the moisture-favorable MS environment, and poor for the stress-prone sites of Alger, Médénine and Santiago del Estero. These sites will produce at least one more year of data in the future, and the possibility to achieve better genomic predictions using yield data over a longer crop cycle was supported by higher predictive abilities obtained for data of the last harvest in Alger and Médénine (Table 1).

**Table 1.** Predictive ability of genomic selection for alfalfa breeding values of biomass yield in managed-stress (MS) environments of Italy and agricultural environments (as correlation of modelled and observed data averaged across 100 10-fold stratified cross-validations, for the best-predictive of two models)

Test environment	Total yield	Last harvest yield <sup>a</sup>
MS, intense drought	0.12	0.22
MS, moderate drought	0.18	0.10
MS, moisture-favorable	0.36	-
Alger (Algeria)	0.05	0.12
Marrakech (Morocco)	0.19	0.23
Médenine (Tunisia)	0.05	0.13
Santiago del Estero (Argentina)	0.10	-

<sup>a</sup> Excluding environments with relatively short experiment duration

One possible reason for less accurate predictions in stress environments was their trend towards higher experiment error than favorable environments, as shown by error coefficients of variations for intense drought ( $CV_e = 27.9\%$ ), moderate drought ( $CV_e = 21.2\%$ ) and moisture-favorable ( $CV_e = 13.9\%$ ) MS environments. One additional reason might be the fact that the reference population derived from stress-tolerant material and, as such, was characterized by only moderate genetic variation for stress tolerance, as suggested by greater genetic coefficient of variation under moderate drought ( $CV_g = 15.7\%$ ) than intense drought ( $CV_g = 10.3\%$ ). However, the adopted genetic base was that of practical regional interest.

The value of genomic predictions ought to be assessed in relation to opportunities offered by phenotypic selection. A comparison of genomic vs half-sib progeny-based phenotypic selection in terms of predicted genetic gain per year equates to comparing ( $i_G r_A / t_G$ ) vs ( $i_P H / t_P$ ), where  $r_A$  and  $H$  stand for genome-enabled predictive ability and the square root of narrow-sense heritability, respectively, and  $i$  and  $t$  are the standardized selection differential and the selection cycle duration in years (including final intermating), respectively, for genomic (G) and a phenotypic (P) selection (Annicchiarico et al. 2017). Since  $H$  lies in the range 0.39-0.45 based on narrow-sense heritability values summarized in Annicchiarico (2015), assuming  $H = 0.47$ ,  $r_A = 0.12$ ,  $t_G = 1$ ,  $t_P = 4$  and same evaluation costs implies about same efficiency of genomic and phenotypic selection. However, considering also the seemingly 7-8 times lower cost per genotype of genomic selection relative to half-sib progeny-based selection (Annicchiarico et al. 2017) implies, for the hypothetical scenario of 1400 genomically-evaluated and 200 phenotypically evaluated individuals and 20 selected parents ( $i_G = 2.54$ ;  $i_P = 1.75$ ), nearly 50% greater efficiency of genomic over phenotypic selection under the assumption of  $r_A = 0.12$ . Hence, most  $r_A$  values reported in Table 1 should not be seen as unfavorable.

An additional assessment focused on genomic selection ability to predict alfalfa breeding values for total yield averaged across all stress environments (i.e., all those in Table 1 except moisture-favorable MS). Phenotypic data were previously standardized to relative yields within environments. We obtained a prediction accuracy of 0.14, which is not unfavorable when considering that predictions related to breeding values for wide adaptation across quite diverse environments. In this case, half sib progeny-based phenotypic selection would have quite low  $H$  value (owing to the outstanding GE interaction), and its cost would be much greater compared with a single-environment phenotypic selection scenario.

## CONCLUSIONS

Alfalfa breeding for the western Mediterranean basin is hindered by outstanding GE interaction that sets a limit to selection for wide adaptation and can hardly be coped with by selecting in MS environments of distant regions. Our preliminary results indicate that genomic selection tends to be more predictive and convenient for favorable or moderately favorable environments than stress-prone ones. In these latter environments, however, also phenotypic selection may lack accuracy because of high experiment error. Genomic selection is particularly promising for selecting resilient varieties with relatively wide adaptation. For the same material, genomic selection proved valuable for key forage quality traits (Biazzi et al. 2017).



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## Genetic mapping of resistance to *Aphanomyces* root rot in alfalfa\*

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**KEYWORDS:** *Aphanomyces euteiches*, genotyping by sequencing, NBS-LRR resistance genes

*Aphanomyces* root rot (ARR), caused by the oomycete *Aphanomyces euteiches*, is one of the most important yield-limiting factors in production of legumes. In Europe, it is the main limiting factor for pea production while in the United States it is one of the most important diseases of alfalfa and pea (Gaulin et al., 2007). The disease causes stunting and death of alfalfa seedlings with complete stand loss occurring in wet and poorly drained soils. Non-lethal damage to seedling roots reduces forage yields and winter survival. The pathogen can infect adult plants during wet periods to cause loss of feeder roots and root nodules, reducing nitrogen fixation, forage yield, and stand life. Varieties with resistance to ARR became widely available in the 1990s; however, failure of resistant varieties identified a second race of the pathogen. Recent reports of failure of varieties with resistance to both race 1 and race 2 suggest that additional pathogenic races are present in alfalfa production fields. The goals of this research were to: (i) determine the prevalence of race 1 and race 2 strains in Minnesota and New York; (ii) identify novel strains of the pathogen; (iii) characterize the race-specific resistance to *Aphanomyces* root rot; and (iv) map resistance genes in order to facilitate breeding for resistance and to clarify race/resistance gene relationships.

From 2012 to 2015, a total of 53 soil samples from alfalfa production fields in the state of Minnesota and 40 soil samples from New York were assayed for the presence of the two races of *A. euteiches*. Although a PCR-based assay is available for identifying *A. euteiches* in plant and soil samples (Vandemark et al., 2002), it does not distinguish races. The only available assay for identifying races is a bioassay using differential varieties. In both states, race 2 predominated over race 1 (Figure 1). Soils with a high level of ARR tended to be rotated frequently to alfalfa while those with a low risk of ARR had not been used frequently for alfalfa production. These results suggest that frequent alfalfa cultivation increases the severity of ARR. Other surveys for ARR on alfalfa found that race 2 is prevalent in the Midwestern United States (Malvick and Grau, 2001; Malvick et al., 2009; Munkvold et al., 2001; Vincelli et al., 1994). The seed rot and damping off pathogens identified from these soils included *Pythium ultimum*, *P. irregulare*, *P. sylvaticum*, *Fusarium oxysporum* and *F. incarnatum-equiseti* (Berg et al., 2017). Many of the *Pythium* strains were resistant to ApronXL (mefanoxam), which is commonly used as a seed treatment, indicating that new strategies are needed for managing seed rot and damping off of alfalfa. A significant number of soils were classified as “atypical” because the race of *A. euteiches* could not be determined with the bioassay. Pure cultures of *A. euteiches* were isolated from diseased alfalfa seedlings grown in nine of the atypical soils and individual strains were used to inoculate the differential varieties. Of the 44 strains tested, 11 were classed as race 1 and 33 were classified as race 2. None of the individual strains could overcome the resistance in the check variety WAPH5 that has resistance to race 1 and race 2.

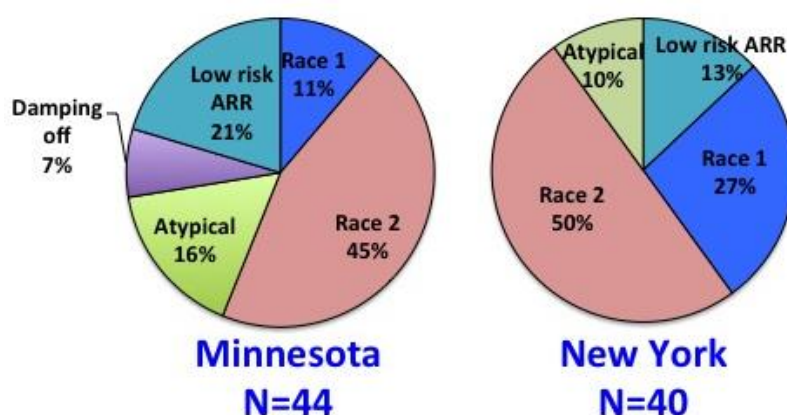
Although resistance to ARR has been incorporated into many commercial varieties, little is known regarding the mechanism of resistance or the genes controlling the trait. Alfalfa seedlings susceptible to ARR, resistant to race 1, or resistant to race 1 and race 2 were used to investigate resistance mechanisms. Zoospores were found to be attracted primarily to the root hair zone of seedlings and penetrated root cells within one hour of inoculation. Cells of resistant plants reacted to penetration with a rapid and localized hypersensitive response characterized by cell death and browning of penetrated cells. Cells infected by the pathogen were limited to epidermal cells or a small number of cortical cells in resistant seedlings. Highly upregulated genes in resistant plants at 24 hours after inoculation included genes involved in the phenylpropanoid biosynthetic pathway leading to phytoalexin (medicarpin), chitinase, and beta-1,3-glucanase production. The production of

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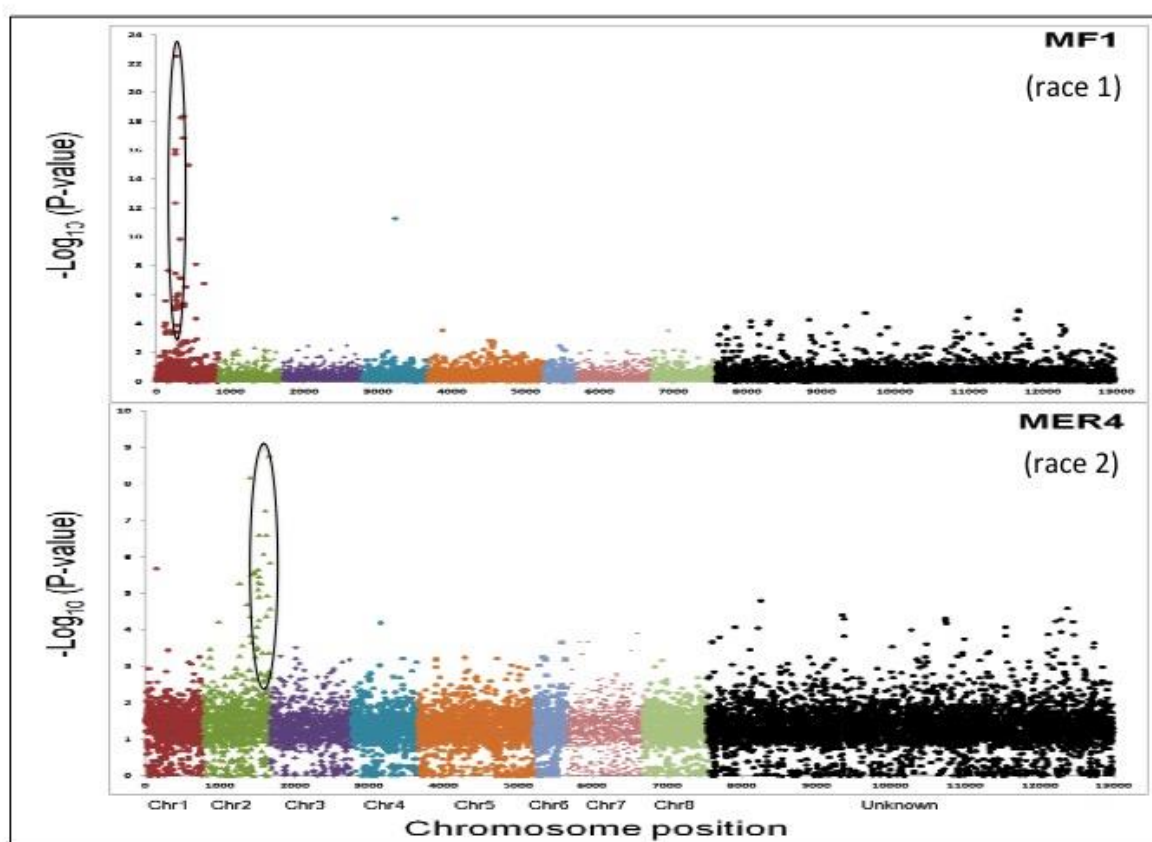
phenylpropanoid compounds and suberization of the stele was seen microscopically in resistant plants at 24 hours after inoculation. Similar responses were seen for resistance to race 1 and race 2 strains. These results suggest that resistance to ARR is triggered by a canonical NBS-LRR resistance gene. Notably, resistant plants prevent completion of the lifecycle of the pathogen. Thus, utilization of resistant varieties can potentially reduce pathogen density in soils.

To gain a better understanding of the genes involved in resistance, plants were identified from the alfalfa variety 53V52 with resistance to a race 1 strain (MF-1) and a race 2 strain (MER4), and single plants were used as parents to produce F<sub>1</sub> mapping populations. Plants were rated for severity of disease symptoms, which were highly correlated with the amount of pathogen DNA and number of oospores in root sections. Resistant plants with a score of 1 to 2 had no oospores in roots and an average of 48 ng of pathogen DNA/g root while susceptible plants with a disease score of 3 to 4 had an average of 23 oospores/mm root and 2744 ng pathogen DNA/g root. Line 85 segregating 3:1 (resistant:susceptible) for resistance to race 1 and 1:3 for resistance to race 2 was selected for disease phenotyping and genotyping. DNA was extracted from 373 F<sub>1</sub> plants from line 85 and used for genotyping-by-sequencing followed by genotype calling using FreeBayes pipelines to the *Medicago truncatula* reference genome sequence. SNP markers significantly associated with resistance to strain MF-1 (race 1) were identified on chromosome 1 (Figure 2). Using the genome sequence for cultivated alfalfa at the diploid level (<https://www.alfalfatoolbox.org>), three candidate NBS-LRR genes were identified within 38 kbp of the most significant marker ( $P=2.76 \times 10^{-23}$ ). Significant SNP markers associated with resistance to strain MER-4 (race 2) were located on chromosome 2 ( $P=1.7 \times 10^{-9}$ ). Resistance to a different race 1 strain mapped to the resistance locus on chromosome 1 and two additional race 2 strains mapped to the resistance locus on chromosome 2, using 265 plants from line 85. These results indicate that a small number of genes are involved in triggering the hypersensitive resistance mechanism in the variety 53V52. Plants with resistance to single strains of *A. euteiches* were identified in this project, suggesting that additional races of the pathogen exist in nature. However, the genes for resistance to these strains appear to be clustered in the 53V52 genetic background.

Strains of *A. euteiches* vary widely in aggressiveness toward alfalfa and previous multilocus sequence typing showed that the pathogen is genetically diverse (Malvick et al., 2009). Ongoing QTL mapping aims to identify markers tightly linked to resistance to ARR that may be useful in marker-assisted selection for breeding alfalfa varieties with high levels of resistance to ARR. The results of this study support the use of diverse *A. euteiches* strains when selecting disease resistant parental materials to achieve durable resistance to ARR.



**Figure 1.** Percentage of race 1 and race 2 isolates of *Aphanomyces euteiches* in soil samples from alfalfa production fields in Minnesota and New York.



**Figure 2.** Manhattan plot showing the location of SNPs associated with resistance to race 1 and race 2 strains of *Aphanomyces euteiches*.

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## Alfalfa dwarf disease, a viral complex affecting alfalfa crop in Argentina\*

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**KEYWORDS:** Alfalfa dwarf disease, *Medicago sativa*, Viral complex, Field distribution, *Aphis craccivora*.

Alfalfa (*Medicago sativa* L.) is considered the most important forage crop in Argentina and an essential component of bovine meat and milk production. It is well adapted to a large region in Argentina due to its high salinity and drought resistance as well as its efficient atmospheric nitrogen-fixing capacity (Basigalup et al., 2007). Alfalfa is the forage crop with the largest cultivated area in the country, over 3.2 million hectares, and it ranks fourth after soybean, wheat and maize (Basigalup pers.comm., 2018; «Bolsa de Cereales», 2018; «Bolsa de Comercio de Rosario», 2017). In 2010, a new viral disease, named alfalfa dwarf disease-ADD, was identified in Argentina. The symptoms include severe plant stunting and leaves with decreased size, distortion, chlorosis and the presence of vein enations and papillae on the abaxial surface (Figure 1). Initial field assays indicated that ADD presented incidences higher than 50%, yield decreases of dry mass of up to 30% and significantly reduced crop life (INTA-Infoma, 2010). Plants infected with ADD were found to be co-infected with *Alfalfa mosaic virus* (AMV) and *Alfalfa dwarf cytorhabdovirus* (ADV). Subsequently, other three viruses were detected in the same plants: *Bean leafroll virus* (BLRV), *Alfalfa enamovirus-1* (AEV-1) and *Alfalfa leaf curl virus* (ALCV). So far, these five viruses (collectively named as Argentine Alfalfa Viruses = AAV) were detected in alfalfa plants, with ADV and AEV-1 being virus species described for the first time worldwide. Methods for AAV detection were adjusted and the complete genome sequences of each virus were reported (Bejerman et al., 2011, 2015, 2016, 2017; Trucco et al., 2014, 2016). Taking into account that little is known about viral diseases affecting alfalfa and considering the importance of this forage crop for cattle production in Argentina, our objective was to generate data to elucidate the situation of the viral disease complex in this crop and the relative importance of each component of the disease complex; meeting this objective will be an important step toward adopting methods for their proper management.

During 2010-2017, the geographical distribution and prevalence of ADD and AAV were evaluated in alfalfa-producing areas of Argentina, with 17 provinces being surveyed: Buenos Aires, Catamarca, Chaco, Córdoba, Entre Ríos, Jujuy, La Pampa, La Rioja, Mendoza, Neuquén, Río Negro, Salta, San Juan, San Luis, Santa Fe, Santiago del Estero and Tucumán. ADD was detected in all surveyed provinces, with a prevalence of 87.0% being recorded (147 alfalfa fields with at least one ADD-plant/169 total surveyed alfalfa fields). Notably, the 22 fields where ADD was undetected correspond to young alfalfa crops (less than one year old) (Figure 2). Except for BLRV in Chaco, all five viruses were detected in at least one of the ADD sampled fields from each surveyed province, with prevalence values ranging from 64 to 100%. Interestingly, AMV was the only one of the five viruses detected in all ADD-infected alfalfa samples, with a prevalence of 100% being recorded.

Alfalfa is also cultivated in Argentina with the purpose of seed production, which is carried out in irrigated arid regions like San Juan province (Basigalup et al., 2007). A preliminary assay was conducted in Guanacache-San Juan; alfalfa plants showing symptoms of enations (characteristic symptom of ADD) were selected and the effect of AAV on seed production and symptom expression was analyzed. A decrease of up to 38% in seed production was observed in plants showing enations, with AMV being the virus with most negative impact, followed by ALCV. This result was confirmed via a principal component analysis, which showed that both

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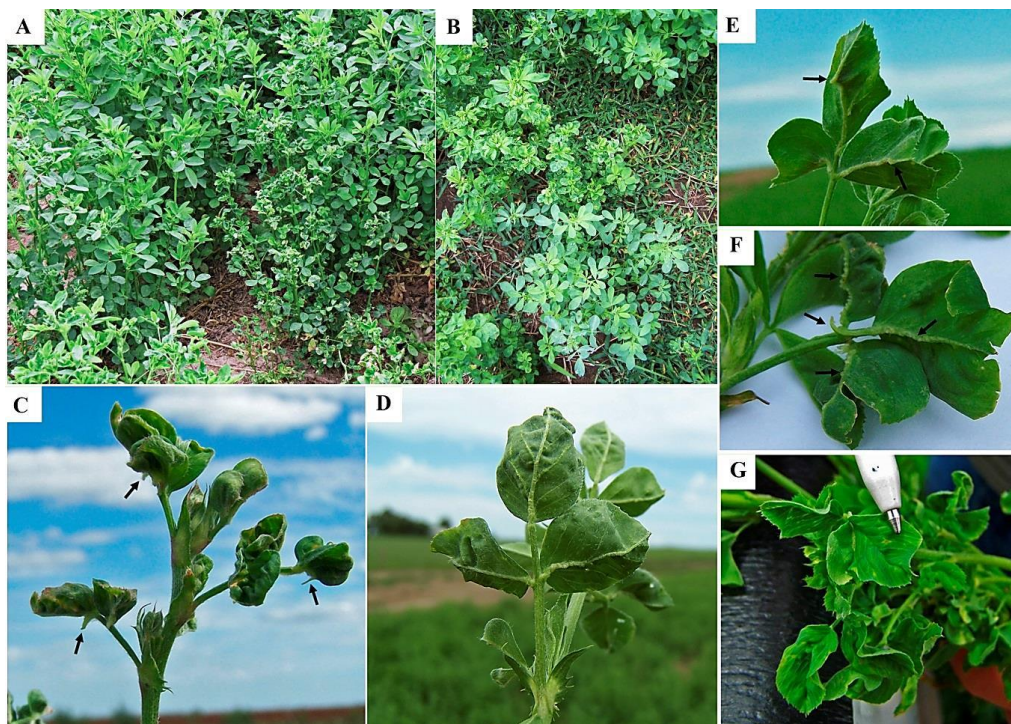


viruses were positively correlated with symptom expression and negatively correlated with seed production; no important correlation was observed with the remaining viruses. On the other hand, this result is supported by the finding that plants naturally co-infected with AMV and ALCV showed symptoms that resemble those of alfalfa dwarf disease, suggesting that their synergism could cause the development of ADD symptoms. Nevertheless, additional assays need to be conducted to confirm the contribution of this co-infection to symptom development (e.g. simple and mixed infection with AMV and ALCV on the same alfalfa genetic base and evaluation both under greenhouse and field conditions).

The viruses can spread to an agricultural crop mainly due to the use of infected seeds, the behavior of vectors and the presence of alternative hosts. Seed transmission of AAV and ADD was evaluated. Seed transmission was recorded only for AMV (3.75%); thus, no seed transmission of ADD was observed. Vector transmission assays were carried out with aphids (*Aphis craccivora*) collected from ADD-infected alfalfa plants in the field. Symptoms of chlorosis and vein enations were recorded in alfalfa plants tested with five and 10 aphids/healthy plant; transmission of ADD by *A. craccivora* was confirmed at a rate of 2 and 10%, respectively. Moreover, all five viruses were detected in aphids collected in alfalfa fields, indicating that aphids are natural carriers of AAV. It is known that AMV, ALCV and BLRV can be transmitted by *Aphis craccivora*; however, the potential of this aphid as vector of ADV and AEV-1 needs to be confirmed. Moreover, the other aphid species that colonize alfalfa crop in Argentina will be evaluated as potential vectors.

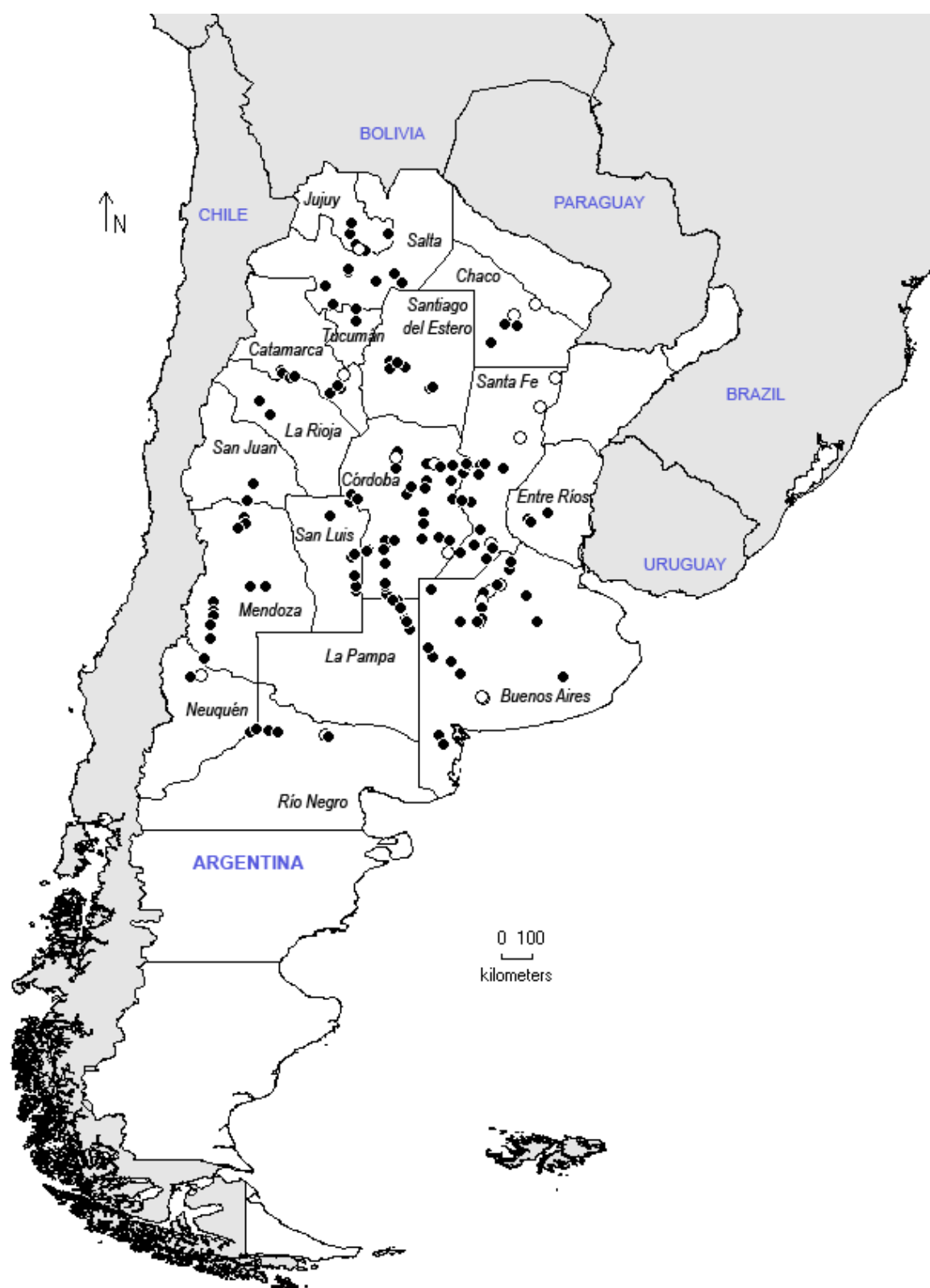
Several weed species (*Trifolium repens*, *T. pratense*, *Dichondra repens*, *Melilotus* spp., *Sonchus oleraceus* and *Talinum paniculatum*) growing adjacent to ADD-infected alfalfa plants and showing virus-like symptoms were collected and evaluated as possible natural reservoirs of AAV. Except for *T. paniculatum*, AMV was detected in all weed species, and both ADV and AEV-1 were detected in *T. repens*. All the other weeds tested negative for the remaining viruses. This is the first record of AMV-infected *T. pratense* in Argentina, and of *Dichondra repens* as natural host of AMV, and *T. repens* of both ADV and AEV-1 worldwide. Additional field sampling including more weed species will be carried out.

The knowledge gained in this work will be useful to design management strategies for this disease and to generate tools that help minimize the negative effect of ADD on alfalfa crops.



**Figure 1.** Symptomatology of ADD. Stunting and chlorosis in affected plants (A and B), decreased size and leaflet deformations (C-G), chlorosis on margins of the leaflet (G), and vein enation and papillae on the abaxial surface of leaves (C-F).





**Figure 2.** Map of Argentina showing the sampling sites of ADD during 2010-2017. Black and white circles indicate the positive and negative detection of ADD, respectively.

## ACKNOWLEDGMENTS

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## Advances of alfalfa breeding for thrips resistance in China\*

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**KEYWORDS:** Alfalfa variety, Thrip resistance, Breeding

As one of main factor which reduce the forage yield and quality, pests control has become an important issue of alfalfa produce and management with the increasing of alfalfa planting area in north China. There are different insect species, different dominant species in different alfalfa planting areas in China. The harms, occurrence and control measures of main pests are varied.

### Investigation and taxonomy of alfalfa pests in China

The investigation and study on alfalfa insect population was started from 1950 in China. Research mainly related to insect species and diversity, biological and ecological characteristics, population dynamics and community structure, biochemistry and molecular biology, pest control and so on. The first publication was "Investigation and research on Alfalfa in northwest China", in which 6 diseases and 4 pests on alfalfa were introduced. And then, 36 alfalfa pests in Xinjiang was identified. And more than 110 alfalfa pests were reported by the Grassland Research Institute of Chinese Academy of Agricultural. As of 2016, the total of 297 alfalfa pest species, belongs to 48 families of 8 orders in China were reported. They are 123 *Lepidoptera* species, 114 *Coleoptera* species, total 34 *Hemiptera* and *Homoptera* species, 11 *Orthoptera* species, 9 *Thysanoptera* species, 4 *Diptera* species, and 2 *Hymenoptera* species.

### Research progress of alfalfa thrips control in China

As one of the main pest of alfalfa in north china, The damage of thrips caused the serious economic loss, especially in Inner Mongolia, Ningxia and Xinjiang. Different thrips control method was applied according to the thrips taxonomy, feeding behavior, life cycle, harmful habits, activity regularity investigation. Thrips are pest which belongs to Order *Thysanoptera*. There are 11 main thrips species harm to alfalfa in China, including *Thrips tabaci*, *Frankliniella occidentalis*, *Odontothrips loti*, *Taeniothripsdi stalis*, *Frankliniella intonsa*, *Haplothrips aculeatus*, *Haplothrips chinese*, *Sussericothrips mililotus*, *Odontothrips phaleratus*, *Thrips valgatissimus*, and *Taeniothrips disatalis*. In Hohhot area of Inner Mongolia, *Haplothrips aculeatus*, *Thrips tabaci*, *Frankliniella intonsa*, and *Odontothrips loti* is the most serious harmful thrips. The whole growth period of alfalfa can be damaged continuously from the beginning of the green period. Thrips can harmful to the leaves, buds and flowers of alfalfa. Leaves were wrinkled and turn yellow after be infected, the plants growth and development become seriously poor. The infection rate of alfalfa varieties in north china was above 70%, and the maximum was up to 100% in warm area. The harm is more seriously in late July and August. Thrip infection seriously reduced the yield and quality of alfalfa and lowering the seed harvest.

As in Inner Mongolia, the loss of alfalfa hay yield was up to 14.9% due to the damage of the thrips, while the nutrient content decreased as showed in table 1 and that cause alfalfa quality declined significantly.

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**Table1:** Forage quality decline of Alfalfa Caoyuan No.1 after thrips infection.

	Amino acid (mg/100mg)	Protein (%)	Crude fat (%)	Carotene (mg/kg)	Calcium (%)	Phosphorus (%)	Crude fiber (%)
CK	13.21a	19.90a	3.35a	189.38a	0.13a	0.19a	27.35a
Infected	8.40b	17.60b	2.25b	154.40b	0.06b	0.16a	30.68a

Note: The different letter in same column indicate significant difference under  $P < 0.05$  level.

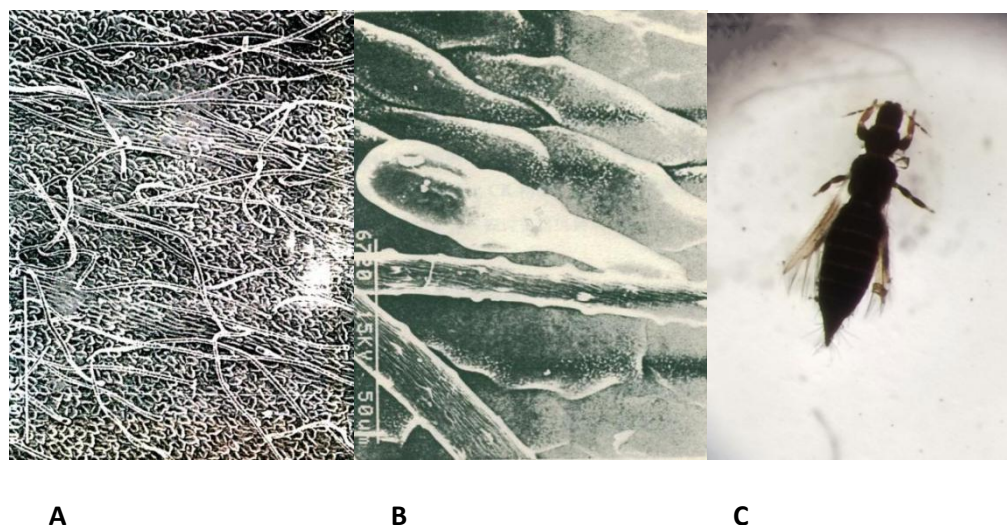
The thrips control methods include chemical control, ecology and biological control and so on. Thrips can be significant controlled by a series of chemical pesticide, for example the 40% pesticide Rogor spray can significant increase alfalfa hay yield (Table 2). But for sustainable agriculture, chemical control supplement become combined with ecology and biological control methods to reduce the environment pollution and lower cost input. The thrips resistance ability of alfalfa was close related to the planting and management conditions. For instance, good water and fertilizer condition can enhance alfalfa's resistance to insects, and the grass-legume mixed planting have less thrips harm than alfalfa one cultivar planting, and the high planting density is beneficial to thrips control. A few *Pseudomonas* and natural enemy insects also can be used in thrips prevention and control.

**Table2.** Alfalfa hay yield under different concentration of pesticide Rogor treatment

concentration	hay yield (kg/hm <sup>2</sup> )	Increase (%)
1 : 500	7092.0 a	25.1
1 : 1000	6874.5 a	21.3
1 : 1500	6093.0 b	7.5
CK	5667.0 c	-

### Morphological characteristics and mechanism of thrips resistance of alfalfa

The defense reaction of alfalfa to Thrips is reflected on the external morphological characteristics, mainly the density and angle of the tomentum on the leaf surface of alfalfa, the layer of epidermal cells, the thickness of cuticle layer and waxy layer. The slight changes of these morphological structures can change the feeding habit, thus affecting the behavior and population density of thrips. Microscopic observation showed that the tomentum density on leaf surface of alfalfa varieties with thrips resistance ability is higher than that of thrips susceptible alfalfa, and the tomentum is shorter and harder and with more vertical angle and more curved shape. The glands on the surface of stems also have high density (figure 1).



**Figure1.** leave and stem surface of thrips resistance alfalfa and main thrips in Hohhot area. A:tomentum on lower surface of leaf (80× ); B:Tomentum and glands on the surface of stem (1000× ); C:Main thrips in Hohhot area.

The biochemical products, nutrients and metabolites in alfalfa plants can directly affect food nutrition and digestibility of insects. Alfalfa with high content of reducing sugar, crude protein and amino acid seems are more attractive to thrips, especially with high concentration of Threonine, glycine, alanine, and isoleucine. The content of total phenol and free proline have significantly difference between different alfalfa varieties, and the content of total phenol and free proline increased significantly in alfalfa with high resistance after infected by thrips. The injured part of leaves can produce higher concentration of Salicylic acid (SA) in high resistance alfalfa varieties. Research indicated both thrips resistant and susceptible alfalfa cultivars can regulate gene expression in the SA and flavonoid biosynthesis pathways to induce defensive genes and protein expression (e.g. polyphenol oxidase, protease inhibitor), which enhances plant defence capacity.

### **Breeding of thrips resistant alfalfa varieties in China**

There were 2 thrips-resistance alfalfa varieties have been registered in China. The first variety named Grassland No.4 (*Medicago sativa* L.) was registered by Inner Mongolia Agricultural University in 2015, and have widely extended in Inner Mongolia China. The second variety named Gannong No.9 (*Medicago sativa* L.) was registered by Gansu Agricultural University in 2017.

A research project for thrips resistance alfalfa breeding was lunched by Inner Mongolia Agricultural University from 1986. The research focused on the morphological characteristics, anatomical structure of above ground vegetative organs, physiological and biochemical products of thrips resistance alfalfa, and aims to reveal the mechanism of thrips resistance and select new varieties. More than 400 alfalfa accessions was collected and evaluated for the thrips resistance ability by infection rate from 1~0. Some accessions were treated by <sup>60</sup>Co radiation. Totally 308 individuals with low infection rate were selected and clone planted. 169 clones were selected and tested for thrips infection rate in 1988. 17 clones with lowest infection rate were selected. A multi-cross nursery was established by these 17 thrips resistance clones, and 20 cuttings of each clone were planted randomly in the nursery. Multiple hybrid seeds were harvested for each clone in 1989. Seed of thrips resistant clones were planted to test the combining ability. 9 clones with high thrips resistance and high combining ability were selected and mixed as the original population. The new strain was established after 3 selecting cycle. The new variety named Caoyuan No. 4 was released after more than 3 years evaluation of forage yield, quality and adaptability in Inner Mongolia.

### **ACKNOWLEDGEMENTS**

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# Global climate change and pests of alfalfa: problems and solutions\*

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**KEYWORDS:** aphids, bollworms, carbon dioxide, lucerne, silicon, warming

## BACKGROUND

Reports from the Intergovernmental Panel on Climate Change (IPCC) consistently show increases in atmospheric carbon dioxide (CO<sub>2</sub>), which are predicted to increase at an accelerated rate in the future. Depending on emission scenarios, elevated CO<sub>2</sub> (eCO<sub>2</sub>) is likely to be accompanied by increases in air temperature ranging from 0.3-1.7°C (low emissions) to 2.6-4.8°C (high emissions) (IPCC, 2013). There is growing concern that such changes will negatively impact crop productivity due to increased attrition by pests and diseases which could seriously undermine future food security (Gregory *et al.*, 2009). Very recent modelling by Deutsch *et al.* (2018), for example, showed that yield losses from insect pests ranged from 10-25% in three grain crops (wheat, rice and maize) for every degree Celsius of warming.

Several studies have reported beneficial effects of eCO<sub>2</sub> on legumes, including alfalfa, via increases in biological nitrogen fixation (Lam *et al.*, 2012). Nonetheless, these tend to be short term effects and studies usually exclude plant antagonists (e.g. insect pests). Moreover, researchers often investigate climatic variables (e.g. eCO<sub>2</sub> and warming) separately which may provide a misleading indication of how important crops like alfalfa are likely to be affected in a CO<sub>2</sub> enriched, warmer world (Johnson *et al.*, 2016). In this talk, I will briefly review the major changes that may occur in alfalfa chemistry and physiology under future climate change scenarios. I will summarise how such changes may affect three key pests of alfalfa in Australia; the pea and cowpea aphids (*Acyrtosiphon pisum* and *Aphis craccivora*, respectively) and the cotton bollworm (*Helicoverpa armigera*). Our most recent research aims to enhance plant resistance in alfalfa using silicon augmentation. Initial results will be discussed.

## METHODS

Glasshouse experiments (Johnson *et al.*, 2014; Ryalls *et al.*, 2015; Ryalls *et al.*, 2017) were used to investigate how eCO<sub>2</sub> and elevated air temperatures (eT) affected pea aphid performance via changes in alfalfa primary metabolism. A subsequent field study (Kremer *et al.*, 2018) investigated whether eCO<sub>2</sub> affected the behavior of organisms (specifically mutualistic ants) that interacted with aphids feeding on alfalfa. In the final example, glasshouse experiments investigated the impacts of eCO<sub>2</sub> on a highly polyphagous herbivore, the cotton bollworm, in relation to compromised defenses in alfalfa. Experiments in which alfalfa is supplemented with silicon (increasingly recognized as alleviating a range of biotic and abiotic plant stresses) are now underway to determine whether exacerbated pest problems can be remediated (Zhang *et al.*, 2017; Coskun *et al.*, 2018).

## RESULTS AND DISCUSSION

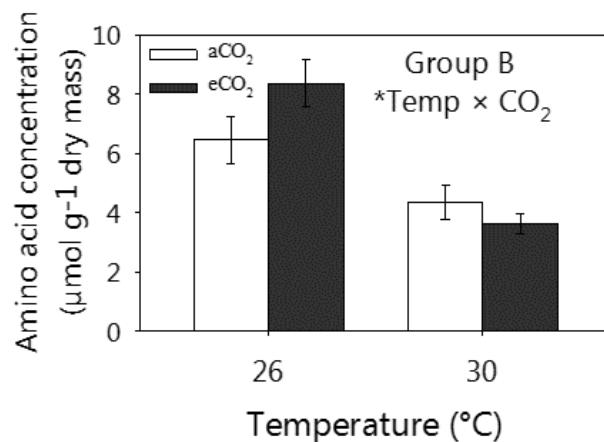
Pea aphid performance increased under eCO<sub>2</sub> conditions which was linked to increases in root nodulation and synthesis of foliar amino acids. These effects were, however, negated under eT conditions. We identified discrete functional groups of amino acids that underpinned the effects of eCO<sub>2</sub> and eT on aphid performance (Fig. 1). Effects of eT and eCO<sub>2</sub> held true across five alfalfa genotypes, demonstrating the generality of their effects. In field experiments, eCO<sub>2</sub> caused aphids to increase feeding behavior and production of honeydew (Fig. 2a). This caused enhanced levels of tending behavior by ants (Fig. 2b), potentially conferring extra

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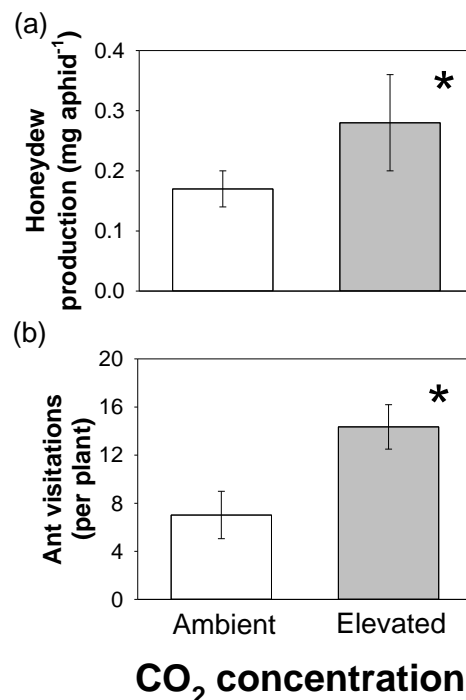
\* **CITATION:** Johnson, S.N. 2018. Global climate change and pests of alfalfa: problems and solutions. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

protection for aphids against their natural enemies. eCO<sub>2</sub> also increased alfalfa susceptibility to the polyphagous cotton bollworm which corresponded to compromised plant defenses under eCO<sub>2</sub> (Fig. 3). Application of silicon had beneficial effects on alfalfa growth under eCO<sub>2</sub> and eT without increasing susceptibility to aphids. Silicon had little impact on plant susceptibility to cotton bollworm (Fig. 3) whether applied in liquid (LSi+) or solid (SSi+) form.

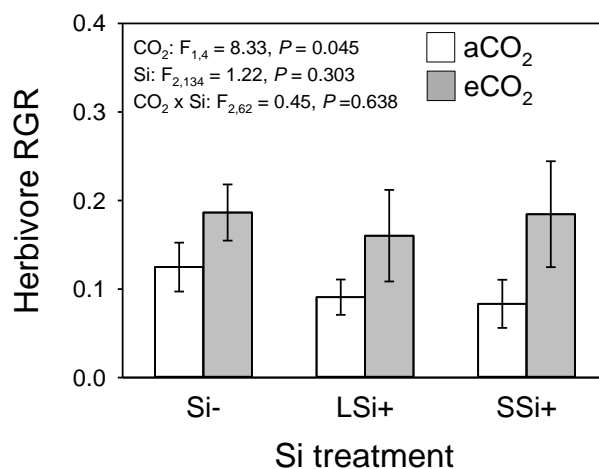
eCO<sub>2</sub> has beneficial impacts on alfalfa productivity and nitrogen accumulation but these also increase plant susceptibility to diverse herbivore pests. eT tended to inhibit some of the effects of eCO<sub>2</sub>, potentially due to inhibitory impacts of higher temperatures on the activity of nitrogen fixing bacteria. Silicon application may confer some benefits on alfalfa, but possibly only when soils are silicon deficient.



**Figure 1:** Five specific amino acids (asparagine, aspartate, glutamate, arginine and histidine) underpinned the positive and negative impacts of eCO<sub>2</sub> and eT, respectively, on aphid performance.



**Figure 2:** Impacts of eCO<sub>2</sub> on (a) honeydew production by aphids and (b) tending behavior by ants.



**Figure 3:** Impacts of eCO<sub>2</sub> on relative growth rates (RGR) of the cotton bollworm in soil which was not supplemented with silicon (Si-) or supplemented with liquid (LSi+) and solid (SSi+) forms of silicon.

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# Restating the role of Alfalfa Integrated Pest Management in Argentina

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## ABSTRACT

The current concepts within the Integrated Pest Management (IPM) framework stem from the contributions by Stern and collaborators (1959), professors of California University, developed precisely for alfalfa crop. At that time, they designed the integration of chemical control with the biological control of the spotted alfalfa aphid. IPM, based on fast and reliable sampling methods, with economic thresholds determined through field research, and the use of selective insecticides that effectively control the target arthropod species but have minor or no impact on beneficial wildlife, is proposed as a sound strategy to reduce economic loss risks without compromising environmental health. IPM integrates concepts from the economic theory and mainly concepts and hypotheses stemming from the ecology theoretical framework, such as the theory of island biogeography (MacArthur and Wilson, 1967); the theory of natural enemies (Doutt & DeBach, 1964); and the resource concentration hypothesis (Root, 1973).

In Argentina, a key IPM workshop was held in 1978, organized by FAO at INTA-Pergamino Experimental Research Station (INTA-FAO, 1978). However, from 1960 to 1985 successful strategies were designed for IPM in cotton by Barral & Zago (1983), leading to a substantial decrease of the number of pesticide applications from almost 15 to 3 through the entire season, an achievement that received international recognition. Following the 1978 workshop, the development of IPM programs were started for wheat, corn, soybean and alfalfa. At that time, the INTA-National Program of Plant Protection had been established and led by R. Parisi (Pergamino), and supported by researchers across the country, such as J. Aragón (Marcos Juárez); J. Ves Losada (Anguil); E. Botto (IMyZA) and J.M. Imwinkelried (Rafaela). They conducted life table and key issues analyses to identify the main natural mortality factors of alfalfa pests with the advice of D. Harcourt (Agriculture Canada). These projects set up the bases for a new approach within the IPM strategy: the conservation of natural enemies. Any activity undertaken on the crop should first be assessed as to how it may affect predators, parasitoids or entomopathogens. Keeping this in mind, for the control of lepidopteran larvae such as those of *Colias lesbia*, *Rachiplusia nu*, *Spodoptera frugiperda*, *Anticarsia gemmatalis* or *Helicoverpa gelotopoeon*, it was recommended to use *Bacillus thuringiensis* formulations or diamide insecticides, which are known to be selective on natural enemies. When aphids are to be controlled, low dosage of pirimicarb was recommended. Nevertheless, since alfalfa is intended for grazing or cutting, perhaps a low cost option is to anticipate the practice without chemical intervention. On the other hand, a successful program for releasing species of *Aphidius* was developed across the cereal and alfalfa production region in the 1980's.

Unfortunately, as it may occur in many other countries, IPM is poorly adopted. However, farmers who practice the IPM philosophy take advantage of difference in control costs by decreasing pesticide sprays. Because alfalfa is a perennial crop, this pasture provides a relatively stable environment during several years, on which beneficial organisms can thrive in more diverse and abundant communities (Zumoffen et al., 2010). No doubt, the agricultural landscapes containing alfalfa may benefit from this biocontrol ecosystem service, receiving enemies of shared key pests.

Monitoring alfalfa fields not only aims to key pests but also to keep records of its natural enemies as follows: Trichogrammatidae and Scelionidae as parasitoid of Lepidoptera or Hemiptera eggs; Aphidiinae and Microgastrinae as aphids or larvae parasites, respectively; Ichneumonidae and Braconidae as parasitoid of larvae and pupae; Chalcidoidea and Encyrtidae polyembryonic wasps, as well as Tachinidae, key parasitoids on *Rachicplusia nu*; Tettigoniidae, long horned grasshoppers, as predators of aphids and soft-body bugs; Carabidae as predators mainly in soil; several predators of thrips and Lepidopteran eggs, such as larvae and

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nymphs of genus *Orius*, *Geocoris*, *Nabis*, *Reduviidae* and *Podisus*; some Diptera, which are generalist predators, like Asilidae or Dolichopodidae; or Syrphidae and Coccinellidae, in the form of adults or immature, as specific predators of aphids. Also, there are Chrysopidae predators as larvae and adults, same as Staphylinidae. *Solenopsis* ants also are important predators. Besides, *Balaustium* mite is a common predator in alfalfa fields. There are several species of spiders representing families such as Araneidae, Thomisidae, Oxyopidae, Salticidae, Lycosidae and Clubionidae. Additionally, under certain environmental conditions, entomopathogenic viruses and fungi (like *Nomuraea rileyi*) can reach epizootic levels on *Spodoptera* spp. and *Rachiplusia nu* and then controlling a very high percentage of the populations.

As an example of the potential of the IPM approach, from 19 alfalfa fields at Rafaela INTA Agricultural Experimental Station, between November 2014 and April 2015, only four fields were totally treated (with 4.8, 11.3 and 11.5 *S. frugiperda* larvae / sweepnet, and 7.8 *C. lesbia* larvae / sweepnet); and three were partially treated (with 9.5 and 7.2 *S. frugiperda* larvae / sweepnet and 7.6 *H. gelotopoeon* larvae / sweepnet). In contrast, it is well known that the dairy farmers of the region apply at least one insecticide spray per summer season just for defoliating larvae.

A question may arise regarding how soybean and corn fields may benefit from alfalfa fields in a region. The spillover or movement of subsidized natural enemies from alfalfa is likely to be an important process affecting insect herbivore populations in surrounding crops. Since natural enemies start to build up population density in early spring, these can pass on flight or by wind to neighbouring fields.

As in any other crop, IPM in alfalfa needs constant revision. All IPM programs, as they are explained on methodological bases, must target the main pests, which are in a dynamic process. For many years, the main defoliators were *R. nu* and *C. lesbia*. However, perhaps *S. frugiperda* has become comparatively more important in the last ten years, due to temperature increase that has favored its earlier development in the growing season.

As previously mentioned, aphids -as key pest of alfalfa- were targeted with several approaches. Firstly, a program of classical plant breeding was established resulting in several alfalfa varieties exhibiting tolerance or antixenosis. Secondly, conservation of ladybug beetles and parasitoids in the field has been crucial for keeping aphid populations at low densities. During July 2018, an unusual density of alfalfa blue aphid (*Acyrtosiphon kondoi*) was present on the entire alfalfa growing region of Central Argentina, where more than 600 aphids / stem were recorded. Perhaps, such extraordinary infestation may have been the consequence of planting seeds with narrow genetic diversity. Indeed, the increase of aphids that broke resistance of previously resistant cultivars is favored by parthenogenesis, an asexual process by which the offspring is an exact replicate of the parent. This scenario resembles what could have happened some years ago when *A. kondoi*-Raf.1990 biotype was detected as a result of the high selection pressure imposed on the aphid population when a very few cultivars were seeding on more than 50% of the region.

Another interesting debate is referred to the need of *Bt*-alfalfa to solve pest problems: Is it really necessary? There are many arguments against the real need of *Bt*-alfalfa. Even though Lepidopteran larvae are a problem, they can be managed in several ways, including anticipating harvest. But one thing should be kept in mind: if farmers failed to adopt refuges in *Bt*-maize and *Bt*-soybean, it could hardly be expected that farmers will adopt refuges in *Bt*-alfalfa. The important issue to discuss is the constant selection pressure exerted by *Bt* gene on insect populations, particularly because alfalfa a perennial crop and so selection pressure will act for more than four years in every field. Besides, *Cotesia ayerza* (Hymenoptera: Braconidae), a gregarious parasitoid of *C. lesbia*, begins its unnoticed control since the end of winter (August) of each year. While the host is considered a summer pest, should we expect to find *C. ayerza* in *Bt*-alfalfa fields? Or concerns about local extinctions are reasonable?

Alfalfa IPM in Argentina is far from reaching an ideal situation. There is a complexity in the dairy production system, where the person in charge of dairy cattle and the milking process coexists with the production of the pastures and the feed stuff. Generally, pest control rests on the use of pesticides. However, IPM and its economic feasibility have already been demonstrated (Imwinkler et al., 1992). On the other hand,

when law prohibits pesticide use in periurban areas there is a potential contribution of alfalfa as a buffer zone to mitigate social conflicts.

Finally, as shown above, there is evidence that alfalfa crops provide stable environments that harbors a community of beneficial insects, like predators and parasitoids, and other organisms like dung beetles, providing real ecosystem services. No doubt then, the conservation of natural enemies of key pest must be the strategy to enhance and promote an alfalfa IPM philosophy in Argentina.

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## Modification of endogenous genes by GE and gene editing techniques\*

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Genetic engineering (GE) refers to the introduction of heterologous or homologous DNA into a plant genome resulting in its stable integration and expression. The use of RNA interference-based GE allows the effective downregulation of endogenous genes. Because transgenic plants obtained by GE usually carry one or more transgenes of several kilobases in length in their genomes, the process for regulatory approval is complicated and costly. In recent years, the development of genome editing technologies, particularly CRISPR, offers the ability to edit plant genomes and alter gene activity with unprecedented precision and reliability. The technology is especially effective for gene knockouts, creating targeted mutations similar to naturally occurred mutants. Because no foreign gene sequences are introduced into the genome, such genome edited plants do not require USDA regulatory oversight. Alfalfa is a tetraploid and outcrossing species, such features form an impediment to generating homozygous knock-out plants. Our initial work employing commonly used CRISPR/Cas9 vectors showed that genome editing efficiency was extremely low in alfalfa. After extensive optimization of the vectors, we successfully developed a very efficient genome editing system in alfalfa. The system allows us to easily obtain tetra-allelic mutations in which all four alleles were simultaneously knocked out, a result that is significant for outcrossing polyploid species. Genome edited alfalfa plants have been produced for several genes that previously only knocked down by RNAi. Progress on genome editing in alfalfa will be reported.

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## GE lignin modification in alfalfa\*

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**KEYWORDS:** Alfalfa, Genetic Engineering, Lignin, HarvXtra

In 2001 a small group of scientists from the U.S. Dairy Forage Research Center (USFDRC), the Noble Foundation, and Forage Genetics International (FGI) began discussions about the potential use of emerging biotech tools for improving forage quality in alfalfa. In 2002 the three organizations formed the Consortium for Alfalfa Improvement (CAI) dedicated to a long-term collaboration bringing together expertise in plant biology, molecular biology, biochemistry, plant breeding and ruminant nutrition. The group was later joined by Pioneer-Hi-Bred in 2005. At the first CAI meeting it was decided that the group would focus on traits that could improve the efficiency of utilization of alfalfa fiber and protein. In this presentation I will report on the work focused on the use of genetic engineering (GE) techniques to the modification of lignin for improved fiber digestibility taking it from a concept to a commercial product. A second presentation at this meeting given by Mark McCaslin will focus on the CAI and FGI research on commercial proof of concept for traits in alfalfa.

### **Reducing Lignin in Alfalfa**

Lignin is a complex polymer that provides mechanical strength to plant secondary cell walls. They are synthesized primarily from Guaiacyl (G) and Syringyl (S) monolignol units although Hydroxyguaiacyl (H) subunits may also be present in small amounts. Lignin makes the cell walls recalcitrant to digestibility for ruminants. The lignin biosynthetic pathway is well understood, and gene suppression techniques have been used to downregulate the expression of all the key genes in the lignin biosynthetic pathway in alfalfa and other plant species. CAI scientists evaluated many of the resulting transgenic alfalfa plants under field conditions collecting data on lignin content, lignin composition, fiber digestibility (NDFD), lodging potential and forage yield. Based on this work two gene targets were identified for further study, caffeoyl-CoA 3-O-methyltransferase (CCOMT) and caffeic acid 3-O-methyltransferase (COMT). Optimization constructs targeting CCOMT and COMT using RNAi technology under the control of the phenylalanine ammonia-lyase (Pal2) promoter from *Phaseolus vulgaris* which directed vascular tissue-specific expression were used to generate additional transgenic alfalfa plants. These were evaluated under field conditions and a subset of the best events were used to produce forage for digestibility trials. Events reduced in both CCOMT and COMT showed improvements in forage quality and performed well in digestibility trials using sheep and dairy cows. However, only events downregulated in CCOMT showed no adverse impacts on yield and resisted lodging.

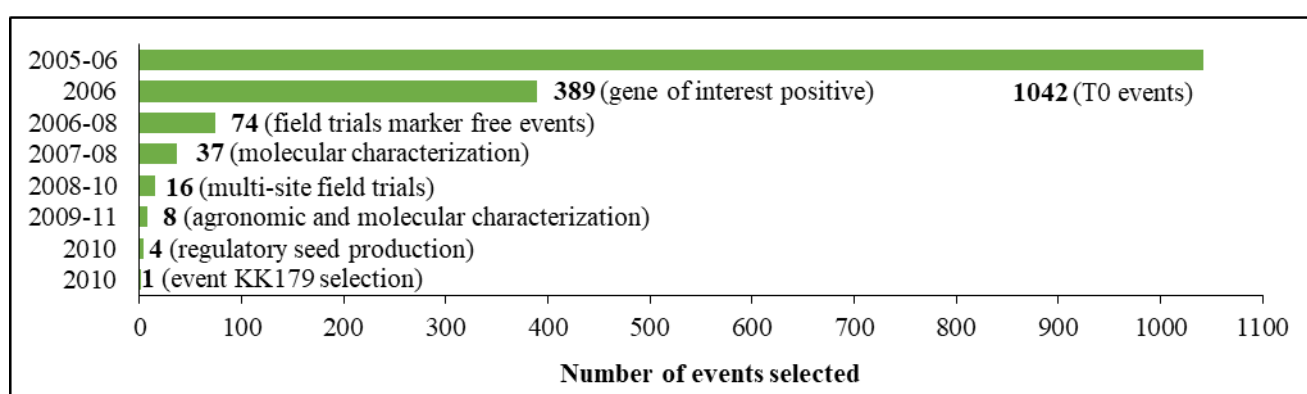
### **Development of the commercial reduced lignin event**

With extensive proof of concept data in place the reduced lignin project moved into the next phase of development. The construct used to produce the commercial reduced lignin event KK179 was a binary vector that contained two T-DNA border sequences. The first contained a CCOMT suppression cassette comprising a 518 bp region of alfalfa CCOMT sequence in antisense orientation and a 299 pb region in sense orientation configured in an inverted repeat. This was placed under the control of the *P. vulgaris* Pal2 promoter and the nopaline synthase transcription terminator. The gene transcript containing the inverted repeat produces dsRNA thus suppressing the endogenous levels of the CCOMT transcript. The second T-DNA contained a cassette that would allow kanamycin selection during plant transformation. *Agrobacterium tumefaciens*-mediated transformation of conventional alfalfa clone R2336 utilizing leaf ex-plants via somatic embryogenesis was carried out using published procedures. A total of 1042 independent T0 generation transgenic events were recovered and transferred to soil for further analysis. PCR based techniques were used to identify 389 events

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\* **CITATION:** GE lignin modification in Alfalfa. Temple, S. J. and Dixon, R. A. 2018. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

that contained the CCOMT suppression cassette (Figure 1). These were crossed with Ms208, a conventional male sterile clone to produce F1 populations tracing to each event. The F1 plants were then screened by PCR to identify plants with an unlinked insertion of the CCOMT suppression cassette a process which allowed for the identification of 74 independent marker-free events, each represented by a small population of plants. These plants were evaluated for general agronomics traits, lignin content and biomass in field trails at the FGI research station in West Salem, Wisconsin during the summer of 2007. Based on single year, single location data 37 events were advanced to a preliminary molecular characterization. Using Southern blot-based technology 16 events were confirmed to contain a single copy insert of the CCOMT suppression cassette and were confirmed to be free of elements from the selection cassette and the vector backbone. An additional round of crossing and genotyping of the progeny allowed multi-location field trails to be established in the spring of 2008. These focused on agronomic evaluation to identify events that met yield and forage quality trait goals. In general, we found that the best events were not the most suppressed for CCOMT and that events with the highest suppression had agronomic problems. This allowed the identification of eight events which based on a combination of additional molecular analysis and agronomic data were subsequently cut to the four lead events.



**Figure 1.** Timeline and activities for the progressive selections leading to the identification of event KK179 used to develop of commercial reduced lignin varieties.

During the 2009 growing season Syn1 seed (75% trait purity) tracing to the four lead events was produced under isolation cages. Following confirmation of event purity this seed was used for regulatory studies and trait integration into FGI reduced lignin alfalfa breeding programs. Reduced lignin event KK179 was later identified as the lead event and was used for the regulatory studies that were ultimately used to petition for the determination of nonregulated status for the event. The regulatory studies contained a detailed molecular characterization of event KK179 that confirmed that the event contains only one copy of the CCOMT suppression cassette that integrated into the alfalfa genome at a single locus. Stability of the insert over four generations of breeding and Mendelian segregation was also confirmed. The sequence of the entire suppression cassette and ~1 kb of adjacent flanking genomic DNA sequence was determined to confirm integrity, confirm the insertion junction and allow mapping of the insertion point. The regulatory package also contains a detailed compositional analysis of event KK179 forage compared to conventional alfalfa varieties looking at levels of key nutrients, anti-nutrients and secondary metabolites. The key conclusion from these studies was that the genetic modification in event KK179 does not result in meaningful changes in composition other than the reduction in lignin, the resulting improvement in neutral detergent fiber digestibility (NDFD) and that the feed, food safety and other nutritional quality traits would be comparable to those found in conventional alfalfa. Reduced lignin event KK179 was also evaluated for any changes in alfalfa plants as a plant pest, assessments of insect damage, changes in susceptibility to common alfalfa diseases and other environmental interactions. The CONCLUSIONS from these studies was that event KK179 was shown to be comparable to the control and would not expected to have an increase in weediness or plant pest potential and that no impacts to current cultivation and alfalfa management practices would be expected following commercial introduction of KK179. Submissions were made to the FDA and USDA-APHIS in August and November 2012. Following an extensive review and public comment period USDA-APHIS published their assessment that alfalfa event KK179

was no longer considered a regulated article under the US regulations governing the introduction of genetically engineered organisms in November 2014.

Starting with a limited commercial release in the spring of 2015, FGI commercialized alfalfa varieties derived from reduced lignin event KK179 under the brand name HarvXtra™ through multiple distribution channels. Event KK179 was subsequently granted deregulated status in Japan, Canada and most recently in Argentina where it will soon be released commercially. Regulatory packages are in preparation or under review in other countries with significant alfalfa cultivation including Mexico and those that are key export markets US produced for alfalfa hay. All HarvXtra™ varieties also contain the Roundup Ready trait present as a breeding stack. Based on the recently issued ISAAA brief 53 on the commercialization of GM crops report 2017 plantings for HarvXtra™ being 80,000 hectares out of a total of 1.14 million hectares of GM alfalfa planted in the US and 3,000 hectares of HarvXtra™ in Canada. When compared to appropriate check varieties HarvXtra™ alfalfa has shown a consistent 15-20% reduction in Lignin and 14-16% increase in NDFD. These differences are far greater than the incremental increases obtained from classical plant breeding. For more details on the potential benefits of HarvXtra™ alfalfa see the paper presented by Mark McCaslin at the II World Alfalfa Congress.

#### **ACKNOWLEDGMENTS.**

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## Transgenic alfalfa tolerant to herbicides, from lab to field\*

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**KEYWORDS:** Alfalfa-transgenic-biotechnology-ammonium glufosinate-herbicides

Alfalfa is the main forage crop produced in temperate regions of the planet due to its strong vitality, high nutritional quality, high yields, high adaptability and multiple uses. Elite alfalfa cultivars not only must have high forage yields but also maintain their productivity and stands over several years to provide substantial economic benefits. Current and predicted climatic conditions pose a serious challenge for agricultural production worldwide, affecting plant growth and yield and causing annual losses estimated at billions of dollars. Transgenic crops provide a promising avenue to reduce yield losses and improve growth. Both economic importance of alfalfa and their biosafety features, make this forage an excellent candidate to conduct research and development of transgenic cultivars that can maintain the quality of this forage under unfavorable conditions.

Our research group uses genetic engineering techniques/biotechnology to develop high performance transgenic alfalfa cultivars. In particular, the group has an extensive experience in alfalfa's transformation and generation of transgenic events. We reported the *Agrobacterium*-mediated transformation of the alfalfa regenerative clone C23 (Garcia et al. 2014) (Figure1) and the high efficiency of the pPZP200BAR binary vector for rapid and low-cost production of large transgenic event libraries (Jozefkiewicz 2016). Then we presented an alternative process for the production of transgenic alfalfa varieties using a single transgenic event containing the BAR transgene for tolerance to the nonsystemic herbicide glufosinate, where this gene was used as a selectable marker and also as gene of interest. We optimized a procedure to develop a synthetic transgenic minimizing time and cost, the strategy combines a high transgene expression of events, RTqPCR analysis to identify individual plants with two copies of the BAR transgene per genome to maximize the expression of transgenic traits into elite germplasm and suitable flowering-triggering greenhouse conditions.

After the introgression process, backcrossing generations (T1–T5) to increase the transgene copy number, T5 plants were randomly intercrossed by hand, producing a T6. T6 individual plants with two copies of the BAR transgene at a single locus were selected as parent plants from the synthetic variety of transgenic alfalfa using RT-qPCR assays. The random intercrossing of plants produces high amounts of the transgenic trait into its progeny. Both, the original regenerative alfalfa clone (C23) and its derivative transformed alfalfa clone, showed a non-elite low-vigor phenotype characterized by uniformly small leaves and short shoot growth, while backcrossing generations led to more vigorous plant growth. These results suggest the success in the incorporation of a transgenic trait into an elite alfalfa germplasm (alfalfa-BAR) (Figure2). The elite performance and the high expression of the transgenic trait of alfalfa-BAR was confirmed, by productivity and glufosinate tolerance assays under field conditions, comparing this herbicide-tolerant transgenic alfalfa variety with the individual conventional elite germplasm used in the introgression process. The levels of yield, regrowth and survival of alfalfa-BAR were similar to those of its genetic-related wild-type varieties, suggesting that this transgenic germplasm is a high-yielding, winter hardiness, healthy variety of alfalfa. These results further support the success of the introgression into elite alfalfa germplasm. In contrast to that observed for weeds and wild-type elite alfalfa plants, alfalfa-BAR transgenic plants showed high tolerance to glufosinate without toxicity effects, even in high doses of herbicide. In addition, exposure to increased doses of glufosinate (0, 2 and 3.5 l/ha) led to enhanced yields in alfalfa-BAR, showing that the use of this herbicide not only does not damage the

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\* **CITATION:** Soto, G.C. 2018. Transgenic alfalfa tolerant to herbicides, from Lab to Field. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA). <http://www.worldalfalfacongress.org/>

transgenic plant but also improves its standard performance (Figure3). This synthetic is now throwing commercial deregulation process (Exp. S05:3543/15 CONABIA) (Jozefkowicz 2018).

The main goal of our group is to develop high performance alfalfa cultivars by introducing biotechnological approaches to conventional improvements programs. Following the full process optimized for alfalfa-BAR synthetic, we identified critical aspects in alfalfa production that could be addressed by transgenic or editing approaches (Stritzler et al. 2018). Regarding weeds control we are working in the develop an alfalfa transgenic cultivar tolerant to ammonium glufosinate; glyphosate and ALS inhibitors.

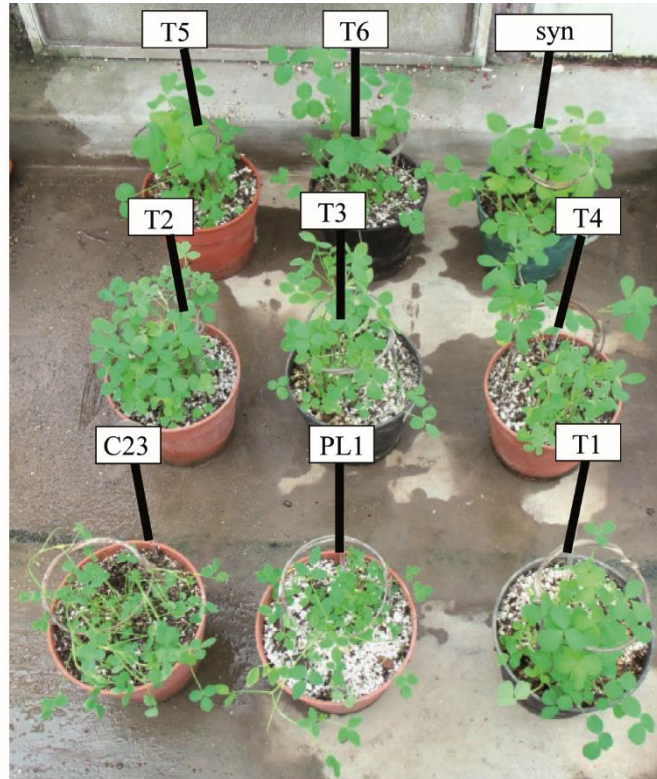
Alfalfa is an integral factor of crop rotation, adding fixed nitrogen to the soil and improving the soil structure for future crops (Lopes et al. 2015). However, similarly to that observed in other forage crops, weeds are often harvested along with alfalfa, reducing its potential protein content and feed digestibility. In addition, weed infestations normally result in a dramatic decrease in alfalfa yield, showing the need to generate novel technologies to control weeds in alfalfa (Rubiales 2014). Over the past 40 years, herbicides have improved crop yield and enabled the development of modern agriculture. However, the extensive use of herbicides has generated a strong selection pressure for the emergence of herbicide-tolerant weeds, threatening the sustainability of current agriculture. This selection pressure is particularly strong in systemic herbicides such as glyphosate, the most important and most widely used herbicide in world agriculture. Currently, it is well known that the success of an individual herbicide is only temporary, and that long-term weed control requires the continuous production of novel herbicide-tolerant cultivars. To this end, it is necessary to have rapid and inexpensive processes to incorporate different herbicide-tolerant traits into important crops, including cultivated alfalfa. Our group is working to develop a genetic modified alfalfa cultivar tolerant with tolerance to ammonium glufosinate, glyphosate and inhibitors of ALS enzyme. The fulfillment of this objective will bring us closer to the general objective of our group and offer a sustainable and effective weed control by allowing the rotation of herbicides with different mode of action, significantly reducing the possibility of the emergence of resistant weeds.

The research and biotechnological advances strongly suggest that it is possible to generate in Argentina Alfalfa elite cultivars with improved performance in fields with high tolerance to herbicides.

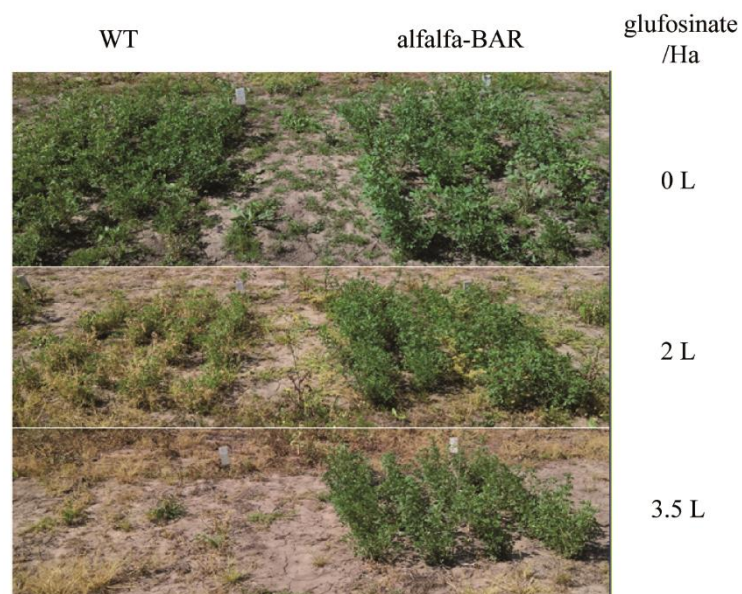


**Figure1:** Agrobacterium mediated alfalfa transformation.





**Figure 2:** Phenotypic analysis of the wild-type regenerative clone C23 (C23), superevent PL1 and representative individual plants from different transgenic generations (T1-syn).



**Figure 3:** Alfalfa-BAR plants were grown without herbicide or exposed to two different doses of glufosinato to determine the expression of the transgenic trait under field conditions. A mix of wild-type elite varieties (WT) was included as an herbicide-sensitive control

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# Produce for any market: coexistence of genetically-engineered and conventional alfalfa hay\*

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## KEYWORDS:

### GE Traits in Alfalfa and the Need for Coexistence

Genetically-engineered (GE) alfalfa became a commercial reality in the US beginning in 2005 with ‘Roundup-Ready’ (RR) alfalfa. A second trait was commercialized in 2014, the ‘HarvXtra’ reduced lignin GE trait. Both are products of Forage Genetics International. The RR herbicide tolerance trait confers the ability to control weeds with a broad spectrum over-the-top herbicide (glyphosate), while HarvXtra is a trait to lower lignin and improve alfalfa fiber digestibility. The HarvXtra trait stacked with RR is currently commercialized for planting in US, Canada and Argentina and approval is pending in other regions. To date, millions of hectares of GE alfalfa have been planted.

The major points of this paper are to put forward the propositions that 1) GE alfalfa traits are likely here to stay, and may include additional traits in the future, 2) Growers and buyers should have the ability to choose and successfully produce alfalfa for any market they wish, conventional or GE, and 3) That coexistence on the farm between those who adapt and those who reject GE crops is necessary and certainly feasible. Full discussion of these issues can be found at Putnam et al. (2016) and NAFA (2018).

*Defining Coexistence.* A recent USDA committee defined coexistence as ‘the concurrent cultivation of conventional, organic, Identity Preserved (IP), and Genetically Engineered (GE) crops consistent with underlying consumer preferences and farmer choices’ (USDA Task Force, 2012). Further, I would define successful coexistence as ‘The ability of diverse systems (GE, organic, non-GE) to thrive without undue influence of neighbors or resorting to extraordinary protection measures.’ (Putnam et al., 2016). Coexistence is complicated by the possibility of gene flow which can occur between alfalfa fields and the possibility of unwanted low level gene presence. Attention to the biology of the crop and requirements of different markets are important to accomplish coexistence.

### Markets Differ in Sensitivity to GE Traits

For many farmers, these GE traits represent a significant advance and opportunity to try new technology and to improve production efficiency. However, not all growers wish to adopt GE crops due to their personal preference or the sensitivity of their markets. Organic growers are required to use non-GE crops. Additionally, export markets may require non-GE crop products either due to regulatory barriers or market preference. Currently, in the US, less than 3% of alfalfa production is certified organic. However, it has been estimated that >12% of alfalfa production in the 7 western US States goes for export (less than 4-5% nationally). In the past 6 years, export hay has proved to be the major market sensitive to GE alfalfa, either in trace amounts or *en total*.

In 2006, I estimated market sensitivity to GE traits to be on the order of 3-5% of US alfalfa hay production markets (Putnam, 2006). However, due to the dramatic increase of export of alfalfa to China since 2006, GE Sensitive’ markets have likely increased, with higher sensitivities for areas dominated by export markets. For

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export growers in western regions, planting decisions must be made for multiple markets (domestic and export), so the hectares impacted by this issue is greater than just the export quantity.

*Most Markets are non-sensitive.* It should be pointed out that the vast majority of the markets for alfalfa hay and forage in the US are not sensitive to GE presence, either as a trait *en total*, or in trace amounts as a low level presence in hay. US hay consumption is dominated by dairy and beef consumers, both of which have widely adopted GE crops as feedstuffs (e.g. >95% of corn and soy). Additionally, research has shown the RR trait in feeds to be safe for animal production (Van Eenennaam and Young, 2014). Currently over 9 billion animals are fed GE crops annually, accounting for up to 90% of the consumption of GE-crop phytomass in the US (Flachowsky et al, 2005; Van Eenennaam and Young, 2014). This represents a massive demonstration of feed safety and of the adaptation of GE crops in the animal sector.

### Defining Non-GE Alfalfa

For the purposes of market assurance, a Non-GE alfalfa forage can be defined as: *“Alfalfa or Alfalfa-Grass Mixtures that have been produced implementing Protocols for Non-GE Management Practices and the hay has been determined to be ‘Non-Detect’ using an appropriate sensitivity threshold.”*

A definition of ‘Non-GE Alfalfa Hay’ is likely to be useful to those producing for sensitive markets. Because the current GE traits (RR and HarvXtra) have been determined to be safe for animal production (USDA-BRS), tolerance levels for low-level presence are determined by market or regulatory factors, not epidemiological factors. Several market categories regarding GE alfalfa can be described (Table 1). These include ‘GE Alfalfa’, non-sensitive ‘Conventional Alfalfa’ and alfalfa produced for GE sensitive markets with some level of sensitivity to low level presence (Non-GE alfalfa – Table 1).

**Low Level Presence.** Unwanted low-level gene presence (LLP) in otherwise non-GE hay can be a problem for sensitive markets. There are three potential sources of an unwanted low level presence of a GE trait in alfalfa hay. Each of these sources represents different levels of risks. These include 1. Gene flow, especially in

**Table 1.** Definitions for GE- and non-GE alfalfa forage grown for markets of varying sensitivity.

	Name of Crop Product	Type of Market	Non-GE Protocol Followed?	Tolerance Level for GE trait	Testing Method
1	GE-Alfalfa (currently RR or HarvXtra)	Non Sensitive GE-	No	Highly Tolerant (100% permitted)	Not necessary
2	Conventional Alfalfa	Non Sensitive GE-	No	Indifferent (no tolerance level)	Not necessary
3	Non-GE Alfalfa (Market Standard)	GE Sensitive Market-based or Organic Sensitivity	Yes	Varying LLP Tolerance at $\leq 0.5\%$ to 5% or statement	QIS Test Strips or quantitative PCR*
4	Non-GE Alfalfa Hay (Regulatory Standard, $ND \leq 0.1$ )	Regulatory Sensitivity (not legally permitted)	Yes	Strict LLP tolerance (generally $ND \leq 0.1\%$ )	Currently PCR*

\*ELISA QIS- Quantitative Immunoassay Strips and Polymerase Chain Reaction (PCR) can be used for desired level of detection. There may be ELISA tests with sensitivity at 0.1% and below available in the market, but generally PCR is preferred.

seed fields, 2) inadvertent movement of hay between fields in equipment, and 3) misidentification or mixing after harvest. These risks can be mitigated by a series of management factors (see below and van Deynze et al, 2006, Putnam, 2016, and NAFA, 2018).

**Non-GE Alfalfa – Market Standard (organic or export)—Category 3, (Table 1).** For organic producers, or for buyers who simply don't want the trait, the standard is a bit fuzzy. There is a demand for non-GE, but are no thresholds for LLP – it all depends upon the market. Some markets simply require a statement of non-GE status, others require testing. Organic rules do not define thresholds or testing. The same is also largely true for export buyers who don't want GE traits but they are permitted by importing countries (e.g Japan). Some buyers rely on statements by growers, but most require routine testing, since significant alfalfa hay goes to China. The non-GMO Project (see <https://www.nongmoproject.org/> ) defines a tolerance level for alfalfa seed (for the Non GMO label) at 0.5%, and 5% for livestock feed and supplements (alfalfa hay).

**Non-GE Alfalfa-Regulatory Standard—Category 4.** This is the strictest threshold for LLP tolerance. Large volumes of exports to China has ushered in a new era of testing for low level presence (LLP). This standard is essentially zero tolerance, but is determined by the level of detection possible by the most sensitive method – PCR (typically  $\leq 0.1\%$ ).

**The Impossibility of a GE-Free Designation.** To analytically and practically declare an agricultural product as 'GE-Free', containing none of a GE trait, is a technical and practical impossibility. In order to be 100% assured that a hay mass is 'GE-Free', every last gram of that mass must be tested, leaving none for its intended use. Thus, declaration of 'Non-GE' status is made within a definition of the threshold of tolerance, sampling protocol, implementation of a protocol to prevent unwanted gene presence, and recognition of the analytical limits of detection and the sampling method. For Category 4, this is typically non-detect at  $\leq 0.1\%$ , and thresholds varies for Category 3.

**A Combination of a Process-based and Testing Approach.** For those interested in satisfying a 'non-GE' market, I recommend a combination of a production protocol and non-GE hay testing. The management practices suggested below are a 'process-based' approach similar to that taken for certified organic production or certified seed.

### **Protocols for Producing Non-GE Alfalfa**

The stewardship of both non-biotech and biotech traits within a region will depend not only on testing, but upon a range of practices, beginning with seed production and purity. Here are the most important aspects of a process for assuring non-GE alfalfa (more fully covered in Putnam et al., 2016 and by NAFA coexistence documents):

**I. Select Certified Cultivars for Seed Purity and Quality.** This is likely the most crucial step to assure trait purity in a hay product. Before planting, seed should be tested to the appropriate non-detection at a given level of market sensitivity (Table 1).

**II. Reduce Possibility of Gene Flow in Hay Fields.** There is some risk of gene flow in hay fields, but this risk is very small (Putnam, 2016, van Deynze et al., 2008). The greatest issues are distances to GE-fields, prevention of excessive flowering or seed, and removal of feral alfalfa from areas in close proximity to GE-sensitive alfalfa fields.

**III. Prevent Inadvertent Transfer of Hay During Harvest.** Clean balers and equipment when moving between fields to prevent mixing of bales which contain unwanted genes.

**IV. Identification of Non-GE Alfalfa Hay/Prevent Mixing of Lots.** Prevent the mixing of hay lots after harvest, maintain identity, and assure customers of that identity for either GE-containing or Non-GE alfalfa hay.

**V. Understand the Sensitivities and Tolerances of the Market.** The threshold of market or regulatory sensitivity must be determined (Table 1).



**VI. Testing to Confirm non-GE status in hay.** Testing for the presence or absence of a GE trait should be used in combination with process-based protocols (1-5) above. The limits of detection of each specific method, and the limitations of sampling should be considered when interpreting laboratory GE tests (Putnam, 2014).

**VII. Cooperation with neighbors and between buyers and sellers.** Working with GE-sensitive or GE-adapting neighbors to understand the risks of gene flow and neighbor influence is important, similar to cooperation on invasive weeds or pesticide drift.

## SUMMARY

Coexistence strategies are a necessary and important component of successful production of both GE- and non-GE alfalfa hay, consistent with consumer preferences and a farmer's right to farm. A number of farmers have produced both GE-sensitive (organic or export) and GE alfalfa hay successfully on nearby farms or adjacent fields. Communication and cooperation between farmers are important components of any coexistence strategy within a region. Common-sense steps for the production of non-GE alfalfa hay include primarily securing of non-detect (tested) seed, preventing accidental mixing of hay lots, and taking steps to prevent gene-flow between alfalfa fields. Market assurance can be further assured by hay testing to meet tolerance of a given market, but the limits of testing and sampling must be considered. A combination of production protocols and testing is recommended to satisfy sensitive non-GE markets.

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## Developing a package of data required for de-regulation of a GE traits \*

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**KEYWORDS:** GMO, regulation, alfalfa.

Despite the fact that specific international guidance and case studies of deregulation for Genetically Modified (GM) alfalfa are still scarce, Argentina has recently joined the group of countries that have already approved the use of a GM alfalfa transformation event for cultivation or food/feed.

This presentation will review the current status of GM alfalfa deregulation worldwide, and the experience gained with the leading case in Argentina, considering the data package needed for biosafety, food safety and trade impact assessment.

Also, a general introduction to the Argentine regulatory framework for GMOs will be provided, including prospective content in the light of upcoming developments such as gene editing and other new breeding techniques that are being applied to alfalfa among other crops, molecular pharming, etc.

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\* **CITATION:** Lema, M.A. 2018. Developing a package of data required for de-regulation of a GE traits. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

# Alfalfa forage quality breeding in France: 30 years of common efforts from seed industry, dehydration industry and public research.\*

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**KEYWORDS:** Alfalfa breeding, forage quality, protein content, ADF, Dehydration

## ABSTRACT :

Alfalfa is an important forage crop in France, where a highly structured dehydration industry has developed in particular. The improvement of alfalfa genetics in France is very active, supported by a dynamic seed sector associated with the involvement of public research. 30 years of joint efforts had led to the improvement of the feeding value of alfalfa at the genetic level without altering genetic progress in yield.

## INTRODUCTION:

Alfalfa (Lucerne ; *Medicago sativa* L.) is a major forage crop in France. It is grown on around 600 000 ha (300 000 ha of pure alfalfa, at least 300 000 ha of alfalfa-grass mixtures), with an average yield of 12 t/ha. From this area of production, a strong dehydration sector produces around 900 000 T of pellets and balls. This sector is well organized and its requirements are determined by Coop de France-Déshydratation. Dehydration plants establish contracts with farmers for forage production, and these farmers are paid as a function of their production tonnage. In 2017, 66752 ha of alfalfa have been contracted for dehydration.

INRA (Institut National de la Recherche Agronomique), the french public organisation in charge of research for agriculture in France, has long time established research program on Alfalfa, involving different labs on agronomy, crop physiology, ruminant nutrition genetics. The institute has also established long time research collaboration with the seed private sector (starting in the 1970's). Seed companies involved in Alfalfa breeding are organized in an association, ACVF (association des Créateurs de Variétés Fourragères) to develop pre-competitive research.

Alfalfa varieties must be listed in the EU catalog to be sold in France. In practice, registration in the french list is mandatory to be recommended by the dehydration sector. Evaluation and Registration of seed varieties in France is conducted by the CTPS (Comité Technique Permanent de la Sélection). CTPS comprise representatives (experts) from three sectors : public research, breeders and end-users, together with administrative staff from the GEVES (the body responsible for evaluation on behalf of the CTPS). The CTPS proposes to the Ministry of Agriculture the registration of valuable varieties, as well as changes to the registration regulations so as to adapt the catalogue to current uses and future needs.

## Alfalfa forage quality breeding in France :

The French Ministry of Agriculture supports the agricultural sector by providing financing under contracts between the private sector and public research organizations. Therefore, given the strong relationship between INRA, seed companies and the dehydration industry, pre and non-competitive research partnerships were initiated in the 1990s to improve the nutritional value of alfalfa. The objective of this partnership research has been to improve the protein and energy value of alfalfa, while maintaining good levels of yield progress.

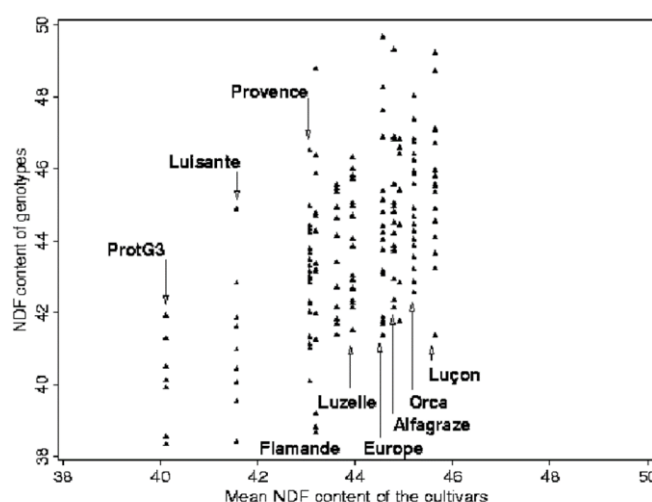
In 1994, INRA's Plant Biology and Breeding Division (BAP) start studies on Energy value trait following studies by INRA's Environnement and Agronomy Division (EA) (agronomy, crop physiology, 1980-1995) and Animal Physiology and Livestock Systems Division (PHASE) (ruminant nutrition, 1995-2000) (Julier, 2015). Indeed, these studies have shown that dairy cows fed with alfalfa forage of good digestibility had milk production

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\* **CITATION:** Béguier V., Guillemot E., Julier B. 2018. Alfalfa forage quality breeding in France: 30 years of common efforts from seed industry, dehydration industry and public research. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

increased by 1 to 1.4 kg of milk per cow per day compared to cows fed with a forage of less digestibility (Emile et al., 1996). Therefore, from 1994 to 2003, different research contracts funded by the Ministry of Agriculture, involving INRA, ACVF and Coop de France déshydratation produced different knowledge and know-how that made it possible to take into account the improvement of feeding value in the seed companies alfalfa breeding programs.

The first achievement was the demonstration that a genetic variability exist within and among alfalfa varieties (Figure 1.), (Julier et al., 2000).



**Figure1:** Within- and among-cultivar variation for NDF content (%) observed for the harvest on 30 May 1996, for 7 to 20 genotypes per cultivar and 11 cultivars. Each value represents the average of three plots of five clones per genotype.

From this result, different studies have allowed to evaluate the genetic variability between varieties and populations regarding digestibility, genetic variability within varieties for digestibility and the most important for the dehydration industry and breeding companies, the development of NIRS equations and testing of their accuracy to predict the digestibility and protein content of dried forage in experimental studies designed to describe genetic variations. For feed value, the ADF (Acid Detergent Fibre) content, which correlates negatively to digestibility, was shown to be the most heritable trait and displayed the most genetic variation.

From the early 2000's, Seed companies involved in ACVF are using these knowhow and knowledge in their breeding program. The challenge to face is to improve feeding value (Energy and Protein) without losing yields and also improved disease resistance (Mainly Stem nemtode, Verticillium and Anthracnose). Genetic correlation is indeed negative between digestibility and forage yield but positive between digestibility and protein content (Julier et al, 2003). And lodging resistance, negatively correlated with digestibility, is particularly important in France where due to the harvest organization of the dehydration plan, the harvesting schedule is around 40-50 days between each cut.

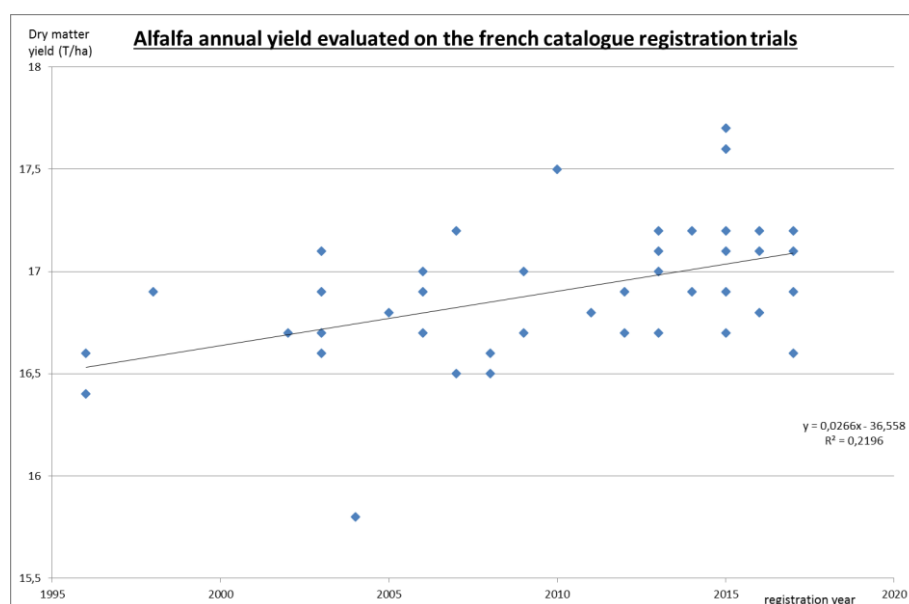
These different results have a direct impact on the regulatory. CTPS has established a list of traits of major interest for alfalfa varieties registration. Since 2000, protein content is measured and included in the score of each variety compared to control varieties, and since 2007, ADF (digestibility) has also been included.

At the same time, the seed industry and the dehydration industry have promoted the fruit of these collaborations by developing a variety assessment. Since 2001, Alfalfa varieties registered in the French catalogue are evaluated specifically for the dehydration sector and a recommended list is published to help farmers who have contracted with dehydration plan. This list is published based on 2 locations – multiannual trials conducted by Coop de France-Déshydratation and the seed sector represented by their union (UFS – Union Française des Semenciers) (Larbre, 2015 - <http://www.luzernes.org/?q=luzerne-et-agronomie/circulaires-agronomiques>).

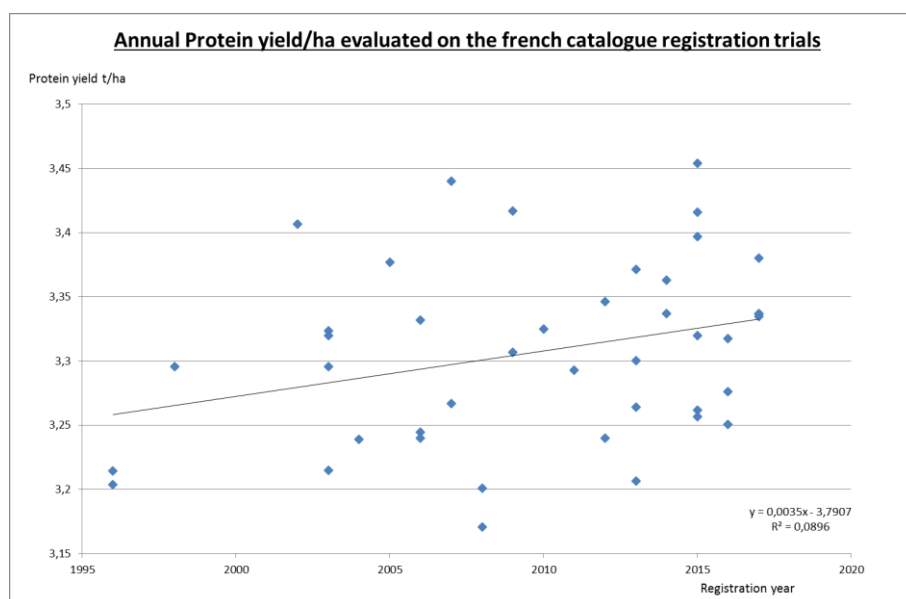
## ACHIEVEMENTS

What impact has this collaboration on varieties registered and marketed in France?

First, continuous genetic progress has been made on dry matter yield as it can be seen in Figure 2. At the same time, there has been a genetic advance in protein content that results in an increase in protein yield per hectare (Figure 3).

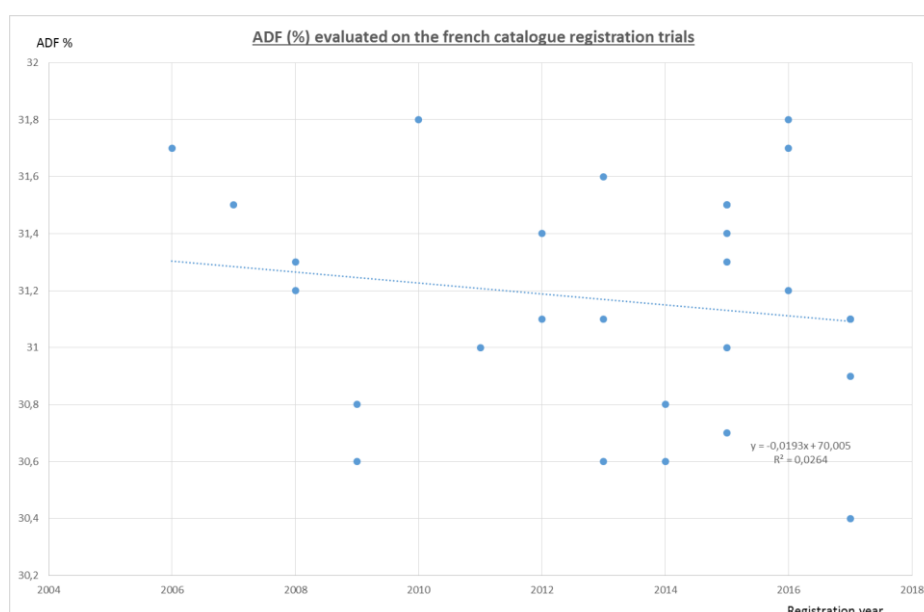


**Figure2:** Illustration of genetic progress in total annual dry matter yield obtained at the time of registration of alfalfa varieties in the French catalog.



**Figure 3:** Illustration of genetic progress in total annual protein yield obtained at the time of registration of alfalfa varieties in the French catalog.

Genetic progress on digestibility is less obvious (Figure 4). But we can see that among the most recent varieties, some have been improved in digestibility (reduced ADF content) while progressing in yield of dry matter.



**Figure 4:** Illustration of genetic progress in ADF content (Acid Detergent Fiber) at the time of registration of alfalfa varieties in the French catalog

At GIE GRASS, we implemented the tools developed during these collaborations and obtained good achievements. For example, we registered a variety in 2015 who illustrate these gains. During the registration trial, it displayed a protein content and ADF content at 104,5% and 98,6% versus the control varieties, respectively (A reduction in the ADF content constitutes a progress). This advance was achieved with a good forage yield (104,7 % of the control varieties).

## CONCLUSIONS :

30 years of collaboration and efforts from public research, the seed industry and the dehydration industry, with the financial support of the French Ministry of Agriculture, have led to the availability of alfalfa varieties to producers improved both in yield and quality (protein, digestibility). The partners of these collaborations are currently evaluating in a new program supported by the Ministry of Agriculture, the VARILUZ project, how these progress can be exploited optimally by the dehydration industry. The final results will be published in 2020.

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## Modification of rate and extent of fiber digestibility in alfalfa\*

Reich, J.M.\*

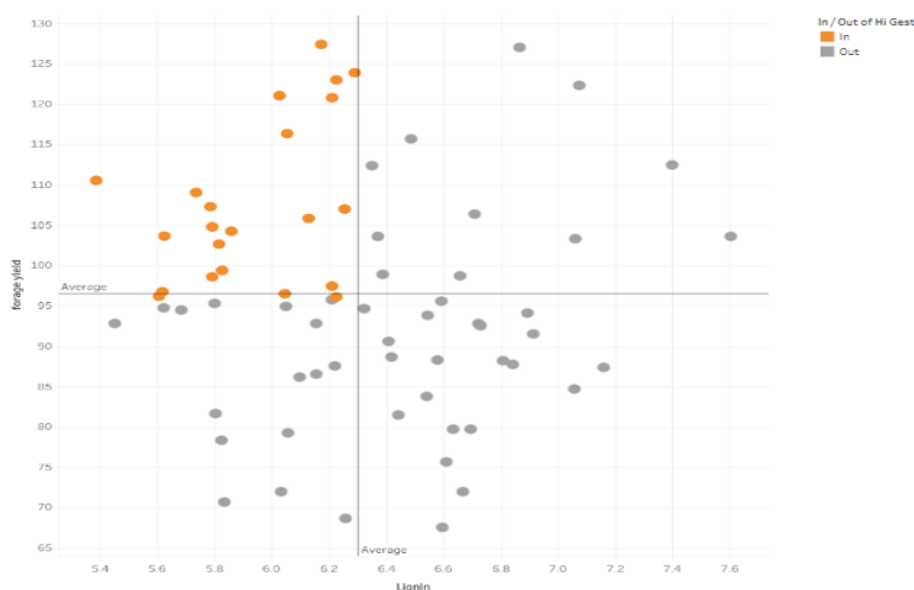
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**KEYWORDS:** Forage Quality, Selection, Digestibility, Lignin

The negative relationship between forage digestibility and lignin concentration has been observed for over 85 years (Woodman and Stewart, 1932). Until the 1950s hay quality was determined primarily by color (Putnam, 2002). Methods of measuring nutritive value in alfalfa and other forages have evolved considerably since the Proximate (Weende) Analysis was first used in the 1800s and include development of TDN (Total Digestible Nutrients) using measures of modified crude fiber, the Detergent Fiber System (Van Soest, 1963), RFV - Relative Feed Value (Rohweder et. al, 1978) and RFQ - Relative Forage Quality (Undersander and Moore, 2002). While each of these represented improvements, especially RFQ which took into consideration fiber digestibility, none considered digestion kinetics and the extent of digestion to more accurately predict animal response. Recent improvements take into account multiple time points to integrate fiber digestion kinetic parameters and also estimate the total amount of potentially digestible fiber in a forage sample to more accurately predict animal response (Combs, 2012). Putnam and DePeters (1995) provided a good synopsis identifying steps where variability is introduced into the measurement of forage quality or feeding value of alfalfa. These steps include but are not necessarily limited to the following: sample procurement, drying, grinding, sub-sampling, storage, and wet chemistry or NIRS analysis. For researchers to have any chance to meaningfully measure forage quality differences or to expect gain from selection in a breeding program, understanding these sources of variation and developing procedures to minimize their impact are necessary.

**Figure 1. Variation for yield and lignin concentration**



Routine, yet rigorous, focused selection for lower lignin in diverse germplasm pools was imposed to exploit existing native trait genetic variability. The scatter plot in Figure 1 illustrates the genetic variation existing in Alforex genetic pools for lignin content and forage dry matter yield.

Selection in segregating spaced plant nurseries was based on multiple harvests per year and was imposed across a wide array of fall dormancy groups. Numbers of parents selected per synthetic varied depending on

\* **CITATION:** Reich, J.M.. 2018.Modification of Rate and Extent of Fiber Digestibility In Alfalfa. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

the pool and dormancy group. Breeder seed (Syn.1) was produced under cage isolation and resulting experimental varieties were entered in standard replicated multiple-location forage yield trials during 2010-2017 which included conventional experimental varieties from the same breeding cycle as well as commercially available check varieties developed by all commercial breeding programs. Forage yield was measured for 3 or 4 years and forage quality samples taken in the 1<sup>st</sup> and/or 2<sup>nd</sup> hay year from a minimum of 3 cuttings. The following forage quality parameters were among those estimated using NIRS: Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), lignin, NDF digestibility (NDFD), Rate of Fiber Digestion (Kd) based on 24, 30, and 48 hour NDFD, undigested NDF at 240 hours (uNDF240), Potentially Digestible NDF (pdNDF240 determined from uNDF240), and Total Tract NDF Digestibility (TTNDFD). Time point studies were also conducted with forage sampled at 21, 28, and 35 days after cutting and sub-samples separated into leaves and stems. Weighted averages of the data are reported herein. Based on the data, varieties in fall dormancy groups 3, 4, 6, 9, and 10 were advanced commercially. During the 2015-2017 growing seasons on-farm forage samples (hay or haylage) produced in all major hay producing regions of the US were submitted by commercial hay growers and dairies to Rock River Laboratory<sup>1</sup> for quality analysis including TTNDFD, Kd, uNDF240, and lignin. Samples were taken by farmers, not researchers, and the resulting data is considered as “crowd sourced”.

Data collected during 2010-2017 in all dormancy groups consistently showed that significant positive responses to selection for lower lignin were achieved. Compared to appropriate control check varieties of the same dormancy group in research trials, Hi-Gest varieties have averaged 7-10% lower lignin, 5-10% faster rates of digestion (Kd), 5-10% greater extent of digestibility (uNDF240), 5-10% higher TTNDFD, and 3-5% higher crude protein. In addition, leaf and stem separation has shown 5-8% higher leaf%. Hi-Gest varieties were measured as having greater leaf% at 28 days than control varieties at 21 days, and greater leaf % at 35 days than control varieties at 28 days. Data presented in Figures 3 through 6 illustrate these responses. Figures 2 and 3 show performance of Hi-Gest 360 and Hi-Gest 660 compared to checks at 28 and 35 days for lignin, uNDF240, pdNDF240, Kd, and TTNDFD (21, 28, and 35 days for Hi-Gest 660).

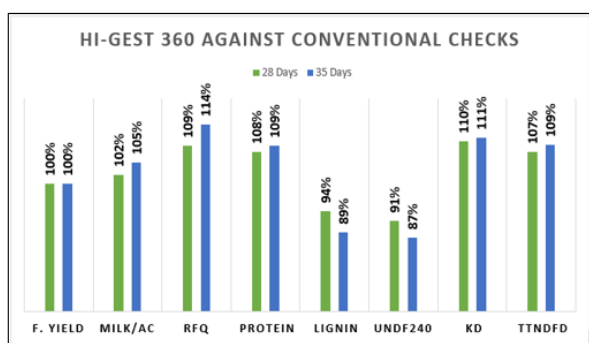


Figure 2. Hi-Gest 360 compared to checks at 28 and 35 days after prior cut for various forage quality parameters.

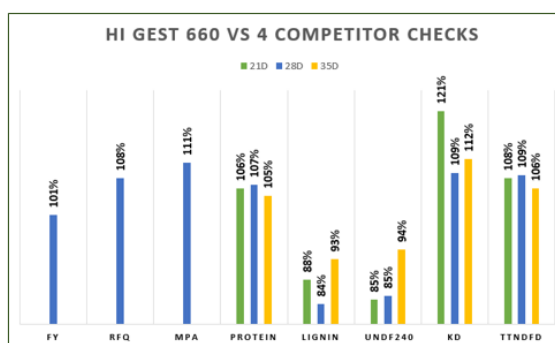


Figure 3. Hi-Gest 660 compared to checks at 21, 28, and 35 days after prior cut for various forage quality parameters.

Figure 4 shows leaf % of AFX 960 compared to checks at 21, 28, and 35 days after the prior cut.

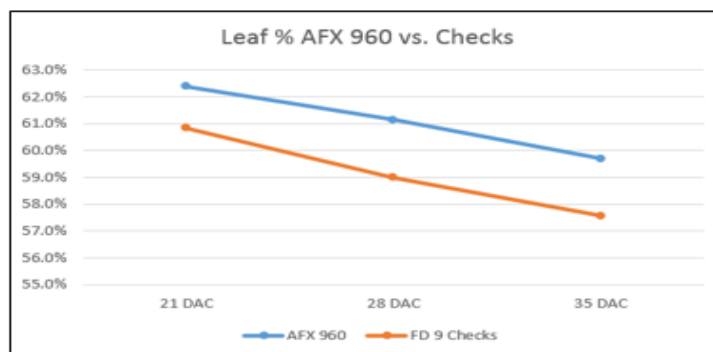


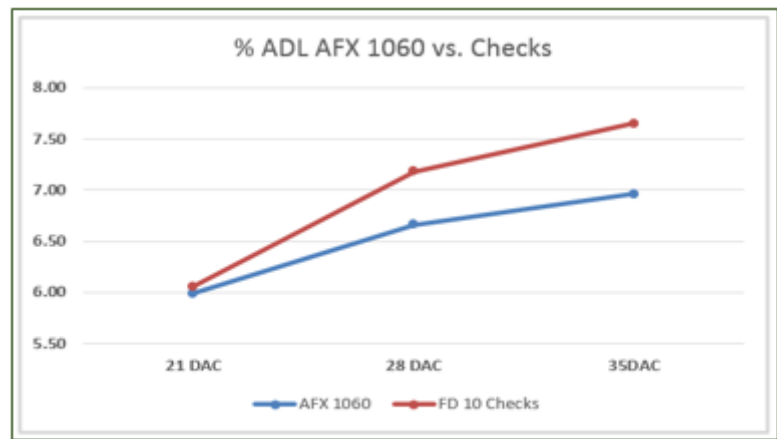
Figure 4. AFX 960 compared to checks at 21, 28 and 35 days after prior cut for leaf % based on physical separation.

Early release of lateral buds is a significant contributor to higher leaf:stem ratio and increased protein content of Hi-Gest alfalfa (Figure 5).

**Figure 5. Early release of lateral buds: samples at 35 days, Cut 4 July 2018**



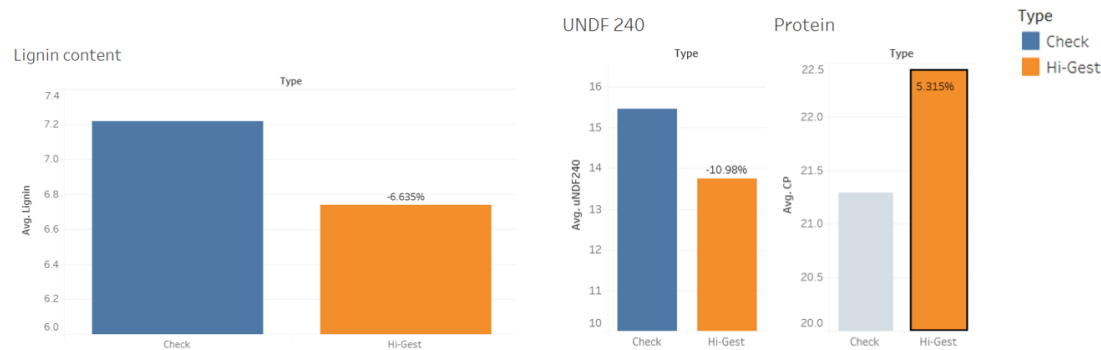
Figure 6 shows lignin content of AFX 1060 compared to checks at 21, 28, and 35 days after the prior cut.



**Figure 6. AFX 1060 compared to checks at 21, 28 and 35 days after prior cut for lignin content.**

Comparison of nearly 200 on-farm alfalfa samples submitted to Rock River Laboratory during 2017, shows that commercially produced Hi-Gest alfalfa averaged 7% lower lignin, had 11% less undigested fiber at 240 hours, and 5% higher crude protein compared to check varieties (Figure 7).

**Figure 7. 2017 on-farm crowd-sourced data (SW USA) – 74 checks, 117 Hi-Gest**



The amount of undigested fiber at 240 hours (uNDF240) is increasingly viewed as an important measure of forage quality and feeding value and remains one of the current focuses of our selection program. Figure 8 illustrates genetic variation for uNDF240 in recent Alforex nondormant and dormant breeding pools.



**Figure 8. Scatter graphs showing Alforex variation for uNDF240 and forage yield in nondormant and dormant breeding pools.**

Research results showing that conventional selection within elite breeding pools for lower lignin content resulted in improved rate of digestion as measured by dynamic Kd, and greater extent of digestion as measured by uNDF240 has been subsequently validated by on-farm studies. In addition, crude protein content has been improved by changing plant architecture resulting in plants with higher leaf-to-stem ratios.

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## A very non-dormant alfalfa (*Medicago sativa* L.) with high multifoliolate expression.\*

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**KEYWORDS:** alfalfa quality, crude protein, leaf/stem ratio, genetic diversity.

Besides forage yield and persistence, forage quality is also very important in alfalfa production. Alfalfa quality depends not only on the environment but also on cultivar and herbage leaf proportion (Lacefield, 2004). Alfalfa leaves have normally three leaflets, and leaves are more digestible and have higher nutritional value than stems. Therefore, one way of improving alfalfa quality can be to increase leaf/stem ratio (LSR) by selecting for a higher frequency of multifoliolate (MF) plants in the population, i.e., plants showing leaves with more than three leaflets (Etzel *et al.* 1988; Volenec & Cherney (1990).

Main objectives of INTA's alfalfa breeding program at Manfredi Exp. Station (Córdoba, Argentina) are forage yield, plant persistence, multiple pest resistance and forage quality. Regarding the latter, a phenotypic recurrent selection (PRS) program for increasing the number of MF genotypes in an extremely non-dormant [Fall Dormancy (FD) 10] population was carried out from 2008 to 2010. Initial breeding population was composed by 83 trifoliolate (TF) genotypes selected under field conditions for vigor, pest resistance and regrowth rate from FD 10 cultivars Ruano, Mireya and CW1010. These selected plants were transplanted to a pollination cage and intercrossed using honeybees in order to produce seed of the initial (C-0) population. C-0 seed was then planted to initiate PRS for increasing MF expression. Selection was performed at two stages of plant development: a) early vegetative stage, by choosing plants with at least one MF leaf; and b) early flower stage on those previously selected plants, by choosing the ones having a MF score of 4 (6-7 MF leaves stem<sup>-1</sup>) and 5 ( $\geq 8$  MF leaves stem<sup>-1</sup>). Scores were assigned according to Sheaffer *et al.* (1995). Each selection cycle started with 1,000 plants. After four cycles of PRS, MF expression (% MF) increased from 6.7% in C-0 to 77.7% in C-4 population (Figure 1)

The effect of high MF expression on yield components and quality along selection cycles (C-1 to C-4) was assessed under field conditions using individual plants and dense stand arrangements. Evaluations on individual plants (25 plants plot<sup>-1</sup>) were performed under two soil moisture conditions (rainfed and irrigated) and two growing seasons (2010-2011 and 2011-2012) using a RCB design with three replications, in which C-1 to C-3 were the treatment populations and C-0 was the check population. Evaluated agronomic traits were forage yield (FY, cumulative kg DM plot<sup>-1</sup> at 10% blooming), number of stems (S), plant height (H), number of nodes

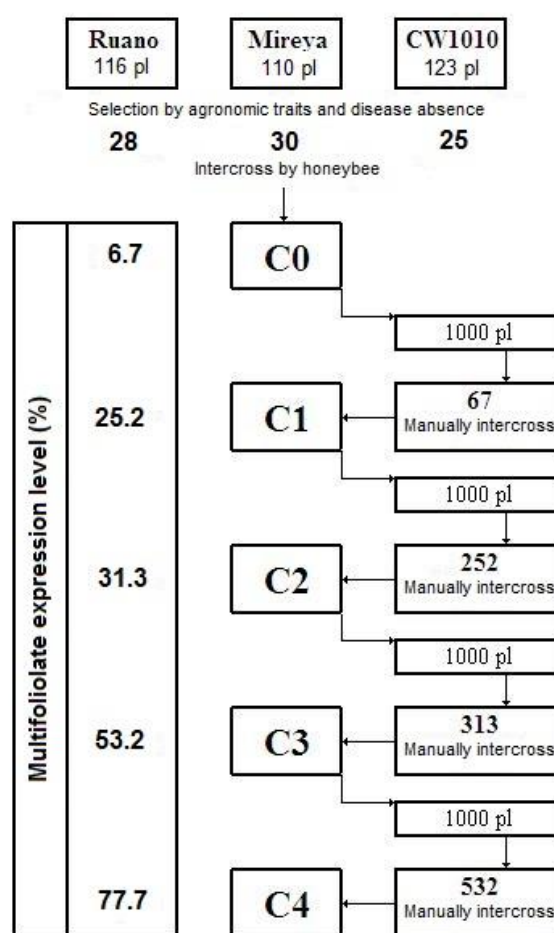


Figure 1 Scheme of phenotypic recurrent selection performed to generate multifoliolate alfalfa populations (C1 to C4). Note: 67, 252, 313 and 532: number of selected plants in each cycle

\* **CITATION:** Odorizzi, A.S., Arolfo V. and Basigalup, D. 2018. A very non-dormant alfalfa (*Medicago sativa* L.) with high multifoliolate expression. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



per stem (N), number of leaves per stem (LN), number of leaflets per leaf (F), multifoliolate expression (% MF) and LSR. Forage quality was evaluated through estimation of crude protein (CP) and neutral detergent fiber (NDF) content and *in vitro* true DM digestibility (IVTDMD) at 48 hours. For dense stand evaluations, 5-m<sup>2</sup> (1 x 5 m) plots were sown at a seeding rate of 10 kg ha<sup>-1</sup> in the fall 2012. Using a RCB design with three replications, populations C-3 and C-4 were compared to C-0 and three TF commercial cultivars. Measured traits (plot means) were cumulative forage yield (metric tons DM ha<sup>-1</sup>), plant height (H), number of nodes per stem (N), leaf/stem ratio (LSR), crude protein (CP), neutral detergent fiber (NDF) and IVTDMD. Results are summarized in Table 1 (individual plants) and Table 2 (dense stand).

**Table 1:** Mean variable comparisons for multifoliolate expression among selection cycles (C-1 to C-3) and initial breeding population (C-0) under individual plant conditions. Values are general means of four environmental evaluations (irrigated 2010/11 and 2011/12 and rainfed 2010/11 and 2011/12) carried out in Manfredi, Córdoba, Argentina.

Traits	Alfalfa populations			
	C-0	C-1	C-2	C-3
FY (kg DM plot <sup>-1</sup> year <sup>-1</sup> )	4.38a	4.11a	3.90a	3.15b
S	75.70a	80.72a	68.00b	60.77b
H (cm)	48.34a	45.64b	43.97b	40.87c
N	9.69a	9.50a	9.67a	9.49a
LN	41.28a	39.64a	41.07a	37.67b
%MF	4.89d	17.08c	39.40b	67.25a
LfN	3.08c	3.28b	3.71b	4.51a
LSR	1.17b	1.30a	1.34a	1.40a
CP (%)	26.60b	28.25a	28.70a	29.25a
NDF (%)	40.47a	39.70b	39.44b	38.91b
IVTDMD (%)	79.29a	80.29a	79.45a	80.72a

Means in the same row followed by the same letter are not significantly different (DGC test,  $p < 0.05$ ). References: FY: forage yield; S: number of stems; H: plant height; N: number of nodes per stem; LN: number of leaves per stem; %MF: multifoliolate leaf percentage; LfN: number of leaflets per leaf; LSR: leaf/stem ratio; CP: crude protein; NDF: neutral detergent fiber; IVTDMD: *in vitro* true DM digestibility.

**Table 2 :** Mean variable comparisons among advances selection cycles for multifoliolate expression (C-3 and C-4) and the initial breeding population (C-0) under dense stand conditions. Values are means of two evaluated growing seasons (2012/13 and 2013/14) in Manfredi, Córdoba, Argentina.

Traits	Alfalfa populations		
	C-0	C-3	C-4
FY (ton DM ha <sup>-1</sup> year <sup>-1</sup> )	12.47a	11.23a	10.70a
H (cm)	45.00a	41.39a	41.05a
N	10.75a	9.57a	9.37a
LSR	1.35b	1.46a	1.49a
CP (%)	23.11b	23.55b	25.00a
NDF (%)	40.93a	39.40a	38.25a
IVTDMD (%)	78.33a	79.89a	80.93a

Means followed by the same letter are not significantly different (DGC test,  $p < 0.05$ ). References: FY: forage yield; H: plant height; N: number of nodes per stem; LSR: leaf/stem ratio; CP: crude protein; NDF: neutral detergent fiber; IVTDMD: *in vitro* true DM digestibility.

Under individual plant conditions, population C-3 had overall lower ( $p < 0.05$ ) forage yield than population C-0 (Table 1). This negative correlation between %MF and DM yield was also reported by Ferguson and Murphy (1973). However, under dense stand conditions, there were no DM yield differences ( $p > 0.05$ ) between MF



populations and TF cultivars (Table 2), even though populations C-3 and C-4 had the lowest DM yields. For the individual plant trials, over the four environments defined by soil moisture and growing seasons, C-0 and C-1 populations exhibited higher ( $p<0.05$ ) number of stems per plant (S) and higher ( $p<0.05$ ) plant height (H) than C-2 and C-3 populations. On the contrary, under dense stand conditions, there were no differences ( $p>0.05$ ) in H among populations. The relationship between H and MF expression was described in various studies and it is controversial (Ferguson & Murphy, 1973; Bingham & Murphy, 1965; Juan *et al.*, 1993a). Regarding the number of nodes per stem (N), there were no differences ( $p>0.05$ ) among MF selection cycles and TF checks, under both individual and dense stands conditions. Number of leaves per stem (NL) was lower ( $p<0.05$ ) in C-3 than in C-0 population and TF checks. Selection for higher MF expression (C-1 to C-3) increased ( $p<0.05$ ) the number of leaflets per leaf (LfN) relative to C-0. However, this may not necessarily correspond to an increase in total plant leaf area because leaflet size might be smaller than in TF plants, as stated by Volenec & Cherney (1990). On the other hand, Etzel *et al.* (1988) concluded that plants with MF expression within the range of 4.1 to 7.3 leaflets leaf<sup>-1</sup> had larger leaf area, faster leaf expansion after defoliation and lesser stems compared to TF plants. In the present work, total leaf area was not measured. Populations C-3 and C-4 showed higher ( $p<0.05$ ) LSR than C-0 under both individual plant and dense stand conditions. This is consistent with the increase ( $p<0.05$ ) of CP content exhibited by MF populations (C-1 to C-4) relative to C-0 under both individual plant and dense stand evaluations. Similar results were reported by Petkova & Panayotova (2007). On the contrary, when Juan *et al.* (1993b) compared forage quality of cultivars with moderate MF expression to “high quality” (HQ) TF cultivars (selected for lower FDN and FDA), they found no advantages from MF alfalfas. In the present study, the more advanced MF selection cycles showed lower ( $p<0.05$ ) NDF than C-0 under individual plant conditions. Under dense stand conditions, FDN also tended to decrease with selection cycles but no significant differences were detected among C-0 and C-1 to C-3 populations. In a similar way, IVTDMD increased as selection cycles progressed, but differences were not significant. In another study, Yancheva *et al.* (2012) detected higher CP and IVTDMD for three MF experimental populations compared to two TF cultivars.

When evaluating MF alfalfa populations, it is important to define the way in which MF expression is measured. In the present work, populations C-1, C-2 and C-3/C-4 were respectively classified as “low expression”, “moderate expression” and “high expression”, according to Sheaffer *et al.* (1995). From a commercial viewpoint, an alfalfa cultivar with  $\geq 60\%$  of the plants having at least one MF leaf is considered as “high expression”. Thus, differences in how MF expression is estimated may explain to some extent the controversial literature results.

When conducting a selection program, it might be important to estimate if the genetic variability is reduced in the resulting selected populations. In this study, intra and inter genetic variability between C-0 and C-4 populations was assessed using SSR markers. Forty genotypes from each one, keeping the original proportion of MF expression (6.7% and 77.7% for C-0 and C-4, respectively), were used. DNA extraction followed the modified CTAB protocol suggested by Doyle & Doyle (1987). SSR analysis was performed on 25 pairs of primers -mainly originated from *M. truncatula* (Julier *et al.*, 2003)- that were successfully amplified and showed clear, strong, single bands for each allele. So, presence or absence of each allele was determined in every genotype. Program ATetra (version 1.2) for autotetraploid species was used to calculate the within- and between-population genetic diversity (Van Puyvelde *et al.*, 2010). This program calculates the expected heterozygosity within-population according to Hardy-Weinberg equilibrium (HE) and Nei's genetic diversity value (Nei 1978). Among-populations, variability was estimated through the population differentiation index or Nei's GST (Nei 1973). Significance level of HE was calculated by the DGC test (Di Rienzo *et al.*, 2002).

For the within-population genetic diversity, 20 markers amplified in a single region and five showed two regions of amplification, which were named ‘a’ and ‘b’ based on molecular weight. These regions were sufficiently distant and did not show any allelic relationship, and so contributed to avoid reading errors when fragments are of similar weight. Between these two regions, only the one that showed greater polymorphism i.e. higher number of alleles (assuming that this would represent a higher discriminative power), was chosen. Overall, the 25 SSR loci gave a total number of 185 PCR fragments (alleles), with molecular weights ranging from 80 to 310 bp. The number of fragments per locus ranged from 3 to 11, with an average of 6.28 alleles locus<sup>-1</sup>. By determining the number of fragments per locus within each population (C-0 and C-4), the loss of alleles during the selection process was assessed. For the vast majority of SSR markers, the numbers of alleles per locus was the same in both populations. Only for four markers, one or two alleles present C-0 were not detected in C-4. These lost alleles in C-4 had in C-0 a frequency lower than 5%. Given the high multiallelic

degree of SSR in alfalfa, this small loss is considered not significant to differentiate the two populations. This is reinforced by the estimation of HE values for each SSR marker, which ranged from 0.565 to 0.889 in C-0 and from 0.491 to 0.877 in C-4. These values are considered moderate to high, and are consistent with the total number of alleles found for each marker. The overall HE estimation was 0.723 for C-0 and 0.726 for C-4. The DGC test did not detect HE significant differences between the two populations. Therefore, no genetic diversity was lost during the selection process.

It is concluded that four cycles of PRS were effective to significantly increase MF expression without producing inbreeding effects. The more advanced selection cycles (C-3 and C-4) had higher forage quality than the initial population (C-0). SSR markers were highly polymorphic and efficiently revealed the level of genetic diversity in C-0 and C-4 populations. Nei's GST value between-populations ranged from 0.002 to 0.033. Overall GST was 0.013, which means that only 1.3% of the total genetic diversity was between-populations and 98.7% was within-populations.

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## Use of GE traits for improvement of forage quality in alfalfa\*

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**KEYWORDS:** alfalfa, biotech, lignin, tannin and flowering

In 2001 a small group of scientists from the U.S. Dairy Forage Research Center (USFDRC), the Noble Foundation, and Forage Genetics International (FGI) began discussions about the potential use of new biotech tools for improving forage quality in alfalfa. The consensus of that group was that conventional breeding methods were unlikely to provide significant breakthroughs in alfalfa forage quality, and that new approaches were warranted. The three organizations formed the Consortium for Alfalfa Improvement (CAI) in 2002, dedicated to a long-term collaboration bringing together expertise in plant biology, molecular biology, biochemistry, plant breeding and ruminant nutrition. Pioneer joined the Consortium in 2005.

At the first CAI meeting it was decided that the collaboration would focus on traits that could improve the efficiency of utilization of alfalfa fiber and protein. In this presentation I will provide updates on the two most promising projects; lignin modification for improved fiber digestibility and production of condensed tannins in leaves and stems to increase the efficiency of protein utilization by ruminants. I will also report on a more recent project to delay flowering in alfalfa. In all of the following examples a GE approach was utilized to modify expression of native genes.

### Reduced Lignin Alfalfa

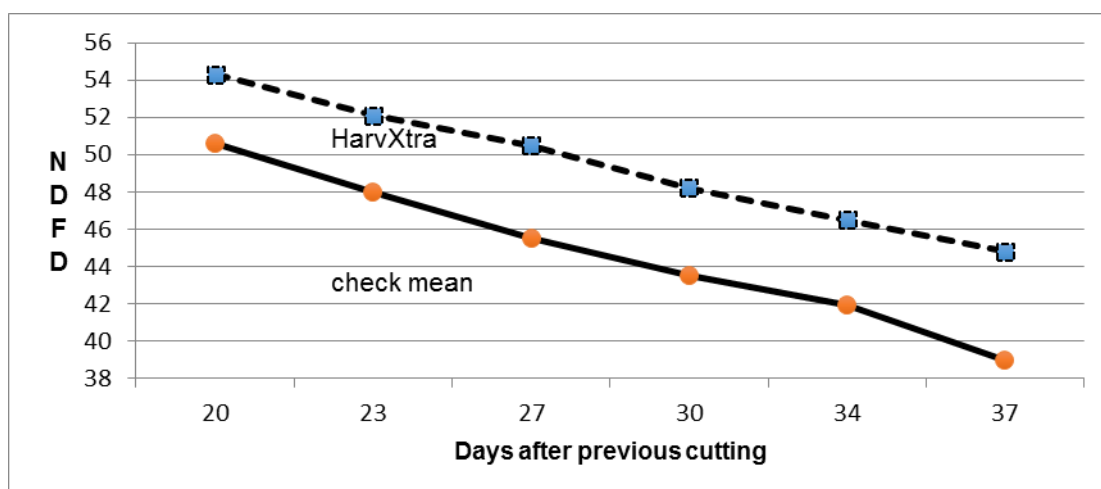
The lignin biosynthetic pathway is well understood, and loss-of-function mutants for specific genes coding for key biosynthetic enzymes have been successfully exploited in brown midrib corn silage and forage sorghum for improved fiber digestibility. Gene suppression techniques were used in alfalfa to suppress each of the genes in the lignin biosynthetic pathway, and transgenic plants were evaluated for lignin content, lignin composition, fiber digestibility (NDFD), lodging potential and forage yield. Only one suppression construct provided the desired outcome – a significant increase in NDFD with no effect on agronomic performance. This construct significantly reduced expression of the gene CCOMT (caffeoyl-CoA 3-O-methyltransferase).

About 1000 transgenic events were produced using a RNAi CCOMT construct, and in field-based event sorting (~70 events field tested) nurseries we found that the best events had moderately strong suppression of CCOMT and that events with very high suppression had agronomic problems. Event KK179 was chosen as the commercial event (brand name HarvXtra™). HarvXtra varieties also containing the Roundup Ready trait (HvX/RR) are now sold in the U.S. and Canada, will soon be sold in Argentina, and are in the deregulation process in Mexico.

Numerous field studies have confirmed a 15-20% reduction in lignin content (ADL) and a 14-16% increase in fiber digestibility (NDFD) in fall-dormant genetic backgrounds. The graph below summarizes changes in forage quality with advancing maturity of a FD4 HarvXtra variety and the mean of several conventional FD3-4 checks. This test was conducted at four locations, for two years, and with 6 sampling dates per cut. HarvXtra had significantly higher ( $p=0.01$ ) NDFD than all of the conventional controls at all sampling times. There was no significant difference in NDFD between the controls when averaged over all locations, both years and all sampling times.

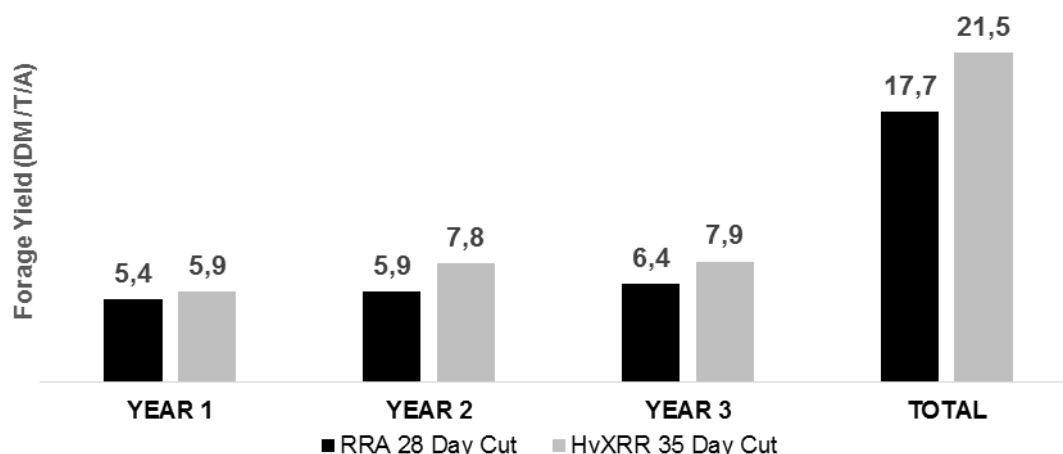
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\* **CITATION:** McCaslin, M.H. 2018. Use of GE traits for Improvement of Forage Quality in Alfalfa. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



**Figure 1:** Changes in Quality with Advancing Maturity

These trials also confirmed that NDFD for the HarvXtra variety was equal to or better than NDFD of the commercial checks harvested 7-10 days earlier. This is consistent with earlier studies, and suggests the potential for a delayed harvest without a decrease in fiber digestibility relative to the controls. The graph below compares data from trials harvested at a 28-day vs 35-day cutting interval. In this trial NDFD for the HarvXtra variety harvested at 35 days was equal to or better than NDFD of the control harvested at 28 days. There was a significant forage yield advantage for the 35-day harvest system vs the 28-day harvest system, demonstrating another potential benefit for the technology. Preliminary data also shows improved stand life with 35d vs 28d harvest schedules.



**Figure 2:** Effect of cutting interval on forage yield

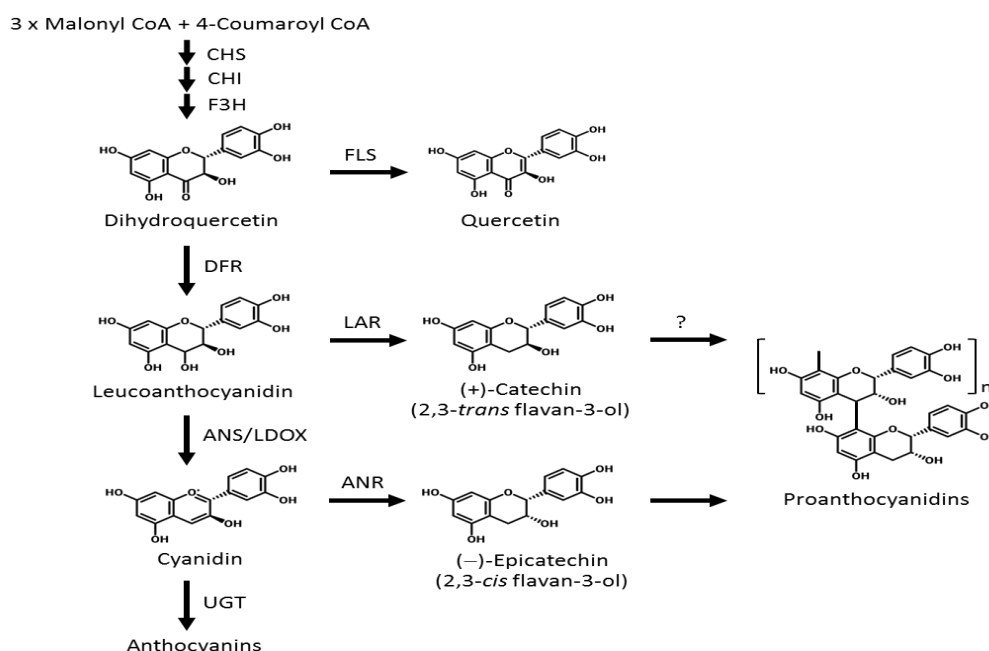
In 2016 sixteen U.S. Midwest dairy producers were recruited to conduct an on-farm feeding trial comparing a HarvXtra variety vs a non-HarvXtra commercial control. Participating producers were required to manage the HarvXtra and control stands similarly and to feed a high forage diet ( $\geq 50\%$  forage), with alfalfa being at least half the forage component. In all cases the ration with the control hay was fed for a 4-week period, after which the HarvXtra ration was fed. Producers measured milk production and milk components for each of the treatment periods. On average the ration with HarvXtra alfalfa gave a 4.1 lbs milk/cow/day increase compared with the ration with control alfalfa.

## Tannin Alfalfa

Forage legumes can be divided into two groups, those that have the potential to cause rumen bloat, and those that do not. In all cases, the non-bloating types contain condensed tannins in leaves and stems. Alfalfa-induced bloat is a common problem for dairy producers that graze alfalfa, leading to substantial economic losses. Condensed tannins also bind with forage proteins slowing the rate of rumen protein fermentation, resulting in an increase in rumen undegradable protein (RUP) – which bypasses the rumen for degradation in the lower tract. When alfalfa forage is fed to high producing dairy cows, the protein fraction is fermented faster than the rumen microbes can use it for growth. As a result, excess nitrogen (NH<sub>3</sub>) is transported out of the rumen and excreted in the urine. Anything that can slow the rate of protein degradation in the rumen should increase RUP, significantly improve the efficiency of alfalfa protein utilization, and decrease nitrogen losses to soil and water on the dairy.

Alfalfa makes condensed tannins, but the biosynthetic pathway is tightly regulated, and condensed tannins are only produced in alfalfa seed coats and in the tips of glandular hairs. CAI scientists agreed on a plan to modify expression of these native genes for condensed tannin biosynthesis, so that condensed tannins would be synthesized also in leaves and/or stems.

In this project various genes, most of which are transcription factors, were evaluated singly and in various combinations to find a gene or gene stack that would provide >1% condensed tannins (dry weight) in alfalfa leaves and/or stems. The first experiments were conducted with breeding stacks of various transformation events, with a very limited number of events evaluated for each transgene tested. Our most promising results were from breeding stacks that include both a transcription factor that increases flux generally through the anthocyanin/tannin biosynthetic pathway, and a second transcription factor that shunts flux of the branched pathway away from Anthocyanin to Proanthocyanidins (condensed tannins).



**Figure 3:** Scheme of the flavonoid pathway leading to proanthocyanidin (PA) production in *Medicago truncatula* (R.A.Dixon).

Our preliminary results allowed us to narrow the 6-7 candidate genes down to the two described above. Although common elements in the initial constructs eventually led to gene silencing instability, we were able to demonstrate accumulation of condensed tannins in alfalfa leaves at up to 2% of dry matter, and show such accumulation resulted in a decrease in protein-induced foaming in a rumen simulator and a decrease in rate of protein fermentation *in vitro*.

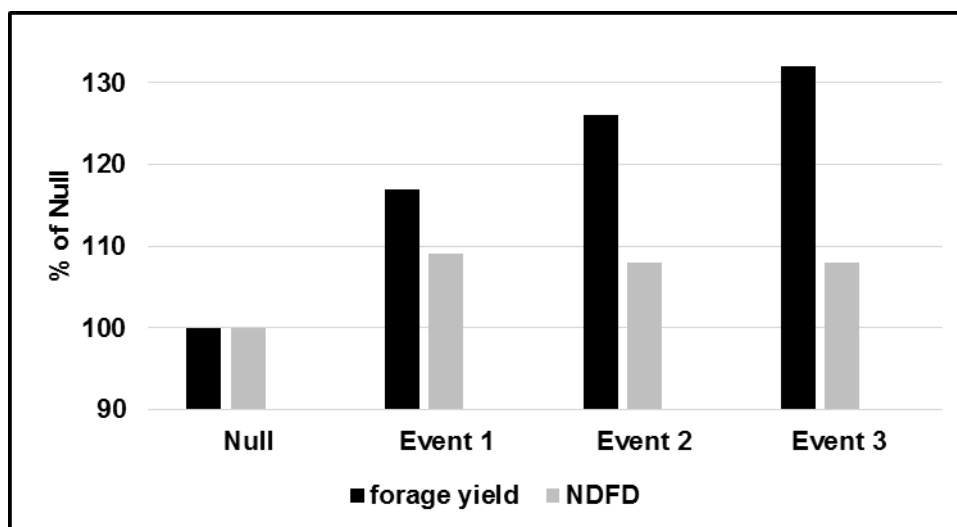


FGI has now produced numerous events of optimized constructs expressing the two genes described above, with the hope of finding plants with sufficient condensed tannins to provide the feed quality benefits and without any significant negative impact on agronomic performance.

### Delayed Flowering

Alfalfa forage quality begins to decline rapidly after initiation of flowering. At about the same stage the rate of dry matter accumulation starts decreasing. Our hypothesis is that changes in forage quality and dry matter accumulation at the onset of flowering are not coincidental, but are controlled by the same developmental “master switch”. In 2014 FGI and the Noble Foundation began a combination of forward and reverse genetic screens in *M. truncatula* to identify mutant loss-of-function alleles that give a delayed flowering phenotype. The mutant phenotype was confirmed for one such gene, referred to here as DF, in transgenic alfalfa with a RNAi construct that suppressed gene expression. Several events from this construct were evaluated in the greenhouse and field in 2017 as T0 clones, and in the field as F1 progeny in 2018.

Flowering delay appears to be related to level of DF gene suppression, and in the 2017 trials appears to range from approximately 14-28 days. In all cases DF suppression and delayed flowering appeared to increase biomass compared to the non-transgenic control. In a 2018 trial both forage yield and forage quality (NDFD) were measured on three DF events compared to a non-transgenic control with harvest 35 days after previous cutting. These results are summarized in Figure 4, and confirm the potential to use the delayed flowering trait to increase both forage quality and forage yield.



**Figure 4:** Forage yield and forage quality of three DF transgenic events compared to a non-transgenic control

All three of the above projects were subject to multi-party research collaborations. In addition to the CAI collaborators mentioned previously, AgResearch NZ and the University of North Texas collaborate on the tannin project, and the delayed flowering project is an FGI/Noble Research Institute collaboration.

## Ideal alfalfa variety - discussion on the breeding direction of alfalfa in China.\*

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**KEYWORDS:** Ideal alfalfa variety, basic characters, special characters, market demand, breeding direction

### CONTENT:

What would an ideal alfalfa variety look like? Due to the large differences in topography, soil and climate in different regions of China, alfalfa growers have different requirements for the varieties, but they have one demand in common: high yield and good quality. However, this can only be considered as basic characters of an ideal alfalfa variety, breeders should focus more on special characters. In the future, the classification of alfalfa varieties will no longer be limited to the indicators such as fall-dormancy level, cold resistance index, insect resistance index and etc. Customization and individualization will become an inevitable trend, so as to meet different demands for alfalfa varieties in the market through optimization and combination. Breeders should play a role of pioneer in the market, starting from the perspective of demanders, jumping out of fixed way of thinking and applying the wings of dreams. Therefore, from the perspective of actual growers in China, the author puts forward bold ideas on wide adaptability, yield and quality, as well as usage method, in the hope of providing some new ideas for breeders.

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## Effects of different levels of alfalfa meal on production performance of sows and pork quality of finishing pigs\*

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**KEYWORDS:** alfalfa meal; replacement gilt; pregnant sow; finishing pig

**OBJECTIVE:** The objective of this study was to explore the effects of different levels of alfalfa meal (AM) in diets on the growth and reproductive performance of gilts and pregnant sows and on production performance and meat quality of finishing pigs.

**Materials and Methods:** 640 healthy replacement gilts (Landrace × Large white) with age of 110 d and body weight of about 45 kg were randomly separated into four treatments, including 0% AM group (control group), 5% AM group, 10% AM group and 15% AM group in the diets, with 4 replicates each treatment and 40 pigs each replicate. The gilts of 5% AM group that had the best daily gain and estrus percentage were selected as pregnant sows and then separated into control group I (0% AM), 10% AM group, 15% AM group and 20% AM group after estrus and artificial insemination. The replacement gilts of control group was still used to act as the control group in gestation period. There were 5 treatments with 4 replicates each treatment and each replicate including 3 pigs. 130 Landrace × Big finishing pigs with body weight ( $60.28 \pm 0.73$ ) kg were randomly assigned to 5 treatments with 26 replicates each group. Those were fed with 0 (control group), 5%, 10%, 20% and 30% AM in the diets, respectively. Auto-feeding system was used in the experiment. The pre-experiment lasted for 10 d, and the formal experimental period lasted for 72d.

**Results:** At the stage of the replacement gilts, the feed intake and daily gain of the AM groups were lower than control group and decreased with increasing of the AM proportion in the diets; there was no significant difference in feed intake among four groups ( $P > 0.05$ ); the daily gain of 5% AM group and the control group were significantly higher than that of 10% and 15% AM groups ( $P < 0.05$ ); whereas, there was no significant difference between 5% AM group and control group ( $P > 0.05$ ); feed and gain rate (F/G) in 10% and 15% AM groups were significantly higher than that of 5% AM group and the control group ( $P < 0.05$ ), while, there was no significant difference between 5% AM group and control group, 10% and 15% AM groups, respectively ( $P > 0.05$ ); compared with the control group, the weight gain cost of 5% AM group decreased and that of 10% and 15% AM groups increased; the estrus rate of sows treated with 5% AM improved, but there was no significant difference among the 4 treatments ( $P > 0.05$ ).

For sows, adding appropriate level of AM in replacement gilts' diet can improve feed efficiency and the estrus rate. But, excessive level of AM may have adverse effects on the growth of replacement gilts.

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\* **CITATION:** Wang, C.Z. Shi, Y.H. 2018. Effects of different levels of alfalfa meal on production performance of sows and pork quality of finishing pigs. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

**Table 1:** Effect of different AM level on production performance of replacement gilts

Items	Control group	5% AM	10% AM	15% AM
ADFI /kg	2.52±0.24	2.34±0.80	2.22±0.39	2.29±0.19
ADG /g	953.10±46.96a	923.75±16.49a	850.72±19.67b	855.12±9.10b
F/G	2.56±0.61b	2.49±0.60b	2.63±0.22a	2.67±0.19a
Weight cost /yuan/kg	6.75±0.24b	6.70±0.44b	7.01±0.39b	7.73±0.17a

Note: In the same row, values with different small letter superscripts mean significant difference ( $P<0.05$ ), and with different capital letter superscripts mean very significant difference ( $P<0.01$ ), while with no letter or the same letter superscripts mean no significant difference ( $P>0.05$ ). The same as below.

**Table 2:** Effect of different AM level on the initial estrus rate of replacement gilts (%)

Group	Estrus rate
Control group	78.67±3.24
5% AM	80.82±1.70
10% AM	78.16±1.36
15% AM	77.80±1.85

At the stage of pregnant pigs, there was no significant difference in the backfat thickness and reproductive performance between the AM groups and the control group ( $P>0.05$ ). Feeding AM during replacement period has a good follow-up effect. Adding appropriate level of AM in pregnant sows' diet can increase the number of newborn piglets and alive piglets, and increase the PSY of sows by about one head, and reduce backfat gain in pregnancy and backfat loss in lactation, improve body condition of sows.

**Table 3:** Effect of different AM level in replacement gilts' diet on reproductive performance of sows

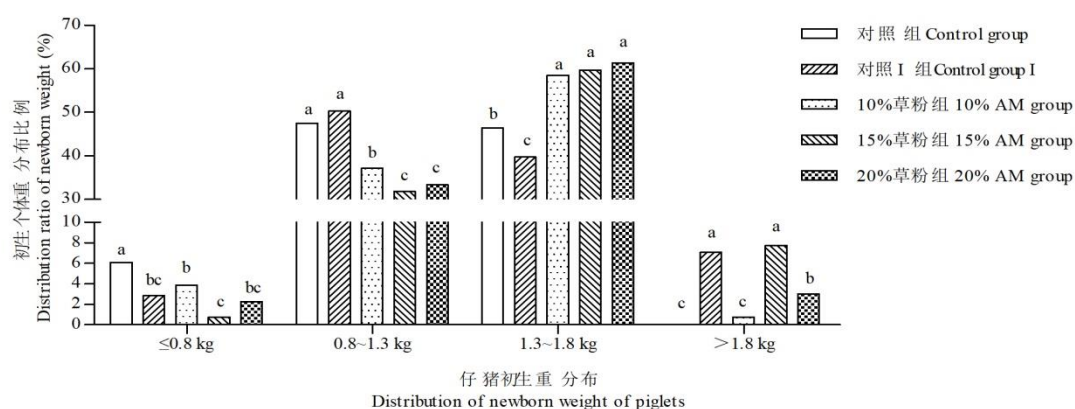
Items	Total number of newborn piglets/Head	Number of alive piglets/Head	Survival rate/%	Newborn litter weight/kg
CK	11.47±1.02	10.48±1.28	91.95±2.92b	13.64±2.27
5%AM	11.99±1.06	11.75±1.13	97.95±2.52a	15.67±1.28

**Table 4:** Effect of different AM level in diet on the backfat thickness of pregnant sows

Items	Day 30 backfat thickness  in gestation	Day 60 backfat thickness  in gestation	Prenatal  backfat thickness	Weaning  backfat thickness	backfat gain  in pregnancy	Backfat loss in lactation
CK	15.55±0.99	17.75±0.82	21.55±1.82	17.95±2.16	5.80±2.01	3.40±1.39
10%AM	15.75±0.91	16.85±0.67	21.10±1.29	18.20±1.05	5.45±1.18	2.85±1.03
15%AM	15.50±0.88	16.45±0.94	21.40±1.23	18.05±1.39	5.30±1.08	2.90±1.25
20%AM	14.85±0.98	16.65±0.81	21.05±1.69	18.55±1.09	5.10±1.37	2.60±1.47

**Table 5:** Effect of different AM level on reproductive performance of pregnant sows

Items	Total number of newborn piglets/Head	Number of piglets/Head	of alive Survival rate/%	Newborn weight/kg	litter
CK	11.90±2.04	11.50±1.98	94.70±4.88	16.92±2.42	
10%AM	12.60±1.60	11.95±1.66	94.90±5.74	16.53±2.07	
15%AM	12.52±2.96	11.75±2.42	93.85±6.83	17.09±3.04	
20%AM	12.20±2.28	11.95±1.90	95.99±5.54	17.03±3.45	



For finishing pigs, there were no significant effects of AM on pH1, cooking loss, marbling and meat color ( $P>0.05$ ) in the pork; however, the pork pH24 significantly decreased ( $P<0.05$ ) with increasing of the AM proportion in the diets while drip loss decreased and meat color improved; compared with control group, 4 delicious amino acid (aspartic acid, glutamic acid, alanine and glycine), 7 essential amino acid (methionine, phenylalanine, valine, isoleucine, leucine, threonine and lysine) and 18 total amino acid significantly increased in pig muscles of 20% AM group ( $P<0.05$ ); the saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents in muscle gradually decreased, while unsaturated fatty acid (UFA), polyunsaturated fatty acid (PUFA), and n-3PUFA contents gradually increased as the AM proportion increases; compared with the control group, the content of UFA, PUFA, linoleic acid, alpha linolenic acid and gamma linolenic acid of 20% and 30% AM groups increased significantly ( $P<0.05$ ), while the ratio of n-6PUFA/n-3PUFA significantly decreased ( $P<0.05$ ).

**Table 6:** Effects of different AM level on growth performance of finishing pigs

Items	Control group	5% AM	10% AM	20% AM	30% AM
Initial weight/kg	60.12±0.58	60.26±0.51	60.38±0.64	60.47±00.53	60.06±0.49
Final weight/kg	100.10±0.54	100.15±0.52	100.24±0.42	100.24±0.59	100.35±0.92
Day/d	49.10±5.68	47.84±5.26	49.47±4.93	50.80±6.29	51.44±6.46
ADFI/kg	2.43±0.23	2.40±0.30	2.41±0.20	2.25±0.42	2.31±0.39
ADG/g	821.94±96.16	840.37±89.98	810.93±76.15	800.74±100.52	774.74±77.56
F/G	2.98±0.30	2.86±0.33	2.98±0.20	2.83±0.48	2.98±0.37
Weight gain cost/(yuan/kg)	7.15±0.71b	7.05±0.80b	7.49±0.52b	7.44±1.25b	8.14±1.01a

**Table 7:** Effects of different AM level on meat quality of finishing pigs

Items	Control group	5% AM	10% AM	20% AM	30% AM
pH1	6.34±0.13	6.37±0.11	6.37±0.15	6.42±0.08	6.44±0.02
pH24	5.87±0.77a	5.62±0.05b	5.67±0.09b	5.68±0.05b	5.69±0.10b
Drip loss/%	2.61±0.39a	2.29±0.33a	2.25±0.53ab	2.00±0.40ab	1.92±0.35b
Cooked meat percentage/%	63.38±2.49	64.93±2.69	61.85±1.81	63.54±2.53	62.69±1.47
Marbling score	3.00±0.35	3.10±0.65	3.20±0.44	3.20±0.37	3.20±0.57
Meat color score	2.9±0.42	3.1±0.42	3.2±0.42	3.3±0.27	3.1±0.45



**Table 8:** Effects of different AM level on amino acid contents in muscles of finishing pigs (%)

Items	Control group	5% AM	10% AM	20% AM	30% AM
Asp <sup>1</sup>	2.14±0.04b	2.16±0.08b	2.16±0.10b	2.31±0.14a	2.27±0.10ab
Glu <sup>1</sup>	3.63±0.06b	3.65±0.17b	3.57±0.20b	3.95±0.31a	3.84±0.19ab
Gly <sup>1</sup>	0.95±0.01bc	0.94±0.03c	0.95±0.04bc	1.04±0.10a	1.03±0.08ab
Ala <sup>1</sup>	1.30±0.02b	1.31±0.04b	1.30±0.06b	1.42±0.10a	1.41±0.07a
Try <sup>2</sup>	0.23±0.00ab	0.23±0.01ab	0.22±0.01b	0.24±0.02a	0.24±0.01ab
Met <sup>2</sup>	0.65±0.01b	0.64±0.03b	0.63±0.03b	0.71±0.06a	0.70±0.03a
Val <sup>2</sup>	1.14±0.05bc	1.12±0.04c	1.14±0.05bc	1.21±0.06a	1.20±0.05ab
Ile <sup>2</sup>	1.10±0.02b	1.11±0.04b	1.11±0.06b	1.20±0.07a	1.19±0.06a
Leu <sup>2</sup>	1.89±0.04b	1.89±0.08b	1.89±0.10b	2.05±0.13a	2.01±0.09a
Phe <sup>2</sup>	1.14±0.02b	1.15±0.03ab	1.18±0.05ab	1.21±0.05a	1.19±0.04ab
Lys <sup>2</sup>	2.19±0.04b	2.20±0.09b	2.19±0.11b	2.38±0.17a	2.32±0.11ab
Thr <sup>2</sup>	1.03±0.01b	1.03±0.08b	1.04±0.05ab	1.11±0.07a	1.08±0.05ab
His	1.04±0.02b	1.05±0.02b	1.05±0.09b	1.12±0.03a	1.13±0.03a
Arg	1.59±0.03bc	1.59±0.07bc	1.58±0.09c	1.74±0.14a	1.71±0.09ab
Cys	0.18±0.03a	0.17±0.03a	0.16±0.02ab	0.13±0.01bc	0.11±0.01c
Tyr	0.87±0.01b	0.88±0.04b	0.88±0.05b	0.99±0.07a	0.97±0.05a
Ser	0.85±0.01b	0.86±0.03ab	0.87±0.05ab	0.91±0.06a	0.89±0.04ab
Pro	0.74±0.06c	0.76±0.02bc	0.76±0.04bc	0.84±0.08ab	0.85±0.06a
EAA	9.37±0.18b	9.37±0.38b	9.40±0.46b	10.12±0.63a	9.94±0.45ab
DAA	8.02±0.11b	8.05±0.33b	7.98±0.40b	8.72±0.64a	8.55±0.42ab
TAA	22.66±0.32b	22.73±0.89b	22.68±1.12b	24.57±1.60a	24.13±1.10ab
DAA/TAA	35.41±0.10	35.42±0.12	35.17±0.14	35.47±0.35	35.42±0.17
EAA/TAA	41.34±0.27	41.21±0.14	41.43±0.12	41.19±0.25	41.18±0.29

Note: Values with 1 mean delicious amino acid, values with 2 mean essential amino acid.

**Table 9:** Effects of different AM level on fatty acid contents in muscles of finishing pigs (%)

Items	Control group	5% AM	10% AM	20% AM	30% AM
Myristic acid ( C14:0 )	1.22±0.15a	1.14±0.08ab	1.12±0.11ab	1.22±0.13a	1.09±0.12b
Palmitic acid ( C16:0 )	22.66±1.88	17.57±8.66	21.20±1.41	21.22±1.32	19.88±0.89
Stearic acid ( C18:0 )	11.60±0.86a	11.76±0.18a	11.38±0.59a	10.55±1.19ab	9.99±1.06b
Eicosenoic acid ( C20:1 )	0.71±0.07ab	0.77±0.06a	0.75±0.18ab	0.63±0.12ab	0.60±0.05b
Palmitoleic acid ( C16:1 )	4.55±0.85	4.86±0.50	4.70±0.88	4.45±0.75	4.21±0.46
Oleic acid ( C18:1n-9 )	44.48±1.76a	44.14±1.50a	42.56±3.91ab	39.45±2.98bc	36.12±3.19c
Linoleic acid ( C18:2n-6 )	8.99±1.74c	7.67±1.18c	9.20±1.85c	14.7±3.01b	17.58±2.34a
α-linolenic acid ( C18:3n-3 )	0.58±0.12c	0.47±0.10c	0.71±0.10c	1.44±0.27b	1.75±0.32a
γ-Linolenic acid ( C18:3n-6 )	1.68±1.34b	2.83±0.64ab	3.17±1.18a	2.53±1.03ab	3.54±1.18a
Arachidonic acid ( C20:4n-6 )	0.74±0.32	0.88±0.13	1.24±0.40	0.89±0.2	1.11±0.27
SFA	35.48±2.80a	34.30±1.65a	33.70±1.59ab	32.99±2.49ab	30.95±1.82b
UFA	61.74±1.57Bc	61.63±1.04Bc	62.19±1.63ABbc	64.08±2.14ABab	64.91±1.04Aa
MUFA	49.75±1.53Aa	49.77±1.43Aa	48.02±3.75ABa	44.53±2.50BCb	40.93±3.10Cc
PUFA	12.00±1.90Bc	11.85±1.38Bc	14.17±3.41Bc	19.55±4.14Ab	23.97±3.21Aa
n-6PUFA	11.41±1.78Cc	11.38±1.29Cc	13.61±3.24BCc	18.11±3.90ABb	22.22±3.00Aa
n-3PUFA	0.58±0.12Bc	0.47±0.10Bc	0.71±0.10Bc	1.44±0.27Ab	1.75±0.32Aa
n-6PUFA /n-3PUFA	19.79±1.70Ab	24.54±2.86Aa	19.79±4.08Ab	12.53±1.04Bc	12.92±2.27Bc

**CONCLUSION:** For sows, the growth performance in 5% AM group was not affected in the replacement gilt diet. At the stage of pregnant pig, the live birth litter size, newborn litter weight and litter health had the best effects by using 20% of AM in the diet. The pregnant pigs that had 5% AM in the replacement gilt stage also produced good reproductive performance. For finishing pigs, 20% AM in the diet significantly increased DAA, EAA, linoleic acid, alpha linolenic acid and gamma linolenic acid contents in the pork and decreased the ratio of n-6/n-3, indicating that the quality and nutritional value of pork were improved.

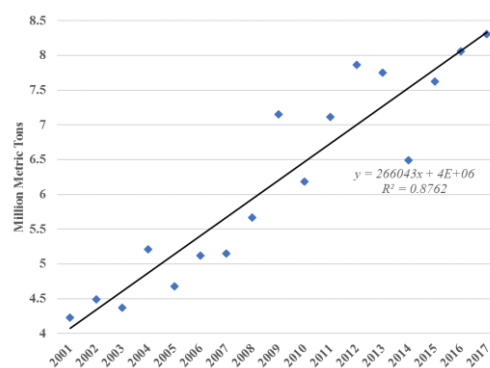
## World trends in alfalfa hay market\*

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**KEYWORDS:** *Medicago sativa*, hay export, USA

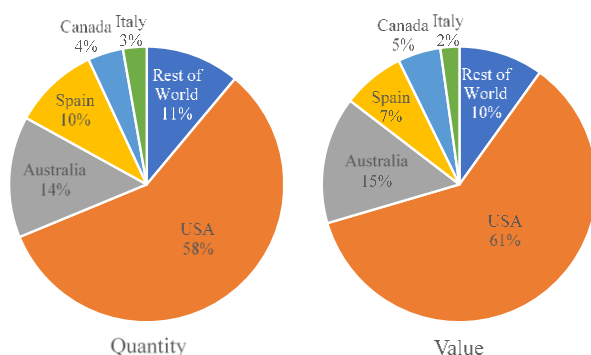
Forages (hay and silage) crops are traditionally consumed locally or within a few hundred kilometers of where they are grown. However, over the past two decades global trade in alfalfa and grass hays has increased substantially, expanding from a minor export crop to a crop much more widely traded on international markets. Over the past 16 years the international hay trade has approximately doubled, increasing at the average rate of about 266,000 MT/year (Figure 1). The demand for hay is driven by limitations in forage production and water issues in Asia and the Middle East, coupled with increases in demand for milk and animal products worldwide. The development of international markets has had a large effect on hay production and markets in several agricultural regions of the world, such as the western United States.



**Figure 1.** Global Exports of Alfalfa & Grass hays, 2001-2017 (Source: ITC Trade Map)

**Forage Sources and Demand.** The United States is the leading hay export country, followed by Australia, Spain, Canada and Italy (Figure 2). While some forage is exported from other countries (e.g. Argentina, France, Germany, Mongolia, Romania), these make up less than 10-11% of the world trade, according to the International Trade Center. While alfalfa dominates hay markets in many countries, high quality grass hays (timothy, sudangrass, bermudagrass, oat hay, kleingrass) make up a substantial portion of exported hay. The major demand for forage crops is in Asia, led by Japan, China and Korea (Figure 3), followed by Middle Eastern countries. The Chinese, UAE and Saudi markets have developed very rapidly only in the past 10-year period, increasing from negligible

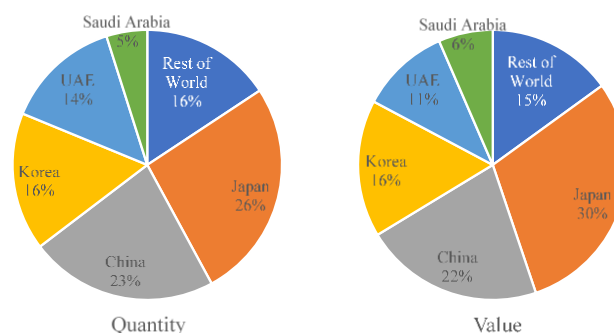
Figure 2. Global Exports of Alfalfa and Grass Hays by Country Share of Quantity and Value, 2017 (Source: ITC Trade Map)



amounts in 2007 to millions of MT per year in 2017. This increase was driven primarily by rapid expansion of modern dairy farms in China and Korea, and limitations of water resources in Saudi Arabia and the UAE.

**Crop Value.** The unit value of the crop varies from source country to source country, and in 2017 ranged from \$200 to \$350/MT. These values can be interpreted as FOB prices at the port, they also reflect variation between alfalfa and grass, between packages, between countries and year-year. Hay generally does not receive government subsidies.

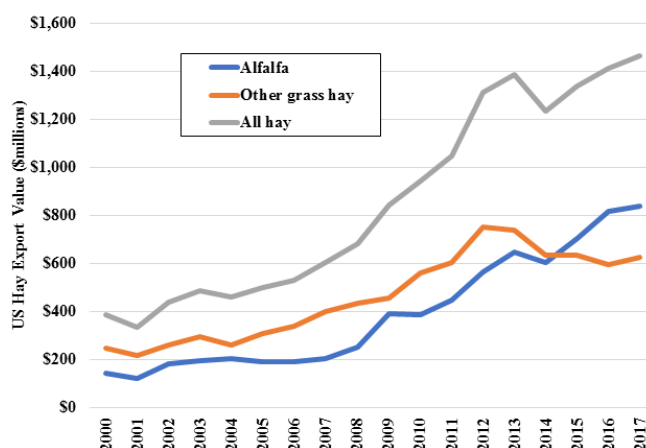
Figure 3. Global Imports of Alfalfa and Grass Hays by Country Share of Quantity and Value, 2017 (Source: WTO International Trade Centre Trade Map)



\* **CITATION:** Putnam, D. H., Mathews, W. A., Hanon, T. M., Sumner, D. A. 2018. Trends in World Alfalfa Hay Trade. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

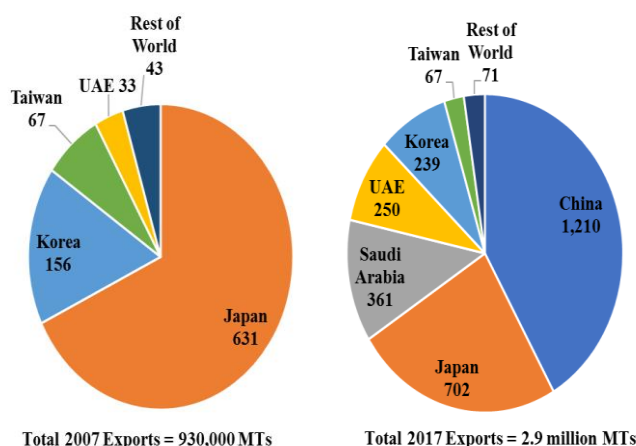
**US Exports.** Over the course of the last two decades, the value of hay exports from the US have nearly quadrupled, increasing from around \$400 million to most \$1.5 billion (Figure 4). Since 2012, grass hay exports have levelled off while alfalfa exports have continued to grow. Alfalfa exports have increased nearly six-fold since 2000. There have been major changes in destination for exported US hay over the past decade (Figure 5). In 2007, about 85 percent of US exports went to Japan and Korea. Over the past decade, China has become the largest importer of US alfalfa, receiving over 40 percent of total US alfalfa exports. Shipments to Japan and Korea have grown as well, but Korea has been surpassed by Saudi Arabia and the UAE. The Middle East is second to Asia as the most important market for exported US alfalfa.

Despite this export growth, alfalfa production in the western US has fallen over the same period. The seven western states of Arizona, California, Idaho, Nevada, Oregon, Utah, and Washington are the primary sources of alfalfa for export and produced close to 22.5 million MT in 2002, a peak



**Figure 4.** Growth in US hay Export Value, 2000-2017 (source: US Dept. Commerce)

year, but in 2017 produced only 18.2 million MT, a decline of nearly 20%. This was due primarily to a steady decrease in California, while production in the other western states has been relatively steady. Competition from other crops and water resource limitations have been key factors in this decline.



**Figure 5:** Volume of US alfalfa Exports by Destination 2007 and 2017. Total grass and alfalfa

exported remains below 6% for alfalfa and below 3% for grass hays, the equivalent percentage of production exported from the 7 western states exceeded 17% for alfalfa and 41% for grass in 2017.

This rapid rise in export demand has been welcomed by cash hay growers, but regarded as a negative by domestic dairy and other livestock hay buyers, who have had to compete for forage supplies with foreign buyers.

#### Impacts on Domestic US Markets.

The combination of increased demand along with reduction in acreage has increased the percentage of hay production which is exported from western US states. While the percentage of the national US crop

**Japan/Korea.** Two of the most important destinations for alfalfa and grass hays are Japan and Korea, both of which have been major export targets. In 2017, Japan imported over 2 million metric tons of alfalfa and grass hays (Table 1), as well as a further 230,000 metric tons in meal, pellet, or cube form. Out of a total of over 1.3 million MT of imports, Korea imported about 1.1 million MT of grass hay and about 212,000 metric tons of alfalfa bales. While the US is the main source of Japanese and Korean hay imports by a wide margin, Australia and Canada are also important exporters to these markets. In particular, Canada is the main source of alfalfa in meal and pellet form for Japan. Oaten hay is a major export product from Australia.

#### Recent US/China Trade Turmoil.

The trade disruption that has developed between the US and China in the summer of 2018 may have an impact on the hay trade. China has become the number one destination for US alfalfa over the course of the last ten years, growing from only 3,000 metric tons of imports in 2007 to over 1.2 million in 2017 (Figure 2). In response to US trade actions, China has recently implemented retaliatory tariffs on US agricultural products, including an increase of 25 percentage points on alfalfa hay, resulting in a 32 percent import tax. We think it is probable that this tariff increase, if it holds, may be sufficient to block imports of US alfalfa. In that case, the impact of these tariffs on the alfalfa market are likely to be substantial. Using an estimate that a one percent increase in the quantity of hay in remaining markets will result in a one percent decline in price (i.e. a demand elasticity of -1.0), the diversion of this alfalfa from China to the rest of the world market would result in a decline in price of about 7.5 percent. That 7.5 percent price decline would result in a loss to alfalfa producers of about \$377 million in the western US region which is the primarily source of US export hay (Table 2). However, this effect could be mitigated if other markets for US forage are identified or the tariffs do not completely block US forage from entering China.

#### Genetic Engineering Issues.

Another substantial challenge for Chinese imports has been the issue of Genetically-Engineered (GE) alfalfa. Two GE traits are now commercialized in the United States: Roundup Ready (glyphosate tolerance) and HarvXtra (a reduced lignin trait). At this writing, neither trait is permitted in China. Thus, growers for export to China must grow a conventional alfalfa variety that is free of the trait, even in a very small amount. Export companies in the US routinely test export hay for Low Level Presence (LLP) of the Roundup Ready trait and will reject hay with even a small amount (e.g. 0.1%) of LLP. This may change in the future if China approves these traits. Other countries either allow a trait to be imported (as with Japan), or do not routinely test. Some importing companies reject GE hay due to sensitivity of their customers who do not want GE traits (even if they are permitted by import regulations).

**Table 1.** Alfalfa and grass Hay Imports into Japan and Korea from USA and other sources, 2017

	Japan	Korea
Quantity Imported (1,000 MT)		
USA	1,540	1,061
Other	581	270
Value of Imports (\$US Million)		
USA	525	311
Other	203	82

Source: Trade Statistics of Japan, Ministry of Finance and Trade Statistics of Korea, Korea Customs Service. Note: The Alfalfa and Other Hay category covers both baled alfalfa hay and other grass hays, as well as some other hay products such as hay cubes. The 121490 HS code also covers fodder roots, but those have been excluded from these values.

**Table 2:** Quantity and Value of Exports to China, and Calculated Potential Revenue Loss from Price Change (2016-2017 Average values).

Measure	Value
Total Production Quantity (Thousand MT)	16,900
Western States Representative Price (\$/MT)	297
Quantity (Thousand MT)	1,181
Value (\$Millions)	351
Estimated Price Decline (%)	7.50%
Estimate Revenue Loss, USA (\$Millions)	377

Source: U.S. Commerce Department, data available from the International Trade Commission Dataweb and author calculations.

**The Water Link.** Water resource availability in many parts of the world plays a key part in the demand, as well as the supply, for international trade in forage crops. UAE and Saudi Arabia, both water-poor countries, have made domestic policy decisions which aim to stop the unsustainable utilization of groundwater for forages. Saudi Arabia s made a decision to stop almost all production of about 4 million MT of hay domestically by 2018-19, following UAE which had made the same decision 6 years earlier. Saudi Arabia imported 685,000 MT in 2016 and over a million in 2017 (up from zero 5 years ago). The demand in China is also driven to some degree by lack of land and water resources for domestic production, as well as proximity to markets and unfavorable weather for hay making. It should be noted that the vast majority of the exported hay from the United States is from irrigated regions, and these regions also face substantial limitations and competition for water resources. This is also true in Australia, Spain, Italy, and North Africa. The export of hay has become somewhat of a political issue as complaints about ‘trade in virtual water’ are raised. Regardless of the politics, it is clear that sustainability of water use must be a key component of forage production wherever grown or consumed.

**Summary.** The international trade in forages (primarily alfalfa and grass hay) has risen from a minor to a major component of hay markets. Hay is increasingly moving from large production areas such as western US, western Canada, Australia, Spain, France, and Italy to Asian and Middle Eastern markets, predominantly China, Japan, Saudi Arabia, and the UAE. US is the lead exporter, and exports are now greater than the equivalent of 17% of the alfalfa and 41% of the grass hays produced in the 7 western US states. Water limitations in different parts of the world, increasing populations and demand for milk products in Asia have been important factors driving this demand. Export markets demand high quality forages, whether alfalfa or grass hay types, which favor exporting production regions with good weather and technology for hay making. Improvements in hay packaging, inexpensive ocean transport systems, and imbalance of world trade have been major components driving this trend. Problems and issues in the hay trade include sensitivity to low level presence of GE traits, narrow margins, dock strikes, and more recently trade disruptions between the US and China. In our view, since these demand trends are likely to continue, international trade in forage crops is likely to increase in importance in the future.



## Research priorities and future of alfalfa in Latin America\*

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**KEYWORDS:** alfalfa, future research, Latin America, network platform

### Content

Future actions aimed at the technological development of Latin American's agrifood and agroindustrial systems should seek to anticipate trends and ensure the permanent adjustment of research and development priorities, viewing innovations inspired by the logic of productive chains, which are increasingly dependent upon knowledge and technology.

To ensure quantity and quality for the growing demand of food while preserving natural resources and adding value to the product, it is necessary to invest in research with a transdisciplinary approach in order to manage systems which are increasingly complex and strikingly bound to convergent technologies. The migration of production systems with few activities for those more complex stands out as a strong trend for the next decades. This will certainly require to handle increasingly dynamic processes that will be part of the agribusiness that opens up for the near future.

This scenario will bring in the upcoming decades new agro-socio-environmental paradigms and will present challenges for Latin American as a whole. The advance of urbanization and the new dietary habits of the population will push for a higher and more sophisticated demand for goods and services, which will exert even more pressure for an efficient use of natural resources. In this context, the agribusiness sector needs to reinvent itself, leading to the development of new production patterns focused on delivering products with higher quality control, innocuousness, traceability and greater diversification.

Potential for this change exists. According to the UN (2017), the world population will reach 9.8 billion inhabitants by 2050, and this will require the production of 70% more food. The number of inhabitants of Latin America and the Caribbean will grow 25%, going from 635 million to 793 million in 2061, according to the Economic Commission for Latin America and the Caribbean (POPULAÇÃO ..., 2015). Latin America holds about a third of the world's freshwater resources and more than a quarter of the world's arable land. Its agricultural production has enormous variation, ranging from subsistence to sophisticated agribusiness and represents 16% of the exports in the world (RABOBANK, 2017). Today, about 50% of the region's food production comes from its 14 million small farmers. While for many this means the importance of small production, for entrepreneurs represents a market – i.e. land areas - to be conquered (AGROLAC 2017).

In this promising scenario, plenty of opportunities, Latin America should move forward to support the expansion of its agrifood and agroindustrial systems, taking alfalfa as the basis for one of those Platforms to direct international networks for generating knowledge and promoting innovations and sustainable technological developments in the region.

Why alfalfa? In Latin America there are about 4 million hectares cultivated with alfalfa, most notably in Argentina, nearing 3.2 million hectares, Chile with 120 thousand hectares, followed by Peru and Uruguay with 120 thousand and 70 thousand hectares, respectively. In Brazil, despite the area with alfalfa being still timid, about 35 thousand hectares, the potential of area expansion from the southern region towards the Cerrado and Caatinga biomes stands out.

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\* **CITATION:** Vilela, D.; Basigalup, D.H.; Juntolli, F.V.; Ferreira, R.P. 2018. Research priorities and future of alfalfa in Latin America. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

In addition, alfalfa is a multifaceted plant and presents an unparalleled potential of use other than animal production, from the pharmaceutical and cosmetic industry to human consumption. Regarding animal feeding, it stands out for the unequaled quality characteristics either as exclusive or complementary feed for cattle, goats, equines, sheep, swine, poultry and small animals (pets).

There are relatively few published research reports on alfalfa under Brazilian conditions, as evidenced by studies on research priorities (Vilela et al., 2008). The first evaluations were conducted by Embrapa in the 1990s (Vilela et al., 1994), proving its economic viability (Rodrigues et al., 2008; Tupy et al., 2015).

In Argentina, where alfalfa has traditionally been used for decades, grazing and harvesting to make hay predominate (Basigalup, 2016; Comeron et al., 2015; Comeron & Romero, 2017). Producing high-quality alfalfa hay is promising throughout Latin America in view of the significant regional and global demand. Improving forage quality using conventional and molecular tools is a recurrent breeding objective that should be prioritized. The application of molecular markers and transgenesis widen the possibilities for alfalfa improvement to a much greater extent (Li et al., 2014; Annicchiarico et al., 2015; Biazzi et al., 2017). Crop management and hay machinery are also important topics that must be included in the research agendas.

In the cosmetics industry (ABIHPEC, 2017), alfalfa extract has been used in the composition of creams for facial rejuvenation and hair treatment. In the pharmaceutical industry (Bora & Sharma, 2011), alfalfa extract is used for its hepatoprotective and estrogenic activities, besides the use as a powerful medicine for treating stomach disorders. In modern cuisine (Ribeiro, 2016), alfalfa sprouts are an excellent functional and healthy food with multiple benefits.

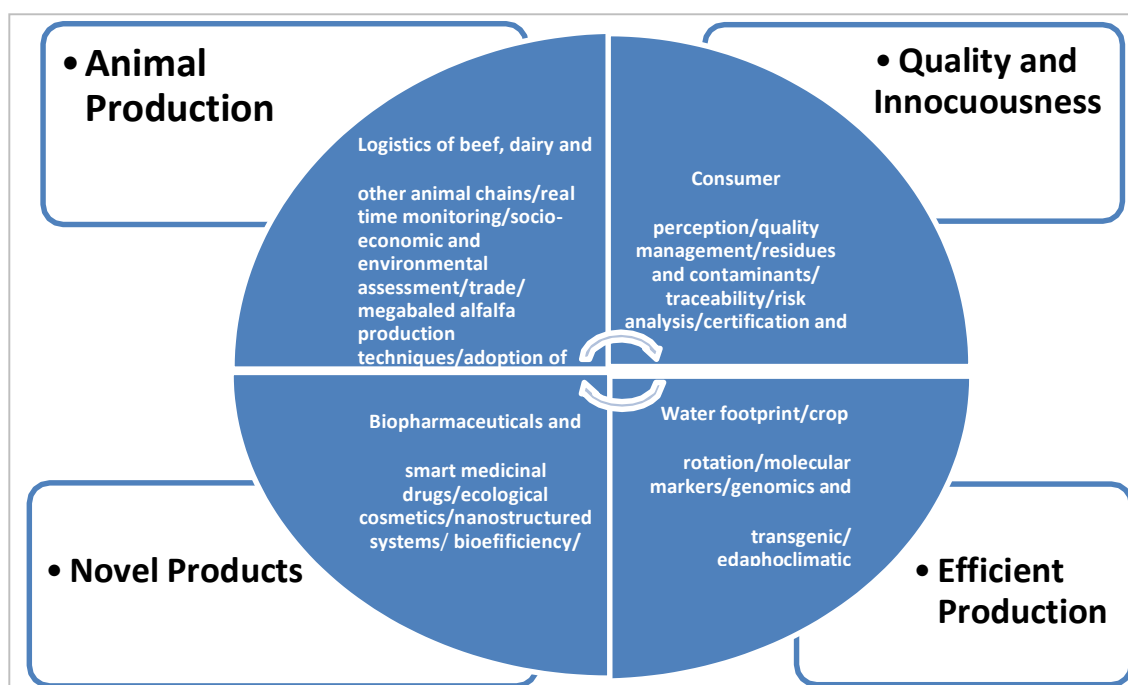
It is widely accepted that no organization or isolated group of scientists hold alone the skills to face an increasingly complex and dynamic environment to compete in a globalized market. As it was mentioned before, production systems are becoming increasingly more complex and therefore require an interdisciplinary approach not only to a domestic level but also to an international level. In this context, the Technological Platforms will be able to aid such a transformation, acting as an inductor in the generation of knowledge, enabling the formation of clusters of researchers and institutions that will accelerate the accomplishment of significant advances in the search for competitiveness and technological modernization. This will enrich the capabilities of every country, or an entire region, to effectively participate in a growing and constantly more demanding globalized market.

A summarized conceptual organization chart of the idea of a Virtual Network Platform that could guide future research on alfalfa in Latin America, coordinated by the Brazilian Agricultural Research Corporation (Embrapa) and the Argentine National Institute of Agricultural Technology (INTA) is shown in Figure 1. Such network would give to educational and research institutions from the other Latin American countries access to information and the opportunity to actively participate in the process of the development of this platform through collaborative research projects and data generation.

Priorities will be classified according to the degree of relevance, since countries like Brazil, with distinctive characteristics of soils and tropical climate, have different priorities from countries like Argentina, Uruguay and Chile, which in addition are in a more advanced stage of knowledge concerning alfalfa. The exchange of information among potential users of the

Network will create a cumulative expertise that will be of fundamental importance for future alfalfa research, avoiding duplication of actions and, at the same time, serving as a database available for the productive sectors.

We propose that the Alfalfa Research and Development Latin American Platform be based on four structural axes: (1) Efficient Production, connected to the agronomic aspects of the crop; (2) Animal Production, including different forms of use; (3) Quality and Innocuousness applied to human feeding; and (4) Novel Products, involving the pharmaceutical and cosmetic industries. All axes will be aimed to add value to the alfalfa productive chain via the application of adapted or generated technological and managerial innovations.



**Figure 1.** Organization chart of the Alfalfa Research and Development Latin American Platform, structured in four axes: Efficient Production; Novel Products; Quality and Innocuousness; and Animal Production. Topics to be addressed are exemplified in each quarter of the chart under their respective axes.

In this context, the book "**Exploração racional da alfafa: do cultivo à sua utilização** (*Rational Exploitation of Alfalfa: from Cultivation to its Use*)", presented in this congress, will be the initial step for the creation of the Platform. The book contains 22 chapters, subdivided into three main subjects: agronomic aspects to improve crop production, alfalfa multipurpose profile and future trends, and prioritized research lines, with the propose of the Virtual Network for Research and Innovation in Alfalfa for Latin America - **REPI- ALfalfa**

The editors, Duarte Vilela and Reinaldo de Paula Ferreira, researchers from Embrapa, Brazil; Dilermando Miranda da Fonseca, professor at the Federal University of Viçosa and Daniel Horacio Basigalup, researcher from INTA, Argentina kindly present this book, which is published in two versions, Portuguese and Spanish and it is edited in two formats, printed and E-book.

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# Promoting the use of alfalfa in the production systems of the Punjab province in Pakistan\*.

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**KEYWORDS:** alfalfa, fodder, dairy industry, Punjab

## Abstract

Fodder is the backbone of dairy industry but land available for fodder production is decreasing at 2% per decade in Pakistan<sup>1</sup>. Therefore, there is tremendous pressure of livestock on available total feed and fodder. Furthermore, there are two major fodder scarcity periods in the country i.e. May-June and November-December that sometimes prolonged to January due to heavy frost. Acute shortage of fodder during these periods severely affects milk yield and animal health. The situation demands the development of high yielding multi-cut fodder to ensure year-round supply of nutritious feed to livestock. The fodder sector had been least priority of the government till the last few years, that is why much progress was not evident in this sector. Furthermore, almost all seed for growing fodders is imported and share of locally produced seed is very low. Now, keeping in view the importance of fodder in dairy improvement, the Government of the Punjab has launched fodder improvement and quality seed production programmes. Public sector Universities and research organization have been involved in production of certified seed of fodder crops. Public-Private collaboration is being encouraged for the development of high yielding and better nutritional quality varieties and introduction of improved varieties of fodder crops from other countries. The characteristics of Alfalfa as fodder legume crop i.e. 18.5% crude protein, 37.4% NDF, high calcium, phosphorous, minerals, low fiber and high digestibility, its tolerance to drought and salinity due to its deep rooting system, and high yield (22.43 t/ha DM) having organic matter digestibility of 55-77% are making it popular and acceptable worldwide<sup>2</sup>. Its good ability for green fodder, hay making, grazing and feed production is also attracting the growers for alfalfa production. Area under alfalfa cultivation around the world is 30 million ha while major producing countries are North & South America and Europe accounting for 90% of world production<sup>3</sup>. During 2016 USA alone produced 58,263,000 tons of alfalfa<sup>4</sup>. Alfalfa is believed to have capacity of resolving the fodder scarcity issue in the province as it is multi-cut perennial leguminous and palatable green fodder which after a small hard work and selection can be made to produce green fodder throughout the year. It could be expected that it will resolve the matter of proteineous, high nutritious and palatable green fodder. The government of the Punjab has initiated the project of production of pre-basic seed of alfalfa and other fodder crops involving public sector universities and research institutes to ensure provision of quality seed to the farmers. Furthermore, private companies like Maxim International, Biotrack, Farm Dynamics Pakistan, and others have started growing alfalfa at large farms and processing in the form of hay.

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## Breeding and field management of alfalfa in climatic conditions of Pakistan\*.

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**KEYWORDS:** alfalfa, fodder, heterosis, dairy industry, Punjab

### Abstract

Livestock contributed 58.92 percent of agriculture value addition and 11.11% to GDP during 2017-18 in Pakistan (Economic Survey of Pakistan, 2017-18). It is predicted that meat and milk consumption will grow at 3.76% per annum, respectively, in developing countries like Pakistan. Our whole system of rural economy has revolved around livestock production. Current livestock-population of the country includes 38.8 million buffaloes, 46.1 million cattle, 30.5 million sheep, 74.1 million goats and 1.1 million camels (Anonymous, 2017-18). Pakistan is among top five milk producer of the world. The human population in Pakistan is expected to reach over 227 million by 2025. Due to high growth rate of population, urbanization has brought a marked shift in the lifestyle of people in feeding habits towards milk products, meat and eggs with resultant increase in demand of livestock products. Peri urban livestock farming near urban centers and emerging fodder markets are indicators of fast changing economic scenario in the livestock sector. But the dark side is that the available feed and fodder is not sufficient to meet the requirements of growing livestock sector and the non-availability of fodder is considered one of the major hurdles in the progress of this sector.

The alfalfa is highly nutritious fodder with 18.5% crude protein, 37.4% NDF, high phosphorous, calcium, minerals, low fiber and high digestibility (Zhang et al 2015). It is an efficient leguminous crop having drought, salinity tolerance, high yield (22.43 t/ha DM and 55-77% digestibility. It is also good for hay & silage purpose. Area under alfalfa cultivation around the world is 30 million hac while major producing countries are North & South America and Europe accounting for 90% of world production (Yuegao et al 2009).

One of the limiting factors for expanding the area under alfalfa is the availability of the seed. Only 11% registered seed is being used, which is produced locally. The missing gap is fulfilled by importing the seed and being sown in all over Pakistan. There is great potential of domestic fodder crops seed production. This situation requires to develop local system for seed production of alfalfa. Because the only variety of Lucerne released by FRI Sargodha is Sargodha lucerne 2002.

Being a tetraploid structure of the alfalfa genome, cross pollination and severe inbreeding depression, cultivars can exhibit different levels of genetic variation (Julier, B *et al.*, 2003). Therefore, information about germplasm diversity and relationships within and among elite breeding material is important for any efficient and successful alfalfa breeding program. Recent studies supported the idea of the semi-hybrid breeding of this crop. The concept involves: breeding alfalfa within a given population, identification of heterotic germplasm, and the production of seed of the population hybrid. The progress of alfalfa breeding has been slow, most notably due to its complex genetic structure (autoteraplod), allogamy and tetrasomic inheritance present in alfalfa, and besides this plant architecture and hermaphroditic flowers. Alfalfa breeding programs are based on recurrent phenotype selection with or without progeny testing, to accumulate desirable alleles at high frequency into a population (Scotti & Brummer, 2010). The tetraploid species usually express severe inbreeding depression. Because of that the process derivation of homozygous plants is very slow or not possible. So, true inbred lines of alfalfa are not available. The effect of heterosis to be partially used for development of free hybrids by crossing lines obtained from 2-3 generations of selfing. The idea on partial utilization of heterosis in alfalfa that emerged in the USA proposed the development of semi-hybrids obtained by crossing genetically divergent germplasm and identifying heterotic groups (Brummer 1999).

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\* **CITATION:** Mahmood, A. 2018. Breeding and field management of alfalfa in climatic conditions of Pakistan. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



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## Spanish dehydrated alfalfa, a different way to produce alfalfa.\*

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**KEYWORDS:** Spanish, dehydrated, alfalfa, forage, alfalfaspain

### -Spanish production

- Cultivation area of 250,000 Ha
- Irrigation production
- 5 or 6 harvests a year (April to November)
- Potential to increase exports

Total production 2017/2018 – 1.453.076 Tn.



### -Dehydrated Process – Phases 6 Steps

Harvest

Processing – Classification

Processing – Dehydration and cooling

Transport

Use/Consumption

Milk production

#### Harvest

No differences between alfalfa hay and dehydrated alfalfa.

- Especially irrigated. 5-year cycles
- Normally 5-6 harvests per season
- Season from April to November
- The cut-off time determines the protein concentration

#### Processing – Classification

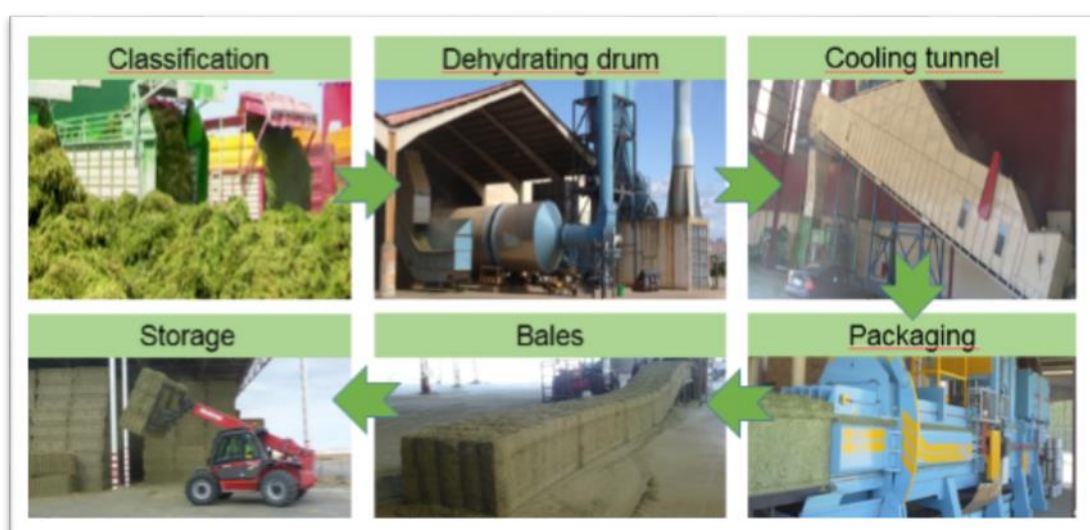
- Upon arrival at the plant its protein and moisture level are analysed

\* **CITATION:** Tabuenca F. 2018. Spanish dehydrated alfalfa, a different way to produce alfalfa.: IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>

- Later it is classified depending on its quality
- It will be dehydrated within 24h from its arrival

### Processing – Dehydration and cooling

- Dehydration is the unique key process of dehydrated alfalfa.
- Alfalfa goes through a dehydrating drum with air at about 250 °C
- This process reduces the humidity of the alfalfa quickly to 8-12% in a very homogeneous way
- Then goes to an extraction tower that filters stones and other impurities
- Then a cooling tunnel returns the alfalfa to environment temperature
- Finally, the bale is produced, packed and stored for transport



### Transport

- It is transported in bales of about +700 Kg.
- However, some companies offer bales 400kg approx. and 40/25 Kg
- In 40-foot containers loading 24tm

### Use/Consumption

- Transition from alfalfa hay to dehydrated alfalfa in 5 to10 days, for example increasing 10%-20% daily up to 100%



### Milk Production

- The nutritional quality will depend on the RFV and appropriate use
- The nutritionist should adjust the mixture as if it was alfalfa hay
- The production of milk will be the same in quantity and quality
- It will allow the farm to take advantage of better prices
- Alfalfa hay and dehydrated can be combine in the same TMR

### -Differences between alfalfa hay and dehydrated alfalfa

Harvest: No differences between alfalfa hay and dehydrated alfalfa

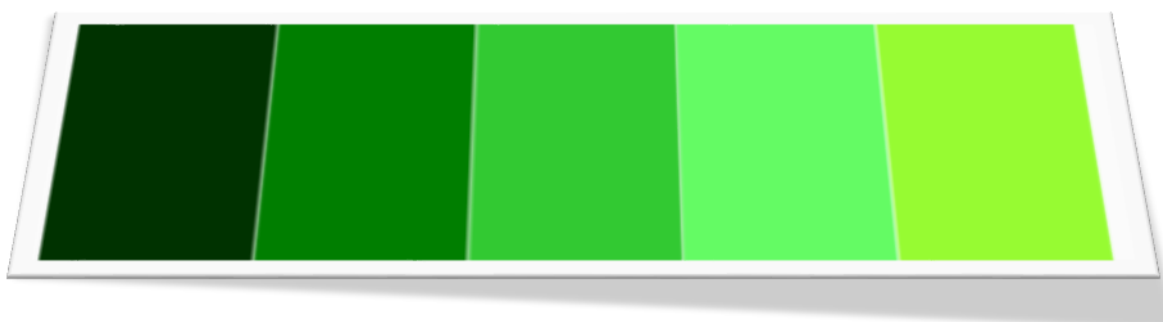
Processing:

- The harvesting machine cuts the fibers between 10 and 20 cm, compared to more than 50 cm of alfalfa hay
- In less than 48h it arrives at the plant, compare to the 5-7 days of drying in the field.
- It is dried by a dehydrating drum within 24 hours after arriving at the plant, in comparison to being dried outdoors for weeks or months.
- This fast dehydrating process using 250°C air leaves the product with a lower humidity level and kills insects and micro-organisms, making the product more stable and homogeneous
- Using the dehydrating drum and cooling tunnel makes it very difficult for stones or heavy materials to enter the bales.

Use/Consumption: Shortest fibers (10-20 cm), enter unifeed mixer later, for about 25% of the time compare to hay

Milk production: No differences between alfalfa hay and dehydrated alfalfa.

### -Green shades do not make a difference on the quality of alfalfa



### **-How to start using Spanish dehydrated alfalfa in bales?**

1. It may be advisable to do a test with part of the cattle to obtain a proof of the results
2. Do not make a sudden change, a progressive 5-10-day transition is necessary, increasing the portion of alfalfa dehydrates by 10%-20% of the total each day.
3. Proper use of the unifeed cart or mixing system. Processed one fourth of the time. For example, 4 minutes vs. 12. Introduce later of the process, not at the beginning.
4. The nutritional benefits are the same in reference to the RFV. The nutritionist should adjust the mixture in the same way as with alfalfa hay
5. The farmer can thus benefit from advantages in price while maintaining quantity and quality
6. The consumption of alfalfa in cows of high genetic quality can be 7 kg/cow/day, although there is a large dispersion from one animal to another, between 2 and 7 kg/day

### **-CONCLUSIONS**

- Spanish dehydrated alfalfa offers the same results and it is used in a similar way.
- It has high level of high-quality protein.
- It can be used as a substitute for part of the soybean meal in the meal concentrate, and production will remain the same.
  - At first look it has some differences with alfalfa hay, but they will not affect production if used correctly.
- It is a durable good which offers high-quality nutrition benefits.
- Its processing method ensures hygienic quality standards.
- Storage for dehydrated alfalfa is safer than storage for alfalfa hay.
- As it needs shorter time in the unifeed mixer to get the appropriate fiber length, users can save time and energy.

## Response of alfalfa to climate change\*

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**KEYWORDS:** alfalfa; climate change; yield; nutritive value; persistence

**INTRODUCTION:** Predicted changes in temperature, precipitation, and atmospheric CO<sub>2</sub> concentration [CO<sub>2</sub>], as well as their interactions (Hatfield et al., 2011) are expected to affect crop growth. Because they cope with climatic conditions in both summers and winters, perennial field crops, such as alfalfa (*Medicago sativa* L.), will be particularly affected by climate change in terms of yield, nutritive value, and persistence (Bélanger et al. 2002; Bertrand, 2012). Approaches used to study and quantify the potential impact of climate change on crops include growth chambers (e.g. Bertrand et al., 2007a, 2007b; Kettunen et al., 2007; Baslam et al., 2014), free-air carbon dioxide enrichment (FACE) (e.g. Ainsworth et al. 2008), open-top chambers (e.g. Sgherri et al., 1998; Messerli et al., 2015), and crop modeling (e.g. Hunt et al., 1991; Parton et al., 1995; Riedo et al., 1999; Thivierge et al., 2016).

Results from those studies indicate that, although the increase in [CO<sub>2</sub>] will likely have a positive effect on alfalfa yield, temperature increases and precipitation changes along with their interaction with the increase in [CO<sub>2</sub>] might result in contrasted effects on alfalfa yield, nutritive value, and persistence. We present here an overview of these potential effects of climate change on alfalfa production along with potential adaptation measures at the farm level with a focus on northern production areas.

### Yield

Temperature increases combined with elevated [CO<sub>2</sub>] is expected to increase alfalfa yield in most regions (Aranjuelo et al., 2006; Sanz-Sáez et al., 2012). This is true in most parts of Europe (Ergon et al., 2018) where alfalfa is expected to perform better than grasses under future climate conditions, with expected yield changes varying from -4% to +27% (Ruguet et al., 2013). This is also the case in eastern Canada where yield increases between 9 and 21% are predicted for pure alfalfa stands in the upcoming three decades (2020-2049) (Figure 1; Thivierge et al., 2016); this expected yield increase is mostly due to the possibility of harvesting additional cuts due to the lengthening of the growing season. Alfalfa yields are also expected to increase in the eastern regions of the United States, but to decrease in the central regions due to more frequent and severe drought events (Izaurrealde et al., 2011).

The response of alfalfa to increasing temperatures and [CO<sub>2</sub>] will be modulated by changes in precipitation regimes and the associated risks of water deficit as can be seen by regional differences in the expected response to climate change in the United States and Europe. Izaurrealde et al. (2011) concluded that the overall effect of climate change on rainfall pattern might affect alfalfa yield across the United States more than changes in [CO<sub>2</sub>] or temperature. In Canada, forage yields of the summer regrowth of alfalfa-timothy mixtures are

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\* **CITATION:** Jégo, G., Bélanger, G., Bertrand, A., Thivierge, M.N. 2018. Response of alfalfa to climate change. IN Proceedings. Second World Alfalfa Congress, Cordoba, Argentina. 11-14 November, 2018. Instituto Nacional de Tecnología Agropecuaria (INTA), <http://www.worldalfalfacongress.org/>



expected to decrease because of a higher occurrence of water stress (Thivierge et al., 2016). Increased  $[\text{CO}_2]$ , however, will likely improve plant water use efficiency by inducing a partial closure of the stomata, resulting in reduced transpiration. In their review, Soussana and Lüscher (2007) concluded that elevated atmospheric  $[\text{CO}_2]$  reduces the sensitivity to low precipitation in grassland ecosystems.

Large increases in temperature and changes in precipitation, however, might offset the positive crop response to elevated  $[\text{CO}_2]$  (Morgan et al., 2004; Hatfield et al., 2011; Izaurre et al., 2011; Lee et al., 2013; Piva et al., 2013). For instance, in eastern Canada, although annual alfalfa yields are generally expected to increase with temperature increases and elevated  $[\text{CO}_2]$ , a yield decrease is expected if changes are more drastic such as those predicted by the distant future scenario of high greenhouse gas emissions represented by the representative concentration pathway 8.5 (RCP 8.5; 2050-2079; Thivierge et al., 2016).

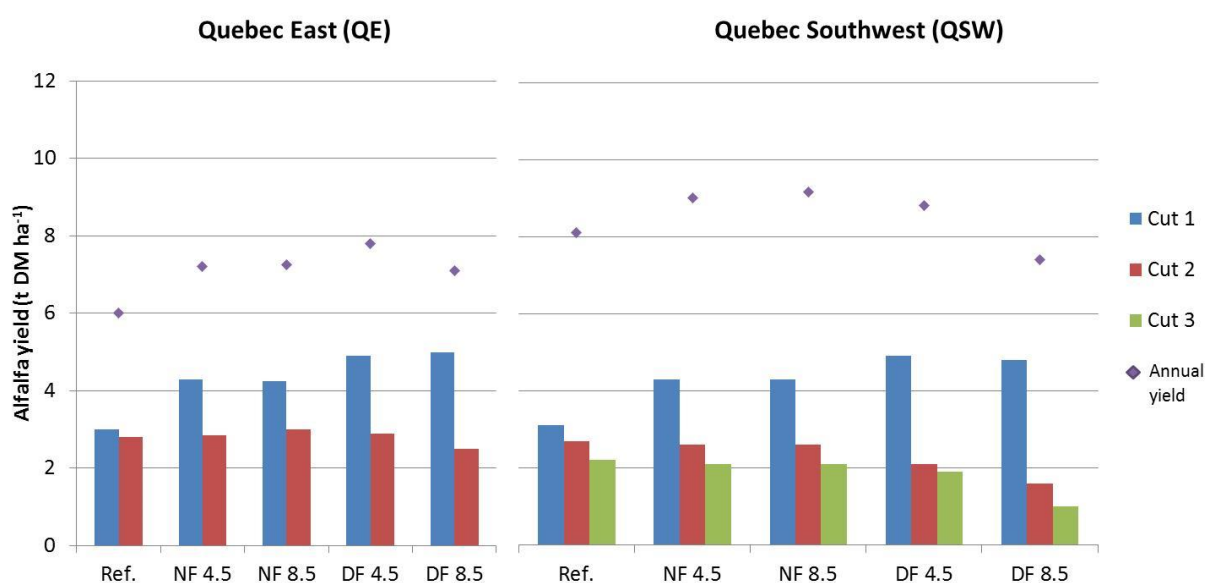


Figure 1. Predicted annual DM yield and DM yield at each cut of pure alfalfa for reference (1971-2000; ref.), near future (2020-2049; NF), and distant future (2050-2079; DF) periods under representative concentration pathways (RCP 4.5 and 8.5) in two contrasted areas of Canada (Quebec East and Quebec Southwest). Values were predicted by the Integrated Farm System Model (IFSM; Thivierge et al., 2016).

### Nutritive value

Forage nutritive value is also likely to be affected by climate change but few studies have been conducted to assess this potential impact. Increased temperatures were shown experimentally to reduce forage or pasture nutritive value (Wan et al., 2005; Thorvaldsson et al., 2007; Lee et al., 2013), including the *in vitro* dry matter digestibility of alfalfa (Sanz-Sáez et al., 2012). Elevated  $[\text{CO}_2]$  is expected to decrease the crude protein concentration and to increase non-structural carbohydrate concentration of forages (Polley et al., 2000; Körner, 2002). On an annual basis, however, crop simulations have shown that increased temperature and changes in precipitation in eastern Canada are not expected to affect significantly the forage digestibility and the crude protein concentration of alfalfa-timothy mixtures if an adaptation strategy consisting of a modified harvest schedule with additional forage cuts is implemented (Figure 2; Thivierge et al., 2016). Changes in temperature and  $[\text{CO}_2]$  might affect the nutritive value of alfalfa on a given day or even for a regrowth period but the integration over the whole growing season along with an adaption of the harvesting schedule tends to offset this effect.

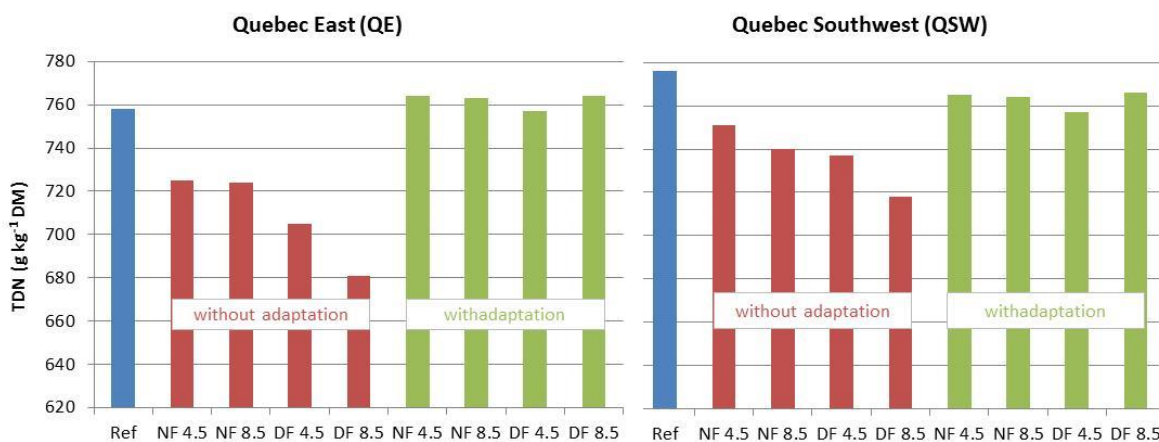


Figure 2. Predicted annual total digestible nutrients (TDN) concentration of an alfalfa-timothy mixture under a reference climate (Ref) and for two representative concentration pathways (RCP 4.5 and 8.5) in near (NF, 2020-2049) and distant (DF, 2050-2079) futures, with and without an adapted harvest schedule in two contrasted areas of Canada (Quebec East and Quebec Southwest). Values were predicted by the Integrated Farm System Model (IFSM; Thivierge et al., 2016).

## Persistence

Persistence is a crucial attribute of perennial forages that enables them to supply feed for animals cost-effectively for several years. Alfalfa tolerates drought quite well (Barnes et al., 1988), as was shown after a severe drought in Australia (2006-2007) and the United States (2011) (Marshall et al. 2008; Bouton, 2012). However, its persistence can be adversely affected by intensive grazing or severe cutting management and by harsh winter conditions. While little information is available on the combined effect of climate change and grazing or cutting management on alfalfa persistence, the expected temperature increase in winter under future climate conditions in northern regions will likely affect winter survival of perennial forage crops such as alfalfa (Bélanger et al., 2002). Alfalfa winter survival can be compromised by unsuitable conditions for winter hardening during the fall, inadequate snow cover during the winter, and/or ice encasement of plants and anoxia damage caused by the formation of an ice layer at the soil surface (Bélanger et al., 2002, 2006; Castonguay et al., 2006). Alfalfa is particularly sensitive to harsh winter conditions (Bélanger et al., 2006; Castonguay et al., 2006). In addition, elevated  $[CO_2]$ , as predicted in future scenarios, reduces alfalfa freezing tolerance (Bertrand et al., 2007a).

## Adaptation at the farm level

Climate change is expected to affect alfalfa management at the field and farm levels. In most regions, forage growth is expected to begin earlier and stop later in the season and the predicted increase in daily temperature might reduce the number of days between forage cuts, hence allowing for an increase in the number of cuts harvested each year (Ruget et al., 2012; Jing et al., 2013, 2014; Thivierge et al., 2016). Therefore, modifying the forage harvest schedule with the possibility of additional cuts is an important climate change adaptation strategy. Alfalfa is often used in mixtures with grasses and climate change could modify the proportion of each species within these mixtures. As a legume with N fixing capacity, alfalfa may benefit more than grasses from the future growing conditions (Thivierge et al., 2016), while the increased uncertainty of alfalfa survival during winter may reduce its persistence in the mixture. This could result in more variability of the proportion of alfalfa and grasses in the harvested forage mixture under the future climate and, thus, in more uncertainty regarding its nutritive value that may make more complex the feeding strategy at the farm level. The increased yield of alfalfa or alfalfa-grass mixtures combined with the adapted harvest schedule could influence crop rotations at the farm

level. A smaller area would then be required to feed the same number of animals, making available areas for other crops (e.g. cash crops) as shown by Thivierge et al. (2017). Adaptation will also be required to address the issues of increased risks of winter damage to alfalfa in northern areas along with the increased possibility of growing annual feed crops such as corn silage in those areas.

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## Role of perennial crops and legumes to the sustainable intensification of agriculture\*

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**KEYWORDS:** fossil fuel energy, non-renewable resources, N<sub>2</sub> symbiosis

Modern agriculture depends heavily on fossil fuel energy – up to 70% of the energy contained in a cereal grain produced using conventional methods of intensive agriculture may come from fossil sources. About 30% of the energy input to the agricultural sector is used in the production of nitrogen fertilisers, basically to break down atmospheric N<sub>2</sub> through the Bosch-Meiser reaction. Feeding an average person in the developed world costs about 1500 L oil equivalents, or about 12 barrels, per year. Peak oil production per capita, in the order of 5 to 6 barrels per person per year, has already been achieved in 1979 and all predictions, public and private, indicate that this amount will not be achieved again in the future (no less because of population growth). This means that food production is heavily dependent on a non-renewable resource that is slowly, but consistently, being depleted. Legume plants, able to fix atmospheric N<sub>2</sub> in symbiosis with Rhizobia, can contribute to curtailing the dependence of agriculture and food production on fossil energy. This is perhaps the greatest contribution that legumes can make to the sustainable intensification of current agriculture. But it is not the only one. Perennial legume species, be them herbs, shrubs or trees are able to grow and fix N in environments where other species – particularly crops and fodders – have difficulties to prosper. They represent key sources of food, feed, fuel and building material, contributing to the efficient use of resources (light, water, nutrients, labour) and to the rehabilitation of degraded soils. I will illustrate these contributions through first-hand cases from marginal environments and degraded soils mostly from sub-Saharan Africa.

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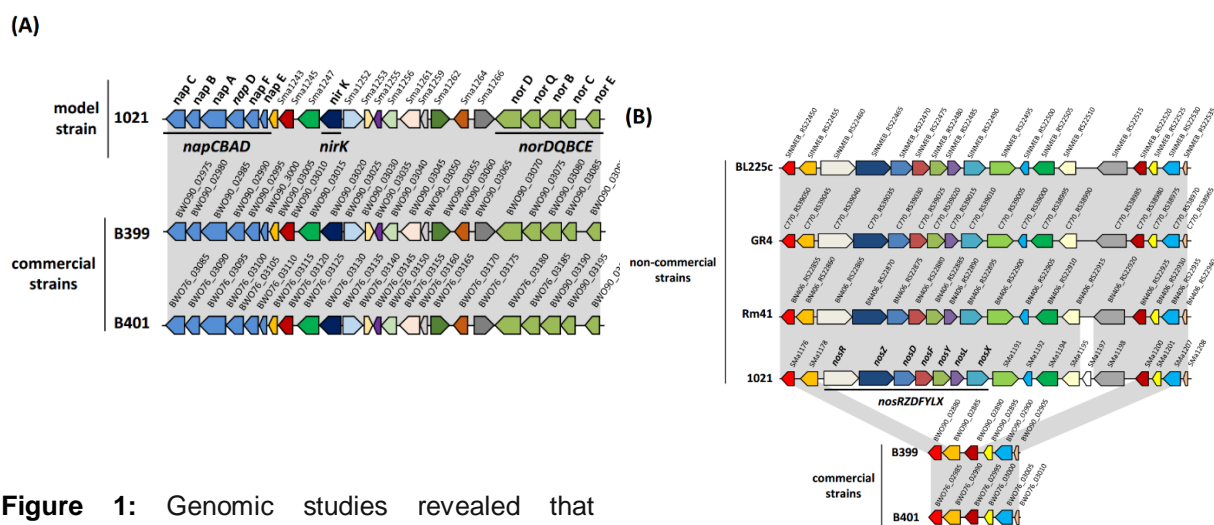
# Maximizing the nitrogen fixation and minimizing the nitrous oxide emissions in alfalfa production using natural inoculants\*

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**KEYWORDS:** alfalfa, rhizobia, nitrogen fixation, nitrous oxide.

Alfalfa is the most important legume crop in cultivated area worldwide (30 million ha) after soybean (110 million ha). Due to the fact that the cultivation of this perennial legume forage constitutes an extensive soil-air interface, as well as to its significant emission of the greenhouse gas nitrous oxide (N<sub>2</sub>O) and the critical role of legume-symbiotic nitrogen-fixing bacteria in N<sub>2</sub>O production via incomplete denitrification, it is important to understand the possible impacts of rhizobial domestication on the evolution of denitrification genes. In fact, the domestication of commercial alfalfa inoculants was prior to the emergence of global environmental change as a critical topic in the research community and society in general, and then, the conservation of their genetic factors related to the mitigation of N<sub>2</sub>O emission was not considered. Genomic analysis focused on denitrification genes revealed that commercial alfalfa inoculants have perfectly conserved the nitrate (NAP), nitrite (NIR) and nitric (NOR) reductase clusters related to the production of N<sub>2</sub>O from nitrate (Figure 1A) but completely lost the nitrous oxide (NOS) reductase cluster (*nosRZDFYLX* genes) associated with the reduction of N<sub>2</sub>O to gas nitrogen (Figure 1B), providing evidences of the environmental risk of the domestication of alfalfa rhizobia (Figure 2). In this context, we have been running screenings for alfalfa-nodulating isolates containing both nitrogen fixation (NIF+) and N<sub>2</sub>O reductase (NOS+) genes for environmental sustainability of alfalfa production. As the results of these screenings, we have been selected several NIF+NOS+ rhizobia from alfalfa nodules (natural microbes without genetic manipulations), which are able to fix nitrogen and reduce N<sub>2</sub>O under nitrogen limiting conditions. In the framework of the 2nd World Alfalfa Congress, we will present some of the results of the research done and the upcoming genomic analyses to understand the diversity of the novel NIF+NOS+ microbial collection (Figure 3). Finally, we will discuss the need of public-private partnership projects to analyze the efficiency and environmental quality of these novel inoculants using different elite alfalfa germplasm in different regions of alfalfa-producing countries.



**Figure 1:** Genomic studies revealed that commercial strains have perfectly conserved NAP, NIR and NOR clusters related to the production of N<sub>2</sub>O from nitrate but completely lost NOS cluster (*nosRZDFYLX* genes) associated with the reduction of N<sub>2</sub>O to N<sub>2</sub>.

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## New services from perennial legumes in agroecological farming systems\*

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**KEYWORDS:** alfalfa, ecosystem services, living mulches

Ecosystem services have been defined in the Millenium Ecosystem Assessment and include provisioning, supporting, regulating and cultural services. Considering this broad analysis, it is possible to identify new uses for alfalfa and other perennial forage legumes in farming systems, based upon agroecology to reach sustainable agri-food systems.

In the present paper, we will present how alfalfa and forage legumes may be used as living mulches in cereal production systems.

Such living mulches make it possible to reach Land Equivalent Ratio higher than 1, through higher biomass production and more regulating services. By avoidance of environmental debt, such new production systems could contribute to a better adequacy between society and farmers expectations regarding production systems. New paradigms such as agroecology, microbioms and chemical ecology offer new prospects for very contrasting production systems such as cereal production with living mulches.

A large dataset of results will be presented, both in experimental conditions and in real farms, on small grains, on maize and in water-limited environments. These French situations are supported by examples from the international literature. On average, the yields of small grain crops are similar when sown in a legume living mulch, with a key attention to be paid to the control of the legume growth in order to avoid competition. There is a slight mean benefit on cereal quality. The economic returns are similar.

In coherence with the ecosystem services approach, a multicriteria evaluation is necessary to document all services. This will enlighten the variability among the various experiments, but also the convergences among all situations. Environmental impacts are clearly improved with less use of nitrogen and pesticides, less consumption of energy, improved soil structure and water management. One peculiar feature will be documented regarding the work load for the farmers and the possibility to drill over larger periods of time due to improved soil structure and soil loading capacity. This offers prospects for larger changes in the evaluation of farming systems.

The physiological and biological mechanisms explaining the observed benefits and some failures will be documented. They include the control of competition between crops and living mulches, and the use of complementarity as species in the swards explore different soil horizons and have different nitrogen sources. The agroecological mechanisms and especially the biological regulations are obtained thanks to an increasing functional diversity of swards, with a special attention paid to water cycle and N and P fertility cycles.

These very contrasting production systems raise many scientific and technical issues. Some will be discussed, such as plant breeding both for the sale crops and the service crops, machinery or decision tool kits. It also raises clear challenges for training and advisory systems as well as for research where more participatory research or new research designs such as Living Labs may be implemented.

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## Alfalfa and its potential in the Argentine dairy systems\*

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**KEYWORDS:** dairy systems, nutritional value, alfalfa grazing, sustainability

### 1. Evolution of alfalfa in dairy systems

Argentina is one of the few countries that maintain most of its livestock production under grazing-based conditions throughout the year, with the benefit of lower production costs; however, there is still a large margin for improving its productive efficiency. In the particular case of the Argentine dairy systems before the '80s, cows fed mostly on annual winter [oats (*Avena sativa*) and summer forages [sorghum and foxtail millet (*Setaria italica*)] without the regular use of concentrates and with insufficient amount of hay. As a result, milk production was very variable and strongly subject to environmental conditions. In the early '80s, an incipient intensification of dairy farming began - or at least the foundations of it - with the introduction of the alfalfa cultivar CUF 101, which was rapidly and widely adopted. This non-dormant cultivar stood out amongst other legumes and grasses due to its higher dry matter production, longer growing season, aphid resistance, adaptation to various environmental conditions and good forage quality (protein, digestibility, palatability, etc.).

As a result, the introduction of non-dormant alfalfas started a dramatic change in the dairy systems due to their ability to be grazed throughout the year (8-9 harvests), provided that an adequate rotational system was used in order to assure productivity and persistence. In doing so, the proper management was founded on one concept: alfalfa tolerates intense defoliation (up to 80% of grazing efficiency with high instantaneous stocking rates, like for instance 160 milking cows ha<sup>-1</sup> day<sup>-1</sup> in daily grazing strips), as long as they are not frequent (i.e., having resting periods of approximately 24 days in summer, 28 days in spring, and more than 40 days in autumn; in winter, the moment of grazing is defined by the development of basal regrowth). Nevertheless, this initial use of non-dormant improved varieties did not immediately translate into a significant increase of productivity (liters of milk ha<sup>-1</sup> year<sup>-1</sup>). Although the high feed value of alfalfa it was widely recognized, the use of a large proportion of this legume relative to the total animal intake originated protein/energy unbalances and bloat problems. In this context, unfavorable consequences on animal health and milk production were commonly observed.

In the '90s, the intensification of the dairy systems was more clearly expressed, presenting an annual 7% increase of the overall rate milk production. This was mainly the result of two facts: a) the large adoption of maize or sorghum silage (whole plant), which allowed a high and stable stocking rate throughout the year; and b) the implementation of a basic crop rotation system in the vast majority of dairy areas that included 3-year alfalfa pastures (60% of the area) + 2-year maize paddocks (both years for silage). To prevent contingencies that could affect the production of alfalfa in its third year, the use of winter (oats for grazing) or summer crops (soybeans for grazing or millet for hay) was implemented in most of the dairy farms as a "safety valve". As a result, not only a greater seasonal and inter-annual stability of forage supply was achieved, but also an increase of the stocking rate during autumn-winter. Complementarily, it was also possible to maintain a higher stability on diet components, and therefore diminishing the overall participation of alfalfa relative to other feed sources. In consequence, dairy production efficiency (liters ha<sup>-1</sup> year<sup>-1</sup>) increased not only significantly (> 30%) but also sustainably.

During the 2005/2015-period, the process of intensification was characterized by adjustments on feeding strategies towards the increasing use of partial or total cow confinement. The latter was generally associated with a larger scale; the purchase of concentrates (i.e. not produced on the farm), that became 25 to 43% of the total DM consumed by dairy cows; and an increase in feed supply expenses (feeding pens), that represented

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about 8% of the gross milk production income. In addition, the trend for decreasing alfalfa participation in the diet was strongly emphasized, going from 50% to 27% of the DM consumed by cows. However, this should not be interpreted as a trend towards alfalfa pastures disappearance in the near future. On the contrary, grazing alfalfa will keep playing an important role on dairy production in Argentina because of two main reasons: 1) it allows an adequate nutrient balance, not only as a source of energy, protein, vitamins and minerals, but also as a great possibility for combining with other feeds; and 2) it reduces feeding costs and therefore provides good economic results for the entire system.

Research generated by the Dairy Unit at INTA Rafaela (Comerón *et al.*, several publications) concluded that using grazing-based dairy systems in combination with supplementation (typical system: 30% concentrate:30% alfalfa pasture:30% corn or sorghum silage:10% alfalfa hay) were able to produce up to 7,000-7,500 liters lactation<sup>-1</sup> and maintain Holstein cows with adequate body condition for 305 days. If these results are combined with a stocking rate of 1.5 cows ha<sup>-1</sup> year<sup>-1</sup>, then productivity levels as high as 11,000 liters ha<sup>-1</sup> year<sup>-1</sup> can be achieved.

The central dairy region of Argentina is the largest in Latin America. In there, and particularly in the Santa Fe and Córdoba provinces, alfalfa finds excellent soil and climatic conditions to grow at the most of its potential as well as it improves soil fertility through the biological Nitrogen fixation along the 3 to 5-year stand persistence in dairy systems. In this context, the use of alfalfa under grazing or chopping (soiling) reduces operation costs and minimizes degradation of natural resources, contributing therefore to environmental and economic sustainability.

## **2. The effect of fresh alfalfa on milk quality, dairy products and antioxidant composition**

As it was mentioned above, alfalfa acts as a source of important nutrients that improve the concentration of beneficial compounds such as vitamins, carotenoids, fatty acids and antioxidant capacity, while diminishing susceptibility to oxidation. When analyzing the inclusion of different proportions of grazed alfalfa in Partially Mixed Rations (PMR), a direct correlation between alfalfa proportion and concentration of beneficial compounds in milk and dairy products -such as cheese and powdered milk- was found. These valuable traits are important for differentiating milk qualities produced under variable presence of fresh alfalfa in PMR strategies.

At INTA Rafaela, after 20 days of feeding 70% fresh alfalfa (fresh weight basis) vs. sorghum silage and hay, higher concentrations of vitamin A (retinol), vitamin E (alpha-tocopherol) and carotenoids (beta-carotene) were found, as well as a higher antioxidant capacity and therefore, less susceptibility to oxidation (Rossetti *et al.*, 2010). In addition, better sensory qualifications for organoleptic traits, such as color and aroma, were also obtained as a consequence of the inclusion of pasture feeding. Since a high proportion of fresh (green) alfalfa on the diet is not always possible, an experiment to compare medium to high alfalfa proportion was conducted at INTA Rafaela (Descalzo *et al.*, 2012). It was concluded that approximately 50% of alfalfa on the diet (fresh weight basis) was enough to obtain the same results in milk quality as with the use of higher alfalfa proportions. As stated before, it is also important to analyze the impact of fresh alfalfa on dairy products. In doing this, composition of whole powdered milk and ripened cheese (*Reggianito*-type) made with milk obtained from the above mentioned studies was assessed. Same as milk quality, results showed a higher better nutritional composition of dairy products when adequate proportion of fresh alfalfa is included on the diet. The overall conclusion is that nutritional value of milk and dairy products can be naturally improved by using fresh alfalfa in the feeding strategies.

## **3. Environmental impact**

Although alfalfa is widely used in Argentine dairy production, very few studies on the environmental impact of grazing dairy systems have been conducted. Tieri *et al.* (2017) analyzed between July 2014 and July 2015 a total of 116 dairy farms in terms of nitrogen use efficiency (NUE) and greenhouse gas emissions. It was possible to identify a number of farms that combined high production and low environmental impact. Regarding NUE, the mean value was  $24.6 \pm 7.7$  %, which is similar to those values reported by Gourley *et al.* (2012) for Australia and USA, but higher than the one estimated by Nevens *et al.* (2006) for dairy farms of the Flemish Region. Nevertheless, 60.3% of the dairy farms in the study exhibited a NUE around 30%, quite close to the acceptable threshold proposed by Powell *et al.* (2010). Total greenhouse gas emissions per unit of product were estimated using the IPCC methodology (Carbon Footprint = CF, in kg CO<sub>2</sub>eq kg milk<sup>-1</sup>). Results indicated that



CF for all farms were within recently estimated ranges for UK and USA, i.e. 0.6-2.8 kg of CO<sub>2</sub>-eq kg of milk<sup>-1</sup> (DairyCo 2012; Thoma *et al.*, 2013). The mean CF (0.92 ±0.24 %) was within the lower range of international evaluations (Opio *et al.* 2013) and was similar to the value of 1 kg of CO<sub>2</sub>-eq/kg of FPCM (fat and protein corrected milk) calculated for Irish dairy systems by studies (Leip *et al.*, 2010; Yan *et al.*, 2013). The mean value found in Argentina is also very similar to the 0.84-0.90 range reported by O'Brian *et al.* (2014) for UK, Irish and USA dairy farms.

## CONCLUSIONS

There are productive and economic evidences that justify the use of alfalfa pastures as a part of PMR (grazed alfalfa + silage + hay + concentrate) in dairy production. These systems imply lower investment and lower operation costs, being therefore more suitable for small to medium ( $\leq 3,500$  liters day<sup>-1</sup>) Argentine dairy farms. In addition, alfalfa pastures contribute to a more stable (less risky) feeding structure and to the improvement of nutritional value of milk and dairy products. Furthermore, they produce a lesser impact on the environment (NUE and CF) compared to confined systems. However, there is still a need for identifying new indicators for a more thorough evaluation of dairy performance, as well as new studies for the improvement of nutrient use efficiency, nutritional composition and sustainability of dairy systems.

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# Predicament and outlet of alfalfa production under water sources scarcity in China\*

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**KEYWORDS:** alfalfa industry, *Medicago sativa* L., groundwater, irrigation

## 1 Introduction

Alfalfa industry, an important part of agriculture, is honored as a gold industry in the world due to its high planting value. In recent years, China also began to attach importance to the development of alfalfa industry. However, alfalfa is considered as a high water consumption plant, to develop alfalfa industry should consume a large amount of water resources. China is a water deficit country, the quantity per capita is only 2300 m<sup>3</sup>, a quarter of the world average. In this situation, to develop the alfalfa industry is bound to increase the pressure on water resources in China. Therefore, it is the key to coordinate the relationship between alfalfa production and water resources for achieving long-term steady development of alfalfa industry in China.

## 2 Relationships between water resources and alfalfa production in China

### 2.1 Water requirement rules

The water requirement of alfalfa in the whole growing season is 400-2250 mm, so alfalfa is a relatively high water requirement plant compared with other crops (Table 1). And the water requirement of alfalfa is also related to cutting cycle, varieties and geographical conditions (Table 2). In addition, the water requirement of alfalfa is variable in different phenological periods (Fig. 1, Table 3). Nevertheless, after branching, alfalfa entered aboveground peak growth period with the high water consumption and intensity.

### 2.2 Water use efficiency

The water use efficiency of alfalfa increases with the increasing growth years. In the establishment year, the water use efficiency of alfalfa is 8-14 kg/ (ha • mm), after the second year, is 12-29 kg/ (ha • mm). The water use efficiency of alfalfa is related to its strong roots system. Compared with common crops and grasses, the roots of alfalfa can absorb shallow and deep soil moisture (Fig. 2). Therefore, in semi-arid and arid regions, alfalfa can utilize soil moisture more effectively than other crops and grasses and has stronger drought resistance.

## 3 Water resources problems of alfalfa production in China

### 3.1 Surface water scarcity in planting area

Alfalfa was mostly planted at arid and semi-arid areas in China, including Xinjiang, Gansu, Ningxia, Inner Mongolia, Shaanxi, Shanxi. The mean annual rainfall is less than 500 mm in these areas, which contrasts with the high water requirement of alfalfa. Therefore, alfalfa need irrigation to improve yields by mining groundwater in these areas.

### 3.2 Mismatch between production planning and regional groundwater resources situations

The carrying capacity of groundwater resources in some areas might not meet the requirements of the production targets. In Ar Horqin Banner, there is 66 667 ha high-quality alfalfa pasture, 60 700 ha of which need to be irrigated. However, studies showed that the theoretical carrying capacity of groundwater resources is only

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37 900 ha. Therefore, the irrational expanding the planting area will accelerate the depletion of groundwater resources.

### **3.3 The decline of regional groundwater table due to unconstrained groundwater irrigation**

The expansion of groundwater irrigation area led to large-scale disorder mining of groundwater and a sharp increase in irrigation water consumption. In Inner Mongolia, from 1982 to 2016, the proportion of groundwater irrigation area in the whole irrigation area increased from 40% to 60%, and the consumption increased from 1.6 billion m<sup>3</sup> to 5.1 billion m<sup>3</sup>. Unconstrained groundwater irrigation eventually caused the decline of regional groundwater table.

### **3.4 The risk of groundwater pollution due to excessive use of phosphate fertilizer**

Phosphate fertilizer is also one of the key factors to improve alfalfa yield. However, there is no unified standard on the amount of phosphate fertilizer used in alfalfa planting in China, which could increase the risk of excessive use of phosphate fertilizer. Excessive use of phosphate fertilizer will accelerate the enrichment of soil phosphorus and pollute groundwater.

## **4 Countermeasures**

### **4.1 Optimize regional water resources allocation, and decide production by water**

Understand the carrying capacity of regional water resources before formulating the alfalfa production targets. Establish the reasonable alfalfa production targets based on the amount of water resources that can be allocated to alfalfa production.

### **4.2 Cultivate water-saving alfalfa, and reduce water consumption**

Study on the water requirement of existing alfalfa cultivars, and then select or breed water-saving alfalfa cultivar by means of cross breeding, induced mutation breeding, biotechnology breeding.

### **4.3 Implement water-saving irrigation technology, and improve the efficiency of water usage**

Apply advanced irrigation equipment and efficient water-saving irrigation methods and systems. Relevant researchers and technicians should guide local farmers or herdsmen to develop effective water-saving irrigation.

### **4.4 Formulate the standard on application of phosphate fertilizer, and promote utilization efficiency**

Research on the relationship between alfalfa growth and phosphate fertilizer for establishing a unified standard for preventing the excessive use of phosphate fertilizer from polluting groundwater resources.

### **4.5 Strengthen water resources management, and improve public water-saving consciousness**

Establish a complete water resources management system, including water permit, paid water usage, and penalty regulations and enhance propaganda of importance of water saving.

## **5 CONCLUSIONS**

In China, alfalfa planting areas are mainly distributed in arid and semi-arid areas where water resources are relatively scarce. Therefore, the sustainability development of alfalfa industry can only be guaranteed by rational utilization of water resources and improvement of water-saving technology.

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**Table 1** Water requirement of crop growing season

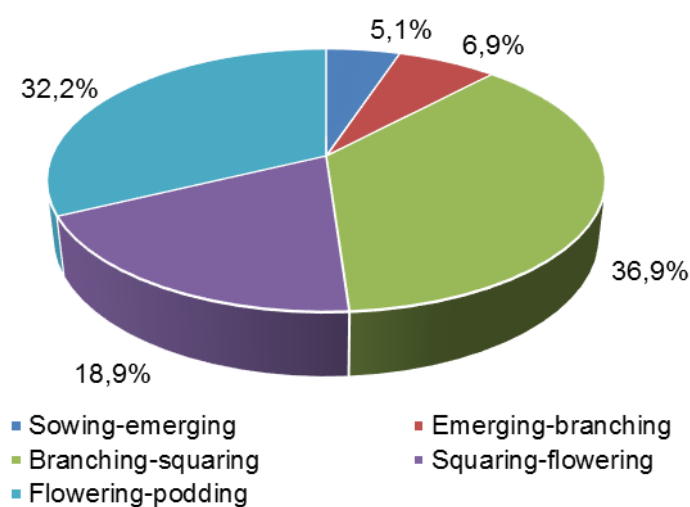
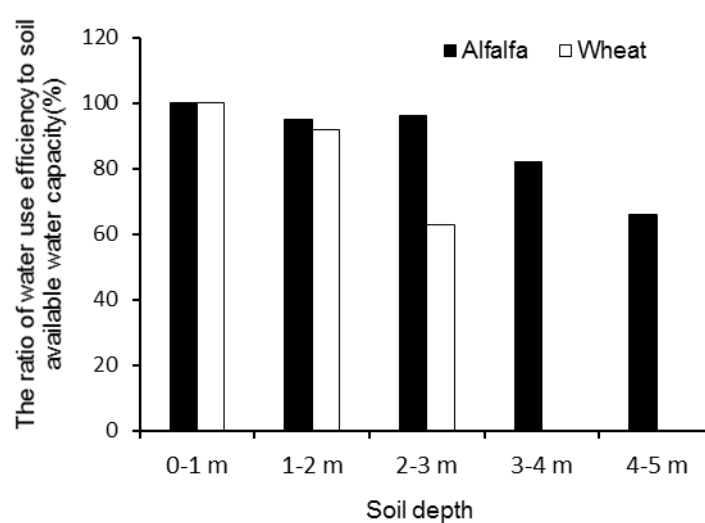
Crop	Water requirement(mm)	Crop	Water requirement(mm)
Rice	525-767	Soybean	450-600
Winter wheat	390-600	Cotton	450-675
Corn	300-450	Alfalfa	400-2250
Sorghum	300-450		

**Table 2** Water consumption of alfalfa with different stubbles

Regions	Cultivars	Stubbles	Days	Water consumption (mm)	Water consumption rate (mm/d)
Hebei	WL323	1	81	297	3.66
		2	36	215	5.97
		3	54	93	1.73
		total	171	605	3.79
Hebei	Algonquin	1	62	243	3.90
		2	30	134	4.50
		3	53	184	3.50
		total	145	562	3.97
Beijing	WL323	1	83	289	3.50
		2	41	230	5.60
		3	83	408	5.00
		total	207	928	4.70
Beijing	Zhongmu <sub>1</sub>	1	83	339	4.10
		2	41	206	5.00
		3	83	364	4.40
		total	207	909	4.50

**Table 3** Water consumption of alfalfa in different phenological periods

Growth stage	Days	Water consumption (mm)	Water consumption rate (mm/d)
Sowing-emerging	6	20.6999	3.45
Emerging-branching	18	38.18981	2.12
Branching-squaring	35	200.864	5.74
Squaring-flowering	14	106.2595	7.59
Flowering-podding	31	180.8391	5.83

**Figure1:** Percentage of water requirement of alfalfa in different phenological periods**Figure. 2:** Comparison of water use efficiency of wheat and alfalfa at different soil depths





## POSTERS PRESENTATIONS

## Response of alfalfa (*Medicago sativa* L.) forage yield to fertilization with nano-particle technology

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**KEYWORDS:** productivity, nutrients uptake, pasture, aerial biomass

**INTRODUCTION:** High alfalfa yields demand high nutrient availability; however, most commonly used fertilizers do not always provide those nutrients in accordance to the pasture needs (Lanyon and Griffith, 1988). New technological alternatives for formulating otherwise traditional fertilizers, such as nanotechnology, open new research opportunities to increase the efficiency of fertilizer application in alfalfa.

**OBJECTIVE:** The objective of this work was to evaluate the impact of fertilization with calcium (Ca), magnesium (Mg) and sulfur (S), formulated through nano-particle technology, on alfalfa dry matter (DM) yields.

**MATERIALS AND METHODS:** Alfalfa plots were sown on May 17, 2017 on an Entic Hapustoll soil at INTA Manfredi Agric. Exp. Station, Córdoba, Argentina (31.5°S, 63.5°W). A pre-planting top-soil (0-20 cm) analysis determined the following values: pH: 6.8; organic matter: 1.92%; extractable P: 58.3 ppm; N-NO<sub>3</sub>: 19.27; Ca: 7.90 cmol kg<sup>-1</sup>; Mg: 2.73 cmol kg<sup>-1</sup>; Na=1.50 cmol kg<sup>-1</sup>; K: 2.67 cmol kg<sup>-1</sup> and CIC: 19.37 cmol kg<sup>-1</sup>. All plots were planted with cultivar Traful PV INTA (FD 9) at the seeding rate of 12

Kg ha<sup>-1</sup>. A randomized complete block design with three replicates and 5-m<sup>2</sup> plots was used. Treatments followed a factorial arrangement in which factors were: a) nutrient source, with three levels: MIST-Ca/Mg + S; MIST-Ca; and MIST-S/Ca+S; b) nutrient level, with two levels: 1.5 l ha<sup>-1</sup> and 3 l of formulated product ha<sup>-1</sup>; and c) moment of application, with four levels: pre-planting; pre-planting + second dose after the 1<sup>st</sup> cut; pre-planting + second dose after the 4<sup>th</sup> cut; and all the above. Combining all factors and levels, the experiment had a total of 25 treatments, included the control (no fertilizer). Plots were kept free of weeds and pests by chemical applications. Plots were cut every time they reached 10% blooming. Along the growing season (October 2017/April 2018), 6 cuts were performed. Adding all cuts, cumulative DM yield (kg DM ha<sup>-1</sup>) for the first growing season was then obtained. Means from each treatment were compared by the DGC test ( $\alpha = 0.05$ ) (DiRiezo et al, 2002).

**RESULTS:** Average cumulative DM yields for each treatment are presented in Figure 1. There were no significant differences among treatments. However, the control plot exhibited the lowest absolute value compared to all the fertilized plots. Among the latter, the use of MIST-S/Ca+S (3 l ha<sup>-1</sup>) and MIST-Ca (1.5 l ha<sup>-1</sup>) produced the highest yield responses in absolute values, even though non-significant ( $p > 0.05$ ).

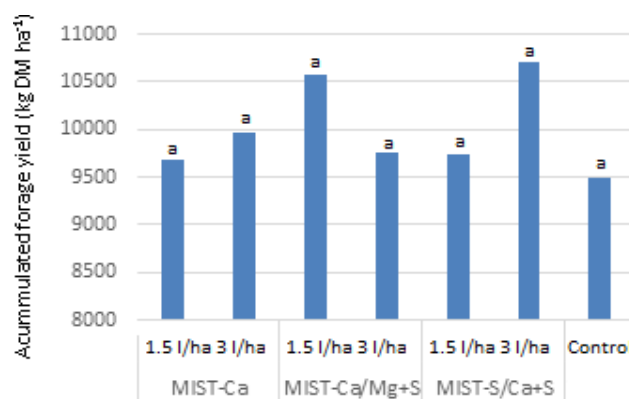
**DISCUSSION:** Pre-planting soil analyses indicated no serious limitations of nutrient available for alfalfa production. Therefore, the lack of significant differences between fertilized and control plots might be expected. Nonetheless, there seemed to be a beneficial response from some of the fertilization treatments compared to the control. In this context, it will important to assess the effect of these applications in the following growing seasons compared to the control without any nutrient reposition. For soils with nutrient deficiencies, nanotechnology fertilizer formulations may be important to increase forage yields, particularly based on their lower costs and easiness of application.

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**Figure 1** – Cumulative (6 cuts) alfalfa yield (kg DM ha<sup>-1</sup>) for fertilizer type and level treatments and control during the first growing season (2017/18). Same letter indicates non-significant differences ( $p > 0.05$ ).

## Pursuing alfalfa's (*Medicago sativa* L.) potential yield in the northern Pampas

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**KEYWORDS:** lucerne, fertilization, production, persistence, L820.

**OBJETIVES:** Alfalfa is the most important forage crop in the Argentinian Pampas, where about 3.2 million hectares are grown and more than 50% is utilized under direct grazing for both beef and dairy production (Basigalup, 2017). Analysis of data from dairy farms demonstrate that significant differences in milk production and profit among grazing systems are highly associated with higher forage consumption. Therefore, to achieve high yields of alfalfa is crucial for the economic sustainability of the farm. However, farm measurements of alfalfa yields indicate that about 10 t DM ha<sup>-1</sup>y<sup>-1</sup> are produced, which is about 40% of the yields achieved in experimental plots. Several experiments in the Pampas demonstrated high response of alfalfa to fertilization. We hypothesize that soil nutrient levels might be a limitation to achieve higher yields in the northern Pampas. Our general objective is to evaluate the productive and economic response to balanced fertilization of alfalfa pastures under rainfed conditions. The specific objectives include: i) to achieve alfalfa yields that exceed 16 t DM ha<sup>-1</sup> y<sup>-1</sup>; and ii) to maintain and/or increase the levels of nutrients in the soil.

**MATERIALS AND METHODS:** Three representative farms of the northern Pampas were selected on a regional soil phosphorus (P) gradient. A soil sampling was carried out in April 2018 and soil organic carbon, nitrogen, exchangeable P, pH, calcium (Ca), magnesium (Mg), soil exchange capacity were measured with conventional laboratory methods. On May 2018, 24 experimental plots of alfalfa L820 (Gentos S.A.) were sown in the 3 farms. On each farm, 4 treatments were performed with two replicates: T0= no fertilization, T1= annual requirement of P, T2= annual requirement of P, sulfur (S) calcium (Ca) and magnesium (Mg), T3= correction of soil to alfalfa optimum (25 ppm P, pH=6.5, Ca/Mg:6) on 5x150 m experimental plots. The fertilizers used were triple super phosphate, Azufertil (18.6% S, 23.4% Ca; Nutrien Ag Solutions) and Granucal (20.5% Ca, 10.7% Mg; Nutrien Ag Solutions) and soil fertilizers were incorporated into the soil shortly before sowing. For T1 and T2 treatments, the level of nutrient requirements was calculated according to IPNI (2016). The fertilization rate will change in the following years according to the expected production, for the first year a production of 16 t DM is expected, 20 t DM for the second year and 18 t DM for the third year of production. Fertilization rates for T3 were according to soil analysis. Initial measurements reported in this abstract were taken 60 days after sowing and included: number of plants, number of nodules per plant and plant cover. Future measurements will include yield for every cut and persistence (number of plants every year) for 3 years at least. Statistical comparisons were made with ANOVA and simple linear regressions (p=0.05) using the software Infostat.

**RESULTS AND DISCUSSION:** Soil P and pH varied among farms from 18 to 30 ppm and from 5.6 to 5.9 respectively. The initial number of plants was related to soil pH, site averages in T0 increased from 183 pl m<sup>-2</sup> to 411 pl m<sup>-2</sup> as soil pH increased (p<0.01). The proportion of plants with nodules also varied with soil pH, with a 12% increase in nodulated plants per 0.1 points increase in soil pH (regression analysis, p<0.01). The number of plants decreased with increasing fertilization rates, which might be explained by phytotoxic effects. However, plant cover, phenology stage and the proportion of nodules per plant were not affected by fertilization treatments.

**CONCLUSIONS:** On the pursuit of Alfalfa's potential yield, we found that initial soil pH is important for an adequate pasture establishment. However, soil pH corrections should be done with sufficient time previous to sowing in order to avoid phytotoxicity. These preliminary results are part of a 3-year experiment, and the impact of fertilization on production and persistence will be evaluated in the future and might overcompensate these initial losses.

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## Water table depth: threat or opportunity for alfalfa crops?

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**KEYWORDS:** lucerne, production, persistence, phreatic level

**INTRODUCTION:** Alfalfa (*Medicago sativa* L.) is one of the most sown perennial legumes in the world. Water table depth can be both an opportunity and a threat for this crop (Mercau *et al.* 2016). The replacement of pastures for grain crops has decreased transpiration rates and increased soil moisture content in the Pampas Region. This has risen the water table and has increased the risk of flooding (Nosetto *et al.* 2012). Alfalfa pastures extract more water from depth than other perennial pastures (Brown *et al.* 2005). Therefore, the use of alfalfa could be proposed as a way to reduce the water table depth. However, many soils in the Pampas Region where alfalfa crops are sown are heavy-textured with slow water movement (Imhoff *et al.* 2016) in which alfalfa root diseases can prosper (Kuan and Erwin 1980). This may compromise the effectiveness of alfalfa roots in these soils and may reduce yield and persistence of this crop.

**OBJECTIVES:** In this research we explored data from a long-term alfalfa experiment to analyze the impact of the depth of the water table on alfalfa yield and persistence.

**MATERIALS AND METHODS:** The data for this study came from a national evaluation network of alfalfa genotypes (Basigalup *et al.*, 2016). The experiment was carried out at INTA Rafaela (-31°12', -61°30'). The soil is a typic argiudoll, with a silty-loam heavy texture. Data was obtained from a comparative trial of alfalfa cultivars (from 1994 to 2016) in which dry matter production (above-ground net primary productivity, ANPP) and persistence (plant cover in the sowing line) under cutting were measured. The trials were planted every two years and lasted 3-4 years. Both group of cultivars semi-dormant (SD) and nondormant (ND) cultivars were sown in 5 m<sup>2</sup> plots, with spacing rows at 0.20 m. Plots were arranged in a complete randomized block design with four replicates. Dry matter (DM) production was obtained by cutting with a rotary mower and weighing all the forage produced in each plot. All cuts were made at 10% flowering, or when the crown sprouts reached 5 cm. Persistence was estimated after 4 years from cover data by counting in each row the number of empty spaces greater than 15 cm at the end of each growth year. Weather data were obtained from a conventional meteorological station located 750 m away from the experimental site. Water table depth was monitored daily with a hydrometer. Regression and correlation analysis were performed to search for significant ( $p < 0.05$ ) associations between variables. Total ANPP and persistence after 4 years were compared to climate (i.e. mean temperature and precipitation) and 4-years-average soil water table depth using non-linear models included in the software Infostat. Model estimates were statistically compared with measured data using linear regression methods. The performance of the tool was evaluated by computing the  $R^2$  and root mean squared error (RMSE). The intercept and the slopes of the observed vs. measured values were tested for deviation from a null hypothesis of 0 and 1, respectively, by a t-test.

**RESULTS:** The average total ANPP after 4 years was 51.8 t DM ha<sup>-1</sup>, with a mean final persistence of 54%. There was no difference ( $P = 0.14$ ) in ANPP or persistence ( $p = 0.96$ ) between SD and ND cultivars. There was a trend ( $P = 0.1$ ) associating productivity and persistence, but both factors were highly correlated with water-table depth ( $p < 0.01$ ). Maximum productivity and persistence were observed when the water table was below 4 m depth. ANPP was estimated as:  $ANPP = a \times y^b \times e^{(-c \times y)} + d \times wt$ , where  $wt$  is the water table depth (m),  $y$  the production year, and the parameter values  $a = 33.00$ ,  $b = 2.09$ ,  $c = 1.16$  and  $d = 1.08$ . The model effectively represented the ANPP using the production year and water table depth as explanatory variables with moderate prediction level ( $R^2 = 0.47$ ,  $p < 0.05$ ).

**CONCLUSIONS:** Water tables above 4 m had a negative impact on alfalfa production under rain-fed conditions. This was observed in heavy-textured soils from the Central Pampas of Argentina. Alfalfa pastures have been suggested as a way to keep water table depths low and avoid flooding risk in hyperplains like the Pampas. However, shallow water tables may weaken its water extraction potential due to poor persistence. These novel results allowed us to quantify a threshold water table depth above which shallow water resistant perennial species might be considered instead of alfalfa. Our results also provide a risk management tool for rain-fed production systems.

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# Nitrogen status of alfalfa pastures (*Medicago sativa* L.) in the north of humid Pampa

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**KEYWORDS:** irrigation; water availability; nitrogen dilution curve; nitrogen nutrition index; productivity

**OBJECTIVE:** analyze the nitrogen status of alfalfa pastures with different water availability in the north of humid Pampa.

**MATERIALS AND METHODS:** the pasture was sown on March 29, 2010 in a Typic Argiudoll at INTA Pergamino Experimental Station, Argentina (Lat: 33°34'S; Long: 60°34'W), with pH=5,4, organic matter=2,9 % and extractable phosphorus=24,9 ppm in the topsoil. Historical average annual rainfall is 986 mm and temperature is 16,6 °C. Alfalfa cultivar was Luján INTA (intermediate dormancy) with a sowing density of 13 Kg.ha<sup>-1</sup> pure seed. Plot size was 120 m<sup>2</sup> with three replicates in a randomized complete block. Fertilization per hectare was applied during sowing (3,5 Kg nitrogen, 20 Kg phosphorus, 3 Kg potassium and 6 Kg sulfur). The experiment was kept free of weeds and insects through pesticides. Cuttings were done when alfalfa begins to flower (spring-summer), and when crown regrowth reaches 5 cm (autumn-winter). The treatments evaluated were natural water availability and complementary irrigation. In the 1<sup>st</sup> cycle the alfalfa was cut 8 times (Jul-Jun), in the second 9 (Jul-Jun) and in the third 5 (Jul-Jan). Sprinkler irrigation was applied empirically to avoid water deficit, during the 1<sup>st</sup> cycle with 250 mm, during the 2<sup>nd</sup> cycle 303 mm, and in the 3<sup>rd</sup> cycle 28 mm. Aerial dry matter (DM) biomass was measured previous each cutting in with two quadrats (each 1m<sup>2</sup>) per plot and a pooled sample of both quadrats was analyzed for N content (Kjeldahl). N absorption was calculated as the product between aerial biomass and its nitrogen concentration, while Nitrogen Nutrition Index (NNI) as the quotient between nitrogen concentration in the pasture and the nitrogen concentration expected from the reference dilution curve (Lemaire *et al.*, 1985), below 1 Mg.ha<sup>-1</sup> it was used 48 g N.kg<sup>-1</sup> as constant. Analysis of variance considering block design was done for biomass and nitrogen accumulated each cycle, and for each cutting for NNI. Treatment average values were compared with Fischer's test ( $p < 0.05$ ).

**RESULTS:** nitrogen accumulation was higher in the irrigated treatment, probably associated with a higher aerial dry matter biomass accumulation (Table 1) as the relative increase was similar for both. Figure 1.a. shows that N concentration was below the reference curve for almost all cutting and treatments, thus suggesting lower absorption and/or lower biological nitrogen fixation. The NNI for alfalfa irrigated was higher for 50% of the cuttings, with an average NNI increase of +21% for those cuttings (Figure 1.b.).

**Conclusions:** alfalfa pasture in north humid Pampa was below nitrogen dilution curve reference suggesting some constraint to achieve potential growth. Irrigated alfalfa had a better nitrogen status than rainfed alfalfa. Limitations in soil or biological N fixation may be involved.

Table 1: Alfalfa biomass dry matter accumulation and nitrogen accumulation as affected by soil water availability

Biomass accumulation (Mg DM.ha <sup>-1</sup> )				N accumulation in alfalfa biomass (kgN.ha <sup>-1</sup> )			
Cycle	Rainfed	Irrigated	(p% increase)	Rainfed	Irrigated	Irrigation value)	(p% increase)
1 <sup>st</sup>	10,0 a*	14,7 b	< 0,0001	46	302 a	460 b	< 0,0001
2 <sup>nd</sup>	11,4 a	16,6 b	< 0,0001	46	380 a	554 b	< 0,0001
3 <sup>rd</sup>	8,4	8,5	0,70 NS	2	271	285	0,22 NS

NS: not significant. \* Different letters in the same row indicate significant differences.

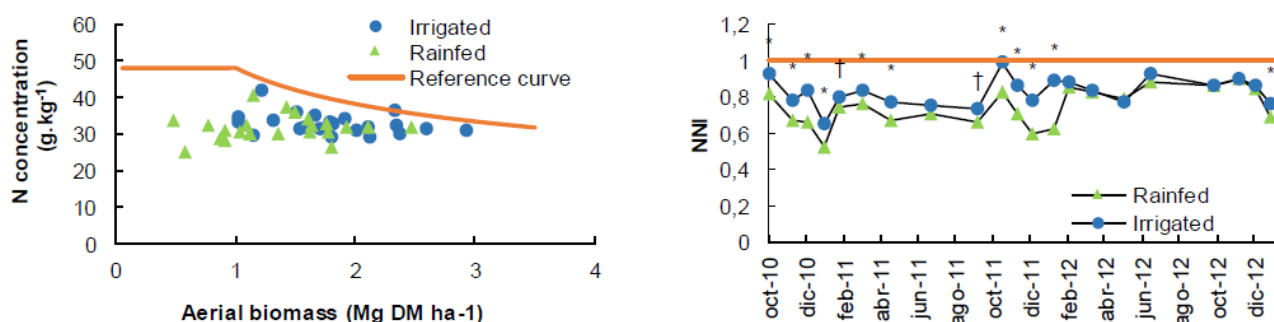


Figure 1.a). Alfalfa pasture nitrogen concentration as a function of aerial biomass for both treatments (rainfed: green triangles and irrigated: blue circles) in relation to the reference curve. b). Nitrogen Nutrition Index (NNI) for all cuttings and both treatments (rainfed: green triangles and irrigated: blue circles). \* Indicate significant difference ( $p < 0.05$ ) and † ( $p < 0.10$ ).

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## Neighbors and defoliation frequencies impact on forage quality and offer of base Lucerne crops (*Medicago sativa* L.)

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**KEYWORDS:** intra and interspecific competition; orchardgrass; leaf/stem ratio; flower buds

Light competition modifies the morphology of the plants and the associated biomass partition between metabolic-leaf-and structural-stem-compartments and with it, forage quality. *Medicago sativa* –Lucerne- intraspecific competition is usually intense. It is unknown if a neighbor grass - interspecific competition - could improve crop quality -the leaf/stem ratio (L/S)- associated with a lower intensity of light competition within the canopy. The aim of the present work was to evaluate the impact of different neighbors on Lucerne forage quality and offer, as a consequence of varying defoliation frequencies during crop establishment. The experiment was carried out in the experimental field of the School of Agronomy, University of Buenos Aires (FAUBA, 34° 35' 29" S, 58° 29' 00" W). The chemical analysis of the upper soil horizon indicated a pH (H<sub>2</sub>O: 1:2.5 in water) of 5.85, electrical conductivity of 0.20 dS m<sup>-1</sup> and total N (%) of 0.17. The content of extractable P showed a spatial distribution so the soil content was increased to 30 ppm. A split-plot factorial arrangement was established in a randomized complete block design (n=4) on March 17<sup>th</sup>. The main plot was the sowing arrangements -3 levels-; I) Lucerne (LUC; cv. Queen, dormancy group 9) in pure stand; sown with 200 viable seeds (vs) .m<sup>-2</sup>; control treatment, II) LUC with twice the density of I; 400 vs.m<sup>-2</sup> and III) mixed crop of LUC + *Dactylis glomerata* L. (orchardgrass) in alternate rows; 400 vs.m<sup>-2</sup> with 50% of each species. The sowing arrangement involves plots with additive + substitutive designs of LUC in order to differentiate the intra- and interspecific competition (Cruz & Soussana, 1997). Each main plot was divided into 2 levels; defoliation frequencies: low -10% flowering- vs. high-visible flower buds (Fick & Muller, 1989). All cuts were made at a height of 5 cm from ground level. Aerial biomass at 0.09 m<sup>2</sup> was harvested and then the rest of the plot was cut. The harvested material -forage offer or biomass- was separated into leaflets + petioles and stems, dried in a stove until constant dry weight and then, weighed. Analyses of variance, mean comparison test (Tukey) and preplanned contrast with a significant level of 5% were performed. We report the results of the first reproductive cut -475 degrees day (°Cd) vs. 287 °Cd- after the previous vegetative cut; October 17<sup>th</sup>- high and low frequency treatments, respectively. Surprisingly, the type of competition did not affect the L/S ratio of LUC (1.16 g.g.<sup>-1</sup>; p> 0.05). Low defoliation frequency decreased 38% the L/S ratio (p <0.05) associated with an increase of 180% in the stem forage offer. The defoliation frequency differentially affected the leaflets + petioles biomass and consequently, total aerial forage offer, depending on the species neighbor (p <0.05). In high frequency, interspecific competition decreased leaflet biomass by 143%, while intraspecific competition increased it by 70% with low frequency of defoliation (Figure 1).

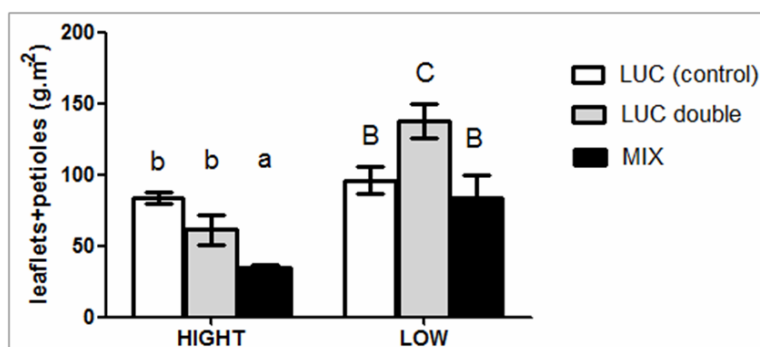


Figure 1: Biomass of leaflets and petioles for the three sowing arrangements under the two levels of defoliation frequencies. Significant interaction between factors: p <0.05; lowercase and uppercase letters indicate the contrast to the control treatment in high and low frequency, respectively.

Crop quality would be directly associated with stem biomass, mainly linked to defoliation frequency. Forage offer would be strongly related to the leaflets biomass; associated to neighbor or type of competition and defoliation frequencies. The consistency of these responses should be analyzed in the rest of the crop growing season.

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# Alfalfa (*Medicago sativa* L) fertilization with “CANPHOS” in the south of Santa Fe, Argentina

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**KEYWORDS:** Alfalfa - Maggiolo - Santa Fe – Fertilization

**INTRODUCTION:** The recomposition of the livestock prices, added to the elimination of exported beef retentions, has generated great development expectations in bovine meats production at low agricultural aptitude areas. In the south of Santa Fe (Arg), at the General Lopez department, INTA is carrying out an intensive bovine rearing project (CBI) of pastoral base with alfalfa's perennial pastures, which are the base of the system and the main component of the diet. The alfalfa's average annual production per ha is 13/14 tons of dry matter in crops without fertilization. The high requirement of nutrients in alfalfa shows, which in high production soil exposures inputs are low, and in the medium / long term the loss of chemical fertility will be very marked.

**MATERIALS AND METHODS:** An assay was made at the GAP establishment in the town of Maggiolo, in the south of Santa Fe, with CANPHOS organic fertilizer in the alfalfa crop. GPS location 33°44'06.3"S 62°14'23.6"W.

A group 9 alfalfa was shown on April 23th, 2015. A grid system was used at 90 degrees with 18 kg per ha of inoculated seed. The field was planted leaving two widths of seeder without CANPHOS. Also, FLUMETSULAM herbicide was used, dose: 600 ml per ha. CANPHOS was applied to the sowing of 200 kg per ha. The CANPHOS composition is N 1,48%, P<sub>2</sub>O<sub>5</sub> 5%, K<sub>2</sub>O 1,3%, SO<sub>3</sub> 30%, CaO 23%, Organic material 23,5%:

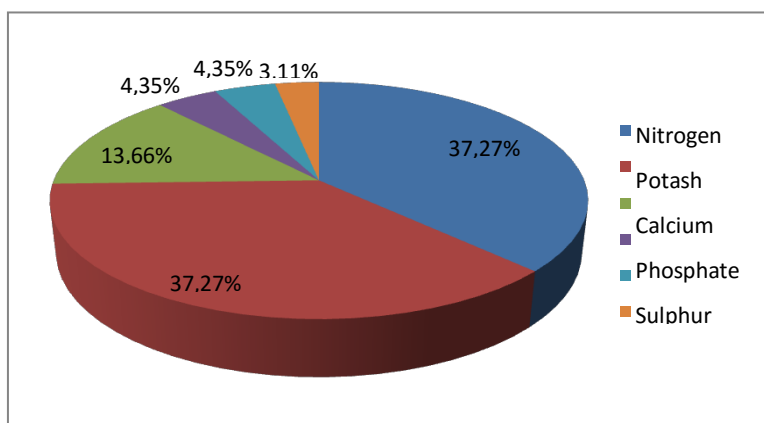


Table 1. Nutrients extraction expressed in kg.

ELEMENT	KILOGRAM
Nitrogen	300
Potash	300
Calcium	110
Phosphorus	35
Sulfur	35
Magnesium	25

Figure 1. Percentage of nutrients extraction based on a production of 10 tons of dry matter per hectare of alfalfa

The 3 nutrients that had most impact in forage production were: calcium, sulfur and phosphorus (Ca-S-P), remembering that alfalfa as a legume fixes nitrogen from the air in symbiosis with specific bacteria's.

**RESULTS AND DISCUSSION:** In the growth, plants were counted per square meter using a hoop, a better emergence was obtained in seedlings with a plus 15% rate in the fertilized part. At December 5<sup>th</sup>, the first cleaning cut was carried out; the forage was weighted per square meter with ring with a 35% difference at the fertilized sector. Afterwards, the crop's condition was inspected and controlled ocularly, the treated crop had a better sanitary behavior and recovery after cutting. Dry matter was evaluated by measuring green forage per square meter and theoretical humidity discount after making alfalfa rolls.

- Fertilized crop with CANPHOS 18,590 kg ha<sup>-1</sup>.
- Control crop 13,850 kg ha<sup>-1</sup>.

The next cleaning cut will be used to make alfalfa rolls.

In the south of Santa Fe, the analysis of soils shows marked deficiencies of Ca, P, S, and N. It is necessary to replace it with fertilizers; in addition, we find an answer to the Magnesium fertilization.

**CONCLUSIONS:** The fertilization with CANPHOS which includes organic matter, phosphorus, calcium, sulphur, potassium and nitrogen as well as organic matter's humic acids and trace elements is profitable, sustainable and recommendable for alfalfa-based pastures high productions in degraded soils.

# Digestible dry matter and crude protein of alfalfa (*Medicago sativa* L.) and double annual forage crops under irrigation condition in North Patagonia

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**KEYWORDS:** Digestible dry matter, crude protein, double annual forage crop, alfalfa, North Patagonia

**INTRODUCTION:** in a previous work (Colabelli et al., 2017) it was shown that the most productive combinations of the double annual crop of a summer (SFC) and winter (WFC) forage crop doubled the annual production of alfalfa forage, perennial pasture widely spread in the northpatagonian region. The objective of this work was to compare the same sequences and alfalfa in terms of digestible dry matter (DDM kg.ha<sup>-1</sup>) and crude protein (CP kg.ha<sup>-1</sup>).

**MATERIALS AND METHODS:** nine sequences resulting from the combination of 3 SFC (maize, sorghum and soybean) and 3 WFC (oat, barley and annual ryegrass), and alfalfa during three cycles (2013/14, 2014/15 and 2015/16) were evaluated. The experiment was carried out in EEA Valle Inferior del Río Negro (INTA). The SFC+WFC sequences were arranged in a split plot design (Main Plot= SFC, Subplots= WFC). All the crops were irrigated and fertilized so as not to limit their growth. In the annual crops, a single cut was made in the appropriate stage for silage. Alfalfa was cut at the beginning of flowering (4 cuts per cycle). In the 2015/16 cycle aliquots of forage were taken from all the crops to determine %CP by Kjeldahl method and %DDM by calculation  $(3.20-0.028*\% \text{ FDA}) / 3.62 * 100$ , Rohweder et al., 1978). The DDM kg.ha<sup>-1</sup> and CP kg.ha<sup>-1</sup> of each sequence were calculated by accumulated biomass\*%DDM or %CP, respectively. For alfalfa, DDM kg.ha<sup>-1</sup> and CP kg.ha<sup>-1</sup> were calculated by the sum of biomass accumulated of each 4 cut \* %DDM or %CP. The results were subjected to analysis of variance and test of significant minimum differences (LSD, p <0.05) with the statistical package InfoStat.

**RESULTS:** the DDM kg.ha<sup>-1</sup> of the maize sequences with oat or barley presented the best aptitude in terms of quantity and quality to produce silage. The sequence of sorghum with oat or barley presented intermediate values, offering an alternative for soils not suitable for maize; the sequences of soybean with oat or barley did not differ from alfalfa, while soybean-ryegrass had the lowest values. In terms of CP kg.ha<sup>-1</sup>, no annual double cropping sequence equaled the alfalfa crop (Table 1).

Table 1. Total Crude Protein (CP, kg.ha<sup>-1</sup>) and Total Digestible dry matter (DDM, kg.ha<sup>-1</sup>) of sequences of double annual forage crop, summer (SFC) and winter (WFC), and alfalfa.

CROP	CP SFC	CP WFC	Total CP	DDM SFC	DDM WFC	Total DDM
Maize-Oat	2400	1125	3525 b	17702	8365	26067 a
Maize-Barley	2400	1129	3529 b	17702	8699	26401 a
Maize-Ryegrass	2400	1251	3651 b	17702	6153	23855 ab
Sorghum-Oat	1311	1125	2436 c	13529	8365	21894 b
Sorghum-Barley	1311	1129	2439 c	13529	8699	22228 b
Sorghum-Ryegrass	1311	1251	2561 c	13529	6153	19681 c
Soybean-Oat	1039	1125	2164 d	3881	8365	12246 d
Soybean-Barley	1039	1129	2168 d	3881	8699	12580 d
Soybean-Ryegrass	1039	1251	2289 c	3881	6153	10033 e
Alfalfa			5282 a			14889 d

Means within columns and components with different letters differ at P<0.05.

**CONCLUSIONS:** for the conditions of the present work, alfalfa showed the highest CP kg.ha<sup>-1</sup> and lower production of DDM kg.ha<sup>-1</sup> than sequences of double annual forage crop which involve maize or sorghum. This allows to have alternatives that satisfy different objectives in the production systems.

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## Improving salinity tolerance of alfalfa (*Medicago sativa* L.) through conventional breeding

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**KEYWORDS:** Lucerne, salt-tolerant, phenotypic recurrent selection

A new cultivar with increased salinity tolerance was obtained from the INTA alfalfa genetic breeding program. The *Medicago sativa* germplasm (Synthetic Santiago=SISA 14) is a salt-tolerant synthetic population derived from three cycles of phenotypic recurrent selection under a semiarid saline natural condition at the Isla Verde, Santiago del Estero, Argentina (28°38'41.9"S, 64°05'03.8"W; soil taxonomy: typic Natracuall). The parental material is derived mainly from cv. Salado (fall dormancy=FD 9), with minor contributions from AZ-97MEC-ST and AZ-97MEC populations (FD 9). The best genotypes at each cycle were selected based on the ability to emerge, grow, and produce forage and seeds under a natural condition of medium to moderate high saline soil. The saline site where the seeds germinated and emerged was the same in the three selection cycles, having an electrical conductivity of saturated paste extract ( $EC_{ex\ 0-5cm}$ ) between 10.6 to 32.8 dS m<sup>-1</sup> and average pH of 7.74 (a minimum amount of water was applied to moisten the soil surface). Selection in cycle I (March 2007): 250 seedlings were selected after emergence. Each seedling was transplanted temporarily to an individual small bag. Later, each plant was transplanted directly to the soil in a nearby paddock ( $EC_{ex\ (0-30cm)}$  oscillated from 4.1 to 20.8 dS m<sup>-1</sup> between April to November, respectively; with increasing salinity in depth) within the same field with the proper isolation to preserve its genetic purity. Plants were grown and intercross until December. The seeds were harvested from selected plants for good vigor, large size, lack of foliar disease and bulked to form the first selection cycle. In March 2008, these seeds were sown in the germination site described before, and a new selection was done after seedling emergence. Thereafter, it followed the same procedure previously described. The site in where second intercross cycle was made had  $EC_{ex\ (0-30cm)}$  oscillated between a maximum of 32.2 in September 2008 to a minimum of 19 dS m<sup>-1</sup> in March 2009. Only 70 genotypes were selected to produce the intercrossing seeds. Seed harvests were done in December 2008 and March 2009 and bulked to form the second selection cycle. These seeds were sown in March of 2010 and 84 seedlings were selected and following the same procedure as before. The site in where third intercross cycle was made had  $EC_{ex\ (0-30\ cm)}$  14 dS m<sup>-1</sup> in average. Seed harvests were done in December 2010 and March 2011, and bulked to form the third selection cycle. These seeds becoming the *breeder* seeds or Syn-1. Intercross where made by native effective pollinators bees *Xylocopa* spp. As it was mentioned, the soil salinity is rarely spatially uniform in field conditions and each of the three cycles has been done under a different level of saline stress. Thus, a long-term study was carried out under controlled conditions of temperature, light and different concentrations and type of salts in the facilities of the USDA Salinity Laboratory, Riverside USA (2011-2012). The irrigation system and closed drainage utilized allow maintaining the levels of stress constant, which allowed lead a comparative evaluation of several alfalfa populations. SISA 14 (Syn-1) combined high tolerance (in relative terms) and higher biomass production (gr pl<sup>-1</sup>), explained in part by a lower concentration of Na<sup>+</sup> in its aerial biomass as one of the attributes associated with one of the mechanisms contributing to the tolerance in alfalfa (Cornacchione and Suarez, 2017). The Syn-1 seeds were sent to the EEA INTA San Juan, Argentina in the fall of 2011 and Syn-2 seed was obtained in April 2012. Subsequently, the Syn-2 was sent in April 2015 and the Syn-3 was obtained in 2016. Syn-2 were used to conformed (in Argentina) evaluation trials of agronomic behavior sowed on fall of 2015 and conducted for 3 seasons (2015/2016, 2016/2017 and 2017/2018) at two locations (EEA Manfredi, EEA Santiago del Estero) and one season at the EEA Rafaela (2015/2016). From Syn-3, the population was conducted as a synthetic cultivar.

**REFERENCE:** Cornacchione, M.V., and D.L. Suarez. 2017. Evaluation of alfalfa (*Medicago sativa* L.) populations response to salinity stress. Crop Sci. 57(1):137–150. doi:10.2135/cropsci2016.05.0371

## Physiological and biochemical responses of alfalfa (*Medicago sativa* L.) to salinity stress

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**KEYWORDS:** Lucerne, salinity, physiological parameters.

Alfalfa is the main forage in dairy cattle feed in the USA, and California is the main producer with almost 100% of the crop under irrigation. High cost of fresh water demands the use of recycled waters that may have high concentration of salts. Our group has reported alfalfa to be moderately-tolerant to salinity while maintaining its nutrient composition and antioxidant activity. In this research, we explored 15 nondormant alfalfa populations (experimental populations from INTA Argentina, and commercial cultivars from the United States), and evaluated the effect of salinity on physiological and biochemical parameters. Plants were grown in sand tanks in a greenhouse located in Riverside, CA (33°58'24" N, 117°19'12" W), and were harvested 10 times during 2011-2012. A randomized design split-plot arrangement was used with two water composition types (salts dominated by Cl<sup>-</sup> or SO<sub>4</sub><sup>2-</sup>) with five salinity levels (ECiw ranging from 0.85 to 24.5 dS m<sup>-1</sup>) as the main plot and 15 populations (P) as subplots. There were six replications (n=6 sand tanks) with three plants per population per tank (total of 2700 plants). Gas exchange, leaf area (LA), and chlorophyll content (Chl a+b, mg dm<sup>-2</sup>) were measured before the 3<sup>rd</sup> harvest at ECiw 0.85, 13 and 18.3 dS m<sup>-1</sup>. Leaf net photosynthetic rate (Pn, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), leaf stomatal conductance (gs, mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), and leaf transpiration (Tr, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were recorded using a portable Li-Cor 6400 Photosynthesis System. Chl was calculated from the equation of correlation between the concentrations of the samples collected from the same leaflet and the readings taken with a portable Minolta Chlorophyll Meter (SPAD-502). New samples were taken before the 8<sup>th</sup> harvest to estimate again the Chl and specific leaf weight (SLW) at all ECiw levels. SLW (g m<sup>-2</sup>) was estimated by the total leaf discs dry weight (g) / total discs area per m<sup>2</sup>. Shoot samples from the Cl-dominated water type were taken to analyze the hydrophilic oxygen radical absorbance capacity (ORAC, in µmoles TEg<sup>-1</sup> DM), and total phenolics (TP, in mg GAE g<sup>-1</sup> DM). Analysis of variance (ANOVA) and treatment comparisons were carried out using InfoStat (Di Rienzo *et al*, 2012). There was no difference (p>0.05) in Pn, gs, and Tr due to water salt type. There was a significant (p<0.01) interaction for ECiw x P for Pn rate. At each ECiw, there were significant differences in Pn among populations (p<0.01). Almost all populations showed a trend to increase Pn under salinity, compared to control. The gs was significantly reduced while Tr rate increased with increased salinity (ECiw p<0.05, ECiw x P p>0.05, and P p>0.05). There were significant differences for both LA and Chl content (both p<0.01) due to water salt type, showing higher LA and lower Chl when plants were irrigated with SO<sub>4</sub><sup>2-</sup> compared to Cl<sup>-</sup>-dominated water. Salinity significantly reduced LA (3.84a, 2.71b, and 2.14c cm<sup>2</sup>, for control ECiw, 13, and 18 dSm<sup>-1</sup>, respectively). The ECiw x P interaction was significant at p=0.05, however the significant difference for LA among populations was observed only for control plants. There was no significant (p>0.05) ECiw x P interaction for Chl or SLW. The Chl content (consistent for both sampling dates) and SLW significantly increased as salinity increased (p<0.01). There was difference among populations (p<0.05) in Chl and SLW. Some populations maintained the highest and the lowest Chl content in both sampling dates (eg, SISA14 and Cibola at the top and SISA 1, SISA 10, and SISA 11 at the end in rank comparisons). There was no significant (p>0.05) ECiw x P interaction for ORAC or TP in Cl-dominated water. In general, according to our data, there were changes in the physiological responses of alfalfa mainly associated to the different salt levels. A highly positive correlation was found between the average of SLW and Chl (y=0.1164x +0.9496, R<sup>2</sup>=0.9653), indicating that under salinity the leaves became thicker (with thicker palisade parenchymas) and could have more chloroplasts per unit of leaf area. Although the Pn increased due to salinity, the reduction in LA with increased salinity suggests that Pn per plant can be reduced under salinity being either "the cause or the effect" (Munns and Tester, 2008) of the decrease on biomass per plant as we showed previously for these populations (Cornacchione and Suarez, 2017). The lack of difference among populations in LA under salinity indicates that the adjustment to salinity occurred in all populations in the same way.

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# The establishment of *Medicago sativa* L. with *Hordeum vulgare* L. as a cover crop

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**KEYWORDS:** *Medicago sativa* L., *Hordeum vulgare* L., establishment, yield

**OBJECTIVES:** The aim of this study was to determine the possibility of alfalfa establishment with spring barley as a cover crop and to analyze the effect of such mixture on barley grain yield and alfalfa annual forage yield in the temperate region.

**MATERIALS AND METHODS:** The field trial was conducted in 2017 at the Center for Organic Production in Selenča, Serbia (45° 25' N, 19° 18' E, 94 m a.s.l.). The climate is characterized as moderate continental with the extreme seasonal variation of temperature and precipitation with the mean annual temperature of 11.4 °C and total annual precipitation of 578 mm. The trial was performed in rain-fed conditions on chernozem, medium deep form and calcareous, gleyed soil (pH-7.45; CaCO<sub>3</sub>-2.53%; OM-2.74%; N-0.14%; P-4.58%). Seeding plot was 15 m<sup>2</sup>. The trial was set up as a random block design with four replicates and included alfalfa (cv. NS-Mediana ZMS V) pure stand (100% - 15 kg ha<sup>-1</sup>) and a mixture of alfalfa and spring barley (cv. NS Marko) in the 100:75, 100:50 and 100:25 seeding ratio of the full seeding rate (240 kg ha<sup>-1</sup>). Seeding was done in April 2017 by a hand driller. In mixed stands, barley was firstly sown at the 25 cm row distance and after that alfalfa was sown between barley rows at the final row spacing of 12.5 cm. The barley grain yield was determined in the harvest maturity and alfalfa dry matter yield was evaluated in the second and the third cut. Differences between the treatments were tested by ANOVA, means were separated by Duncan's multiple range tests and statistical significance was evaluated at p ≤ 0.05. Means of each cut followed by the same letter are not significantly different from each other at p ≤ 0.05.

**RESULTS:** The barley grain yield ranged from 2.8 t ha<sup>-1</sup> in the mixture with 25% of barley to 4.1 t ha<sup>-1</sup> in the mixture with 75% of barley (Figure 1). In the second and third cut alfalfa pure stand had significantly higher yield compared to mixtures (Table 1). Between the mixtures with 75% and 50% of barley there was no significant difference in alfalfa total dry matter yield, and the highest was obtained with the lowest barley ratio in the mixture.

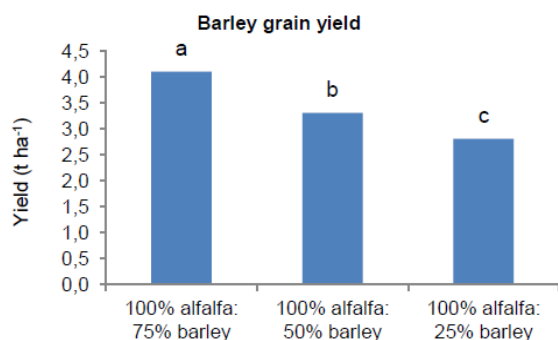


Figure 1. The effect of the mixture of alfalfa and different barley proportion on barley grain yield (t ha<sup>-1</sup>)

Table 1. The effect of the mixture of alfalfa and different barley proportion on the alfalfa dry matter yield (t ha<sup>-1</sup>) in the second and third cut of the establishment year

Treatment	Seed ratios	Alfalfa dry matter yield (t ha <sup>-1</sup> )		
		II cut	III cut	Total
Alfalfa	100	1.7 a	3.3 a	5.0 a
Alfalfa:barley	100:25	1.3 b	2.5 b	3.8 b
Alfalfa:barley	100:50	1.0 c	2.1 c	3.1 c
Alfalfa:barley	100:75	1.0 c	1.9 c	2.9 c

**CONCLUSIONS:** The results indicate that alfalfa could be successfully established with grain barley as a cover crop in the areas with similar soil and climate conditions as this research. Such kind of intercropping offers both stable forage yield of alfalfa in the establishment year as well as grain barley yield. In order to obtain an adequate re- growth and yield of alfalfa, it is recommended to use the lowest seed ratio of cover crop (25%), having in mind that this combination enables satisfying barley grain yield (more than 2.5 t ha<sup>-1</sup>), and high productivity of the perennial with the lowest cover crop seeding costs.

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# Forage production of Alfalfa (*Medicago sativa* L.) with different densities of sowing under irrigation in the North Patagonia

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**KEYWORDS:** Valle Inferior, río Negro, dry matter, seedlings.

**INTRODUCTION:** in the production systems of the irrigated valleys of the Negro river, alfalfa is a fundamental component in the forage chain due to its potential high yield and quality per unit area. This aptitude is considered both for direct grazing and for the preparation of reserves to be used in times of lower forage supply (autumn- winter). To achieve high yields and persistence over time, aspects such as planting density are important because they determine the initial stand of the pasture.

**OBJETIVE:** the objective of the present work was to compare forage production using different sowing densities and varieties.

**MATERIALS AND Y METHODS:** the experiment was sowed on april 8<sup>th</sup>, 2008 in the EEA Valle Inferior del río Negro (40° 48' S; 65° 05' W), on a Vertisol soil series "Chacra" (pH: 7,5; P asimilable (ppm): 24; MO: 5%). Three varieties (V) of alfalfa were evaluated: G 969, Super Monarca INTA and Trinidad 87 of use fall dormancy 8-9 combined with four sowing densities (D) 10, 15, 20 and 25 kg / ha of viable seeds inoculated. A split-plot design with randomized blocks with four repetitions was used; where V were the main plots and D the sub-plots. Their size was 2.10 m<sup>2</sup>, in 6 rows at 17.5 cm between rows. The cycles evaluated were 2008/09 (C1) and 2009/10 (C2). It was fertilized when sowed with 100 kg / ha of mono ammonium phosphate. The accumulated precipitations during C1 and C2 were 195 and 281 mm respectively, while the water applied by irrigation for those cycles was 1,300 and 1,100 mm. Sixty days after sowing, a seedling count was carried out to determine the initial stand in each treatment (Figure 1). The accumulated forage production of C1 and C2 was evaluated where 5 and 4 harvests were made respectively. The harvests were made in 1,60 m<sup>2</sup> with a lawnmower at 5 cm height when the crop reached 10% flowering or 3 cm of basal regrowth. Aliquots of forage were taken to determine dry matter (DM) content. On this information, ANOVA and a significant minimum differences test (LSD, p <0,05) were performed.

**RESULTS AND DISCUSSION:** only in C1 interaction was found between V \* D (p=0,0055), where it was observed that D20 increased production between 11 and 33% (Figure 2). In C2, there was no interaction between the D\*V factors,

although there were differences between V (Fig. 3).

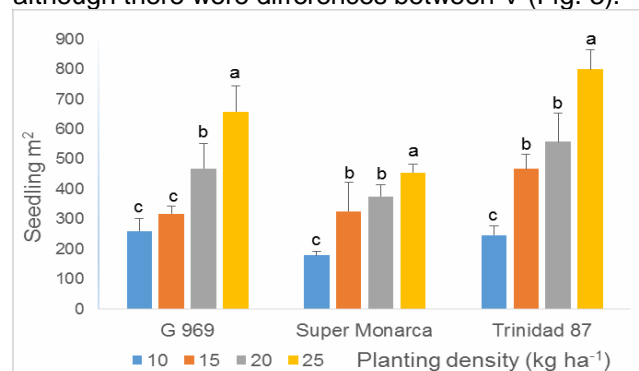


Figure 1. Number of seedlings m<sup>2</sup>. Different letters indicate significant differences (p<0,05).

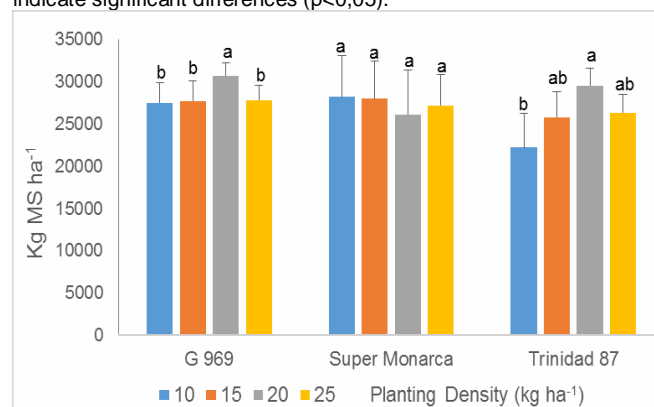


Figure 2. Accumulated forage production of C1 according to D (kg MS ha<sup>-1</sup>). Different letters indicate significant differences (p<0,05).

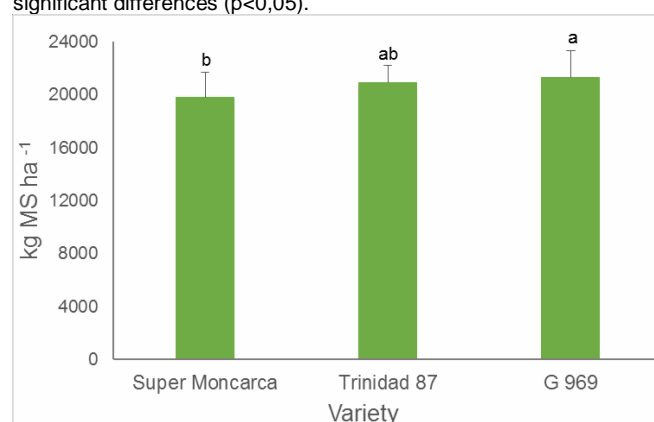


Figure 3. Accumulated forage production of C2 (kg MS ha<sup>-1</sup>). Different letters indicate significant differences (p<0,05).

**CONCLUSION:** although the number of established seedlings is higher when D increases, forage production only increases in the first cycle and stabilizes during the second cycle for the evaluated densities.



# Productive behavior of alfalfa (*Medicago sativa* L.) in Argentina different regions: cultivars evaluation networks

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**KEYWORDS:** alfalfa, varieties, localities, production, forage.

**OBJECTIVE:** to compare the forage production, persistence, and period of use of non-dormant alfalfa cultivars in different argentine environments.

**MATERIALS AND METHODS:** the study was performed using information from the INTA's Cultivar Evaluation Network (RECA) published by Spada (2011; 2012; 2013 and 2014), referred to dry matter production (DM), persistence (P), and duration of the period of use. Thirty-two cultivars were evaluated during 4 growing seasons from 2010/11 to 2013/14 in the localities: Santiago del Estero (SdE), Reconquista (Rec), Rafaela (Raf), Manfredi (Man), Marcos Juárez (MaJ), Paraná (Par), Concepción del Uruguay (CdU), Gral. Villegas (GVi), Bordenave (Bor), Hilario Ascasubi (HiA), Villa Mercedes (ViM), Anguil (Ang) y Viedma (Vie). All localities followed the same protocol: the trials were planted in autumn of 2010, the cultivars were sown in 5 m<sup>2</sup> plots, with spacing rows at 0.20 m. Plots were arranged in a complete randomized block design with four replications. The trials were performed in rainfed conditions except at SdE, HiA and Vie where gravitational irrigation where applied. Forage production was determined by cutting with a motor mower and all forage produced in each plot were weighted. The cuts were made at 10% flowering or when the crown sprouts reached 5 cm. Results of production were expressed as t DM ha<sup>-1</sup>. The persistence (%) of the crop was calculated by: 100 - [(initial coverage - final coverage)/ initial coverage] \* 100. The duration of the period of use (days year<sup>-1</sup>) in each locality was calculated as the average of the period that extends between the date of the first and the last cut of the growing seasons. The accumulated forage production of the 4 growing seasons, persistence and period of use were analyzed by main components analysis (PCA) and a Biplot diagram were carried out in order to describe the environmental similarities respect to the variables.

**RESULTS:** CP1 represents 51% of the total similarity and is associated with forage yield and harvesting period while CP2 (32%) is associated with persistence (Figure 1). The accumulated forage was different among localities (Table 1). The environmental controls (such as temperature, radiation, evapotranspiration and soil field capacity -data not shown) plus the total annual precipitation and their distribution during of the growth period, explain the annual growth of each locality ranging between 22,1 (MaJ, with also phreatic water supply) and 5,8 t DM ha<sup>-1</sup> Yr<sup>-1</sup> (Ang). The irrigation in those localities as SdE, Vie and HiA determine the high DM production ranging between 22,4 and 17,8 t DM ha<sup>-1</sup> Yr<sup>-1</sup> Excessive precipitation occurred during the wet period in the Rec and CdU localities played in detrimental of the DM production. For different reasons, some trials were comparatively less long-lasting (Man, VM, Bor, CdU, and Ang).

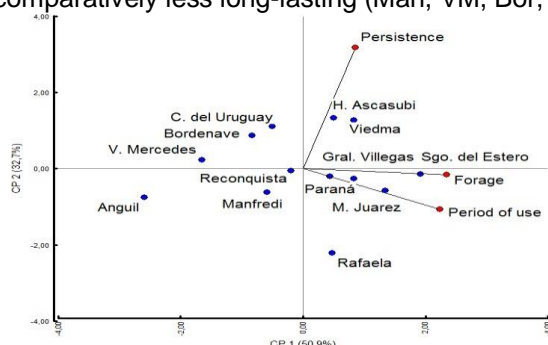


Figure 1. Main components analysis for accumulated forage yield, persistence and period of use for all localities.

**CONCLUSIONS:** RECA from INTA provide sufficient data every year to facilitate to the producers to select the best-adapted varieties based on the productive performance in one specific environment.

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Table 1. Annual precipitation, Accumulated forage yield, persistence (P) and period of use of the studied localities (2010-2014). (SD: standard deviation)

Localities	Annual precipitation mm year <sup>-1</sup> (SD)	Accumulated Forage t DM ha <sup>-1</sup>	P %	Period of use days
Sgo. del Estero*	554 (157)	89,7	75	281
Viedma*	228 (91)	75,9	96	194
H. Ascasubi*	416 (134)	71,2	95	177
M. Juárez	860 (217)	88,5	60	248
Gral. Villegas	681 (122)	73,0	65	232
Rafaela	1002 (187)	57,3	24	276
Paraná	983 (183)	50,8	67	244
Manfredi	749 (90)	30,1**	53	214
V. Mercedes	540 (56)	29,2**	60	116
Bordenave	506 (48)	21,1**	83	182
Reconquista	1230 (193)	19,0	71	254
C. del Uruguay	863 (94)	18,8**	92	207
Anguil	543 (77)	17,5**	33	87

\* With irrigation

\*\* Accumulated forage during 3 growing seasons (idem average)

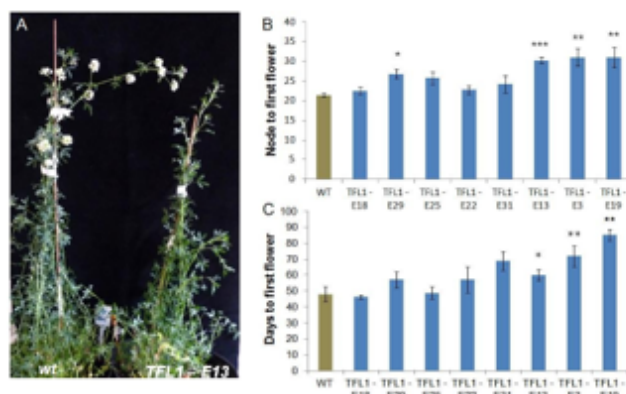
# Overexpression of *TERMINAL FLOWER 1 (TFL1)* genes in *Medicago sativa* leads to delayed flowering and modified plant architecture

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**KEYWORDS:** Alfalfa, late flowering, *msTFL1*

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops worldwide. This is mainly due to several traits such as its adaptability to varying external conditions, its excellent high-quality forage yield and its capacity to fix nitrogen. Alfalfa constantly increases in yield during spring and until summer's onset and reaches its peak when alfalfa plants start to flower. From this point onwards, the quality of the forage decreases due to morphological changes, such as an increase in the proportion of stems and a reallocation of photosynthetic resources to the development of reproductive structures. Therefore, this decrease in quality represents a considerable loss of nutritive value. In alfalfa, flowering onset depends mainly on day-length and temperature. It has been studied that members of the *TERMINAL FLOWER 1 (TFL1)* family of genes play a major role as prominent flowering repressors in several plant species. The main objectives of this study involved the identification of *TFL1* orthologues genes in alfalfa (*msTFL1s*) and the development of transgenic alfalfa lines overexpressing *msTFL1s* in order to delay flowering, extend the vegetative phase and therefore increase biomass yield without compromising the nutritive values.



**Figure 1:** Late flowering transgenic plants overexpressing *msTFL1*. A) Phenotype of transgenic plant late flowering compared to a WT. B) Flowering time measured as Total nodes to first flower and C) Total days to first flower

Asterisks represent different levels of significance ( $p < 0.05 = *$ ,  $p < 0.01 = **$ ,  $p < 0.001 = ***$ ) ( $n = 5$ ).

**Table 1:** Plant architecture parameters measured for 5 transgenic lines overexpressing *msTFL1*.

Table 1	Parameters		
Lines	Height	Branch N°	Height/Node
WT	65.13	8.667	2.715
E3	41.83***	11.33*	1.88***
E13	83.38*	8.571	2.9
E19	66.6	8.75	2.642
E25	74.56	8.444	3.013
E29	62.82	8	2.876

Asterisks represent different levels of significance ( $p < 0.05 = *$ ,  $p < 0.001 = ***$ ) ( $n = 7$ ).

We identified a *msTFL1* like gene (*msTFL1L*) with roles in flowering repression and vegetative phase extension. *msTFL1L* was overexpressed in alfalfa under the 35S promoter and 8 transgenic lines were obtained and then tested in growth chambers under inductive flowering conditions (24°, 16:8 photoperiod). Results were analyzed by a one-way ANOVA with posterior Tukey test. Out of the 8 lines, E29, E13, E3 and E19 showed significant delayed flowering times (measured as both nodes to flower and days to flower) when compared to their WT lines regenerated from calli (Fig 1). Additionally, lines E13 and E3 (the latest lines to flower) showed an altered plant height (Table 1), and particularly line E3 showed an increase in the number of lateral branches and a reduced height/node relation.

These results show that *msTFLs* genes have conserved roles in alfalfa as flowering repressors and that their use as transgenic tools can lead to later flowering times and modified plant architecture. This could possibly translate to an increase in alfalfa yield and quality.

## Physiological and productivity trait estimation in alfalfa (*Medicago sativa* L.) from VIS-NIR reflectance data

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**KEYWORDS:** isotope composition, spectral reflectance index, multivariate regression

**INTRODUCTION:** In rainfed Mediterranean environments of Central Chile, alfalfa cultivars from Australia and USA have exhibited high productivity and allowed to extend the growing season into early summer and autumn (del Pozo et al., 2017). The selection of genotypes of high yield potential and persistence after long period of drought (4-6 months) is a major objective for alfalfa breeding programs in Mediterranean environments. Remote sensing could help to evaluate physiological and productivity traits in alfalfa germoplasm under water deficit conditions and facilitate and speed-up alfalfa breeding programs. Spectral reflectance or the spectrum of energy reflected by the plant is closely associated with absorption at certain wavelengths that are linked to specific characteristics or plant conditions. The spectral VIS-NIR (350–2,500 nm) signature can be related to physiological and agronomic traits using spectral reflectance indices (SRIs) and multivariate regression models (Garriga et al., 2017).

**OBJECTIVES:** the aim of this work was to assess the feasibility of using spectral reflectance data to estimate physiological and productivity traits in alfalfa under rainfed conditions.

**MATERIALS AND METHODS:** A set of nine varieties of alfalfa from Australia and USA were assessed in four locations in the Mediterranean central-south Chile, between 2012 and 2014. The four sites differ in their Mediterranean type climate, annual rainfall and soil type. At each season several physiological and productivity traits were evaluated. Some of them were assessed at different times during the growing season such as dry matter (DM), stomatal conductance (gs), water potential (WP) and leaf area index (LAI), and others one time, at the end of the growing season (December-January), such as nitrogen content (N) and isotope composition ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) of the dry matter. The spectral reflectance assessments were carried out three times along the experiments, October 2013 and at the end of the growing seasons 2013 and 2014, using a FieldSpec 3 (ASD, Boulder, CO, USA) spectroradiometer. Spectral data was used to estimate the traits assessed at these three times, through SRI and four multivariate regression methods: Principal Components Regression (PCR), Partial Least Square Regression (PLSR), Ridge Regression (RR), and Support Vector Machine Regression (SVR). All models were validated by 10-fold cross-validation (10xCV) and their performances evaluated by the coefficient of determination ( $R^2$ ).

**RESULTS:** The estimation of each trait was variable between assessment times regardless of the prediction method. For the SRI-based estimations, the  $R^2$  values for each assessment time were lower than 0.65. However, in traits like LAI, WP, gs and N the  $R^2$  values were higher when the data of the different assessments were combined. In general, regression-based estimation showed similar or higher power prediction (coefficient of determination) than SRI-based ones. The highest  $R^2$  values were for  $\delta^{13}\text{C}$  (0.78 and 0.65 in 2013 and 2014, respectively), but for most traits, the combination of data from different assessments led to higher trait estimation; for examples, the  $R^2$  values for LAI, DM, WP and gs were 0.67, 0.75, 0.63 and 0.85, respectively. Among regression methods, the best estimation were achieved by using SVR and to a lesser extent with RR.

**CONCLUSIONS:** Although more studies are needed, the use of spectral reflectance data collected at field level and multivariate regression models has a great potential to estimate physiological and productivity traits in alfalfa under water deficit and could be useful in alfalfa breeding programs.

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## Improving alfalfa (*Medicago sativa* L.) cultivar selection by GIS Mapping of fall dormancy and winter survival index classes and modeling seasonal and annual yield

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**KEYWORDS:** Lucerne, fall dormancy, winter survival index, GIS

**INTRODUCTION:** There are hundreds of alfalfa cultivars within 11 fall dormancy (FD) and 6 winter survival index (WSI) classifications. Currently, cultivar selection is sub-optimal due to the inability to match cultivar characteristics with planting site conditions. This project is quantifying climatic and soil conditions, FD and WSI requirements, and using GIS tools to map parameterized functions and crop modeling to predict yield.

**OBJECTIVES:** (1) To improve cultivar selection through matching location climatic and soil conditions with cultivar FD and WSI classes. (2) To improve potential yield prediction through crop simulation modeling.

### **MATERIALS AND METHODS**

- Assemble existing agro-ecological/alfalfa zone maps from scientific literature and seed companies.
- Review yield data and expert recommendations from field trial data in each alfalfa production zone.
- Create logistic response functions for T-min and T-max parameterized for each cultivar class.
- Develop suitability maps using GIS layers and response functions and validate in each growing zone.
- Develop seasonal and annual yield maps from APSIMX-Lucerne crop model and verify from yield data.
- Create extension and journal manuscripts and web-based materials for cultivar selection.
- Conduct professional development workshops for outreach personnel.

### **RESULTS**

- Collaborators identified for USA, PRC, New Zealand, and Australia.
- Project planning sessions held at national and international forage meetings.
- Quantitative tolerances developed and mapped for example FD/WSI class.
- Logistic functions parameterized for 8 clover species demonstrated the improved approach to be used.
- Prototype selection process flowchart and web application developed.
- APSIMX-Lucerne crop simulation model shows good agreement between predicted and observed values.

### **CONCLUSIONS**

This project will: (1) connect alfalfa scientists and seed industry specialists in several countries leading to faster, more efficient research progress; (2) create a quantitative database of alfalfa cultivars that will assist alfalfa research projects; (3) improve alfalfa cultivar selection leading to higher yielding, more persistent stands and increased profitability; (4) demonstrate integration of research tools (crop simulation modeling and GIS), and web-based information delivery.

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## Role of private sector in alfalfa (*Medicago sativa* L.) production and promotion in Punjab, Pakistan

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**KEYWORDS:** Biotrack Enterprises; Alfalfa Hay; Farmer training; Organic manure

The rural economy system in Pakistan especially Punjab province mainly depends on livestock sector that is the vital sub-sector of Agriculture, contributing 11.8% to GDP which is 56.3% of the Agriculture share to GDP. It is predicted that milk and meat consumption will grow at 2.8% per annum in developing countries like Pakistan. The ever-increasing human population in Pakistan demands more milk and meat production to meet the nutritional requirements. At present total livestock is 182.2 million heads and is increasing @ 3% per annum for the last three years. Milk and meat production of livestock in Pakistan is very low compared to those in developed countries. The main reason is that available fodder for livestock does not meet the nutritional requirements allowing them to express full genetic potential. Land available for fodder production is decreasing @ 2% per decade, therefore, there is tremendous pressure of livestock on available total feed and fodder. Furthermore, there are two major fodder scarcity periods i.e. May-June and November-December that sometimes prolonged to January due to heavy frost. Acute shortage of fodder during these periods severely affects milk yield and animal health. Traditional fodders do not provide enough nutrition to the dairy animals for either milk production to their capacity or sustain a good health. In this situation alfalfa may be a better option to provide nutritious fodder throughout the year. Alfalfa is being cultivated in Pakistan for green fodder as well as for hay & haylage purpose. Low germination, and slow initial growth of alfalfa is a problem due to various soil related problems in most of the alfalfa growing areas in Punjab. Biotrack Enterprises is focused to sustain role as a Leader in the market by introducing new technology and services to enhance per acre yield of crops especially alfalfa at lowest cost of production keeping in view the international standards and demands. The motto of the company is capacity building of the farmers for profitable farming at their doorsteps. The company deals with pesticides, seeds, and fertilizers, along with growing alfalfa for hay and haylage purpose. The Biotrack company has also introduced an organic manure with the trade name of 'Bionic' that improves the soil properties helping the enhanced germination and early growth establishing a good crop stand. We are harvesting up to 8-9 cuts of alfalfa in a season that are used for making hay and haylage. The company also offers trainings to the farmers regarding best practices for growing alfalfa and making hay and haylage without minimum loss of nutritional quality. It is believed that with the growing interest of private sector in growing alfalfa will improve the fodder production in the country leading towards a better feed for livestock enhancing production of daily products.



# Tetraploid annual medics (*Medicago truncatula*) offer improved growth rates for climate change adaptation in Mediterranean environments

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**KEYWORDS:** lucerne, alfalfa, pasture

**INTRODUCTION:** In the next few decades it is anticipated that climate change will result in higher temperatures and more variable rainfall. The whole crop production system will be challenged in Mediterranean environments, with less and more variable rainfall impacting on yield, risk and profitability. Later seasonal breaking rains will force germination of annual pastures into cooler winter months with shorter day lengths. As a consequence, improved seedling vigour and winter production will be important adaptive traits for these changing environments.

**OBJECTIVE:** To evaluate the potential of tetraploid annual barrel medic (*Medicago truncatula*) lines for climate adaptation traits in Mediterranean environments.

**MATERIALS AND METHODS:** Tetraploid annual plants (Tet-BM) were produced from diploid barrel medic cv. Sultan SU by submersing the cotyledons in 0.075% colchicine. Ploidy of experimental lines and controls was confirmed using squash technique (Dubrovsky & Contreras-Burciaga 1998). Experimental entries were evaluated in 2018 at Adelaide, Australia (-34.9670° S, 138.6360° E) on a loam soil with pH<sub>Ca</sub> 6.0. A partially replicated field design was employed, where cv. Sultan SU was replicated six times, 5 Tet-BM lines were replicated 3 times, and a further 12 Tet-BM lines were un-replicated. Plots were 1m<sup>2</sup> with 24 plants in six columns and four rows. Growth rates were estimated using development of green canopy cover with Canopeo phone application (V2.0 Oklahoma State University). The youngest fully emerged leaf from four plants in each plot were scanned and analysed with Image J software (Rasband 2018) to estimate average leaf size for each experimental unit (plot). Means of fixed effects for green canopy cover and leaf size were calculated using spatial linear mixed models, performed by GenStat 18.

**RESULTS:** The Tet-BM plants had greater green canopy cover development and 2.3x the leaf area of their diploid parents when grown under field conditions in Adelaide, Australia (Figures 1 and 2).

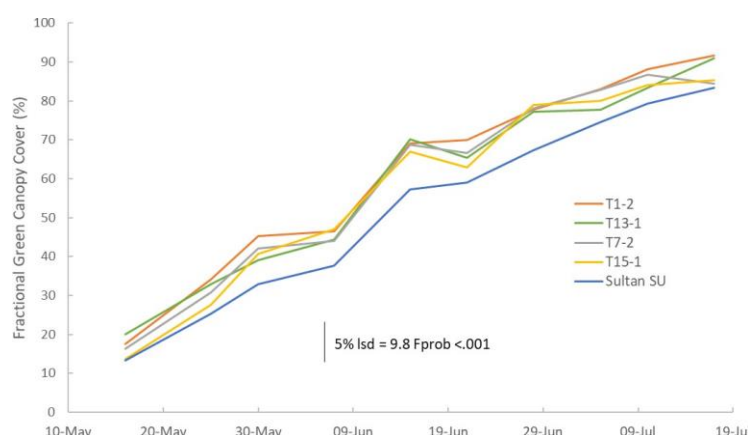
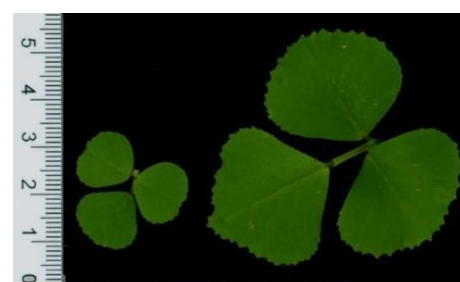


Figure 1. Tetraploid annual medics have quicker increases in Green Canopy Cover over time and larger leaves than their diploid parents.



Leaf Area (mm <sup>2</sup> /leaf)	30-May	10-July
Sultan SU	1002 <sub>bcd</sub>	1122 <sub>abc</sub>
Tet-BM7-2	1270 <sub>ab</sub>	1455 <sub>a</sub>
Tet-BM13-1	869 <sub>cde</sub>	961 <sub>cd</sub>
Tet-BM15-1	929 <sub>cd</sub>	1425 <sub>a</sub>

Figure 2. Leaf size of diploid parent Sultan SU (left) compared to tetraploid line Tet-BM7-2 (right). Table shows differences in leaf area between Sultan SU and four tetraploid BM lines with 5% LSD of 354 and var. date Pvalue 0.015.

**CONCLUSIONS:** Tetraploid annual medics have greater increases in canopy cover and larger leaves in comparison to their diploid barrel medic parent cv. Sultan SU. Further work will investigate their herbage and seed yield, and their breakdown pattern of hard seededness. Improved plant development rates and leaf size are two important traits to reduce the negative impacts of climate change in Mediterranean environments.

**ACKNOWLEDGEMENTS:** The authors would like to acknowledge support from the AW Howard Memorial Trust Fund and the Crop Trust. This work is part of an honours thesis by Lauren Innes at the University of Adelaide.



# RGB-images derived vegetation indices for estimating alfalfa dry matter production in rainfed Mediterranean environments

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**KEYWORDS:** diversity panel, broad sense heritability, phenotypic correlation

**INTRODUCTION:** Dry matter (DM) production is the most important agronomic trait in perennial forage species like alfalfa. Multiple trials and seasons are required by breeders to obtain reliable estimates of DM production. In earlier stage of a breeding program, visual scores are frequently used. Today, digital image analyses offer high-throughput and reliable techniques for plant phenotyping. The objective of this work was to evaluate, in an alfalfa diversity panel, the phenotypic relationship among some vegetation indices (VIs), obtained with a conventional RGB-digital camera, and DM production.

**MATERIALS AND METHODS:** Sixty three alfalfa populations provided by the Global Crop Diversity Trust Program (ID GS15014) were planted in 1x2.5 m plots. Seeds were germinated in germination trays and plantlets were established in 20 cm spaced rows every 10 cm. Alfalfa populations are originally from 16 countries across the globe and they belong to the complex *sativa* (62%), *varia* (35%) and *coerulea* (3%). The alfalfa diversity panel includes landrace, cultivars and advanced genetics lines. Two experiments were established in Cauquenes Research Station of INIA-Chile (S35°57', W72°19'). Experiments were managed under irrigated and rainfed conditions. The environmental conditions were mostly influenced by the Mediterranean climate (523 mm rainfall) and the Alfisol (2.7% organic matter). Both experiments were arranged in a RCB experimental design with four replicates. Dry matter production was measured in two harvest during the first growing season. One day before the biomass harvest, digital images were taken with a conventional RGB-digital camera (Canon EOS Rebel t5i). Image analyses were performed with the software BreedPix (Casadesús et al., 2007) and vegetation indices (VIs) were calculated. The VIs are based on either the average color of the entire image, in diverse units related to its "greenness" (HUE, Intensity, Lab and Luv), or on the fraction of pixels classified as green canopy relative to the total number of pixels of the image (GA and GGA; Table 1). A phenotypic linear mixed model was implemented to estimate the best linear unbiased prediction (BLUP) using the restricted maximum likelihood method within the ASReml-R package in R software. Phenotypic correlation between DM production and VIs were calculated with corplot-R package.

**RESULTS:** Most VIs exhibited a significant association with DM production ( $P < 0.05$ ; Table 1). The Lab-a index showed the highest  $r$  value. Additionally, some greenness related VIs exhibited a medium-high genetic control with  $H^2$  values around 0.60.

**Table 1.** Broad sense heritability ( $H^2$ ) and correlation coefficient (DM-r) between dry matter production and some vegetation indices calculated in an alfalfa diversity panel grown in a Mediterranean environment.

Vegetation índices	DM-r coefficient	$H^2$
HUE	0.58***	0.63±0.09
Intensity	-0.40**	0.18±0.20
Lab-a	-0.62***	0.41±0.05
Lab-b	0.56***	0.67±0.06
Luv-u	-0.38**	0.29±0.17
Luv-v	0.55***	0.57±0.10
Green area (GA)	0.36**	0.54±0.11
Greenest area (GGA)	ns	0.26±0.18
DM production	-	0.29±0.06

\*, \*\*, \*\*\* for  $P < 0.05$ , 0.01, 0.001; ns: no significant

**CONCLUSIONS:** Preliminary results allow us to conclude that VIs are phenotypically associated to the expression of DM production. Furthermore, some of them exhibited higher genetic control than DM production.

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## Effects of potassium, cultivar, and harvest time on sustainable alfalfa production

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**KEYWORDS:** Low lignin alfalfa, optimum growth, nutritive value, potassium requirement, stand longevity

**INTRODUCTION:** Alfalfa (*Medicago sativa* L.) is the most important perennial forage crop grown in the U.S. and many parts of the world. Alfalfa production is heavily reliant on soil nutrients. Continuous production of crops has resulted in depletion of most soil nutrient reserves. These nutrient deficits must be met through fertilization. For increased production of alfalfa, it is often recommended to use potassium (K) and phosphorus (P) fertilizers. In alfalfa production, K is a key element and removed in high quantities with hay harvesting. Many studies suggested that fertilizing alfalfa stands with K and P improved yield and increased stand longevity. However, whether fertilizing alfalfa with K in an un-limiting or limiting soil will result in any yield increase is elusive. Our knowledge on the effects of application of K to soils with adequate residual amounts and soils with declining K is still rudimentary. Recent reports suggest that median K levels have declined in many states of USA.

**OBJECTIVES:** Determine the effects of K, cultivar, and harvest time on productivity of alfalfa.

**MATERIALS AND METHODS:** The study was laid out in a randomized complete block design with four replicates at the University of Wyoming Sustainable Agriculture Research and Extension Center, near Lingle, Wyoming, USA (42°14'N, 104°30'W; 1272 masl) under irrigation. There were three factors in the study namely cultivar of alfalfa, K rate, and harvest time. Cultivars included Hi-Gest 360 (low lignin alfalfa) and AFX 457 (conventional alfalfa). There were four rates of K: 0 (control), 56, 112, and 168 kg K<sub>2</sub>O ha<sup>-1</sup>. The source of K was muriate of potash (50% K<sub>2</sub>O). Harvesting treatments included harvest at optimum growth stage (late bud to 10% bloom) and 7 days after the first harvest. A clean weed free land was prepared in early September of 2016 during which P was incorporated as a blanket dose of 84 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as triple super phosphate (46% P<sub>2</sub>O<sub>5</sub>). Potassium fertilizer was applied and thereafter alfalfa seeds (22 kg pure live seeds ha<sup>-1</sup>) were planted using a research grade Hege cone planter on September 8, 2016. Four harvests in 30 days interval (June to October) were made under each harvest time in 2017. Data collection included dry matter yield, nutritive value (e.g., crude protein, acid detergent fiber, neutral detergent fiber, in vitro dry matter digestibility, relative feed value), and K uptake. Forage samples were oven dried at 60°C for 72 hours to determine forage yield. Forage nutritive value was determined using near infrared reflectance spectroscopy. Data was analyzed by SAS Proc MIXED and mean separation was done using Least Significant Difference at 0.05 level of significance.

**RESULTS:** No visible K deficiency symptoms were observed in alfalfa. Results showed that K affected ( $P < 0.05$ ) forage production in alfalfa cultivars. For example, Hi-Gest 360 and AFX 457 produced the highest total forage yield at 168 (8372 kg ha<sup>-1</sup>) and 112 (8283 kg ha<sup>-1</sup>) kg K<sub>2</sub>O ha<sup>-1</sup>, respectively. This indicates that a moderate level of K is needed for high yield of AFX 457, while a high level of K is needed for similar yield of Hi-Gest 360. Late harvest time had higher forage yield (2260 kg ha<sup>-1</sup>) than early harvest time (2058 kg ha<sup>-1</sup>). Nutritive value was not affected ( $P > 0.05$ ) by the application of K, however cultivar influenced the nutritive value (e.g., crude protein, 277 and 267 g kg<sup>-1</sup> for Hi-Gest 360 and AFX 457, respectively). Potassium uptake followed a similar trend as dry matter production (87 vs. 81 g kg<sup>-1</sup>, late harvest vs. early harvest).

**CONCLUSIONS:** Potassium application affected forage yield at different harvests but did not affect the forage nutritive value. There was difference in potassium requirement in cultivars of alfalfa used in the study. In general, potassium uptake by alfalfa was high. Overall, the study results suggested that K application and harvest time could impact sustainable alfalfa forage production in long-term. Continuous and long-term monitoring is needed to accurately determine the effects of K, cultivar, and harvest on alfalfa growth, yield, nutritive value, and stand longevity.

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## Managing the USDA-ARS National Plant Germplasm System's temperate-adapted forage legume genetic resources

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**KEYWORDS:** germplasm, alfalfa, clover, trefoil

Temperate-adapted forage legumes (TFL) are among some the most significant agricultural crops in the world. Alfalfa, clover and trefoil are among these because of their high yields and nutritive quality as well as their ability to fix nitrogen. Changing climatic conditions and emerging pests and diseases threaten world agriculture production. The same constraints, and many others, imperil crop plant germplasm in centers of genetic diversity and domestication. Therefore, ex situ plant germplasm collections continue to be collected and conserved serving as potential sources of 'new' and improved traits for plant breeding. A team of scientists, technical and support personnel in both Pullman and Prosser, WA manage the USDA-ARS National Plant Germplasm System's (NPGS) TFL germplasm collection. The collection presently consists of over 13,347 accessions in five priority genera with some initial collections dating back to the early 1900s. The *Medicago* genus consists of 80 taxa and 8,614 accessions and the *Trifolium* genus contains 99 taxa with 3,736 accessions. Trefoil accessions belong to three genera *Lotus*, *Acmispon*, and *Hosakia* with 997 accessions in 64 taxa. A systematic approach using weighted factors including quantity and viability of seed as well as age and improvement status are used in determining what germplasm is increased yearly. Most regenerations require the use of alfalfa leaf cutter bees and insect-proof isolation cages. Maintaining genetic integrity in accessions is a high priority and a DNA barcoding approach to correctly voucher taxa and to reduce mislabeling is being used. Since the deregulation of transgenic alfalfa, sentinel plots at field regeneration site are now regularly utilized to monitoring gene-flow and potential adventitious presence. Requests are for small quantities of freely-distributed seed to support research and education purposes and a clear justification for the need is required. Proper documentation must be supplied by the requestor on international shipments. All the TLF germplasm, and its accession-associated information, can be freely accessed via the Germplasm Resources Information Network (GRIN)-Global webpages.

# Evaluation of different fall dormancy varieties of alfalfa (*Medicago sativa*) in southern Chilean Patagonia.

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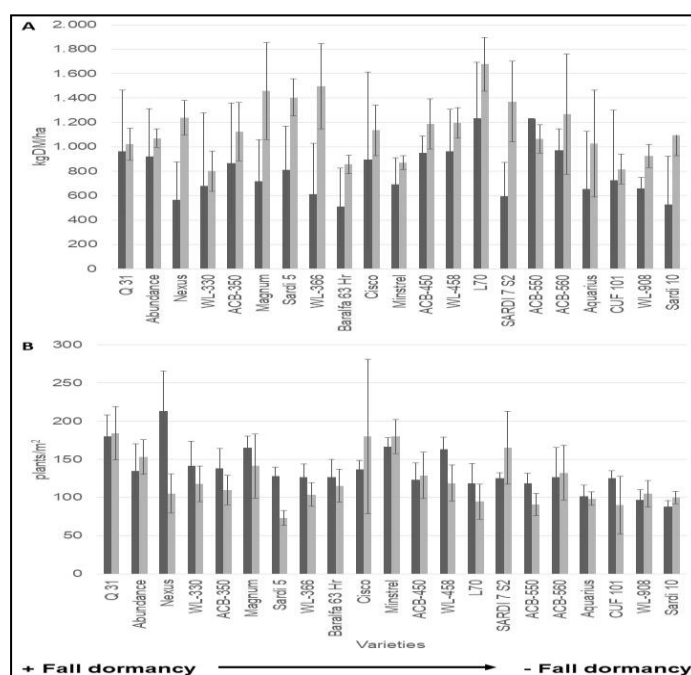
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**INTRODUCTION:** Alfalfa is the most successful forage crop in the Chilean Patagonia due to its high yields and persistence (at least 10 years), which allows to have high quality forage for winter and to be used under grazing in summer, allowing to rest large areas of Tussack grass (*Festuca gracillima*). Varieties with fall dormancy levels of 3 and 4 are currently used in this environment, but Climate Change and the need to have forage in an early spring make it necessary to look for varieties of different fall dormancy. In this experiment we evaluate a range of alfalfa cultivars to determine the most appropriate fall dormancy for Chilean Patagonia, with reference to a trend for increasing temperatures resulting from climate change.

**MATERIAL & METHODS:** The trial was established in September 2016 at the Kampenaike experimental station located in the transition zone of Magallanes (52°42'05.26"S 70°55'57.84"O). The climate is Cold Steppe with an average annual precipitation of 300 mm. In general, the soil was sandy clay loam (64.3% sand, 15.4% silt and 20.2% clay), pH H<sub>2</sub>O 6.8; 10.3% organic matter and a particle density of 2.3 g\*cm<sup>-3</sup>. Twenty-one varieties were established with different fall dormancy levels (Tables 1) in a completely randomized block design with three replications. The sowing date was in September 2016 and the sowing rate was 15 kg\*ha<sup>-1</sup>. The plots were never irrigated. There was only one cut per year when the growing rate stop (the plants never reach 10% flowering). Total yield (kg DM\*ha<sup>-1</sup>) and persistence (plants per m<sup>2</sup>) was evaluated over two years. The statistical analysis was ANOVA taken in to account the factor of varieties and blocks. **RESULTS:** There were no significant differences in yield and plants per m<sup>2</sup> between varieties for each year (Figure 1). **CONCLUSIONS:** It seems to indicate that Alfalfa 6-7 adapt better to the area. However, it is needed to extend the period of evaluation the cultivar that adapts best to these conditions with high yield and persistence, considering that potentials yield is obtained only in the third year.

**Table 1.** Varieties and fall dormancy evaluated

Fall dormancy level	Varieties
3	Q31
4	WL-330
	ACB-350
	ABUNDANCE
	Nexus
5	Magnum
	WL-366
	SARDI 5
6	ACB-450
	WL-458
	BARALFA 63 HR
	CISCO
	Minstrel
7	SARDI 7 S2
	L70
8	ACB-550
	ACB-560
9	WL-908
	CUF 101
	Aquarius
10	SARDI 10



**Figure 1.** A) Yield (kg DM\*ha<sup>-1</sup>) and B) Persistence (plants\*m<sup>-2</sup>) of the varieties during the first year (black bars) and second year (gray bars). Bars indicate  $\pm 1$  standard error.  $p$ -value > 0.05.

# Optimization of alfalfa crop through forage mixtures in the Southern Patagonia of Chile

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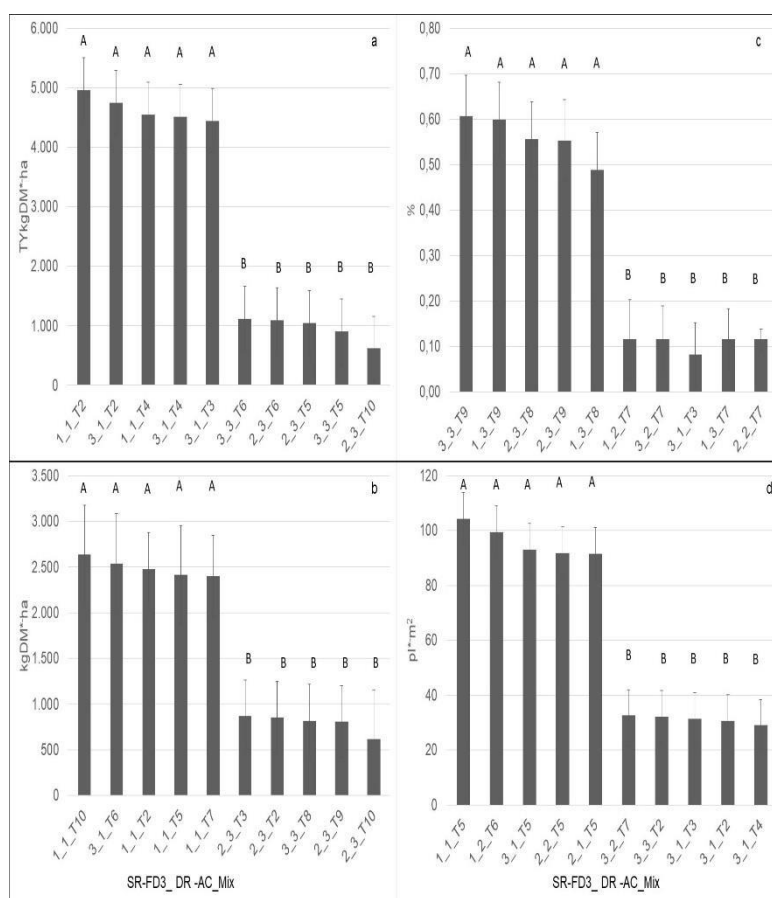
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**INTRODUCTION:** Alfalfa with fall dormancy level (FD) 3 is the most successful forage crop in the Chilean Patagonia due to its high yields and persistence. However, the potential yield is expressed at the third year. Therefore, the use of forage mixture (Alfalfa FD 3 plus another forage species) will allow high yields for the first two years and maintaining the third year yield of alfalfa FD 3 alfalfa. The general objective is to evaluate the productive parameters of different forage mixtures in the transition zone of Magallanes.

**MATERIAL & METHODS:** The trial was established in October 2016 at the Kampenaike experimental station (52°42'05.26"S 70°55'57.84"O) The climate is Cold Steppe with an average annual precipitation of 300 mm. The soil was sandy clay loam (64.3% sand, 15.4% silt and 20.2% clay), pH H<sub>2</sub>O 6.8; 10.3% organic matter and a particle density of 2.3 g\*cm<sup>-3</sup>. Different forage mixtures were analyzed, alfalfa FD3 accompanied by another species (AC). Both FD3 and AC were seeded in three different seeding rates (SR: 1-high; 2-medium; 3-low). The experimental design was split plots with three repetitions, where the main plot was SR of FD3 (15x14 m = 210 m<sup>2</sup>), sub plot was SR of AC (14 x 5 m = 70 m<sup>2</sup>) and the sub-sub plot was AC (1,4 x 5 m = 7 m<sup>2</sup>) wich was randomized in the plots and sub plots. The AC species where Annual Ryegrass (*Lolium sp*; T1), Bialual Ryegrass (*Lolium multiflorum*; T2), Hibryd Ryegrass (*Lolium hybridum*; T3), Perennial Ryegrass (*Lolium perenne*; T4), Alfalfal (*Medicago sativa*) fall dormancy 6 (T5) and 9 (T6), Oat (*Avena sativa*; T7), Cocksfoot (*Dactylis glomerata*; T8) and Tall fescue (*Festuca arundinacea*; T9) and Alfalfa without any AC was used as control (T10). Total yield (TYkgDM\*ha-1), yield of FD3 (kgDM\*ha-1), percentage participation of alfalfa (%) and plants\*m<sup>-2</sup>, during two seasons were evaluated.

**RESULTS:** Figure 1 shows only the five best and the four worst results. The mixtures with annual and Perennial Reygrass have the best yields, while the mixtures with Alf9 and Alf6 did not show high yield. Alfalfa showed a greater participation in the mixtures with Tall fescue and Cocksfoot. T5 and T6 had a higher Yield and density of plants.

**CONCLUSIONS:** It is not recommended to make mixtures with Ryegrass since, despite having higher yields, they compete in greater magnitude with the alfalfa. It must be emphasized that the objective is to get to the third year with a good number of plants and yield.



**Figure 1.** a) Total Yield (TYkgDM\*ha), b) Yield of FD3 (kgDM\*ha), c) Percentage of alfalfa participation (%) and d) plants per square meters (plants\*m2). Letters show highly significantly difference (p < 0,01)



# Yield gap analysis of lucerne (*Medicago sativa* L.) in the Argentinian Pampas

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**KEYWORDS:** Yield potential, Alfalfa, Argentina, Milk production

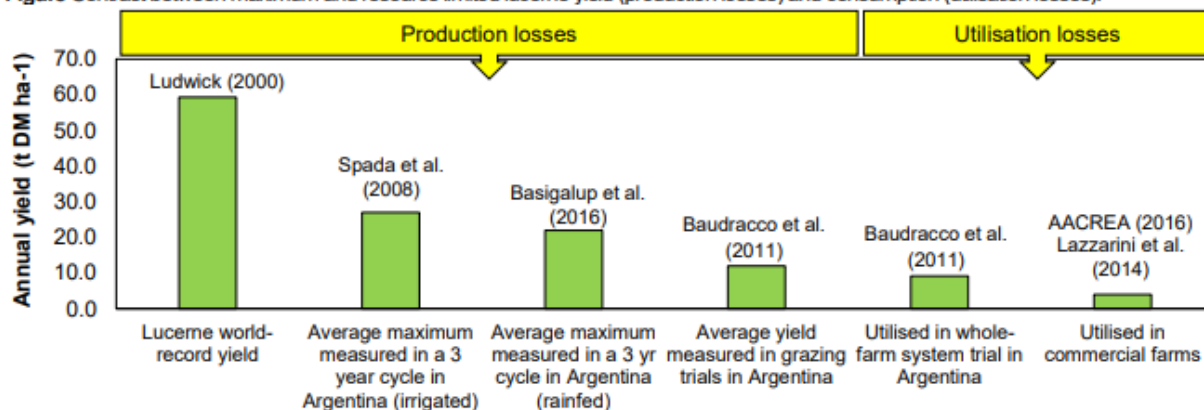
**INTRODUCTION:** The amount of pasture grown, consumed and converted into milk on dairy farms is associated with their profitability. Lucerne is the main grazed forage in the Argentinian Pampas and plays a key role by contributing to both milk production and sustainability of dairy systems. However, in the last two decades dairy farms have increased the use of summer crops for silage, winter grazing crops and concentrates. Such change resulted in an intensification of dairy production, *i.e.* the production of more milk per unit of cultivated land, but at the expense of a reduction in total pasture consumption. This scenario could increase risk and vulnerability of dairy farms. Increasing pasture production and consumption could reduce risk and increase profitability. The difference between potential and actual yields achieved on farm is defined as yield gap, *i.e.* the difference between potential or theoretical maximum, resource limited and actual yield of crops. Such gap indicates the biophysical scope to intensify yield on a given area (van Ittersum *et al.* 2013). The profitability of dairy systems in the Argentinian Pampas could increase by reducing the lucerne yield gap in these environments.

**OBJECTIVES:** to explore potential and actual lucerne yields and consumption levels to identify, in the future, the main drivers that could reduce production and utilisation losses.

**MATERIALS AND METHODS:** A literature review was conducted to identify gaps between current and potential production and consumption of lucerne pastures on dairy farms in the Argentinian Pampas.

**RESULTS:** The world record for lucerne yield is from Yuma Valley, Arizona (USA), with ~59 t ha<sup>-1</sup>. Such yield was achieved under potential conditions: arid climate, sandy soils with irrigation (~9400 mm), high fertilisation (515 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 570 kg N ha<sup>-1</sup>) and under cutting. In Argentina, considering only maximum short-term growth rates (which occur in spring) and full irrigation, lucerne yield could potentially reach 47 t ha<sup>-1</sup>. However, low temperatures and short photoperiods reduce aerial biomass accumulation. When the whole growth cycle is considered, lucerne average yield is reduced to 27 and 22 t ha<sup>-1</sup>yr<sup>-1</sup> for irrigated (Catamarca) and rainfed (Marcos Juárez) trials, respectively. Such yields are higher than observed values in whole-farm grazing trials in Rafaela, Argentina (12 t ha<sup>-1</sup> yr<sup>-1</sup>). Heavier soils might play a role in this reduction. Grazing could also act as a factor that diminishes yield. Nevertheless, in Rafaela, total consumed pasture reached 9 t ha<sup>-1</sup> yr<sup>-1</sup>, 125% more than the average values for the Argentinian Pampas under dryland conditions (4 t ha<sup>-1</sup> yr<sup>-1</sup>).

Figure Contrast between maximum and resource limited lucerne yield (production losses) and consumption (utilisation losses).



**CONCLUSIONS:** We identified a gap of ~100% between potential and actual lucerne yield. Such difference could be due to the effects of irrigation, fertilization and grazing. Adequate grazing management could more than double the amount of consumed pasture per ha. Such findings show a large potential to reduce the cost of milk production.

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# Effect of a thermal time grazing management system on productivity and persistence of Alfalfa (*Medicago sativa* L.) of contrasting fall dormancy groups

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**KEYWORDS:** Lucerne, biomass, persistence, degree days, grazing

**OBJECTIVES:** In the grazing systems of Argentina, both in cattle and dairy operations, it is observed that forage resources are underutilized even though an efficient grazing management system is critical to have high conversions of biomass into meat or milk. The common practice in the grazing management of a pure alfalfa stand is to graze at 10% flowering during spring and summer. This recommendation is based on the idea that at this stage there is an adequate balance between biomass production and quality. However, during this season if grazing starts at 10% flowering the pasture will lose quality rapidly due to favorable environmental conditions such as temperature and rainfall that promote alfalfa growth at high rates (Sardiña et al. 2015). The objective of this research work is to test under real conditions of use a grazing management system which can be adopted by cattle and dairy producers to maximize the efficiency in the conversion of biomass into meat or milk without compromising productivity and persistence of the alfalfa pasture. A thermal time model which considers the seasonal pattern of accumulation and remobilization of reserves that occur throughout the year in the crown of alfalfa has been proposed in other parts of the world as an efficient way to utilize alfalfa (Moot et al. 2003). On the first stage of this work the objective is to validate this technique on the warm temperate climate and environmental conditions of Argentina focusing on biomass productivity of two alfalfa cultivars with contrasting fall dormancy and the effect on root and crown biomass.

**MATERIALS AND METHODS:** The trial is currently taking place at the GENTOS Experimental Station in Pergamino, Bs As Province, Argentina (33°55'15.3"S 60°23'28.9"W), on a typical Argiudol soil, a well-drained, deep silt loam (OM 2.7 %, P 11 ppm and pH 5.9). The climate is temperate, mean annual temperature of 16.4°C and mean annual rainfall of 946 mm. On March 29<sup>th</sup>, 2016, L820 (FDG 8) and Nobel 620 (FDG 6), two alfalfa cultivars from Gentos, were sown in a split plot design with four replicates and two grazing treatments during spring-summer: T1= grazing at 500°d, T2= grazing at 400°d. Base temperature used was 5°C. The 10 x 20 m experimental plots of each cultivar were split in half plots (10 x 10 m) to apply the two grazing treatments. Sowing rate was 15 kg ha<sup>-1</sup> and 80 kg of DAP were used at sowing. Additional fertilization is carried out every year on spring and autumn. During autumn both treatments are grazed at 500°d to allow reserves replenishment in the crown. Measurements taken in this trial include: initial stand (plants/m<sup>2</sup>), dry matter production (DM) per grazing event, root-crown biomass (RCB) and persistence two years after sowing. DM is determined cutting biomass manually in a 50 x 50 cm frame, weighing and placing the sample in a stove at 80°C until constant weight is reached. RCB was determined two years after sowing digging out plants in a 30 cm row with no gaps, cutting the aerial portion of the plant and washing before placing in the stove at 30°C until the sample reached constant weight. Statistical comparisons were made with analysis of variance (ANOVA) using the software Infostat.

**RESULTS AND DISCUSSION:** The initial number of plants was on average 170 plants /m<sup>2</sup> with no differences between cultivars. The ANOVA of DM production through the two years shows higher production, 15,5 tn ha<sup>-1</sup> yr<sup>-1</sup> when grazing at 500°d for both FDG and 13,9 tn ha<sup>-1</sup> yr<sup>-1</sup> when grazing at 400°d (p<0.05). Using the 400°d model the alfalfa was grazed 18 times (with lower biomass accumulation in each grazing) vs. 16 grazings using the 500°d model with higher biomass accumulation. Different percentage of alfalfa utilizations, mainly in spring and summer, was observed between 400°d and 500°d (data not shown). Root-crown biomass was measured in mid-winter. Although the results were not significant, higher RCB/ m<sup>2</sup> was observed in the 500°d treatment (8,44 tn ha<sup>-1</sup>) vs. 400°d (7,92 tn ha<sup>-1</sup>) and the FDG 6 cultivar had higher RCB than the FDG 8 cultivar, 8,45 tn ha<sup>-1</sup> and 7,91 tn ha<sup>-1</sup> respectively. After the second year, no differences in % cover were found in any treatment (degrees to grazing or dormancy group).

**CONCLUSIONS:** These preliminary results are part of a 3-year experiment, and the effect of these contrasting grazing systems on the production and persistence of the alfalfa stand of different fall dormancy groups will continue to be evaluated. Future measurements will include differences in animal consumption and remnant forage after grazing.

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## Research in Alfalfa (*Medicago sativa* L.) – grass mixtures in grazing systems for contrasting environments

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**KEYWORDS:** Lucerne, biomass production, persistence, companion species

**OBJECTIVES:** Argentina is the second alfalfa pasture grower in the world of which approximately 60% is pure sown while the remaining 40% is grown in mixtures, mainly with temperate perennial grasses (Basigalup, 2014). In the Buenos Aires Province its predominant use is in grass mixtures under grazing (Basigalup, 2004). Gentos is committed to providing solutions that maximize earnings for the farmers, for this reason, a research program was started to find the best grass companion for alfalfa of contrasting fall dormancy groups (FDG) under real conditions of use. Preliminary work done in Gentos in Argentina and Uruguay showed that fall dormancy FDG 6 alfalfa is suitable for growing with summer dormant grasses due to complementary seasonal growth that minimize inter-species competition while maximizing forage production all year round. In contrasting parts of the world, such as NY, USA, alfalfa-grass mixtures increase biomass production stability with higher yields than pure stands (Cherney J.H., 2017). The objectives of this research are to find alfalfa-grass mixtures with complementary seasonal biomass production and persistence under grazing.

**MATERIALS AND METHODS:** Two alfalfa cultivars FDG 6 and FDG 9 were sown in a split plot design with three replicates in two locations: Gentos Experimental Station in Pergamino, Bs. As. Province, Argentina (33°55'15.3"S 60°23'28.9"W) on March 14<sup>th</sup>, 2018 and Gentos Experimental Station in Colonia Valdense, Uruguay (34° 20'S 57° 13' W) on May 21<sup>st</sup>, 2018. Nine treatments were randomized: T1= pure alfalfa; T2= Mediterranean tall fescue (*Festuca arundinacea*) cv. Med100; T3= Med. tall fescue exp. Line GFM29; T4= Continental tall fescue cv. Royal Q200; T5= Med/Cont tall fescue blend cv. Colona; T6= Phalaris (*Phalaris aquatica*) cv. Mate; T7= Orchardgrass (*Dactylis glomerata*) cv. Poseidon; T8= Med. tall fescue/Orchardgrass blend; T9= Phalaris/Orchardgrass blend. The alfalfa cultivars as main plots were drilled first, and the 8 grass treatments, subplots, were drilled in a square pattern at an angle of 90 degrees on the same day. Sowing rates were adjusted for each mixture. Plot size is 50 m<sup>2</sup> so that each alfalfa-grass mixture can be individually grazed with sheep according to individual growth rates (three leaves in grasses in autumn and winter and 8-10 nodes in alfalfa in spring and summer) and resting periods. Future measurements will include biomass production of the individual components of the mixture, seasonal total forage production and persistence under grazing for 3 years.

**RESULTS AND DISCUSSION:** Every autumn each mixture will be characterized considering the whole year to determine which companion maximizes alfalfa yields stabilizing the pasture for different environments. The analysis of variance will be used to analyze data from the two locations in multiple years to increase knowledge in the use of alfalfa-grass mixtures in these regions.

**CONCLUSIONS:** Persistence after three years is going to be a key information of the trial. We expect to find that different mixtures will produce forage at different times in the year and that after three years some mixtures that may have been high yielding at the beginning may be outperformed by others that may have produced less biomass but with more stability. This will provide technical tools for selecting the most adapted species for different climatic and edaphic conditions and to support the needs for any cattle and dairy operation.

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## **Development, implementation and extension of technologies to increase pasture production and consumption in Argentina. Vision from GENTOS Seed Company**

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**KEYWORDS:** innovation, technology, forage production, R&D

### **INTRODUCTION:**

Due to changes in land use that have occurred world-wide, livestock production has moved to less productive areas. This generated the need not only to develop technologies adapted to these conditions but also to extend key learnings and improvements so that farmers in those areas can adopt them and benefit from them. These technologies are associated to work protocols that guarantee success in these high-risk production systems through economic earnings that will allow the development of the farmers and the area.

### **OBJECTIVES:**

Gentos, and Argentine forage seed company, has put in place an innovative work flow to develop new technologies that create real opportunities to grow in areas that are generally considered marginal or non-productive. By increasing the understanding of the pastures and improving its use efficiency it is possible to increase, in a sustainable way, the productivity of forage resources. To achieve this goal, Gentos' technicians work side by side with key farmers in each area. Work protocols are developed and shared through agriculture extension ensuring the new technologies can be adopted by other farmers in the area of influence. Prior to starting the on-farm trials a strong research is done to identify regions that can be technified and to select farmers who are interested in changing farming practices and are eager to share their experiences with others. The trials that are carried out in each environment have specific objectives according to the challenges defined for each region and to general objectives related to pasture production. Measurements include: recommended crops prior to pasture establishment, cover crops, sowing dates and time to first grazing, sowing systems, selection of species and cultivars for different environments, sowing rate in terms of plants/m<sup>2</sup> aimed, variables that affect establishment, fertilization, seed treatments, grazing systems, biomass production before and after grazing and persistence. Trial establishment, maintenance and data collection is done in cooperation with the farmers. In many opportunities some research topics may involve teams from farmer associations or supply companies. Conclusions and work protocols are shared through on-farm workshops, extension agencies, meetings and social media.

### **RESULTS AND CONCLUSIONS:**

Since Gentos took this hands-on approach more than 500 farmers have participated in workshops every year learning through the experience of other farmers. We have witnessed the transformation of hundreds of non-productive hectares into high quality pastures. Part of this research was published in an unprecedented handout titled "Pasture Establishment" with 20,000 copies printed. In addition, the School of Shepherds, founded in 2014, teaches the people who are every day at the farm how to utilize the available forage resource efficiently and to manage the pasture adopting good practices that will guarantee its persistence and best use. As a key tool the School started its 5<sup>th</sup> edition in 2018. To increase biomass production and utilization, there is a need for local breeding programs linked to technologies of use that are easy to implement and people who are willing to generate and adopt new work protocols. As an R&D company with deep roots in our region and strong associations with foreign companies and institutes we are currently evaluating innovative grazing systems, fertilizing options and highly adapted mixtures in alfalfa to provide solutions to the cattle and dairy farmers.

# Use of Canopeo to determine light interception and yield of alfalfa (*Medicago sativa* L.) crops

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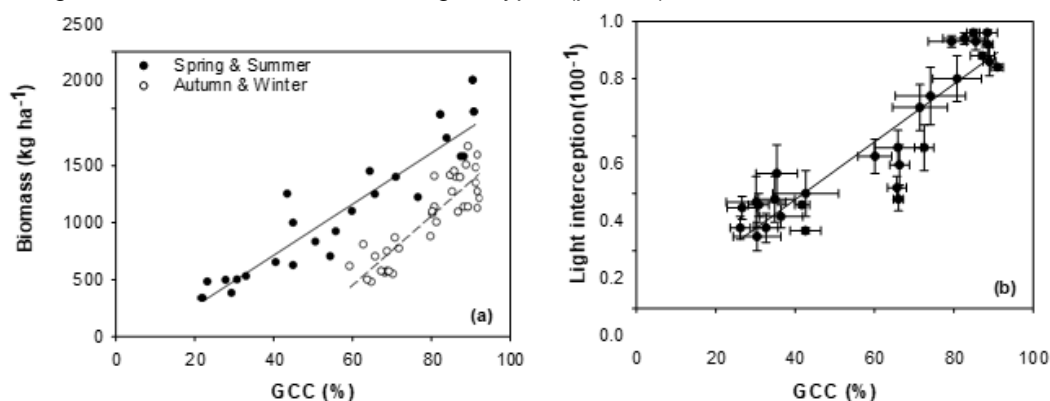
**KEYWORDS:** Digital image analysis, Plant cover, Biomass accumulation

**INTRODUCTION:** The amount of light intercepted by a crop constitutes a key variable in ecophysiological studies. Traditional methods to measure light interception require using expensive equipment with little portability (*i.e.* LI-190SA, AccuPAR, SunScan), making it difficult to carry them to remote areas. Light interception is also linked to accumulated biomass (Lati *et al.* 2011), which is crucial to develop proper feeding budgets on dairy and beef farms. In this work we present data to validate the use of Canopeo (<http://www.canopeoapp.com>) as a tool to determine light interception (%) and yield of alfalfa crops. Canopeo is an image analysis tool which uses color values in the red–green–blue (RGB) system (Patrignani and Ochsner 2015). Canopeo analyzes and classifies all pixels in the image according to ratios of R/G, B/G, and the excess green index, and then reports the average percentage of green canopy cover (%GCC) of the image.

**OBJECTIVE:** To validate the use of Canopeo App as a tool to determine yield and light interception of alfalfa crops.

**MATERIALS AND METHODS:** A field experiment with rainfed alfalfa was established in August 2017 in Esperanza, Argentina (-31°27', -60°55'). The experiment was set up as a complete randomized block design with four replicates. Treatments consisted of three alfalfa genotypes with fall dormancy ratings of 10, 9 and 6. Each plot was 25m<sup>2</sup>. Plant growth and development were measured during the establishment year (Aug 2017- Jul 2018). On each plot, 3 images were taken with a cell phone using Canopeo. The cell phone was held ~1 m above the canopy and pictures were taken at a straight angle. Simultaneously, light interception was measured with a Line Quantum Sensor (LI-Cor LI190SA) by making one measurement above and 4 measurements below the canopy per plot. On the same spots where images were taken and light interception was measured, lucerne biomass was determined. This was done by cutting to ground level a 1.5m<sup>2</sup> area in each plot. Samples were forced-oven dried (65°C) until constant weight. Linear regression models were constructed to determine the capability of Canopeo to predict biomass and light interception of alfalfa crops. Models were evaluated using t-test analysis ( $\alpha=0.05$ ) and their goodness of fit ( $R^2$ ). For biomass accumulation, two different regressions were constructed according to seasons (Spring & Summer data refers to 1 September -1 March, while the rest of the dates correspond to Autumn & Winter).

**RESULTS:** The overall goodness of fit for biomass was 0.86 for Spring & Summer ( $y=22.44X$ ) and 0.77 for Autumn & Winter ( $y=-1361.5+30.4X$ ) ( $p<0.05$ , Figure 1a). In the case of light interception, Canopeo was also able to predict values with very good accuracy without the need to consider seasons ( $y=0.0101X$ ,  $R^2=0.83$ ,  $p<0.05$ ; Figure 1b). Regressions did not differ between genotypes ( $p>0.05$ ).



**Figure 1 (a)** Biomass accumulation (kg ha<sup>-1</sup>) and **(b)** light interception (100<sup>-1</sup>) of alfalfa crops growing in Esperanza, Argentina (-31°27', -60°55') as related to green canopy cover (GCC %) measured with Canopeo. Horizontal and vertical bars in **(b)** represent the standard error of the mean.

**CONCLUSIONS:** We identified a fast and reliable method to estimate light interception (%) and yield of alfalfa crops using an Android application. This tool could be used to determine (1) light interception in remote areas or when more expensive equipment is not readily available and (2) biomass of alfalfa crops. Canopeo could be a tool to both scientists on a low budget and farmers and farm advisors working on alfalfa-based systems. Special attention should be placed if weeds are present as the software cannot discriminate against them.

**ACKNOWLEDGEMENTS:** This research was partially funded by Universidad Nacional del Litoral (CAI+D ORIENTADO 006.022.000 and PEIS 643662).

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# Shoot and root biomass of lucerne (*Medicago sativa* L.) were affected by sowing dates and inoculation treatments

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**KEYWORDS:** Radiation use efficiency, Leaf appearance, Planting date, N fixation

**INTRODUCTION:** Understanding the factors that affect the establishment of lucerne crops is critical to increase productivity and persistence. Choosing appropriate sowing dates maximises shoot and root growth (Teixeira *et al.* 2011). Sowing lucerne in a season with optimum temperatures (~20°C; Moot *et al.* 2012) results in faster accumulation of biomass and an early establishment process. Lower temperatures (5-15°C; Wynn-Williams 1976) and short photoperiods (Sim *et al.* 2015) prolong the establishment phase. Sowing is also when inoculants introduce rhizobia to the plant. In soils with no previous history of lucerne, positive effects of inoculation have been reported (Berenji *et al.* 2015). Yet, controversy exists with regard to the effectiveness of commercial strains and the need for inoculation in soils with a previous history of lucerne (Moot 2012). This experiment tested the effects of different sowing dates from summer into autumn and different inoculation treatments.

**OBJECTIVE:** The aim of this work was to understand how different sowing dates and inoculation treatments affected crop physiological responses during the seedling stage and for the first two growing seasons.

**MATERIALS AND METHODS:** A field experiment with rainfed 'Force 4' (fall dormancy 4) lucerne was established as a split plot design with three replicates at Lincoln University, New Zealand. Soils were N deficient (~4 kg N ha<sup>-1</sup> to a depth of 150 mm). Main plots were sown on: (i) 26 Jan; (ii) 21 Feb; (iii) 15 Mar; and (iv) 3 Apr in 2012. Subplots (6.3 x 10.0 m) comprised five inoculation treatments: (i) coated seed (NZ industry standard; CS); (ii) peat slurry (PS); (iii) Nodulator (ND); (iv) ALOSCA® (AS); and (v) bare seed (used as a control; BS). *Sinorhizobium meliloti* RR128 was the bacterial strain used in the inoculated treatments. Plant growth and development were measured during the first two growing season (Jan 2012- Sep 2013). ANOVA was used to analyse all variables in a split plot experimental design. Fishers' LSD test was used to determine variation between different levels of a factor when the ANOVA was significant ( $\alpha=0.05$ ).

**RESULTS:** Shoot biomass accumulation was 90% higher ( $P<0.05$ ) in the earliest sowing date ( $19.8\pm0.14$  t ha<sup>-1</sup>) compared with the latest ( $11.9\pm0.15$  t ha<sup>-1</sup>). Yield variability was explained by differences in light interception and its conversion efficiency ( $RUE_{shoot}$ ) in the first spring. A 40% reduction in  $RUE_{shoot}$  was estimated for March and April (autumn) sowing ( $0.93\pm0.05$ ). This was probably caused by greater continued partitioning of biomass to roots. A longer ( $P<0.05$ ) phyllochron for autumn sowings reduced leaf area expansion rates. Root biomass was higher ( $P<0.05$ ) from early sowing dates, but by the end of the experiment all treatments had  $>3$  t ha<sup>-1</sup> of perennial biomass. Inoculation increased ( $P<0.05$ ) shoot yield by 40% compared with the uninoculated control ( $16.6\pm0.1$  vs.  $13\pm0.2$ ) in the first year. Bare seed treatments fixed 50% less ( $P<0.05$ ) nitrogen during the winter and early spring of 2012. This was linked to less nodulation and reduced nitrogen content in shoots of the uninoculated treatment. However, after late spring, nitrogen fixation was similar among all treatments. This was probably linked to the presence of a small proportion (~18%) of RR128 strains in nodules from the bare seed treatment, as indicated from DNA analysis. Same analysis also showed a large proportion of other bacteria (particularly *Erwinia* spp.) in those nodules. A 20% decrease in  $RUE_{shoot}$  was measured in uninoculated crops compared with inoculated ones, probably due to reduced photosynthesis due to a lower shoot N%. Root biomass was unaffected by inoculation and all treatments reached  $>3.5$  t ha<sup>-1</sup> by the end of the experiment. Uninoculated lucerne crops appeared to have similar accumulation rates of perennial biomass despite lower N fixation and N content in shoots. This supports the idea that perennial biomass accumulation is driven by ontogeny and less affected by the environment (Sim *et al.* 2015).

**CONCLUSIONS:** Early sowings and inoculation are tools to reduce the duration of the seedling stage and thus increase the chance of successful lucerne establishments. From a physiology perspective these results provide a framework for understanding crop responses to sowing date and inoculation and could be included in simulation models. Agronomically, any delay in seedling canopy development makes the crop more vulnerable to weed competition.

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# The establishment of alfalfa with field pea-oat mixture as a cover crop

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**KEYWORDS:** alfalfa, pea, oat, establishment, yield

**OBJECTIVE:** The aim of this study was to determine the suitability of field pea and their mixture with oat as a companion crop for alfalfa establishment and weeds control.

**MATERIAL AND METHODS:** The trial was conducted at the Experimental field of the Institute of Field and Vegetable Crops at Rimski Šančevi-Novı Sad, Serbia (45° 20' N, 19° 51' E and 84 m asl) with an average annual temperature of 11 °C and rainfall amount of 611 mm. The trial comprised two sowing years, 2008-2009, and also the first cut in the first full harvest year in 2009 and 2010. The trial was set up as randomized block design in three replicates and included alfalfa pure stand (cv. NS Medijana ZMS V) and row intercropping of alfalfa with different proportions of field pea (P) (cv. Javor) and oat (O) (cv. Dunav) (100% P, 90% P/10% O, 80% P/20% O, 70% P/30% O, 100% O). The sowing was performed in April 2008 and 2009. The plot size was 6 m<sup>2</sup>. In intercropping, field pea and field pea-oat mixtures were firstly sown in 20 cm row spacing and then alfalfa was sown between cover crop rows reducing the distance to 10 cm. Basic seeding rate of alfalfa was 15 kg ha<sup>-1</sup>, of field pea 200 kg ha<sup>-1</sup>, and of oat 180 kg ha<sup>-1</sup>. When the field pea had reached the harvestable stage all plots were cut. At that time, alfalfa was in the vegetative stage (approx. 10 permanent leaves) and oat was in the heading. No herbicides were applied in the trial. The analyses included dry matter yield and weed proportion in the first cut and dry matter yield in other two cuts of alfalfa in the establishment year and the first cut in the following year. Differences between the treatments were tested by ANOVA in STATISTICA 13 software, means were separated by Duncan's multiple range tests and statistical significance was evaluated at p≤0.05.

**RESULTS:** The interaction year and treatment was not significant. Based on a two-year average, dry matter yield in the first cut ranged from 1.69 t ha<sup>-1</sup> in pure alfalfa stand to 6.30 t ha<sup>-1</sup> in intercropping alfalfa and 100% oat and the yield decreased with reduced oat proportion in the mixture (Table 1). The lowest total yield in intercropping was recorded in the mixture of alfalfa and 100% pea and the highest in the mixture of alfalfa with 70%P+30%O. The total annual yield of alfalfa pure stand was significantly lower than other treatments (5.53 t ha<sup>-1</sup>). In the full harvest year, the highest yield was obtained in the treatment alfalfa + 70%P+30%O (6.49 t ha<sup>-1</sup>). The highest weeds proportion, more than 40%, was in the pure alfalfa stand (Figure 1). In intercropping weeds proportion was less than 10%, reaching the minimum in the intercropping of alfalfa with 70%P+30%O.

**Table 1.** The effect of pea-oat mixtures in intercropping with alfalfa on the average dry matter yield in the first cut of the two sowing years (t ha<sup>-1</sup>) (2008-2009), and in the first cut of the following year (2009-2010).

Treatments	2008-2009		2009-2010
	First cut	Total yield	First cut
A + 100% P	3.51d	7.08c	4.92b
A + 90%P+10%O	4.31c	7.63bc	6.39a
A + 80%P+20%O	5.01bc	8.54ab	6.47a
A + 70%P+30%O	5.56ab	8.87a	6.49a
A + 100%O	6.30a	8.35ab	5.18b
100% A	1.69e	5.53d	5.25b

A - alfalfa, P - field pea, O – oat

Means of each cut followed by the same letter are not significantly different from each other at p ≤ 0.05.

**CONCLUSION:** The obtained results indicate a potential for development of a new, reliable and environmentally friendly method of the alfalfa establishment, without negative effect on the yield in subsequent cuts and the yield in the full harvest year. Intercropping of alfalfa with 70:30 pea-oat proportions is recommended for obtaining good and stable alfalfa crop without suppressive effect on alfalfa re-growth and with the highest effect on weeds control. When sown as the cover crop, an annual forage legume and their mixture with oat, provides an economic yield during the perennial forage crop establishment.

**ACKNOWLEDGEMENT:** This research is supported by "H2020 SERBIA FOR EXCELL" project. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 691998.

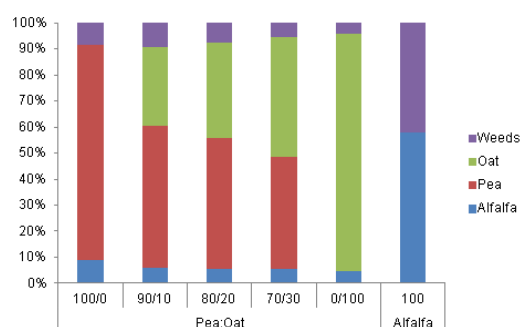


Figure 1. Weeds proportion (%) in the first cut of alfalfa established with field pea-oat mixtures as cover crops.



## Seed production mechanisms in alfalfa (*Medicago sativa* L.) populations

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**KEYWORDS:** yield components, seed weight, path coefficient, Semiarid Pampean region, dry matter.

**Introduction:** Seed production potential has received sporadic attention by alfalfa breeders. Search for greater production of seeds in addition to forage uses natural genetic variability of alfalfa for the development of new varieties (Rodríguez, 1983). For this reason, it is important to consider the relationship between seed and forage production in breeding populations (Rodríguez, 1983), taking into account that seed production and its components show high inter-and intra-varietal genetic variability (Bolaños-Aguilar *et al.*, 2000).

**OBJECTIVES:** We evaluated seed production and related characters and underlying genetic mechanisms in six alfalfa populations with different fall dormancy group (FDG) in the Semiarid Pampean Region.

**MATERIALS AND METHODS:** in September 2015, plants from six alfalfa populations (one for FDG 6, 7, 9 and 10 and two for FDG 8) planted in 2013 were collected in four provinces of the Semiarid Pampean Region of Argentina. Six isolated cages were set up at Anguil Experimental Station (INTA) geographical coordinates with 300 plants each and pollination was made using honey bees. At mature pod stage, ten plants from each cage were randomly selected, identifying the tallest stems, on which the following variables were measured: dry matter (DM), number of pods (CV), number of seeds per pod (CS), pod weight (PV), and total seed weight per plant (PS). Descriptive statistics were calculated to characterize the populations, and then the relationships between characters were analyzed using phenotypic correlations and path coefficient method (Sengul, 2006).

**RESULTS AND CONCLUSIONS:** Phenotypic correlations between DM, CV, CS, PV and PS were not significant with each of the six populations. These results can be compared with those treated by Hutmacher *et al.* (1991) who pointed out that DM production is detrimental to seed production. Correlation coefficients between CV and PV and between CS and PS were positive and highly significant for the six populations. The other correlation coefficients were positive and highly significant for three of the populations with FDG 6, 7 and 9 while for populations with FDG 8 and 10 they were not significant. Direct effects of DM on seed production were low in all populations, and negative in some cases with values between 0.04 and -0.08. The direct effect of the CV variable had negative values between -0.43 and -0.08, except for populations 3 and 6 with values of 0.08 and

0.18 respectively. The direct effect of CS was very high in all populations. Finally, the direct effect of the PV was positive and variable for the populations, with values between 0.55 and 0.06, except for population 3 that presented a negative value of -0.19. Regarding the indirect effects, the effect of CV via CS showed high values between 0.33 and 1.25, except for population 3 that had a very low value and even negative of -0.03. The indirect effect of PV via CS was important in all populations with values between 0.30 and 1.18 and again the exception was population 3, whose value was low (0.12). The rest of the indirect effects were low or zero values, except for DM via CS in population 5 that had a value of 0.54, and population 1 whose indirect effects CV via PV and CS via PV presented values of 0.54 and 0.51, respectively. In summary, the CS variable was the one that had the greatest direct effects on seed production. The mechanisms of seed production in alfalfa were similar in populations with different dormancy.

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# Identification of genetic loci associated with resistance to drought and high salinity in alfalfa (*Medicago sativa* L.) using genome-wide sequencing and association mapping

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**KEYWORDS:** linkage disequilibrium, biomass yield, drought, genotyping by sequencing, SNPs.

**OBJECTIVES:** Alfalfa is a worldwide grown forage crop and is important due to its high biomass production and nutritional value. However, the production of alfalfa is challenged by adverse environmental factors such as drought and high salinity. Developing drought resistance alfalfa is an important breeding target for enhancing alfalfa productivity in arid and semi-arid regions. Our objective is to identify alfalfa DNA markers associated with drought and salt tolerance to clearly define the genetic basis of resistance to these stressors and accelerate breeding programs.

**MATERIALS AND METHODS:** A panel of 200 alfalfa accessions was used for evaluating drought and salt resistance in the field and greenhouse. An integrated framework that merges a QTL mapping approach called 'genome-wide association studies' with high-throughput next-generation sequencing.

**RESULTS:** A total of 28 markers at 22 genetic loci were associated with yield under drought, whereas only three markers associated with the same trait under well-watered condition. Comparisons of marker-trait associations between water deficit and well-watered conditions showed non-similarity. Most of the markers were identical across harvest periods within the treatment, although different levels of significance were found among the three harvests. The loci associated with biomass yield under water deficit located throughout all chromosomes in the alfalfa genome agreed with previous reports. Our results suggest that biomass yield under drought is a complex quantitative trait with polygenic inheritance and may involve a different mechanism compared to that of non-stress. BLAST searches of the flanking sequences of the associated loci against DNA databases revealed several stress-responsive genes linked to the drought resistance loci, including leucine-rich repeat receptor-like kinase, B3 DNA-binding domain protein, translation initiation factor IF2 and phospholipase-like protein. Marker-trait association identified a total of 42 markers significantly associated with salt tolerance. They were located on all chromosomes except chromosome 2 based on the alignment of their flanking sequences to the reference genome (*Medicago truncatula*). Of those identified, 13 were associated with multiple traits. Several loci identified in the present study were also identified in previous reports. BLAST search revealed that 19 putative candidate genes linked to 24 significant markers. Among them, B3 DNA-binding protein, Thiaminepyrophosphokinase and IQ calmodulin-binding motif protein were identified among multiple traits in the present and previous studies. With further investigation, those markers closely linked to drought and salt resistance can be used for MAS to accelerate the development of new alfalfa cultivars with improved resistance to drought and high salinity.

**DISCUSSION:** It has been reported that a number of genes and pathways involved in the yield and yield components under abiotic stress in plants (Mickelbart et al., 2015). Our result of identification of multiple loci genome-wide supports that biomass yield under stress in alfalfa is complex traits with polygenic inheritance. Multiple loci associated with yield trait were consistent at different harvesting periods under water deficit. For instance, among 28 markers identified with drought treatment, 18 and 6 were identified by three and two harvests, respectively. Only 4 were identified by single harvest. It may suggest that the experimental data and statistical procedures used in the present study are reliable.

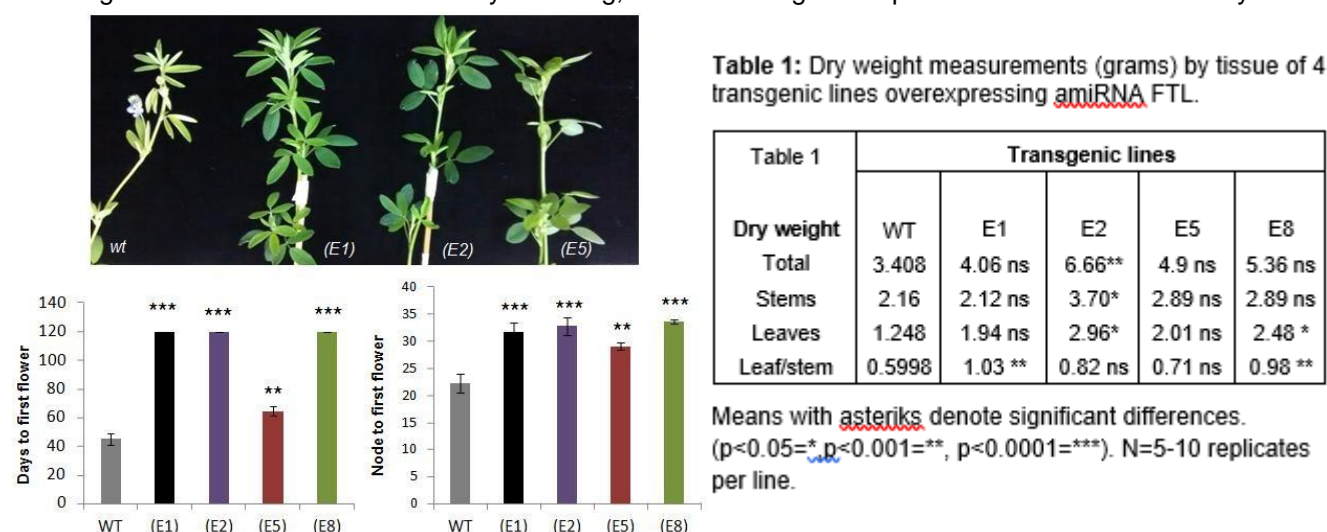
# Downregulation of *FLOWERING LOCUS T* genes in *Medicago sativa* leads to delayed flowering and increased yield

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**KEYWORDS:** *Medicago sativa*, late flowering, *msFTL*.

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops worldwide. This is mainly due to several traits such as its adaptability to varying external conditions, its excellent high-quality forage yield and its capacity to fix nitrogen. Alfalfa constantly increases in yield during spring and until summer's onset, and reaches its peak when alfalfa plants start to flower. From this point onwards, the quality of the forage decreases due to morphological changes, such as an increase in the proportion of stems and a reallocation of photosynthetic resources to the development of reproductive structures. Therefore, this decrease in quality represents a considerable loss of nutritive value. In alfalfa, flowering onset depends mainly on day-length and temperature. The combined cues of long days (LD, 16:8) and temperatures above 18°C are the primary signals that trigger flowering. It has been studied that both signals induce the expression of a gene known as *FLOWERING LOCUS T* (*FT*), one of the most prominent flowering inducers in plants. The main objectives of this study involved the identification of *FT* orthologues genes in alfalfa and the development of transgenic alfalfa lines with downregulated *FT* levels in order to delay flowering, extend the vegetative phase and increase biomass yield.



**Figure 1:** Late flowering transgenic plants overexpressing *miRNAFTL*. A) Phenotype of apical stems at the moment of wt flowering B) Total nodes to first flower and C) Total days to first flower. N= 5-10 replicates per line. Means with asteriks denote significant differences. (p<0.05=\*, p<0.001=\*\*, p<0.0001=\*\*\*).

In order to accomplish this, a *FT* like gene was identified in alfalfa (*msFTL*) and based on its DNA sequence a specific microRNA (*miRNA FTL*) was designed to downregulate. This microRNA was transformed into Alfalfa through *Agrobacterium tumefaciens* (INDEAR) and 4 independent transgenic lines were obtained. All lines were then tested in growth chambers under inductive flowering conditions (24<sup>o</sup>, 16:8 photoperiod) in order to asses flowering time. Alfalfa plants overexpressing *miRNA FTL* under the 35S:CAMV promoter showed a clear delayed flowering time determined as both, nodes to first flower and days to first flower compared to wt controls regenerated from calli (Fig 1). In particular transgenic lines E1, E2 and E8 did not show any flowering signs at the moment harvest. Additionally, dry weight measurements from each tissue (leaves and stems) were performed at the moment of flowering in order to analyze biomass distribution (Table 1). All lines show a clear tendency for a higher biomass accumulation, with E2 and E8 lines presenting a significant increase in leaf biomass and in the leaf/stem ratio.

Our data support the use of the *FT* family of genes as a flowering regulator and how its downregulation successfully leads to an effective delayed flowering with a concomitant increase in biomass yield.

# Seasonality and forage accumulation in alfalfa (*Medicago sativa*) and tall fescue (*Schedonorus arundinaceus*) pure pastures and its mixtures

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**KEYWORDS:** seasonality; biomass; grass; legume; competition

## OBJECTIVE:

Analyze biomass dynamics accumulation in contrasting canopy structure pastures

## MATERIALS AND METHODS

At INTA Pergamino Experimental Station (Lat: 33°34'S; Long: 60°34'W), Argentina, they were evaluated different canopy structure pastures including pure pastures and its mixtures in plots (7,5 m<sup>2</sup>) with a randomized block design and 5 replicates. The pastures were sown 04/05/2016 in a Typic Argiudoll (pH=6; organic matter=2,9 % and extractable phosphorus=24,7 ppm). Treatments consisted in seven different pastures: i) AA: alfalfa, ii) FC: north Europe tall fescue, iii) FM: Mediterranean tall fescue, iv) AA-FC: alfalfa and north Europe tall fescue, v) AA-FM: alfalfa and Mediterranean tall fescue, vi) FC-FM: north Europe and Mediterranean tall fescue, vii) AA-FC-FM: alfalfa, north Europe and Mediterranean tall fescue. The alfalfa cultivar used was Bar Pal 9242 without winter dormancy. Tall fescue cultivars differ in their phenology, concentrated during winter-spring for Mediterranean type. The cultivars used were north Europe type cv. Palenque plus INTA and Mediterranean type cv. Flecha. Mixtures were sown in alternate rows. Drought conditions were avoided through occasional sprinkler irrigation. Aerial dry matter biomass per component (alfalfa and tall fescue) was measured cutting a quadrat per plot (1m<sup>2</sup>) at 5 cm height considering 500 ± 50 degree day sum for each period, except during establishment with a higher degree days accumulated. The statistical analysis for biomass dynamics was performed with a mixed model with repeated measures in time considering blocks, pasture, cutting and the interaction between pasture and cutting. Total biomass per each component was analyzed with ANOVA. Treatment average values were compared with Fischer's test (p< 0.05).

## RESULTS:

Seasonal distribution of biomass was different between pastures (Figure 1.a.), being the interaction between pasture and cutting highly significant (p< 0,0001). In the first spring pure FC and FC-FM had significantly higher biomass, and continue till the third cutting for all pastures that include FC. Just since the fourth cut (summer) that pure AA had greater biomass, during the first autumn and winter pure AA was similar to mixtures which include FM. Again, during spring mixtures with FC stand out, after that AA and its mixtures with FM produced more. Remarkably, pure tall fescue pastures had very low yields after the initial good behavior, probably related with the absence of nitrogen fertilization. Total forage biomass accumulation in two production cycles (15 cuttings) was similar between AA pure stands and its mixtures (average=26205 kg DM.ha<sup>-1</sup>) and higher than tall fescue pure pastures (average=8952 Kg DM.ha<sup>-1</sup>) (Data not shown, p value < 0.05). AA contribution in mixture decrease as tall fescue increases in mixtures, as associated with FC participation (Figure 1.b.) suggesting a more intense interspecific competition. When FC was present higher tall fescue biomass was produced than with only FM.

## CONCLUSIONS:

Different canopy structure pastures affects forage total biomass accumulation and also in its seasonality. Alfalfa pure stands and its mixtures produced more and no differences in total biomass were detected between them. With higher FM participation in the mixture increased alfalfa biomass (AA-FC < AA-FM-FC < AA-FM).

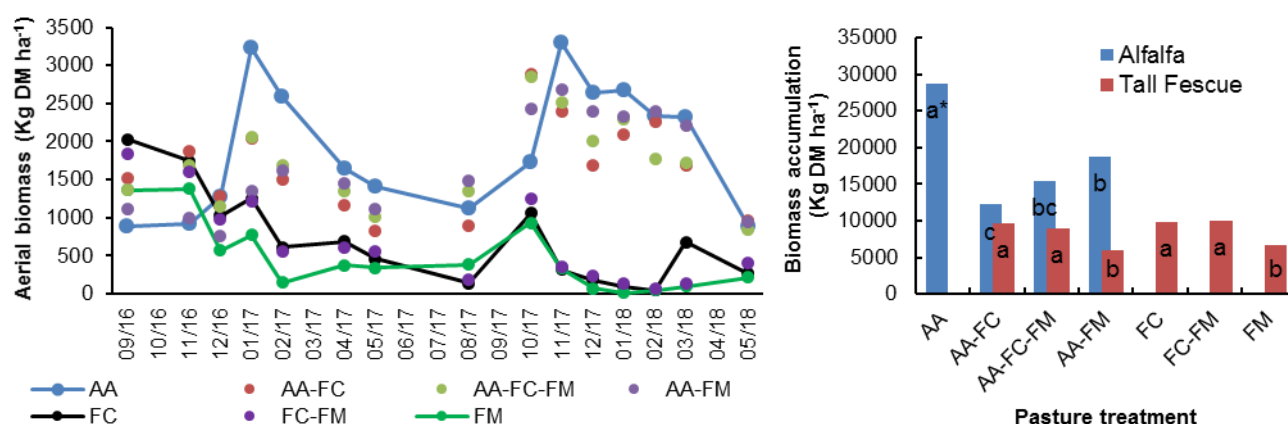


Figure 1.a). Biomass dynamics in contrasting canopy pastures throughout two cycles of evaluation. b). Total aerial biomass accumulated for each species (alfalfa and tall fescue) in pure and mixed stands. Different letters indicate significant differences for each component in pure pastures and its respective mixtures.



## The productivity of alfalfa accessions in contrasting environments in Kazakhstan

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**KEYWORDS:** lucerne, accessions, evaluation

**Introduction:** Global climate change is increasing the surface temperature of the planet, elevating the importance of drought tolerance as a trait in forage breeding. Crop wild relatives (CWR) of alfalfa are adapted to arid environments and offer the potential to contribute novel alleles for drought tolerance selection. Kazakhstan is rich in flora ecotypes of wild species of alfalfa: tetraploid *Medicago sativa* subsp. *falcata* (syn *M. tianschanica*, *M. falcata*), *M. s. varia* and diploid species *M. s. caerulea*, (syn. *M. s. trautvetteri*), and *M. s. falcata* (syn *M. s. difalcata*). In this paper we illustrate the targeted evaluation of varieties, landraces and CWR in low (Almaty) and high drought x cold stress (Kokshetau) environments in Kazakhstan.

**MATERIAL AND METHODS:** Almaty (south Kazakhstan) is characterized by a climate with hot summers and milder winters. Most alfalfa genotypes are sufficiently adapted to the average annual +7.50°C temperature and 417 mm rainfall. More than 70% precipitation occurs in the spring months April – June and there is a winter snow cover 25 – 35 cm. The fertile loam soil has a neutral pH. Kokshetau is characterized by severe climate in the winter months with minimum temperature reaching -35 – 45°C. Here, winter hardiness is one of the important criteria in the evaluation of varieties. The average annual temperature is +2.1°C and annual rainfall of 320 mm is dominated in summer. Winter snow can reach 50 – 70 cm. The soil is a leached chernozem with neutral pH. Forty-eight diverse lines were evaluated from the Crop Trust CWR alfalfa project. Information on these lines can be found at <https://ics.hutton.ac.uk/cwr/alfalfa/#home>. Forage yield was cut by hand shears and oven dried. Means of fixed effects for forage yield were calculated using spatial linear mixed models using variety x site as the treatment structure, performed by GenStat 18.

**RESULTS:** In Almaty, the entries APG 38688, Kokbalausa, SARDI Grazer, K 271, Titan 7, Darkhan 90, Alta Sierra 2, Q 75, Kokorai, Stamina 5, SARDI 7 Series 2, APG 19018 produced an average yield of over 13500 kg/ha (Figure 1). In Kokshetau, APG 45644, APG 38688, Force 5, Chiza, APG 58577, APG58575, KWP809317 with dry matter yield greater than 2500 kg/ha. Entries APG35169, APG45677, Alta Series 2, K267 and K266 were not winter-hardy and, consequently, their productivity (influenced by survival and recovery) was the lowest. The CWR accession APG 38688 (*M.s.falcata* collected from Alakol in southeastern Kazakhstan) showed the best combination of yield across the two sites of Almaty and Kokshetau, showing high ductility to different environments.

**CONCLUSIONS:** The evaluation of 48 diverse alfalfa varieties, landraces and accessions to Almaty and Kokshetau will be an important step in the development of more drought tolerant accessions for future climate scenarios in Kazakhstan.

**ACKNOWLEDGEMENTS:** The authors would like to acknowledge support from the Crop Trust.

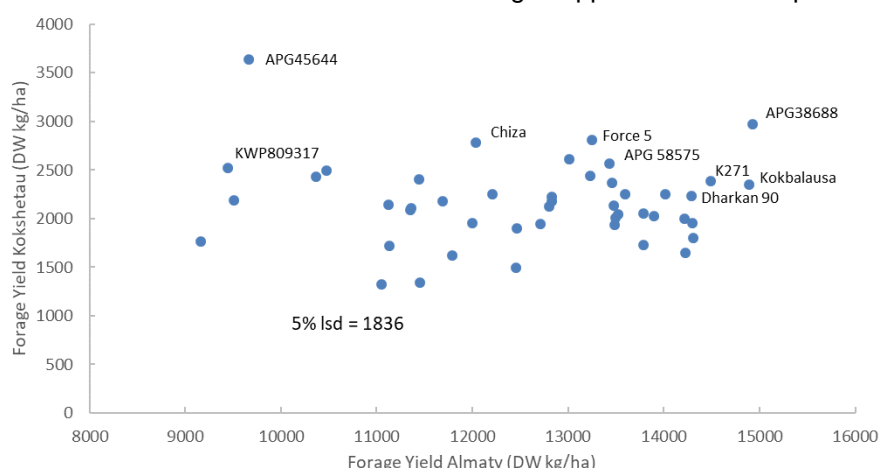


Figure 1. Average annual forage yield production at Almaty (low stress environment) and Kokshetau (high stress environment) for 48 diverse *Medicago sativa* populations.

# Sheep liveweight production from dryland alfalfa (*Medicago sativa* L.) and alfalfa/grass mixes over five years

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**KEYWORDS:** *Bromus* spp., cocksfoot, *Dactylis glomerata*, *Medicago sativa*, orchardgrass

**INTRODUCTION:** To be both productive and persistent direct grazed pasture mixes require the component species to offset timing of the periods of peak resource (light, water, nutrient) demand. Grass dominant pastures are N deficient throughout the year while alfalfa is recognised for its ability to maintain growth as droughts develop by accessing water from lower in the soil profile which offsets the delayed start to spring grazing. Can alfalfa/grass mixes provide early spring feed to equal or exceed LWt production from an alfalfa monoculture?

**OBJECTIVE:** This experiment compared the annual liveweight (LWt) production of sheep direct grazing alfalfa monocultures compared with alfalfa/grass mixtures over five growth seasons.

**MATERIALS AND METHODS:** A randomised complete block experiment with six replicates and three pasture treatments (alfalfa monoculture, alfalfa/brome, alfalfa/orchardgrass) was established at Ashley Dene (43°39'0.07"S; 172°19'24.57"E; 39 m a.s.l.), Canterbury, New Zealand in autumn 2012. Plots ranged from 0.6 to 1.3 ha in size. In most years lucerne was initially set stocked for about 2 weeks before animals were mobbed into treatment groups and a 6-paddock rotational grazing programme was initiated. Grazing continued, with various stock classes, until water stress restricted pasture growth and pastures were destocked. Graze days per paddock and a seasonal mean weighted LWt gain were used to create replicated data for LWt analysis. Liveweight production was not measured during maintenance grazing events for sward management. Measurements ceased in Feb 2017. Data were analysed by ANOVA in Genstat and means were separated at the  $\alpha=0.05$  level.

**RESULTS:** Over five years total LWt production ranged from 405 to 883 kg LWt/ha (Fig. 1). In all years LWt production from the monoculture was greater than, or similar to, mixtures. In two out of five years (2014/15 and 2015/16) mixtures were able to be stocked earlier than the monoculture.

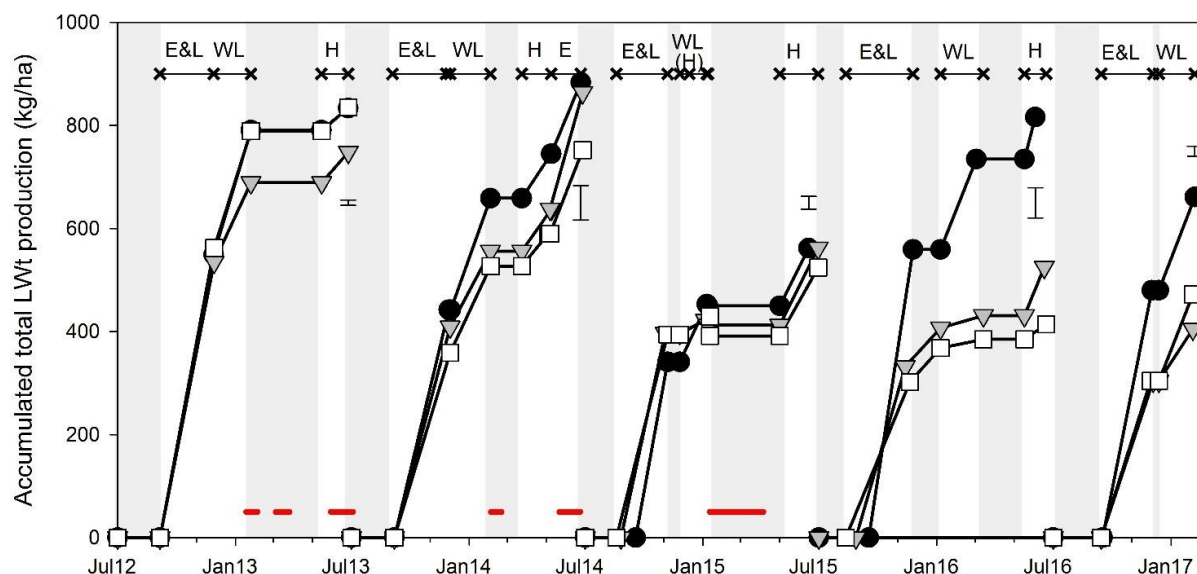


Fig. 1 Net annual accumulated LWt production (kg LWt/ha) from ewes with twin lambs at foot (E&L), Weaned lambs (WL), Hoggets (H) and dry ewes (E) grazing alfalfa (●) alfalfa/brome (□) or alfalfa/orchardgrass (▼) pastures. Grey vertical areas indicated periods when pastures were destocked. Red lines indicate "maintenance" grazing events. Error bar is SEM for total accumulated LWt production.

**CONCLUSIONS:** In three out of five years the highest LWt production was from alfalfa monocultures. Earlier growth from alfalfa/grass mixes did not increase total LWt production.

## ACKNOWLEDGEMENTS

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## The Alfalfa (*Medicago sativa* L.) Breeder's Toolbox integrates genomics to advance breeding applications

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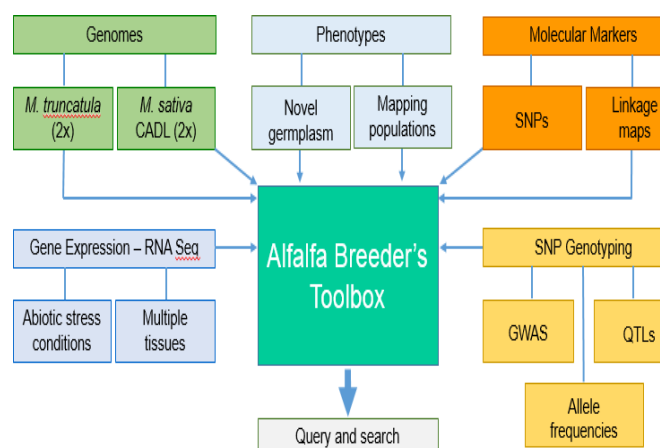
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**KEYWORDS:** genome, transcriptome, breeding, allele frequency, marker-assisted selection, gene expression

**OBJECTIVES:** Alfalfa (*Medicago sativa* L.) is a perennial forage legume with global agronomic importance. The objectives of this project were to generate an assembly of the diploid alfalfa genome, proceed with gene annotation and develop the Alfalfa Breeder's Toolbox (ABT, available at: [alfalfatoolbox.org](http://alfalfatoolbox.org)) as a resource for molecular breeding strategies to enhance plant breeding activities aimed at cultivar development.

**MATERIALS AND METHODS:** PacBio® and Dovetail® approaches were used for sequencing the genome of 'cultivated alfalfa at the diploid level' (CADL) (Fajardo et al. 2016). The MAKER and SPADA pipelines generated high-confidence gene models based on alfalfa RNA Seq data (Dai et al. 2017). Tripal, Drupal and Chado provided the framework toolkit to integrate genome sequences (CADL and *M. truncatula*), molecular markers distributed throughout the genome, and gene expression data visualized using JBrowse (Fig. 1). The gene expression atlas integrates RNA Seq data from different alfalfa subspecies (O'Rourke et al. 2015), tissue types and from those contrasting for tolerance to soil pH, aluminum and drought stress and enables discovery of differential gene expression and co-expression networks. Phenotypic data from diverse alfalfa accessions obtained from GRIN and evaluated in the field using 10 plants per replication in a randomized complete block design with four replications was obtained for agronomic and forage quality traits.

**RESULTS:** CADL genome (V1.0) includes 5,753 scaffolds and 87,892 non-redundant genes representing 95% of the conserved plant gene orthologs. SNPs distributed throughout the genome can facilitate tracking shifts in allele frequencies in tetraploid alfalfa resulting from selection during the breeding process. Differentially expressed genes between different tissues (leaves, stems, roots, flowers) and between tolerant and susceptible alfalfa plants can provide useful insights on stress tolerance mechanisms and facilitate selection for target traits. The ABT also enables *in silico* PCR and BLAST searches of target sequences against the genome assembly.



**CONCLUSIONS:** The ABT is a user-friendly website that includes genomic resources, information and functionalities to advance alfalfa research, trait integration and breeding to increase genetic gains as part of the cultivar development pipeline.

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# Summary of liveweight gains and rotational grazing methods used for sheep grazing alfalfa (*Medicago sativa* L.) in New Zealand

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**KEYWORDS:** Grazing management, Lucerne, Liveweight production

**INTRODUCTION:** New grazing management guidelines for alfalfa have been promoted in New Zealand for the last 15 years (Moot et al. 2003). These maximize consumption of high quality leaf and soft stem, particularly in spring and summer. A period of extended growth in autumn is used to recharge underground reserves to maintain persistence of the alfalfa stands. This paper summarises the grazing rules, liveweight gains and on-farm experiences of farmers adopting them.

**OBJECTIVE:** To quantify animal production by sheep from rotationally grazed alfalfa in New Zealand.

**MATERIALS AND METHODS: Data source.** Data were sourced from three field experiments (Moot et al. 2016) and two on-farm case studies. In each case alfalfa was rotationally grazed from early spring until water deficits stopped growth in summer. A period of 6-7 weeks regrowth is advocated in autumn which is the only time plants are allowed to flower. Grazing in spring commences when the first paddock is 10-15 cm tall and is stocked at ~12 ewes+twin lambs per ha. These animals complete the first rotation through six paddocks in 28-35 days depending on weather conditions. The second rotation aims for all paddocks to be 35-40 cm tall on entry. This equates to about 3 ton of dry matter (DM) per ha. In Canterbury, liveweight gain was measured 2-4 weekly on-station with 3- 5 years of data analysed. On-farm production gains are reported over a 10 year period from Bonavaree farm in Marlborough (550 mm average annual rainfall) and a 9 year period at Bog Roy Station in the Mackenzie District (450 mm average annual rainfall).

**RESULTS:** On-station experiments consistently showed twin lambs growing >300 g/hd/d from birth to weaning (Fig 1a) which allowed them to reach killing weights ( $\geq 17.5$  kg carcass weight) in 100-120 days. On-farm production at Bonavaree has increased meat production per hectare by 94% over 10 years through increased lambing percentage (117 vs. 143%), and individual lamb weights (13.3 vs. 19 kg/hd). At Bog Roy total lamb weight weaned has increased 50% from 20% more ewes (Fig. 1b).

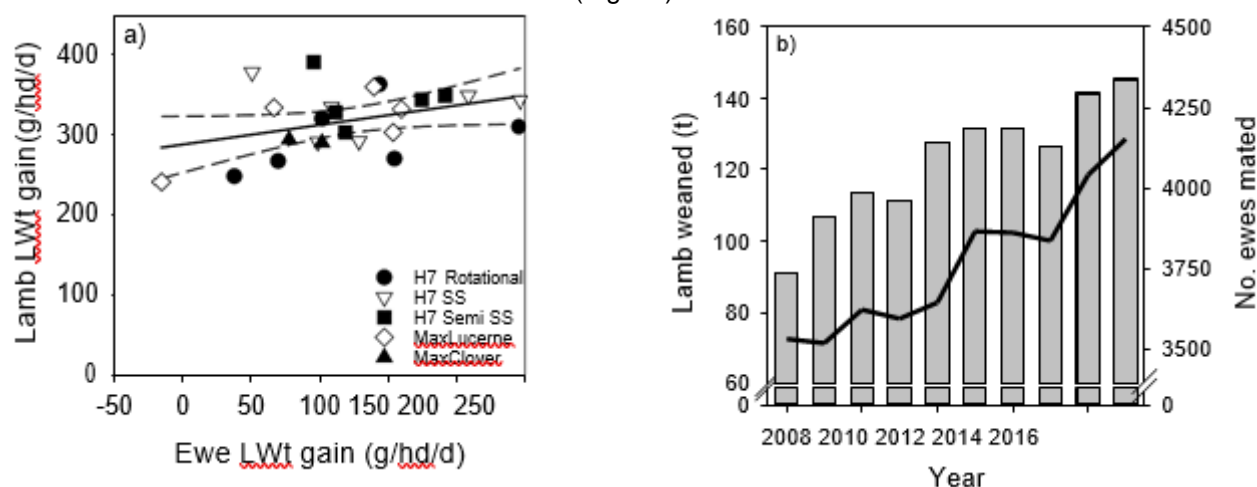


Fig 1. a) Correlation between LWt gain (g/hd/d) of ewes and suckling twin lambs grazing dryland lucerne for three dryland alfalfa grazing experiments in Canterbury and b) changes in lamb weaned (t) and number of ewes mated at Bog Roy Station in the Mackenzie District.

**CONCLUSIONS:** Adoption of changes in grazing management for alfalfa has resulted in increased animal production per head and per hectare on-farm. Changed grazing management has transformed over 200,000 ha of low rainfall areas in New Zealand.

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# Water use efficiency in pasture-crop rotation of intensified livestock systems in the semiarid pampa region of Argentina

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**KEYWORDS:** Biomass production – Alfalfa – Silage maize

**OBJECTIVE:** Assess forage production and water use efficiency in a pasture-crop rotation of the semi-arid pampa region of Argentina.

**MATERIALS AND METHODS:** The study was carried out on a petrocalcic Paleustoll, located at the Experimental Station of National Institute for Agricultural Technology (INTA) at Anguil, La Pampa, Argentina (S 36° 36'37.95"; W 63° 58' 48.22"). Annual rainfall average is 783 mm with mean temperature of 15°C (1973-2018). Crop sequence was four years annual crops (vetch-rye mix in winter as cover crop (*Secale cereale* L. and *Vicia villosa* ssp. *dasycarpa*), maize (*Zea mays*) in summer destined to silage) followed by four-year Wheatgrass (*Thinopyrum ponticum* var. Hulk) – Alfalfa (*Medicago sativa*) pasture (25 and 10 kg ha<sup>-1</sup> respectively). Experimental design was a completed randomized 4- block design. Experimental units and treatments were fully described in Fernández *et al.* (2017) and Oderiz *et al.* (2017). Soil water content (gravimetric method) was determined at the beginning and end of all crop growing seasons. Rainfall was also measured. Crops biomass production was determined by manual harvest and oven- dried at 60° C. Hence, water use efficiencies (WUE) were estimated as the relation between biomass production and consumptive water use. Analysis of variance was carried out using mixed linear models. Means were compared using Fischer Test (p≤ 0.05). All statistical analyses were carried out using InfoStat software.

**RESULTS:** Vetch-Rye biomass production varied across years. However, the year with the lowest values (1<sup>st</sup>) exceeded 2500 kg ha<sup>-1</sup> cited as threshold for evidencing cover effects on soil. Dry matter production of silage maize was superior every year respect of the pasture and also varied among years, showing the highest values in the second year. The WUE of annual crops followed the same way, in spite of last year when rainfall was only 200 mm. However, Wheatgrass-Alfalfa dry matter production and WUE were higher the first year of the growing season (5<sup>th</sup> year of rotation) showing a reduction the rest of the years (Table 1). This drop was 50% between the first and second year and 25% between the second and third year, stabilizing towards the fourth year. This tendency could be associated to losses in the productivity of the pasture, since the consumptive water use (data not shown) did not have a decreasing tendency as did dry matter production. Thus, WUE decreased throughout years.

Table 1: Dry matter production and water use efficiency in Vetch-Rye, Maize and Wheatgrass-Alfalfa during 8 year rotation. Different letters indicate differences between years (p<0.05).

Dry matter production (kg ha <sup>-1</sup> )				
	1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year	4 <sup>th</sup> year
Vetch-Rye	4301 c	10620 a	7976 ab	6076 bc
Maize	16731 b	25422 a	11722 b	12250 b
	5 <sup>th</sup> year	6 <sup>th</sup> year	7 <sup>th</sup> year	8 <sup>th</sup> year
Wheatgrass-Alfalfa	11169 a	5568 b	4222 b	4199 b
Water use efficiency (kg ha <sup>-1</sup> mm <sup>-1</sup> )				
	1 <sup>st</sup> year	2 <sup>nd</sup> year	3 <sup>rd</sup> year	4 <sup>th</sup> year
Vetch-Rye	29.1 b	53 a	24 b	13 c
Maize	40.4 b	43.4 b	17.6 c	62.1 a
	5 <sup>th</sup> year	6 <sup>th</sup> year	7 <sup>th</sup> year	8 <sup>th</sup> year
Wheatgrass-Alfalfa	16.1 a	11 b	7.9 c	7.8 c

**CONCLUSIONS:** Silage maize and pasture biomass yield varied among years in the semiarid region, as well WUE did. On average, annual crops produced higher than pasture and had greater WUE. Future studies should consider beneficial effects of perennial pasture on soil health, as well as the impact of mechanical harvest on cation exchange complex saturation because of bases-nutrient high extraction.

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# Is alfalfa a pasture option in dryland livestock systems in Mediterranean Chile?

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**KEYWORDS:** perennial legumes, Mediterranean pastures, dryland

**INTRODUCTION:** The Mediterranean region of Chile presents a strong concentration of rainfall in autumn and winter (May to September), and a prolonged dry season of 5-6 months. The strategy to improve pasture productivity of dryland has been the introduction and use of annual legumes, like subterranean clover, burr medic, balansa clover and arrow leaf trefoil, as well as mixtures of the above species. However, the distribution of the production of these species is very concentrated in spring. We hypothesize that perennial deep-rooted legumes, like alfalfa can improve pasture productivity by extending the production period and increasing the water use efficiency.

**MATERIALS AND METHODS:** The experiment was conducted at the INIA Experimental Center of Cauquenes (35° 58' S, 72° 17' W; 140 m. above sea level), in the sub-humid Mediterranean zone of Chile. The soil was an Alfisol of pH 6.8, the organic matter content was 1.6% and phosphorus in the top 20 cm was 12 mg kg<sup>-1</sup>. The average annual temperature in this region is 14.7 °C, the minimum average is 4.7 °C (July) and the maximum 27 °C (January). Long-term average annual precipitation is 695 mm with 5-6 month without rainfalls. Nine cultivars of alfalfa from Australia and USA (Table1) were evaluated between May 2012 and January 2018. Two months seedlings previously inoculated and lime pelleted with *Sinorhizobium meliloti* strain WSM2141 were planted in two 3 m long rows separated by 40 cm (60 plants per plot), in July 2012. The experimental design was a randomized complete block with four replicates. Plant survival (%) was assessed at the end of the summer period of each year by counting the number of green plants per plot and expressing the result as a percent-age of the establishment density. Biomass was evaluated annually at the end of winter (August), at flowering (November) and before entering summer dormancy (January) by harvesting 3 m row.

**RESULTS:** Alfalfa presented a high survival rate (varying from 98% in the first year to 50% in the sixth) under the severe water restriction during the summer period. The effects of cultivar and year, and the interaction cultivar\*year were significant. Cultivars with low or no fall dormancy were the most productive during the winter period and in the whole growing season (Table 1). Indeed, the most productive ones reached 2,500 kg DM ha<sup>-1</sup> in late winter, which is not possible to achieve by any of the annual legumes options currently used in dryland Mediterranean areas of central Chile. Total annual production was significant higher in cultivars Sardi Ten, Aquarius, Genesis and Sardi Seven (Table 1).

**Table 1.** Fall dormancy and winter and total dry matter production (average of six years) of nine cultivars of alfalfa in a rainfed Mediterranean site of central Chile.

Cultivar	Fall dormancy	Winter production	Total production (kg DM ha <sup>-1</sup> )
WL 326 HQ (USA)	4	1.611 bc*	6.565 c
Venus (Australia)	5	1.977 b	7.120 b
SARDI Five (Australia)	5	1.817 b	7.104 b
WL 458 HQ (USA)	6	1.718 bc	7.377 b
SARDI Grazer (Australia)	6	1.992 b	7.537 ab
Genesis (Australia)	7	2.496 a	7.733 ab
SARDI Seven (Australia)	7	2.441 a	7.693 ab
Aquarius (Australia)	8	2.428 a	8.365 a
SARDI Ten (Australia)	10	2.514 a	8.875 ab

\* P<0.05, Test Duncan's

**CONCLUSIONS:** These results show that alfalfa has a great potential of as a forage crop in the interior dryland of central Chile. The most cultivars were Sardi Ten, Aquarius, Genesis and Sardi Seven.

## Wide area evaluation of yield and forage nutritive value of a reduced lignin alfalfa (*Medicago sativa* L.) cultivar

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**KEYWORDS:** fiber, digestibility, transgenic trait

Striking a balance between yield and nutritive value of alfalfa forage has always been a challenge for forage producers; as the crop matures yield increases but nutritive value declines. The indigestible polyphenolic compound, lignin, is known to increase with plant maturity and reduce cell wall (i.e. neutral detergent fiber, NDF) digestibility, and thereby limit forage intake and livestock performance. Transgenic alfalfa varieties with less lignin have been developed and made commercially available under the brand name HarvXtra™ in the USA in 2015. The objective of this research was to determine impact of harvest interval on nutritive value and yield of a reduced lignin (RL) alfalfa cultivar compared to two conventional cultivars.

Three alfalfa cultivars, 'HarvXtra-008' (RL) and conventional non-RL 'WL355RR' and '54R02' were sown at 20 kg ha<sup>-1</sup> in spring 2015 in six states (California, Kansas, Michigan, Ohio, Pennsylvania, and Wisconsin). Fertilizer application was made at each location according to state recommendations, based on soil test results. Herbicide, insecticide, and fungicide treatments were applied as needed to control weeds, insects, and foliar diseases, respectively. The experimental design was a randomized complete-block with four replications with a split plot restriction on treatment randomization, where harvest interval (28-, 33-, 38-day) was the whole plot factor and cultivar was the sub plot factor. First growth in 2015 was clipped off and no data collected. Beginning in the second growth cycle of the seeding year and through 2016, plots were harvested based on harvest interval treatments. Before harvest a 500-g sample was collected by hand, dried, ground, and later analyzed for nutritive value using calibrated near infrared reflectance spectroscopy equations for acid detergent lignin (ADL), NDF, ash, NDF digestibility (NDFD) and crude protein. A forage plot harvester was used to cut and weigh plot fresh weights that were converted to dry weights for determination of dry matter yield.

The overall linear model for cultivar response to harvest intervals was significant for nutritive value factors and all cultivars responded similarly across harvest intervals and events. Nutritive value of HarvXtra-008 was always greater than 54R02. Neutral detergent fiber digestibility for HarvXtra-008 was greater ( $P < 0.05$ ) by 4% to 10.4% compared to non-RL cultivars. Neutral detergent fiber was lower ( $P < 0.05$ ) for HarvXtra-008 by 5.4 to 8.3 than the non-RL cultivars. Acid detergent lignin was also lower ( $P < 0.05$ ) by 3.8 to 9.5 for HarvXtra-008 than non-RL cultivars. Ash concentrations were greater in HarvXtra-008 than the conventional cultivars and reason for this needs further investigation. Crude protein concentration was slightly greater in HarvXtra at each harvest, and significantly ( $P < 0.05$ ) greater when analyzed across all harvest events. HarvXtra-008 was slightly less mature at any given harvest, based on mean stage count procedure, than the other two cultivars and also had slightly greater leaf proportion than P54R02. It is not known if maturity and morphological differences among cultivars is related to the down-regulation of lignin pathway enzymes in RL alfalfa.

Differences in yield among cultivars within events and harvest intervals were non-significant; however, HarvXtra-008 produced consistently lower total annual yield than the other two cultivars averaged across harvest intervals. Harvesting on longer intervals resulted in greater annual yield; there was a linear yield increase for all cultivars with increasing harvest interval in 2015 and for the 2015-16 total.

It is concluded that the transgenic reduced lignin alfalfa cultivar HarvXtra maintained lower lignin and NDF concentrations and greater NDFD than non-RL cultivars across a wide range of environments and harvest intervals. The results with HarvXtra-008 are promising for alfalfa growers who want to harvest less frequently and still maintain adequate nutritive value, or when weather delays harvest.



## Evaluation of alfalfa (*Medicago sativa* L.) genetic resources of Epagri - Experimental Station of Lages, Santa Catarina, Brazil

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**KEYWORDS:** germplasm bank, "Alfafa Crioula", fall dormancy

Alfalfa is a forage crop with potential to intensify dairy and beef production in Brazil. This leguminous forage has a great genetic diversity; the called "Alfafa Crioula" populations have emerged in Brazil as result of both, natural and farmers selection. The aim of this work was to evaluate fall growth of alfalfa accessions maintained in the Epagri's forage germplasm bank. Seeds of 84 alfalfa accessions were germinated using paper test, normal seedling were counted after 10 days. Fourteen accessions of "Alfafa Crioula" with good germination rate were transferred to field for fall dormancy evaluation. The Monarca SP INTA (nondormant) and Victoria SP INTA (intermediate dormant) cultivars were used as controls. The experiment was carried out in the municipality of Lages, SC, located at latitude 27° 47' S and longitude 50° 19' W and altitude of 917 m. The region climate is Cfb type according to Köppen classification. The experimental design was a randomized complete block design with four replications. Each experimental unit was composed of eight lines with 4.5m spaced at 0.20m. The fall dormancy rating was determined according to Teuber et al. (1998), with the evaluation carried out in the first year of the establishment. To verify the similarity among accessions a hierarchical cluster analysis was performed using the euclidean distance and Ward's clustering criterium. Seeds of alfalfa accessions had germination ranging from 0 to 98%. Based on the results of the field experiment, it was possible to classify the accessions of "Alfafa Crioula" in three groups of fall dormancy. Figure 1 shows that two accessions were similar to the Monarca SP INTA cultivar and were classified as nondormant, 10 accessions that formed a grouping together with Victoria SP INTA were considered as intermediate dormant. Two accessions formed an isolated group considered as dormant.

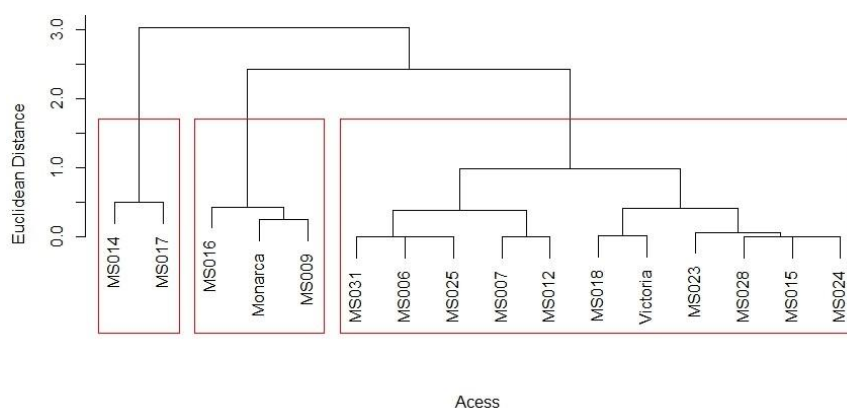


Figure 1 - Dendrogram generated by Ward's method of cluster analysis among the 14 alfalfa accessions from Epagri's forage germplasm bank and 2 commercial controls.

The "Alfafa Crioula" presents a range of dormancy during the fall/winter in the Highlands of Santa Catarina, with the majority of accessions belonging to the intermediate group. Since Brazil has both tropical and subtropical climate, this information will help breeders to select parents for composing synthetic varieties adapted to specific regions.

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## Use of artificial neural networks in an alfalfa (*Medicago sativa*) breeding program

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**KEYWORDS:** Tai index, *Medicago sativa*, computational intelligency

Alfalfa breeding programs aim to develop cultivars that, in addition to high yield, present high nutritive value and tolerance to biotic and abiotic stresses. For this reason, alfalfa breeding is based on a set of important traits, which requires the adoption of selection techniques that maximize the selective accuracy. Artificial Neural Networks (ANN) can be trained to reproduce the same answer that is given by selection techniques applied on alfalfa breeding. This work proposes the use of ANNs in the selection of alfalfa genotypes, based on a selection index. Data from 77 cultivars evaluated in four cuts were used. The evaluated traits were divided into two main groups (yield and nutritive value) and then further subdivided into classes, according to the Tai index scores. After obtaining the scores, the genotypes were sorted in descending order according to the value obtained in the index. From this, four subgroups for production and four for nutritive value were determined, where the genotypes were classified as optimal, good, medium and bad. Two ANNs were established, one for the forage yield group and another one for traits of nutritive value group. The network conformations were defined from the test of different topology possibilities. In all cases, the architecture used the multi-layer Perceptron. The number of hidden layers tested ranged from one to four and the number of neurons in each layer ranged from three to six neurons. The output layer was composed of a single neuron which represents a vector of response (classification of the genotypes). After testing the topologies, the networks that presented the lowest apparent error rates were chosen. The ANNs were processed by Matlab from the scripts of software GENES. For the forage yield group, the topology of lower apparent error rate included six neurons in the first hidden layer, three in the second and six in the third, and the activation functions were tansig, tansig and logsig, respectively. For the nutritive value group, the topology of lower apparent error rate included six neurons in the first hidden layer, six in the second and three in the third, and the activation function for all of them was the logsig. The error rates for the production and nutritive value networks reached values of up to 15%. The classification obtained in the ANN was similar to that obtained in the index for each group of traits. Even in the cuts where the ANN presented the worst performance, it was possible to observe its potentiality for classification of alfalfa genotypes. Once the researcher establishes a great index that considers satisfactory weights of the traits, it is possible to use the ANN successfully to automate the selection in alfalfa breeding programs. The information obtained by the index can be used to train an ANN that is able to classify new observations with the efficiency required in the selection process. In doing so, it is fundamental that there be an effective training of the network, providing examples that characterize several situations. The ANNs obtained in this work can be used in future cuts to classify alfalfa genotypes a breeding program.

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# Alfalfa (*Medicago sativa* L.) coumestrol phytoestrogen content in response to genotypes, cultivar and viral disease

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**KEYWORDS:** Lucerne, severity index, viral complex

**OBJECTIVE:** the objectives of this work were to compare coumestrol phytoestrogen (COU) content in two alfalfa varieties under two viral infection conditions [infected with *Alfalfa mosaic virus* (AMV) + *Alfalfa dwarf virus* (ADV) and non-infected] and to explore the relationship between disease severity and COU production.

**MATERIALS AND METHODS:** plant material: seven genotypes from cv Monarca SP INTA (M) used as susceptible and six genotypes from cv Traful PV INTA (T) used as tolerant were cloned and divided into two groups: 3 infected clones and 3 non-infected clones (NI). The trial was conducted in the greenhouse under controlled conditions. Plants were covered with an anti-aphid fabric to prevent virus infection by insect vectors. COU content in both conditions (I and NI) was measured by HPLC in 6 cuts in ppm (18 measures for each genotype). Disease symptoms in I treatment were visually assessed using a 1 (very light symptoms) to 4 (very affected, nearly dead plant) scale. This, later was used to calculate the Severity Index (SI) for each genotype according to this equation:

$$SI = \frac{\sum[(n^{\circ}PG1 \times 1) + (n^{\circ}PG2 \times 2) + (n^{\circ}PG3 \times 3) + (n^{\circ}PG4 \times 4)]}{n^{\circ} \text{ of cuts in each genotype} \times 4} \times 100 \quad \text{where: } n^{\circ} P = \text{number of plants per genotype, } G = \text{severity grades (1 to 4).}$$

The relationship among COU and SI by genotype was calculated as the sum of the differences of COU content in I and NI in each cut for each genotype, divided by the number of cuts. All data for SI and COU were analyzed using the Linear Mix Model test and genotype means were compared by post-hoc comparison DGC test <sup>(1)</sup>. Differences between I and NI conditions for each genotype were assessed through student t-test.

**RESULTS AND DISCUSSION:** no differences in COU content among cultivars were detected (Table 1). Most of the genotypes showed a general trend to increase coumestrol production in response to viral infection; however, only four genotypes (M18, M29, T140 and T124) significantly ( $p < 0.05$ ) increased COU content relative to their NI counterparts (Table 1). As expected, M behaved as more susceptible ( $p < 0.05$ ) than T, with mean SI values 73.57% and 50%, respectively (Table 1). A negative association ( $p < 0.05$ ) between SI and COU differences (I relative to NI) was detected for M (Figure 1) but not for T ( $p > 0.05$ ).

Table 1: Mean content of coumestrol (COU) and severity index (SI) in 7 and 6 clones of cultivars Monarca (M) and Traful (T) infected (I) and non-infected (NI) with AMV and ADV.

G	NI-COU	I-COU	SI
M10	381 ± 39,6 <sup>a</sup>	337 ± 41,0 <sup>a</sup>	86,1 ± 5,0 <sup>A</sup>
M11	1107 ± 40,2 <sup>a</sup>	1125 ± 40,2 <sup>a</sup>	70,8 ± 8,3 <sup>A</sup>
M18	382 ± 39,7 <sup>a</sup>	524 ± 39,8 <sup>b</sup>	79,2 ± 4,8 <sup>A</sup>
M23	784 ± 39,7 <sup>a</sup>	835 ± 41,0 <sup>a</sup>	77,1 ± 2,1 <sup>A</sup>
M29	695 ± 39,7 <sup>a</sup>	795 ± 39,7 <sup>b</sup>	56,3 ± 2,1 <sup>A</sup>
M36	444 ± 39,7 <sup>a</sup>	521 ± 40,1 <sup>a</sup>	58,4 ± 8,4 <sup>A</sup>
M37	448 ± 40,2 <sup>a</sup>	527 ± 39,8 <sup>a</sup>	86,1 ± 1,4 <sup>A</sup>
T112	nd	nd	41,7 ± 4,8 <sup>B</sup>
T124	727 ± 40,2 <sup>a</sup>	856 ± 40,2 <sup>b</sup>	34,7 ± 5,5 <sup>B</sup>
T129	941 ± 39,7 <sup>a</sup>	950 ± 39,8 <sup>a</sup>	25 ± 0,0 <sup>B</sup>
T140	650 ± 39,6 <sup>a</sup>	759 ± 39,8 <sup>b</sup>	75 ± 4,8 <sup>B</sup>
T141	728 ± 31,2 <sup>a</sup>	741 ± 31,7 <sup>a</sup>	73,6 ± 2,3 <sup>B</sup>
T152	nd	nd	57 ± 5,0 <sup>B</sup>

References: G: genotypes (M: Monarca SP INTA and T: Traful PV INTA), COU (coumestrol) mean values expressed in ppm ± EE (on DM basis) from mean peak areas in HPLC measures. NI = non-infected and I = infected with AMV+ADV. SI = severity index. Different lower case letters indicate significant differences ( $P < 0.05$ ) (unpaired t-test) among between I and NI conditions by genotype. Different capital letters indicate significant differences ( $p < 0.05$ ) between cultivars (M and T). nd = non detected COU.

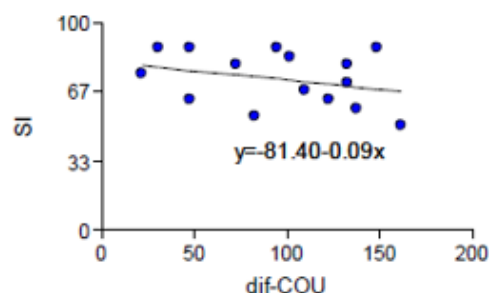


Figure 1: Relationship between differences in COU (ppm) content (I relative to NI) and severity index by genotype (SI) in cv Monarca.

**CONCLUSION:** our results suggest: 1) viral infection seems to stimulate coumestrol accumulation independently of the tolerant or susceptible cultivar condition; 2) this COU increase may be heterogeneous among genotypes; 3) disease severity index (SI) confirmed that cv Monarca SP INTA was more susceptible than Traful PV INTA; and 4) a negative association between genotype differences in COU production (I-NI) and SI was detected in Monarca.

<sup>1</sup>Di Rienzo, et al., 2002. A Multiple-Comparisons Method Based on the Distribution of the Root Node Distance of a Binary Tree. J. of Agr. Biological, and Environmental Statistics, Vol. 7, No. 2 pp.129142.

# Proposal for an alfalfa (*Medicago sativa* L.) hay quality classification system in Argentina

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**KEYWORDS:** alfalfa, hay quality classification system, organoleptic evaluation, Argentina

Currently, Argentina does not have an official and standardized classification system for the commercialization of alfalfa hay. Thus, the National Institute of Agricultural Technology (INTA) proposes that the alfalfa hay quality classification system to be used for both domestic and international markets could be in accordance to the one that it is being used in the USA (Orloff & Putnam, 2015) and in many other countries worldwide. The system includes five hay quality classes: **1) Superior, 2) Premium, 3) Primera, 4) Segunda** and **5) Tercera**, which in comparison to the USA system are equivalent to *Supreme, Premium, Good, Fair* and *Utility*, respectively.

The belonging to a particular class is determined by the chemical composition of the hay, defined by the content of neutral detergent fiber (% NDF), acid detergent fiber (% ADF), relative feed value (RFV), crude protein (% CP) and total digestible nutrients (% TDN). Proposed values for each class are expressed on a dry matter basis and are as follows:

CLASS	ADF %	NDF %	RFV	TDN %	CP %
Superior	< 27	< 34	> 185	> 62	> 22
Premium	27-29	34-36	170-185	60,5-62	20-22
Primera	29-32	36-40	150-170	58-60	18-20
Segunda	32-35	40-44	130-150	56-58	16-18
Tercera	> 35	> 44	< 130	< 56	< 16

ADF: acid detergent fiber, NDF: neutral detergent fiber, RFV: relative feed value, calculated as  $[88.9 - (0.779 \times \%FDA)] \times [(120/\%FDN)]/1.29$ , TDN: total digestible nutrients, calculated as  $82.38 - (0.7515 \times \%FDA)$ , CP: crude protein. All % values are on dry matter basis.

An additional and very important trait is the moisture content, which must not be higher than 14% for both, domestic and export markets.

Besides the chemical composition, each class is also accompanied by an organoleptic description, which can be summarized as follows:

CLASS	ORGANOLEPTIC TRAITS
Superior	Late vegetative to early bud stage; soft, fine stems; very leafy; bright green colour; no evidence of damage.
Premium	Mid bud to late bud stage; fine stems; very leafy; green colour; no evidence of damage.
Primera	Early bloom (10% flower) stage; fine to medium stems; leafy; no evidence of damage other than slight discoloration.
Segunda	Mid-bloom stage (50% flower); moderate leafiness; medium to coarse stems. If evidence of damage, type of and degree must be described.
Tercera	Full-bloom (100% flower) to mid-pod stage; low leafiness; coarse stems. If evidence of damage, type and degree must be described.

Damage may include discoloration (light green to yellow) caused by excessive sunlight, presence of weeds, dirt or other foreign materials, brown colour due to rainfall during drying period, white colour and/or unpleasant smell due to mold growth, and dark/black colour due to fermentation and high temperature caused by excessive moisture at baling.

In order to correctly estimate the real quality of a hay lot, it is essential to obtain a representative sample. For doing that, we strongly recommend to follow the Alfalfa Hay Sampling Protocol defined by Urrets Zavalía *et al.* (2018).

## REFERENCES:

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## Modelling alfalfa (*Medicago sativa* L.) phenological development

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**KEYWORDS:** Lucerne, APSIMX, Node number, Flowering

**INTRODUCTION:** Predicting alfalfa phenological development is important for optimising defoliation scheduling and other management events. Primary drivers of phenological development are temperature and photoperiod (Pp). This project quantified the response of alfalfa to these factors to create algorithms for the APSIMX model.

**OBJECTIVE:** To quantify and simulate alfalfa phenological development in seedling and regrowth crops.

### **MATERIALS AND METHODS:**

**Data source and experimental design.** Data were assembled from two field experiments conducted from 2002 to 2018 at Lincoln University, Canterbury, New Zealand (43°38'S, 172°28'E, 11 m a.s.l.). For both experiments, treatments were imposed as a complete randomized block design with four replications: 1) two cutting frequency and 2) fall dormancy and cutting frequency. Reported data are from fall dormancy class 5 and 42-day cutting frequency, with 7 growing years, each with 3-7 regrowth periods.

**Thermal time calculation and base temperature determination.** Phenological development parameterization involved calculating thermal time (Tt; °Cd) and selecting a base temperature (Tb; °C). For Tt calculations, “broken-stick”, Fick framework, and the WE model were compared. For Tb determination, X-intercept, least variable, and regression coefficient methods were compared.

**Phenological measurements and calculations.** At the beginning of each regrowth cycle, five shoots were marked and node appearance, height, and flowering were measured every 7-10 days. Nodes were counted as primary leaf attachment and height (mm) was measured on fully extended stems. Time of 50% flowering was recorded. Phyllochron was calculated as the slope of Tt against node number. “Heightchron” was defined and calculated as the slope of Tt against height.

**Statistical analyses.** Regression analyses, analysis of variance (ANOVA), and Fisher’s least significant difference (LSD) were determined with RStudio (R. 3.4.0). For model performance evaluation, coefficient of determination ( $R^2$ ), Nash-Sutcliffe efficiency (NSE), and relative root mean square error (R\_RMSE) were calculated. To quantify the causes of deviation, the error was further segmented into components, including standard bias (SB), non-unit (NU) slope, and lack of correlation (LC).

### **RESULTS:**

- Statistical evaluation of methods for calculating Tt indicated that the “broken-stick” model to define cardinal temperature most accurately computed Tt. A Tb of 1 °C had the lowest CV% and highest *P* value.
- The relationship between Tt and primary node number was a positive linear response ( $R^2=0.84-0.99$ ).
- Phyllochron was constant at 35 °Cd/primary leaf in increasing Pp condition. Phyllochron increased from 32 to 51 °Cd/primary leaf as Pp decreased from 16.7 to 10.5 h.  $R^2$ , R\_RMSE, and NSE values indicated strong agreement between predicted and observed values.
- The relationship between accumulated Tt and height was a positive linear response ( $R^2=0.79-0.99$ ).
- “Heightchron” and mean Pp displayed a strong polynomial relationship ( $R^2=0.89$ ) in which “heightchron” decreased as Pp increased from 2.2 to 0.6 °Cd/mm, with highly significant agreement between predicted and observed values.
- Tt to 50% flowering decreased as Pp increased; being 1000 °Cd at 12.3 h and about 500 °Cd at 16.7 h, with highly significant agreement between predicted and observed values.

### **CONCLUSIONS:**

- The “broken-stick” model with a Tb of 1 °C was the most accurate approach for calculating Tt.
- Phyllochron was only responsive to Pp in decreasing Pp conditions (autumn). Greater Tt was required for leaf appearance in short Pp conditions.
- There was a strong polynomial relationship between “heightchron” and mean Pp.
- Tt to 50% flowering decreased as Pp increased.
- There was close agreement between predicted and observed values of node number, height, and time to 50% flowering.

## Effect of different cutting frequencies on the production of *Medicago sativa* L. under irrigation in La Pampa, Argentina

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**KEYWORDS:** forage yield, dry matter, hay production, alfalfa.

**INTRODUCTION:** The alfalfa (*Medicago sativa* L.) is the most important perennial forage in the world, and in Argentina is used extensively for beef and milk production. It is important to consider both forage yield and quality for grazing or hay systems of production. The frequency of cuts is determinant in dry matter production, and also modifies nutritional qualities of the pasture. The objective of this work was to determinate the effect of different cutting frequencies on the accumulated forage production during a season of alfalfa production.

**MATERIALS AND METHODS:** Trial was conducted at Curacó farm (Latitude 37,91° S y Length 67,79° O), located at the irrigation area of La Pampa (Argentina) named "Sistema de Aprovechamiento múltiple de 25 de Mayo" Sección V, using a randomized complete block design with four replications and plots of 6 m<sup>2</sup> (1 x 6 m), with five rows spaced 0.20m from each other. Trial has a 3x5 factorial structure with three varieties and five frequencies of cuttings. Varieties were CW 194 (Fall Dormancy 9), Monarca SP INTA (Fall Dormancy 8) and Pastora (Fall Dormancy 7). Cutting frequencies were every 21, 28, 35 and 42 days, and when plot reached 10% of flowering. Soil in the irrigation area is alluvial, with marked texture heterogeneity from sandy to loamy franc, and presence of rolled boulder in the profile. Climate is continental, with an average annual temperature of 15 °C, and high daily and annual thermal amplitudes. The average annual precipitation is 261.2 mm. The trial was sowed on March 11, 2013 with 20 kg ha<sup>-1</sup> seed density. 80 kg ha<sup>-1</sup> of monoammonium phosphate was applied to sowing, and later 600 kg ha<sup>-1</sup> of the same fertilizer was used after the first irrigation. Irrigation was carried out following farmer protocols (flood irrigation). The crop was irrigated each 15 days from August 30 until the end of the cycle, applying approximately 1,300 mm of water. The three central rows were harvested in each cutting, and the two borders rows and 0.5m in each header were left uncut. Forage production was evaluated as dry matter (kg ha<sup>-1</sup>). Data from season 2015-2016 were analyzed by ANOVA and Tukey test ( $\alpha=0.1$ ) with Infostat software.

**RESULTS:** There was no evidence of interaction between cutting frequency and alfalfa variety ( $p=0.16$ ), so both factors were analyzed separately. Varieties do not show differences in dry matter production ( $p=0.25$ ). The accumulated production of dry matter for each variety were: 13,218.83 kg ha<sup>-1</sup> for CW 194, 13,571.82 kg ha<sup>-1</sup> for Monarca SP INTA and 12,659.41 kg ha<sup>-1</sup> for Pastora. Instead, cutting frequency had significative effect in dry matter production ( $p<0.01$ ). The highest production was achieved when cutting was done at 10 %, and production decreases when the cutting frequency increases (Table 1). No differences were found among cutting treatments at 10% flowering and cutting every 42 and 35 days. Cutting frequency every 21 days is significantly lower than the other treatments, and cutting every 28 days differs significantly from 35 days, 42 days and 10% flowering.

**Table 1.** Accumulated production of dry matter (kg ha<sup>-1</sup>) for each cut frequency, number of cuts for each treatment and phenological stage.

Frequency of cuts	Number of cuts	Dry matter production (kg ha <sup>-1</sup> )	Phenological stage
% 10 flowering	5	17,011.39 a	10% flowering
42	4	15,946.92 a	Early seed pod
35	5	15,425.41 a	Late flower
28	7	11,617.09 b	Late bud
21	8	8,130.61 c	Late vegetative

Different letters indicate significant differences between treatments. (Test: Tukey  $\alpha = 0, 01$ ).

**CONCLUSIONS:** The delay in cutting frequencies (every 35 or 42 days) do not differ from cutting when crop reaches 10% flowering. This is important for hay production plans where yield per hectare is maximized, since cutting every 42 days implies fewer operational difficulties in the production system. However, it should be taken into account how the different cutting frequencies would affect nutritional qualities of the crop.