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Pest incidence and biological regulations in an apple tree-based agroforestry system

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Abbreviations

AC: Agricultural Control

AEI: Agroecological Infrastructures

AF: Agroforestry

AFIR: Agroforestry Inter-Row

AFR: Agroforestry Row

AFS: Agroforestry Systems

AUC: Area Under the Curve

FT-AFS: Fruit-Tree based Agroforestry Systems

GLM: Generalized Linear Models

GLMM: Generalized Linear Mixed Models

INRA: French National Institute for Agricultural Research

N: Nitrogen

NCI: Neighbourhood Crowding Index

NE: Natural Enemies

NS: Not Significant

RAA: Rosy Apple Aphid

SYSTEM: the joint research unit “Tropical and Mediterranean Cropping System Functioning and Management”

Introduction

AgroForestry (AF) represents an opportunity to increase the resilience and sustainability of current production systems, and promote pest regulation by increasing the plant diversity, especially for fruit production. Inspired from multi-strata tropical AgroForestry Systems (AFS) with perennials and based on the assumption that shade trees can benefit to fruit trees by mitigating microclimatic stresses, an INRA innovative system have been developed by the joint research unit SYSTEM Tropical and Mediterranean Cropping System Functioning and Management. Apple trees were planted as an intermediate stratum under a walnut tree upper stratum. Impact of such design on pest dynamics, Natural Enemies (NE) and biological regulations have not been addressed for the moment. This work is integrated in this effort of knowledge acquirement. We especially focused on *Dysaphis plantaginea*, *Aphis sp*, *Cydia pomonella* for pests, and on NE present in apple tree canopy and *D. plantaginea* colonies.

This work is divided into two parts that are dealt separately:

1) Assessment of the impact of AF context on pests, NE and biological regulations.
2) Proposal of enrichment in AgroEcological Infrastructures (AEI) in order to enhance pest control.

After presenting the knowledge background of this study, the context, scientific question and hypothesis are presented. For part one, the material and methods developed to answer the question is explained. Results are then presented and discussed. To finish, part two makes the diagnosis of existing AEI in the current AFS and propose enrichment of AEI.

Bibliography

1) Challenges for apple production

Apple (*Malus domestica*) is among the main fruit produced in temperate regions (Lauri and Simon, 2018), with about 83 M tons of fruit produced in 2017 (FAO, 2019). Sustainability and resilience of conventional apple production systems are questioned (Parisi et al., 2014; Gliessman and Engles, 2015; Simon et al., 2016). Protection from pests and diseases is a core challenge, intertwined with the reduction of pesticide use fostered at European and National levels (*eg* Ecophyto in France) (Ministère de l'Agriculture et de l'Alimentation, 2018; Labeyrie et al., 2018). These products have known detrimental effects on environment and generate risks for human health (Aubertot et al., 2005). In France, the Frequency of Treatment Index is about 35 for apple, as one of the most elevated among temperate fruit-trees (Labeyrie et al., 2018). Redesign of current cropping systems is needed, especially considering agroecological principles that aim at enhancing Ecosystem Services' (ES) provisioning (*eg* biological control), diversifying production and decreasing the reliance on external inputs and negative impacts on

Figure 1: Cydia pomonella adult (left, ©Ephytia, 2015) and larva exiting apple fruit (right, ©Washington State University)

Table 1: Main characteristics of the predominant aphids occurring on apple tree (Ricard et al., 2012)

Species	**Reproduction**	**Number of host**	**Primary host**	**Secondary host**
Aphis pomi	Holocyclic (1)	Monoecious (3)	Apple, Pear	-
Aphis spiraecola	Anholocyclic in Europe (2) and Holocyclic in the rest of the world	Dioecious (4)	Spirea, Citrus	Various species (20 botanical families) including Apple, Pear, Citrus
Dysaphis plantaginea	Holocyclic	Dioecious	Apple	Plantago sp
Eriosoma lanigerum	Anholocyclic	Monoecious	-	Apple, Hawthorn
Rhopalosiphum insertum	Holocyclic	Heteroecious	Apple, Pear, Hawthorn	Grasses

(1) Holocyclic: species having a complete biological cycle with two types of reproduction (sexual and asexual)

(2) Anholocyclic: species which lost totally or partially the ability to reproduce through sexual reproduction and that multiplicates parthenogenetically

(3) Monoecious species: species achieving the totality of its lifecycle on plant from the same species

(4) Heteroecious or dioecious species: species requiring two host plant from different botanical families to achieve their lifecycle

environment (Hill and MacRae, 1996; Wezel et al., 2009; Simon et al., 2011; Gliessman and Engles, 2015) .

2) Implementing Agroforestry Systems with apple trees

Implying a deliberate integration of trees in agricultural systems, or considering already established ligneous species, AFS represent an opportunity for agrosystem redesign (Dupraz and Liagre, 2011). Van Noordwijk and Sinclair (2016) redefined AF as a "type of land use combin[ing] aspects of agriculture and forestry, including the agricultural use of trees", highlighting 1) the increase in diversity and complexity, 2) the diversity among AFS (planted species, spatio-temporal arrangement, management and purposes). AFS are assumed to enhance various ES among which provisioning, regulating (*eg* pest control) and maintenance, and cultural ones (Jose, 2009; Lovell et al., 2018; Haines-Young and Potschin, 2017).

With their high value for AF, fruit trees are the main component of many AFS in tropical areas (Nair, 1991 ; Jha et al., 2011). Yet, in temperate countries, Fruit Tree-based AgroForestry Systems (FT-AFS) represent a tiny fraction of the area covered by orchards (5.98% of the total area planted with fruit in Europe) (Lauri et al., 2019). Lovell et al. (2018) proposed the concept of "Multifunctional Woody polyculture" to name the association of different fruit tree species (and shrubs) and/or with a forest tree species, emphasizing the potential for production, as well as ES provisioning. Though woody polycultures represent 20% of the FT-AFS area, intercropping of fruit trees with forest trees is very scarce (Lauri et al., 2019). In AFS, apple trees are mainly used as the upper stratum, in silvopastoral systems (*eg* 'pré-verger'), or associated with annual crops (Lauri et al., 2016 ; 2019).

Taking the example of tropical AFS, the integration of fruit-trees, and especially apple trees as an intermediate layer, under an upper stratum composed of timber/nut tree, might be promising for Mediterranean areas (Lauri et al., 2016). In these regions with high solar irradiance and dry climate, even if competition for light and nutrients can affect fruit tree growth and cropping, the main expected benefits are related to the mitigation of microclimatic stresses for apple tree (Lauri et al., 2016), *eg* light and heat excess, that can alter fruit quality (sunburn) and leaf functioning (photooxidative damage) (Correlli-Grappadelli and Lakso, 2007; Racsko and Schrader, 2012). Then, it is necessary to assess pests and diseases' dynamics and possible biological regulations in such systems.

3) Pests and diseases: core challenge in apple production

Various pests and diseases attack apple tree. Key pests of apple, on which I will focus, are the codling moth *Cydia pomonella* (Lepidoptera, Tortricidae) (Dorn et al., 1999) and aphids (Hemiptera, Aphididae) (Blommers et al., 2004).

C. pomonella larvae causes direct damage to fruit cropping (Kuhrt et al., 2006b) (figure 1). In Mediterranean regions, three successive generations of *C. pomonella* can occur each year, which substantially increases the potential for fruit damage (Trillot et al., 2002) (appendix I).

Figure 2: Aphis pomi (left) and Aphis spiraecola (right) in their apterous forms (© INRA Bernard Chaubet)

Figure 3: Dysaphis plantaginea in apterous (left) and winged (middle) forms (© INRA Bernard Chaubet), and example of damages caused on a floral shoot (leaves curling and depigmentation) by a colony of D. plantaginea

The apple tree hosts different aphids (sap-sucker insects), the main being: *Aphis pomi*, *Aphis spiraecola*, *Dysaphis plantaginea*, *Eriosoma lanigerum* and *Rhopalosiphum insertum* (table 1). *A. pomi* and *A. spiraecola* are problematic in case of severe attack or on young trees, leading to leaf curling, depigmentation and sooty mold fungi development (Lathrop, 1928). *A. pomi* is totally dependent to apple tree whilst *A. spiraecola* has a wide range of hosts (table 1). They have different lifecycles (appendices II and III), occur during all the growing season of apple tree and are difficult to distinguish (figure 2): molecular tools are required (Naaum et al., 2012).

D. plantaginea – Rosy Apple Aphid (RAA) – is the most damaging aphid on apple tree (figure 3) (Blommers et al., 2004). Holocyclic and dioecious, it spends autumn, winter and spring on its primary host, *Malus domestica* (Rosaceae), and migrates by the end of Spring/onset of Summer on its secondary host, *Plantago* sp (Lamiales, Plantaginaceae) (Bonnemaison, 1959). Return migration in autumn from plantain to apple tree is associated to the unique sexual generation, giving overwintering eggs on apple trees. Parthenogenetic reproduction occurs the rest of the year (Bonnemaison, 1959). Information on the lifecycle of RAA is available in appendix IV. Rapid outbreaks of *D. plantaginea* cause severe damages on apple tree, impacting tree growth and production performances the year of the infestation but also the following years (Blommers et al., 2004; Lathrop, 1928). They generate leaf curling and thickening, and shoots malformations (figure 3), leading to shoot growth arrest (Trillot et al., 2002) and impairing flowering the following year (Bonnemaison, 1959). Finally, honeydew promotes the development of sooty mold fungi (Angeli and Simoni, 2006). As *A. pomi*, *D. plantaginea* is myrmecophilous, *ie* involved in mutualistic interaction with ants: while ants benefit from honeydew, less mortality of aphid colonies and lower predation in presence of ants are reported (Stadler and Dixon, 1999; Stewart-Jones et al., 2008; Miñarro et al., 2010). After inadequate control, 20 to 40% (Blommers et al., 2004), even 80% (Qubbaj et al., 2005) of damaged, non-marketable, fruits are reported. Its high precocity and fecundity lead to a low threshold of tolerance (1 aphid/100 shoots) (Trillot et al., 2002), especially in organic farming (Wyss et al., 1999) where available control levers are restricted (contact insecticides, kaolin…).

4) Biological regulation of apple pests

In this report, we distinguished auxiliaries, Natural Enemies (NE) and predators. Auxiliaries correspond to the fauna beneficial to the agrosystem, including natural enemies (NE) and pollinators for example. NE regroups predators and parasitoids.

Biological regulation represents an opportunity for pest control in orchards. In this report, regulation designate the existence of a “beneficial action of parasites, pathogens and predators in managing pests and their damages (University of California Agriculture and Natural Resources, 2014). As perennial, diverse and multi-strata systems, orchards have great potential for pests and diseases’ populations build up, but also for NE promotion (Nicholls and Altieri, 2004; Simon et al., 2010; Demestihas et al., 2017).

Table 2: Main natural enemies reported as exerting aphid control in fruit trees in Europe (adapted from Ricard et al., 2012). L: Larvae; A: Adult; Juv.: Juvenile. NA: Classification not relevant. For some taxa (eg Carabids, Spiders, Heteroptera), potential for aphid predation depends on the species.

Order	**Family**	**References citing the taxa**	**Example of Species**	**Type of predator**	**Active stage in the predation**
Coleoptera	**Coccinellidae**	(Miñarro et al., 2005 ; Dib et al., 2010)	*Adalia bipunctata, Coccinella septempunctata, Harmonia axydris*	Specialist	L + A
	Carabidae	(Simon et al., 2007 ; Albert et al., 2017)	*Harpalus sp , Bembidion sp…*	Generalist	L + A
	Staphylinidae	(Wyss et al., 1995 ; Simon et al., 2007 ; Albert et al., 2017)	*Tachyporus spp*	Generalist	L + A
	Cantharidae	(Ricard et al., 2012 ; Albert et al., 2017)	*Cantharis livida*	Generalist	L + A
Heteroptera	**Anthocoridae**	(Wyss et al., 1995 ; Simon et al., 2007 ; Albert et al., 2017)	*Anthocoris sp, Orius sp*	Generalist	L + A
	Miridae	(Wyss et al., 1995 ; Simon et al., 2007 ; Marliac et al., 2015 ; Albert et al., 2017)	*Phytocoris, Malacocoris, Heterotoma, Deraeocoris sp, Pilophorus*	Generalist	L + A
	Nabidae	(Wyss et al., 1995 ; Simon et al., 2007 ; Albert et al., 2017)	*Himacerus mirmicoides, Himacerus apterus*	Generalist	L + A
	Geocoridae	(Dib et al., 2010)		Generalist	L + A
Diptera	**Syrphidae**	(Wyss et al., 1995 ; Miñarro et al., 2005 ; Dib et al., 2010 ; Albert et al., 2017)	*Episyrphus balteatus, Eupoedes corollae, Syrphus sp*	Specialist	L
	Cecidomyiidae	(Wyss et al., 1995 ; Miñarro et al., 2005 ; Ricard et al., 2012)	*Aphidoletes aphidimaza*	Specialist	L
Nevroptera	**Chrysopidae ; Hemerobiidae**	(Simon et al., 2007 ; Dib et al., 2010)	*Chrysopa perla, Chrysopa formosa ; Microsomus sp*	Specialist	L
Dermaptera	**Forficulidae**	(Miñarro et al., 2005 ; Simon et al., 2007 ; Dib et al., 2010 ; Marliac et al., 2015)	*Forficula auricularia*	Generalist	L + A
Arachnida (canopy and soil)	**Theridiidae, Philodromidae, Thomisidae, Clubionidae, Lycosidae, Linyphiidae, Salticidae, Miturgidae, Araneidae**	(Simon et al., 2007 ; Lefebvre, 2016 ; Marliac et al., 2016)	*Cheiracanthium mildei, Anyphaena Accentuata, Philodromus cespitum/aureolus, Icius hamatus*	Generalist	A + Juv.
Hymenoptera	**Ichneumonidea : Braconidae**	(Simon et al., 2007 ; Brown and Mathews, 2007 ; Bribosia et al., 2005)	*Aphidius colemani, Ephedrus persicae*	NA (Parasitoids of aphids)	L
	Chalcidoidea : Aphelinidae	(Simon et al., 2007)	*Aphelinus mali*	NA (Parasitoids of aphids)	L

This topic has been extensively studied in orchards (Miñarro et al., 2005; Brown and Mathews, 2007; Simon et al., 2010; Dib, 2010), especially on how to promote natural regulation targeting aphids such as RAA (Wyss, 1995; Albert, 2017; Cahenzli et al., 2019). In orchards, various arthropods have been reported as predating or parasitizing aphids, more especially in the colonies on apple trees (table 2). These NE are parasitoids or predators – the latter categorized as specialists (*eg* Syrphidae) or generalists (*eg* spiders) (Box 1). According to their phenology and ecology, the different taxa of NE can act complementary at different periods in *D. plantaginea* lifecycle either on apple trees (Miñarro et al., 2005) or on *Plantago* (Ricard et al., 2012). In RAA colonies in South-Eastern France, Syrphidae were the earliest in spring, followed by ladybirds and earwigs (Dib, 2010). Most of the studies conclude that NE exerted predation on RAA, but insufficient to maintain populations under the economic acceptable threshold of 1 aphid colony over 100 shoots (Blommers et al., 2004; Miñarro et al., 2005; Brown and Mathews, 2007; Dib, 2010).

5) Questioning the arthropod prevalence in multi-strata FT-AFS

AFS participate to the spatial and temporal diversification of crop and non-crop habitats, which promotes associated diversity such as arthropods and more especially NE (Stamps, Linit, 1998; Hatt et al., 2018). In a meta-analysis, Pumariño et al. (2015) concluded that NE were more abundant in AFS, and that pest abundance was lower only in AFS with perennials (coffee, cacao and plantain). Though AFS is proposed as a lever to enhance biological control (Altieri and Nicholls, 2004; Jose, 2009; Hatt et al., 2018), results depend on environmental context, system design, management practices, insect pest and NE species (Schroth et al., 2000; Chaplin-Kramer et al., 2011). In AFS with perennial crops, impact of shade trees (upper layer) on pests and diseases of the shaded crop is widely investigated in tropical systems (mainly with cocoa and coffee crops) (DeClerk, 2012; Andres et al., 2016). Contrasting results have been obtained in this area, with shade trees reported either as promoting or hampering pests and diseases. According to Andres et al. (2016), this is linked to different levels of shade cover and management between studies.

This topic has been poorly addressed for FT-AFS in temperate regions, and to our knowledge no evidence exists on pest and NE dynamics and regulations with apple trees grown under a shade tree.

Box 1: Generalities about specialist and generalist predators

Specialist predators are generally density-dependent: their populations increase or decrease in synchrony with that of their prey, and these predators rely totally on one or few taxa of prey – for at least one period of their lifecycle. For some predators, not all the stages feed on aphids, *eg* larvae of Syrphidae feed on aphids while adults are pollinators (Ricard et al., 2012). Populations of generalist predators are less synchronized with that of their prey, and these predators can act at tipping points in aphid lifecycle (Ricard et al., 2012). For example, spiders are reported to regulate RAA population in autumn during migration return on apple tree (Wyss et al., 1995), and to exert predation on winter eggs and newly hatched fundatrices during Spring onset (Boreau de Roincé et al., 2012). Synchronicity between a prey and its predator is crucial (Boyer et al., 2017): the earliest their predation activity in the pest lifecycle, the most efficient the control by NE is expected (Ricard et al., 2012).

Context of the study and scientific question

1) System studied and context of the study

Based on the assumption that shade trees can benefit to apple trees in the Mediterranean zone by mitigating microclimatic stresses, an INRA (French National Institute of Agricultural Research) innovative and unique system is currently tested by the joint research unit SYSTEM, located in the Restinclières AF Platform, South of France (website UMR SYSTEM, 2018). The GAFAM (Growing AgroForestry with Apple-Montpellier/Mediterranean) system consists of forest trees (25-year-old walnut trees - upper layer), apple trees (4-year-old - intermediate layer) and herbaceous legume groundcover (2-year-old sainfoin) associated in an alley cropping AFS. The initial question addressed was the impact of walnut trees on apple tree architecture, ecophysiology and fruit production, related to competition for light. It is also important to integrate the effect on pests and diseases, and on mechanisms of biological regulation.

Pests dynamics and natural regulations topic has not been assessed for such innovative system. To address this question, the system was integrated in the five-year research program ALTO ("Systèmes en ArboricuLture et Transition agrOécologique") "Arboriculture Systems and Agroecological Transition" - launched in 2018 (Le Bars et al., 2018). Part of Ecophyto call, this project regroups three system experiments, and aims at designing innovative, low- to no-input apple production systems, evaluating them and sharing the knowledge (Le Bars, 2018). The work presented here takes part in this project, addressing biological regulations in this multi-strata Apple tree-based AFS.

2) Scientific question

2.1) Work investigated

This work was divided into two parts: first, it aimed at studying the impact of AF context on apple tree pests, NE and regulations (part 1). Then, the goal was to propose to enrich the already existing AEI, targeting improvement of biological regulations (part 2).

2.2) Studying pest incidence, natural enemies and natural regulations, hypothesis (Part 1)

I first studied if the AF context, through walnut tree shade and presence, affect the incidence of 3 main apple pests – *D. plantaginea*, *Aphis* sp (dynamic) and *C. pomonella*. Then, I concentrated more precisely on RAA population dynamics along with NE – particularly predators – and ants, in marked colonies, to determine if the AF context impact NE and to study the interactions within this ecosystem. NE in apple tree canopy were surveyed with static sampling - beating, strap strips -, and sentinel-prey card method was tested to estimate pests' predation activity (exploratory work, appendices V to VII).

A continuous variable characterizing the environment in walnut – proxy of AF context – was introduced as explanatory variable (Neighbourhood Crowding Index, see Material and Methods). This variable tightly correlates with ecophysiological and production responses of apple tree: one may wonder if it has also a meaning in terms of arthropod dynamics.

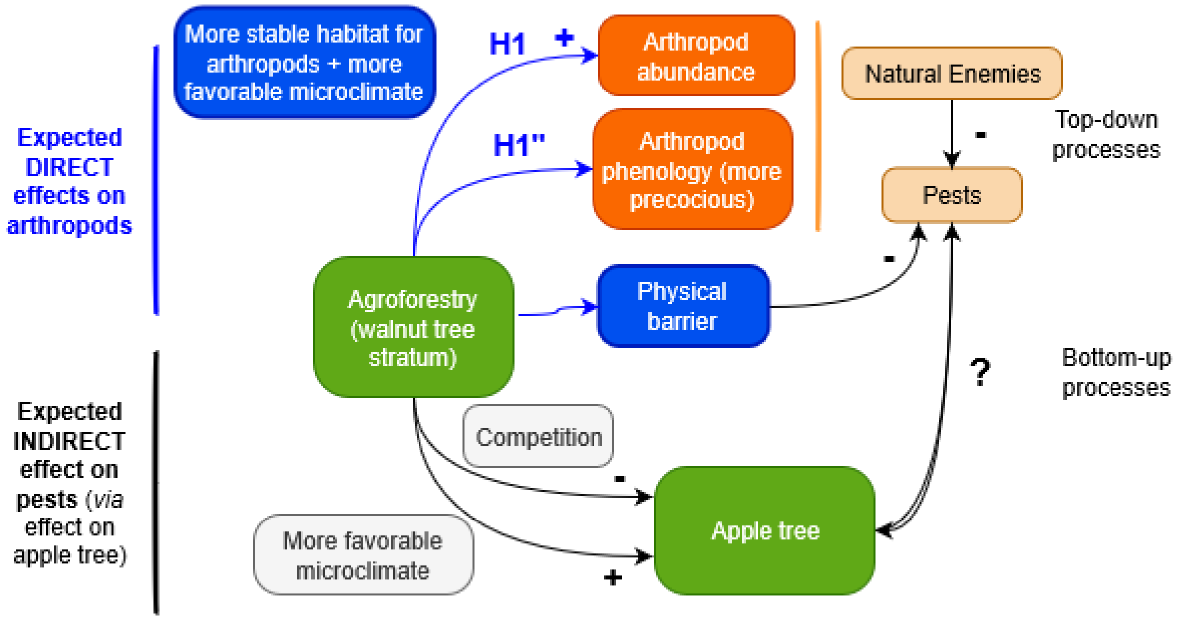

Figure 4: Main hypotheses of the effect of walnut tree presence on arthropods. H1 and H1'': hypotheses that are going to be tested

Box 2: Agroecological Infrastructures (AEI)

Implementation of AEI is an approach integrated into the framework of "Conservation Biological Control", which "aims to enhance pest control, by 1) improving resources for natural enemies through habitat enhancement, and 2) reducing pesticide-induced mortality in NE population" (Holland et al., 2016). Maintenance or implantation AEI or Semi-Natural Habitats are a lever to achieve this goal. AEI can be defined as "any agroecosystem's habitat in or around which a spontaneous vegetation […], or a sown cover crop intentionally not harvested, develops" (Sarthou, 2016). It regroups a diversity of habitats (hedges, wood, grass strips, rocks, water pond…). AEI aim at increasing the diversity and abundance of NE in the crop by increasing floral diversity and providing complementary, supplementary resources such as food, oviposition site and shelter from adverse conditions (*eg* overwintering, agricultural disturbances…) (Landis et al., 2000; Ricard et al., 2012).

Some hypothesis can be proposed regarding the effect of AF context on the studied pests and their NE (figure 4). Only the hypotheses H1 and H1" are going to be tested in this report.

First, it can be supported that walnut tree presence positively impacts arthropods abundance (H1) - either NE or pests (especially the incidence of the latter) by creating a more favourable microclimate for them - typically in AFS (Altieri and Nicholls, 2004) -, and by providing them an undisturbed shelter (Stamps and Linit, 1998; Altieri and Nicholls, 2004). For these reasons, AF context might also lead to changes in the phenology of these arthropods (H1"). Walnut trees can also act complementary as a physical barrier to pest colonization (*eg C. pomonella*). As hybrid, they are not reservoir for this pest (Ephytia, 2015).

On the other hand, while microclimate alteration has a slight positive effect on apple tree, light and nutrient competition impact negatively its growth (*eg* leading to more frequent and longer shoot growth cessation) and cropping (Pitchers, unpublished results). This is expected to negatively affect aphid dynamics (bottom-up processes, figure 4). Indeed, these latter are assumed to attack more severely the most vigorous plants (Grechi et al., 2008; Stoeckli et al., 2008).

Top-down processes (*ie* predation or parasitism by NE, figure 4) are also expected as regulating factors of pests.

In this current report, after presenting the methods used, the impact of AF - in the specific context of the experiment - on the incidence of the 3 pests followed, at apple tree scale, is presented. Then, I focussed on predators and biological regulations in *D. plantaginea* colonies.

2.3) Proposal of Agroecological Infrastructures (Part 2)

After presenting and discussing the results of part 1, I broaden the scope of my analysis with the proposal of enrichment in AEI that aim at promoting biological regulations in the specific context of the experiment (Box 2) - after a first diagnosis step.

Part 1: Studying pest incidence, natural enemies and biological regulations

1) Material and Methods

1.1) Description of the experimental site and design of the system experiment

1.1.1) Experimental site, climate and soil characteristics

The system experiment is settled in the Domaine de Restinclières, in Prades-le-Lez town. This town is located at 20 km at the North of Montpellier, South of France (figure 5). The Hérault County Council owns this Domain which covers 220 ha. More than 40 ha are dedicated to agronomic research in AF and managed by INRA, since 1995 - date of tree plantation. The experimental plot 'A1' is situated in the South of the Domain (figure 5).

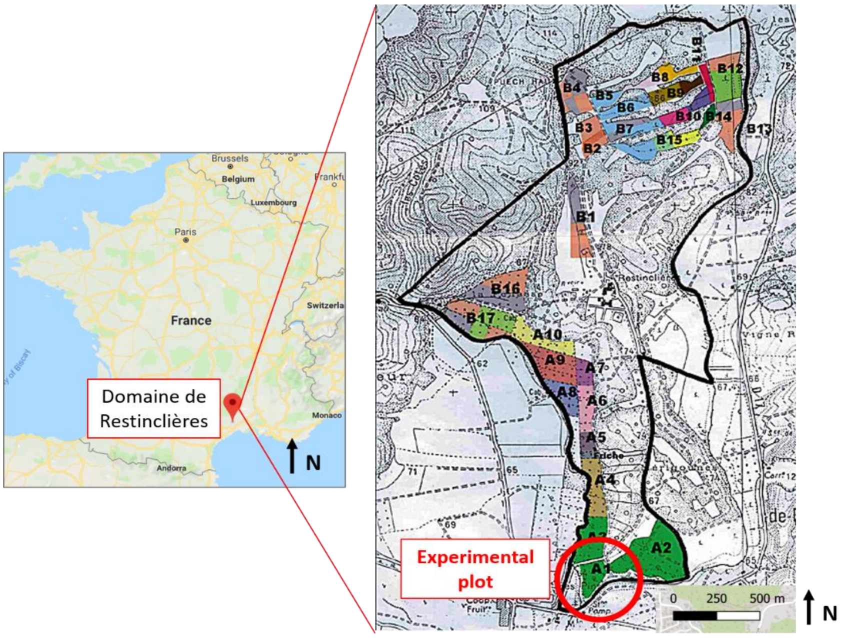

Figure 5: Domaine de Restinclières and experimental (or GAFAM) plot location (© Google Maps, UMR System)

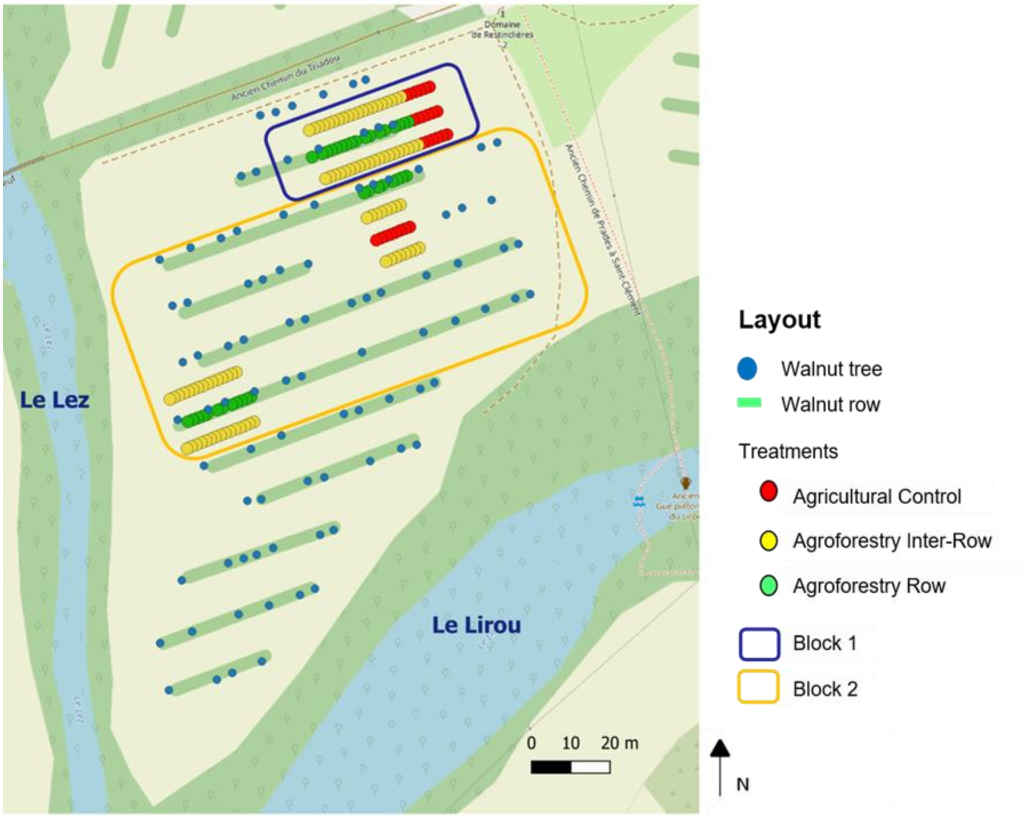

Figure 6: Experimental design. AC: Agricultural control; AFIR: Agroforestry inter-row; AFR: Agroforestry row

Table 3: Number of apple trees per treatment and block. AC: Agricultural Control; AFIR: Agroforestry Inter-Row; AFR: Agroforestry Row

Block	**1**			**2**		
Treatment	**AC**	**AFIR**	**AFR**	**AC**	**AFIR**	**AFR**
Nb of apple trees per treatment	18	40	25	8	45	12
Nb of apple trees per block	83			65		
Nb total	148					

Prades-le-Lez has a Mediterranean climate and is situated 70 m above sea level. In Prades-le-Lez, mean minimal and maximal temperatures were respectively of 8.7°C and 18.8°C. Mean precipitation was about 745 mm. More information regarding on microclimatic variables on the plot A1 - temperature and radiation taken from a meteorological station - during the monitoring course in 2019 is provided in appendix VIII. Soil presents a clay loamy texture.

1.1.2) Plantation design and plant material

The plot A1 is bordered by hedges and riparian trees of Le Lez and Le Lirou rivers (figure 6). Information regarding the flora surrounding apple trees is provided in the 2nd part of this report.

In the plot, hybrid walnut trees (*Juglans regia* x *Juglans nigra*) were planted with cereals in 1995, for wood production - with 13m between the rows and 4m between trees within the row. This was an intercropping design, with an East-West row orientation (figure 6). A thinning operation in 2004 let half of these trees, unevenly. In 2011, alfalfa *Medicago sativa* replaced cereals.

Apple trees of 'Dalinette' variety grafted on G202 rootstocks were planted in April 2016, at a density of 1,125 trees/ha, separated by 1.3m on the row.Roughly, this variety is recommended in organic farming and has been selected for its resistance to scab (*Vf* gene). The rootstock, vigorous, presents a good ability to grow in poor soil conditions especially in replantation and a tolerance to telluric diseases (Lauri et al., 2019). The variety 'Story' was chosen as tree pollinator. In total, 148 apple trees were planted – 141 trees of 'Dalinette' and 7 trees of 'Story'. Three treatments were distinguished (section 1.1.3). Sainfoin *Onybrichis viciifolia* replaced alfalfa blocks in 2018. Apple tree rows and mixed walnut-apple tree rows are separated by 6.5m. Walnut trees constitutes the upper stratum, covering apple trees at the intermediate stratum and the legume groundcover at the lower stratum.

1.1.3) Core explanatory variable: treatment and Neighborhood Crowding Index

Initially, three treatments, were distinguished (figure 6, table 3), as discrete explanatory factor:

- Agricultural Control (AC): apple trees that receive little shade from nut trees, constituting the control (internal to the experiment)
- Agroforestry inter-row (AFIR): apple trees planted at 6.5m of the walnut tree rows
- Agroforestry row (AFR): apple trees planted on the walnut tree rows

In order to have a better proxy of the heterogeneity in light interception brought about by walnut trees and of their influence on each individual apple tree, the Neighbourhood Crowding Index (NCI) has been introduced. This type of index is commonly used in forestry to assess competitive effects of neighbouring trees on a focal tree, assuming that competitive effects of these trees – partly related to light interception - increase with neighbouring trees' size and proximity (Pretzsch, 1997; Canham et al., 2004; Fichtner et al., 2017; Andrés et al., 2018). According to preliminary results of B. Pitchers, this variable tightly correlates with some apple tree traits: architecture (*eg* number of branches), growth dynamics, and ecophysiology (unpublished results), and seems consistent to capture competitive effects of walnut tree on apple trees.

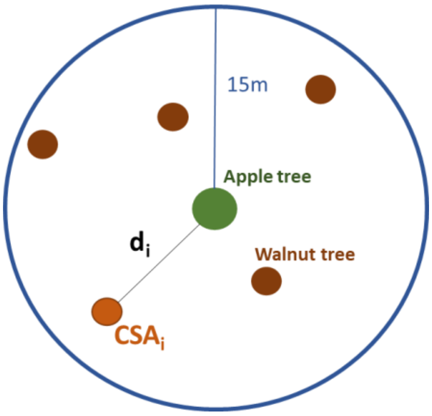

Figure 7: Scheme of Neighbourhood Crowding Index construction

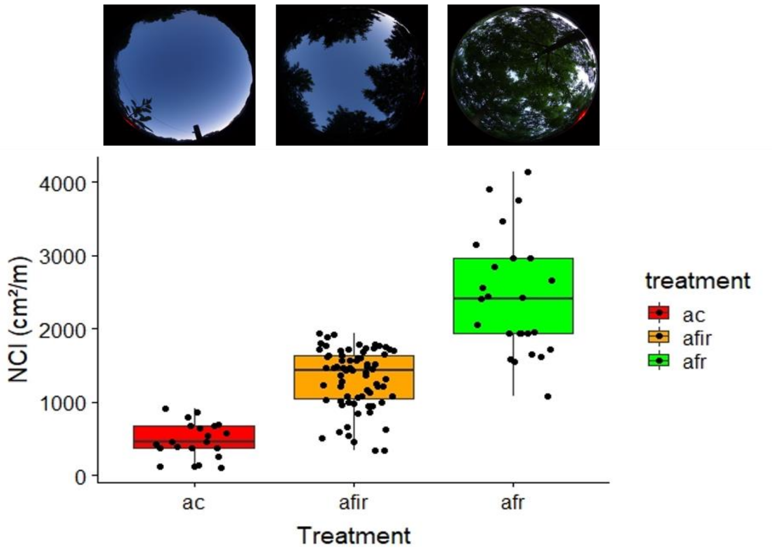

Figure 8: Neighbourhood Crowding Index variability of the apple trees in the three treatments. Hemispherical pictures provide an objective measurement of the forecasted walnut tree shade variation in each treatment. For each treatment, boxplot figures the median, the interquartile range and the complete range of data variation (ac: Agricultural control, afir: Agroforestry Inter-row, afr: agroforestry row)

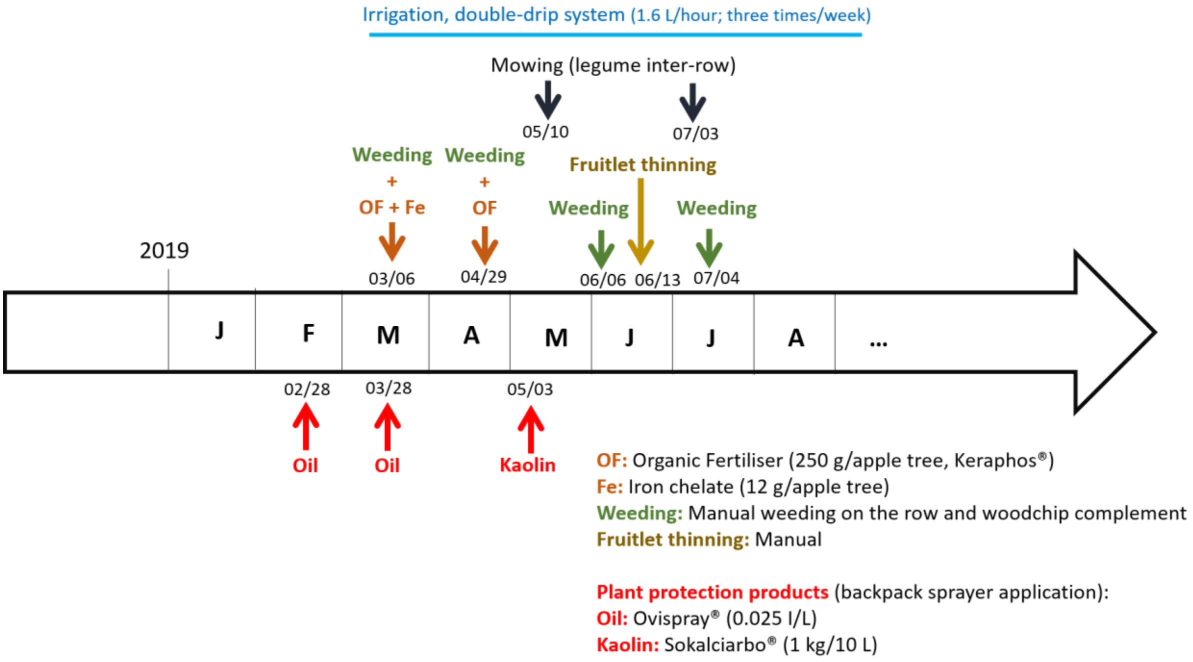

Figure 9: Cropping Management Practices on the apple-tree based AFS in 2019

Here, this index has been calculated for each apple tree, as a continuous variable characterizing the "environment created by walnut trees". Inspired from Canham et al. (2004), NCI was constructed as the sum of the cross-section area of each neighbour walnut tree within a 15m radius area around the apple tree, weighed by their distance to the apple tree (see equation 1; inspired from Canham et al., 2004) (figure 7). The 15m-radius has been reported by Canham et al. (2004) as enabling to catch the effective range of competitive interactions between neighbouring trees and corresponds approximately to the height of walnut trees. The cross-section area at breast height is used, as a proxy of walnut tree vigour, height, crown projection and forecasted shade at the equinox, and a scaling parameter for competitive effects (Canham et al., 2004; Fichtner et al., 2017).

Equation 1: $NCI = \sum_{i=1}^{n}(CSA_i \times \frac{1}{d_i}) = \sum_{i=1}^{n} (\mathrm{DBH}_i^2 \times \pi \times \frac{1}{4} \times \frac{1}{d_i})$ *(Within a 15m radius)*

With CSA_i: Cross Section Area of the walnut tree i
DBH_i: Diameter at Breast Height of the walnut tree i
d_i: distance between the apple tree and the walnut tree i

Most of pests and NE monitoring were made on a sample of 45 'Dalinette' apple trees (15 per treatment) also followed by B. Pitchers. These trees were selected as having a relatively homogenous basal diameter for sap-flow measurements and then as being distributed along a gradient of NCI.

Initial treatments correspond to NCI classes that represent the gradient of competition, though they are overlapping slightly (figure 8). To render figures more readable, results on the evolution of arthropod abundances across the season were represented based on treatment distinction, while statistical analyses were done considering NCI as the independent continuous variable that represents more precisely AF context.

1.1.4) Cropping management practices

Apple trees are under organic and "low-input" management (Parisi et al., 2014), with advices from the CETA Hérault-Vidourle. No pruning or training operation on the apple trees themselves have been made. Figure 9 presents Cropping Management Practices on the plot in 2019.

1.2) Arthropods survey: aphid pests and natural enemies

Monitoring had to be stopped from May 31st to June 17th.

1.2.1) *Dysaphis plantaginea* and *Aphis* sp incidence

Incidence of *Aphis* sp[1] and *D. plantaginea* was followed dynamically at apple tree scale. It was assumed that not only NCI impacted the proportion of infested shoots, but also the type of shoots (floral vs vegetative) and the side of the tree (South vs North). From 0 to 4m high, shoots were browsed for absence/presence of each type of aphid. This monitoring was done on a sample of 45 apple trees (15 per treatment). A shoot was considered as infested when at least one aphid was noticed.

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[1] : It was not possible to determine according to morphological criteria whether the aphid *Aphis* spp infesting the plot was *Aphis spiraecola* and/or *Aphis pomi*.

The variable measured was thus the number of infested shoots – per aphid taxa, azimuth (*ie* North/South) and type of shoot (*ie* vegetative or floral). This survey was made weekly from the 10th of April to the 27th of June. *D. plantaginea* migrating in June (Bonnemaison, 1959), by the end only *Aphis* sp was followed.

Total number of vegetative and reproductive shoots was counted in June – per azimuth and type of shoot – in order to calculate the proportion of infested shoots for each apple tree.

1.2.2) Incidence of *Cydia pomonella*

At the end of the 1st generation of *C. pomonella* (July 8th), according to the BSV of the CETA Hérault-Vidourle, *C. pomonella* incidence on fruit was measured: on all the apple trees, all the fruit – after manual fruitlet thinning – were browsed for *C. pomonella* stings.

1.2.3) *Dysaphis plantaginea* colonies: population dynamics, ants and natural enemies

Marked colonies of *D. plantaginea* were monitored visually, weekly from April to the migration on plantain, based on the method used in previous studies. This allowed to access to aphid population dynamics and study the link with NE and ants (Dib, 2010; Albert, 2017).

Infested shoots were marked - between 0 to 1.7 m (for practical reasons) - at the phenological stage F/G of the apple tree, found by browsing all the trees of Dalinette variety planted in 2016 (119 trees), during 2 successive weeks. The 1st week, one colony on floral shoot on each tree detected as infested was marked. Initial idea was to have 30 shoots/treatment to cover the gradient of NCI. Vegetative shoots were first discarded, assuming dynamics to differ between inflorescence and vegetative shoots. At this first marking date, the effective in AC (n=14) and AFR (n=6) treatments were low. Thus, the 2nd week, it was decided to increase the number of shoots marked in AC and AFR, by adding one colony (when found) per infested tree and including vegetative shoots in the marked sample. Finally, the number of shoots marked was the following: 19 in AC, 41 in AFIR, 24 in AFR .

When one colony disappeared, it was replaced by another, ideally in the same tree or in a neighbour tree which had not already 2 marked colonies.

In the marked colonies, were counted the abundances of:

- *D. plantaginea* (wingless and winged individuals)
- *Aphis* sp, if present
- Ants
- Abundance by taxon of predators[2] observed previously (Dib, 2010; Albert, 2017), and their stage (*ie* egg, larva, adult): Coccinellidae, Anthocoridae, Miridae, Nabidae, Syrphidae, Cecidomyidae, Chrysopidae, Hemerobiidae, Forficulidae, Hymenoptera, Arachnida.

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[2] : Number of parasitoids was not counted though few mummies were observed by the end of *D. plantaginea* migration, as their occurrence is reported as varying between studies (Albert, 2017) and delicate to assess

Table 4: Type of analysis/Model equations tested for each type of monitoring

Variables studied		**Analysis**	**Model equation**	**Distribution family**	**Link function**
Incidence *D. plantaginea* and *Aphis spp*	**Incidence at tree scale**	GLM	Infested tree (0 or 1) ~ NCI (scaled)	Binomial	Logit
	Proportion of infested shoots	GLMM	cbind(number of infested shoots, nuber of uninfested shoots) ~ NCI (scaled) + Azimuth + Type of shoots + (1 | apple tree)	Betabinomial	Logit
Incidence *C. pomonella*		GLM	cbind(nb of fruits damaged, nb of fruits undamaged) ~ NCI (scaled)	Betabinomial	Logit
***D. plantaginea* colonies**	**logAUC of *D. plantaginea***	GLMM	logAUC *D. plantaginea* ~ NCI (Scaled) + (1 | apple tree)	Gaussian	Identity
	Abundance of ants and Natural Enemies	GLMM	Nb of individuals for a given taxa ~ NCI (Scaled) + AUC *D. plantaginea* (Scaled) + (1 | apple tree)	Poisson	Log
	Phenology of ants	GLMM	Nb of ants ~ NCI + Abundance of *D. plantaginea*	Poisson	Log
	Regulations	PCA	Illustrative variables: NCI and relative growth rate of *D. plantaginea* . Active	-	-
		LM (first date) and GLMM	Relative growth rate of *D. plantaginea* ~ Predator + Ants + (1 | apple tree)	Gaussian	Identity

1.3) Statistical analyses

R software was used for statistical analyses (R Core Team). Generalized Linear Models (GLM) and Generalized Linear Mixed Models (GLMM) were mostly used (glmmTMB Package; Magnusson et al., 2019) with the choice of statistical laws depending on the studied variables (see below). Also, PCA was used (FactoMineR package; Husson et al., 2019). GLMM was used to introduce tree as a global random factor. For these models, normality and homoscedasticity conditions of the residuals are not expected (Zuur et al., 2009). Yet, model validation process is still debated, especially for GLMM (Zurr et al., 2009; J. Peyhardi, J.B Durand and C. Della Vedova, personal comm.). NCI was used as core explanatory continuous covariable in all these models. Based on Schielzeth (2010) recommendations, quantitative covariables (NCI, Area Under the Curve *D. plantaginea*) were first scaled (centred and standardized), except for models designed to study interactions between organisms (it would have modified the true estimates).

Binomial distribution was assumed to fit proportion and binary variables, Poisson distribution to fit count variables and normal distribution to fit continuous variables. Then, independence on the residuals, as well as overdispersion, were checked. Betabinomial (binary and proportion variables) and Negative binomial (count variables) distributions were used to tackle overdispersion (Zuur et al., 2013).

Considering the number of explanatory variables, and potentially the high number of possible interactions, even when restricting to order-two interactions, the choice was made to concentrate our analyses on the proper effects of explanatory variables.

Non-significance (NS) threshold was fixed at 5%.

1.3.1) Dysaphis plantaginea and Aphis spp incidence

1.3.1.1) Evolution of the incidence at tree scale

For each aphid taxa a new variable was created, corresponding to whether the tree was infested (1; at least one shoot with aphid) or not (0). The effect of NCI on the probability of a tree to be infested was assessed with a logistic regression, at each date (table 4). For RAA this was made on all dates while for *Aphis spp* this was possible only for the first and last two dates (all the trees infested the other dates).

1.3.1.2) Evolution of the proportion of infested shoots

This analysis was made on a sub-sample of trees (all infested; exclusion of not infested trees). Proportion of infested shoots were only calculated for dates for which the number of infested trees was above 5 trees per treatment (n = 15 trees per treatment). Model outputs were only interpreted for dates for which median proportion of infested trees were above 5%, to minimize observation bias. Four observations per tree were made (combination between azimuth and type of shoots), implying dependency in the data: observations within a same tree were more linked than observations between different trees. To include this dependency, a GLMM was used (Zuur et al., 2009), with apple tree as random effect, assumed to be a constant (random intercept).

All other factors were fixed (table 4). The aim being to compare between dates the effect and significance of the different variables, the same model was tested on each date separately, and no model selection was made.

1.3.2) First generation *Cydia pomonella* incidence

A GLM was tested on the number of fruits damaged over the total number of fruits (table 4)

1.3.3) Predators, *D. plantaginea* and biological regulation of *D. plantaginea* in *D. plantaginea* colonies

1.3.3.1) *D. plantaginea* and predators' abundances

For RAA, thanks to splinefun R function, a curve of the evolution of the number of *D. plantaginea* was fitted, to access the Area Under the Curve (AUC) on all the survey period for each colony. For ants and predators, the sum of each taxa over the entire monitoring period was computed for each colony. Then, a model GLMM was implemented for each taxon (RAA and predators) to test the effect of NCI (table 4). RAA AUC was integrated as a covariable in the model testing the effect on predators' taxa, as RAA conditions the presence of predator (Albert, 2017). GLMM was necessary to account the fact that, in some apple trees, 2 colonies were surveyed: apple tree was integrated as intercept random effect (hypothesis of constant effect).

1.3.3.2) Phenology of ants

The analysis of the effect of AF on arthropod phenology could be made only on ants (not enough individuals in the other taxa of predators). For that, each date was analysed separately with a GLMM model, with ants' abundances at each date explained by the NCI and the abundance of *D. plantaginea* in each colony (table 4). When median number of individuals were equal to 0, model outputs were not considered.

1.3.3.3) Interactions within the colonies: regulations and myrmecophily

For this analysis, the last date was discarded, being the end of *D. plantaginea* migration (June 27th). First, the relative growth rate of *D. plantaginea* was calculated thanks to a curve fitting (splinefun function, R) on the evolution of the number of *D. plantaginea* individuals in each colony (Equation 1 and 2; Albert, 2017). This latter can be considered as representing RAA population dynamics (Albert, 2017).

Equation 1: $\frac{dN(t)}{dt} = \mathrm{r(t)} \times N(t)$

with r(t) the relative growth rate at t : $r(t) = \frac{dN(t)}{N(t) \times dt}$

Equation 2: $r(t) = \frac{dN(t)}{N(t) \times dt}$

with $\frac{dN(t)}{dt}$ the first derivative of the evolution of the number of RAA at t
$N(t)$ the number of RAA observed at t.

Then, our aim was to have an overview of the potential predation and myrmecophily within the colonies (Albert, 2017). A first visualisation was made thanks to PCA (FactoMineR package), on each

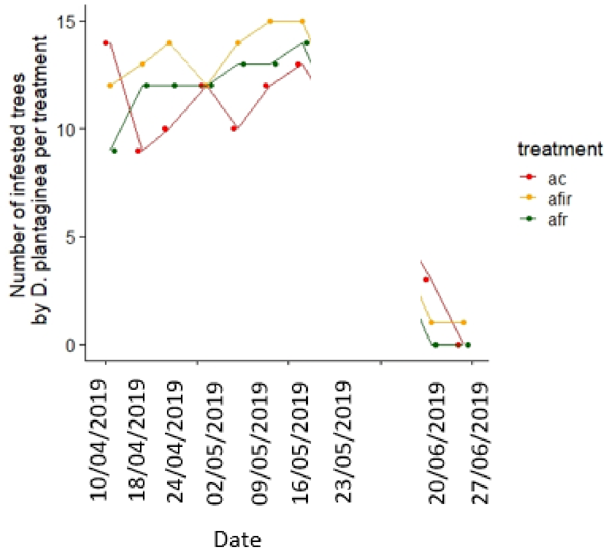


Figure 10: Evolution of the number of infested trees by D. plantaginea according to the treatment. Monitoring had to be stopped between May 27th and June 17th. *n = 15 trees per treatment*

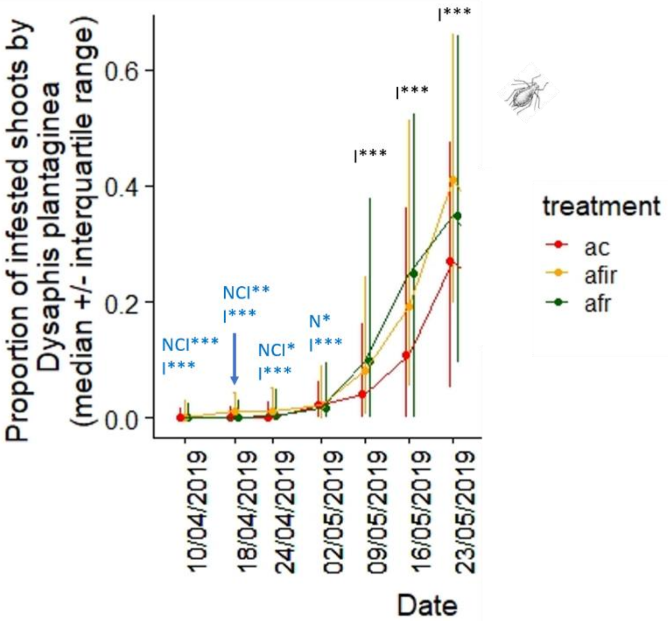


Figure 11: Evolution of the proportion of infested shoots by D. plantaginea per tree (median and interquartile range by treatment).
Figure and analysis were made excluding not infested trees. Results of significance using GLMM date per date are provided (n=45 trees). In blue: all the date with a median less than 5% of infested shoots are hardly interpretable.
ac: Agricultural control; afir: Agroforestry Inter-row; afr: Agroforestry row.
NCI: NCI effect; N: More infested shoots on the North side (Azimuth effect); I: Inflorescence more infested (Shoot type effect).
*NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*

Table 4: Evolution of the proportion of infested shoots by D. plantaginea per tree.
Effects of NCI, azimuth and shoot type on this proportion (n=45 trees). For each factor, the estimate (with significance) is given as a difference between a treatment indicated after the name of the variable and the reference treatment indicated between parentheses using GLMMs. Analysis was made excluding not infested trees. In blue: all the date with a median less than 5% of infested shoots are hardly interpretable.
*In bold: significant effects. NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*

Date	NCI	Azimuth: South (North)	Shoot type: Vegetative (Inflorescence)
	Estimate (significance)		
10/04/2019	**0.50 (***)**	-0.294(NS)	**-1.78(***)**
18/04/2019	**0.372 (**)**	-0.303 (NS)	**-1.26 (***)**
24/04/2019	**0.278 (*)**	-0.212 (NS)	**-0.788 (***)**
02/05/2019	0.235 (NS)	**-0.362 (*)**	**-0.983 (***)**
09/05/2019	0.340 (NS)	-0.301 (NS)	**-0.707 (***)**
16/05/2019	0.259 (NS)	-0.156 (NS)	**-0.802 (***)**
23/05/2019	0.175 (NS)	-0.173 (NS)	**-0.917 (***)**

date separately (from the 15th of April to the 27th of May) with the number of individuals in each taxon of arthropods (active variables), the relative growth rate of *D. plantaginea* and NCI (illustrative variables). For each date, only taxa observed more than 5 times were included as active variables (if not, they were put as illustrative variable). Significance threshold for correlation with the dimensions was fixed at 5%. To go further, interactions were analysed based on the hypothesis that the population of RAA within a colony evolves according to its intrinsic growth rate r, and to the number of predators and ants. It can be assumed that predators (P) will eat a number of aphids that will depend on the total number N of aphids (e), e being expected to be negative (Equation 3 and 4). Similarly, ants (A) might have the same kind of effect but either positive or negative (h) according to the period (Equation 3 and 4).

Equation 3: $\frac{dN(t)}{dt} = rN(t) + eN(t) \times P(t) + hA(t) \times N(t)$

which gives

Equation 4: $\frac{dN(t)}{N(t) \times dt} = r + eP(t) + hA$

Except for the first date were LM was required (a unique colony per apple tree), a GLMM was performed (several colonies per tree) on this hypothesis at each date separately (to be consistent with the PCA). Relative growth rate was assumed to follow a gaussian distribution, and apple tree as being a constant random effect (random intercept).

2) Results

2.1) Incidence of the 3 main apple pests

2.1.1) Incidence of *D. plantaginea*

2.1.1.1) Evolution of the incidence at tree scale

Regardless the date, the proportion of infested trees (*ie* having at least one shoot with *D. plantaginea*) seemed to vary but not to be linked to the AF context (figure 10) (n = 15 trees per treatment). At some dates, almost all trees were infested (*ie* May 16th). Probability to be infested for a given tree was not significantly correlated to the NCI, as confirmed by the GLM performed (appendix IX).

2.1.1.2) Evolution of the number of infested shoots at tree scale

The evolution of the proportion of infested shoots was similar over the three treatments and variable within treatment (figure 11). Only three dates were interpretable, *ie* having a median proportion of infested shoots above 5% (from May 9th to May 23rd). The analysis date per date outlined no significant effect of the NCI after May 2nd, as for the Azimuth (figure 11 and table 4). For all the dates, vegetative shoots were significantly less infested in proportion than floral ones (negative estimates).

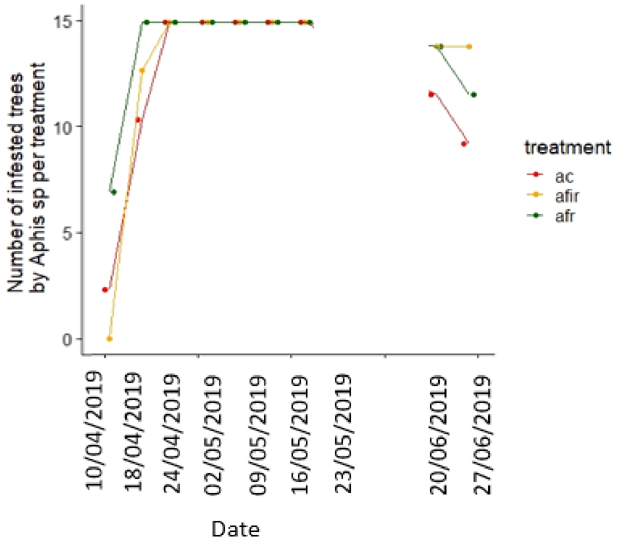


Figure 12: Evolution of the number of infested trees by Aphis sp *according to the treatment. Monitoring had to be stopped between May 27th and June 17th. n = 15 trees per treatment*

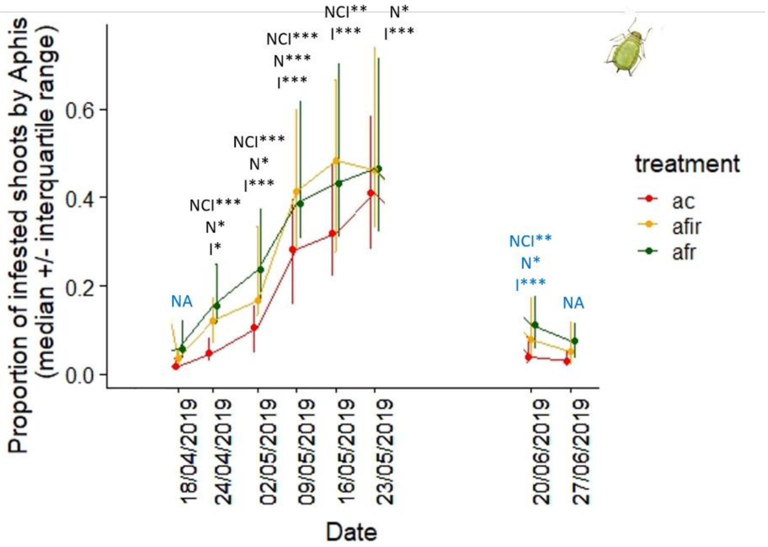


*Figure 13: Evolution of the proportion of infested shoots by Aphis sp per tree (median and interquartile range by treatment). Figure and analysis were made excluding not infested trees. Results of significance using GLMM date per date are provided (n=45 trees). In blue: all the date with a median less than 5% of infested shoots are hardly interpretable. Monitoring had to be stopped between May 27th and June 17th. ac: Agricultural control; afir: Agroforestry Inter-row; afr: Agroforestry row. NA: date for which there were not enough observations to run the model. NCI: NCI effect; N: More infested shoots on the North side (Azimuth effect); I: Inflorescence more infested (Shoot type effect). NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*

Table 5: Evolution of the proportion of infested shoots by Aphis sp per tree. Effects of NCI, azimuth and shoot type on this proportion (n=45 trees). For each factor, the estimate (with significance) is given as a difference between a treatment indicated after the name of the variable and the reference treatment indicated between parentheses using GLMMs. Analysis was made excluding not infested trees. Monitoring had to be stopped between May 27th and June 17th. In blue: all the date with a median less than 5% of infested shoots are hardly interpretable.

Date	**NCI**	**Azimut: South (North)**	**Shoot type: Vegetative (Inflorescence)**
	Estimate (significance)		
10/04/2019	Not analyzed: less than 5 trees infested per treatment		
18/04/2019	NA - Not analyzed: 84 observations only (model failure)		
24/04/2019	**0.486 (***)**	**-0.244 (*)**	**-0.263(*)**
02/05/2019	**0.473(***)**	**-0.311 (*)**	**-0.591 (***)**
09/05/2019	**0.306 (***)**	**-0.529 (***)**	**-1.06 (***)**
16/05/2019	**0.224 (**)**	-0.178 (NS)	**-0.976 (***)**
23/05/2019	0.111 (NS)	**-0.241 (*)**	**-1.06 (***)**
20/06/2019	**0.365 (**)**	**-0.173 (*)**	**-0.733 (***)**
27/06/2019	NA - Not analyzed: 84 observations only (model failure)		

2.1.2) Incidence of *Aphis* sp

2.1.2.1) Evolution of the incidence at tree scale

The effect of NCI at tree scale for *Aphis* sp (probability of a given tree to be infested) could be analysed for the two first and two last dates only, since for the other dates all the trees of the sample were infested (*ie* having at least one shoot with *Aphis* sp) (figure 12). Evolution of the number of infested trees showed a similar pattern between treatments. For the dates analysed with GLM, the probability of being infested for a tree was not significantly affected by NCI (appendix IX).

2.1.2.2) Evolution of the number of infested shoots at tree scale

The proportion of infested shoots followed a similar trend between treatments, with variability within each treatment (figure 13). Only 5 dates were interpretable *ie* having a proportion of infested shoots above 5%: April 24th to May 23rd. There was a positive correlation with the NCI except on May 25th (figure 13 and table 5). Also, North side was significantly more infested for 4 dates between April 24th and May 23rd. Eventually, vegetative shoots were significantly less infested than floral ones, as for *D. plantaginea* (figure 10 and 11).

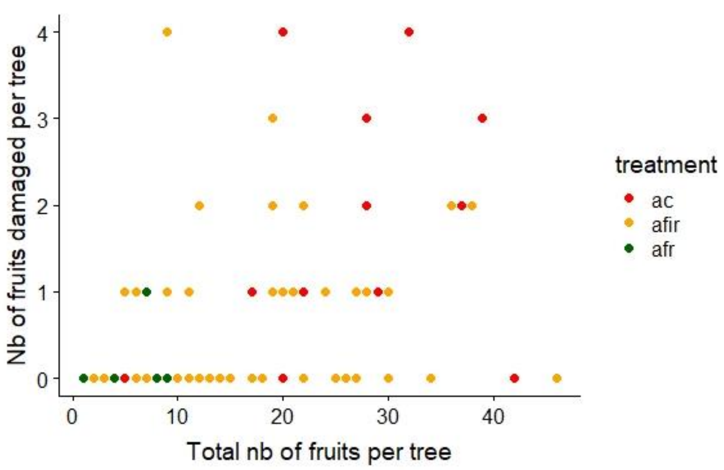


Figure 14: Cydia pomonella *damages on fruit at the end of the first generation.*
Number of fruits damaged per tree according to the total number of fruits in the tree. ac: Agricultural control; afir: Agroforestry inter-row; afr: Agroforestry Row

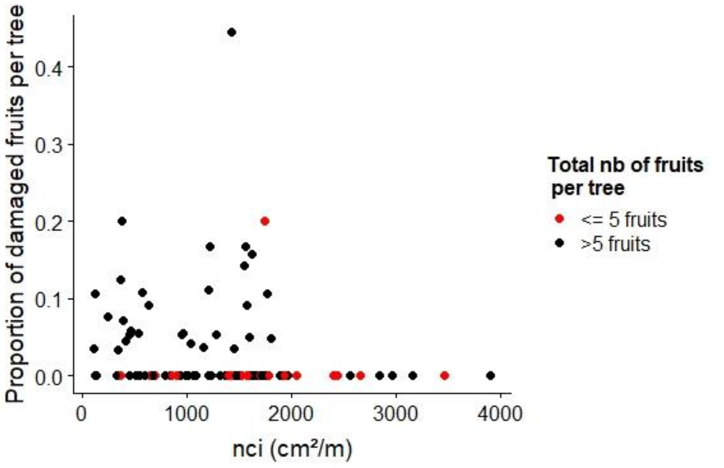


Figure 15: Cydia pomonella *damages on fruits at the end of the first generation.*
Proportion of damaged fruits per tree according to the NCI (n=117 trees). In red: trees with less than 5 fruits. In black: trees with more than 5 fruits.

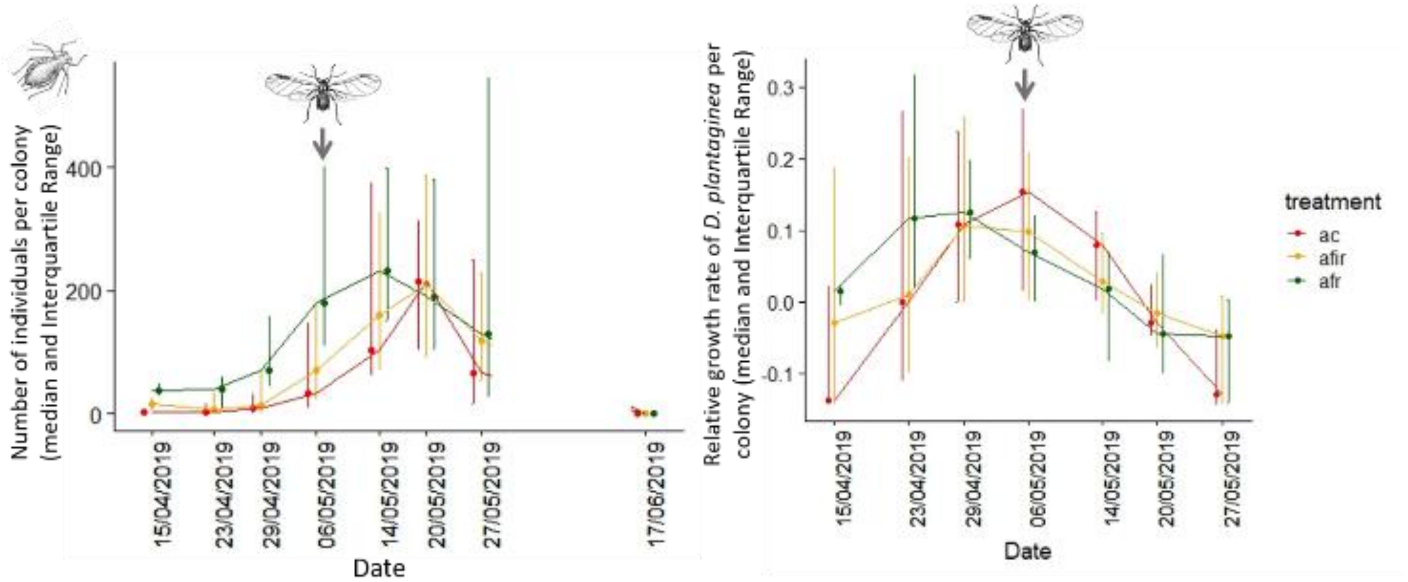


Figure 16: Evolution of the number of D. plantaginea individuals per colony (left) and the relative growth rate of D. plantaginea colonies (right) (median and interquartile by treatment). Monitoring had to be stopped between May 27th and June 17th.
n = 84 colonies at each date. First alate were observed on May 6th (arrow and alate D.plantaginea *drawing above the curves)*

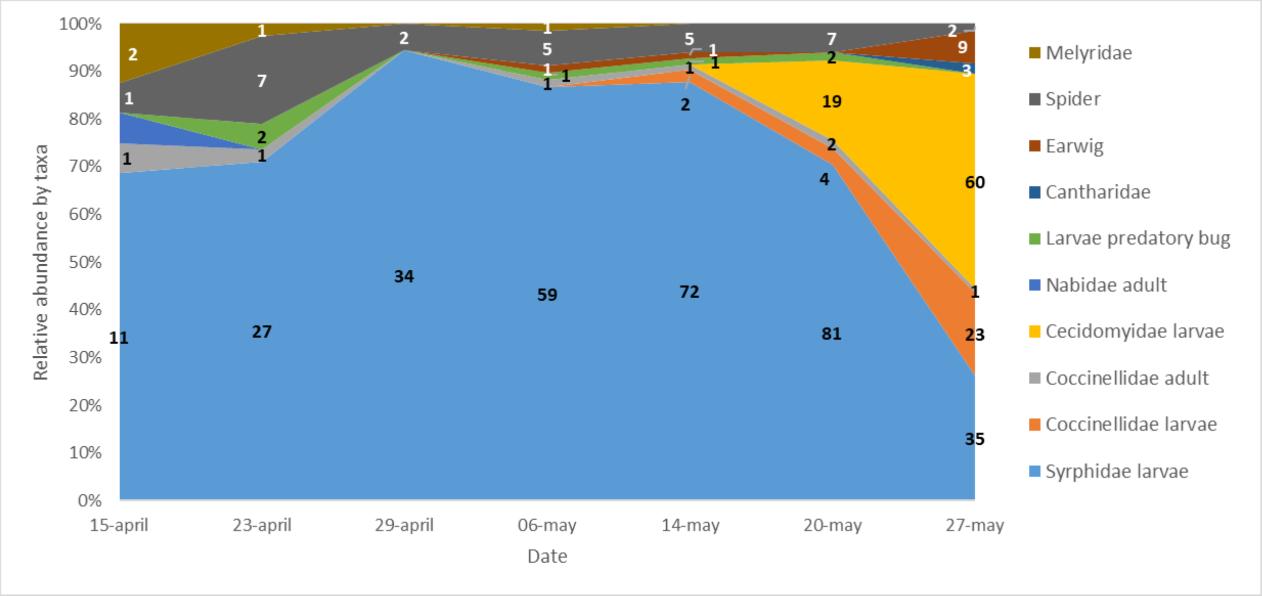


Figure 17: Evolution of the relative abundances of the different taxa of predators observed in D. plantaginea colonies.
Absolute number of individuals observed are provided for each date (n= 767 observations)

2.1.3) Incidence of the first generation of *C. pomonella*

Few trees were observed with fruit damaged from *C. pomonella* (30 trees with damages over 117). Several trees had less than 5 fruits (25 trees), especially in AFR, with high NCI (> 2,000 cm²/m) (figures 14 and 15): for these latter, calculation of damages proportions might be biased. 1,606 fruits were browsed. 419, 1052 and 135 fruits belonged to AC, AFIR and AFR treatments, respectively. This corresponded to 5.49%, 2.76% and 0.741 % damaged fruits in each treatment, respectively. For trees with a NCI between 0 and 2,000 cm²/m, there was no clear trend between NCI and the proportion of damaged fruits (figure 15).

The GLM confirmed an absence of effect of NCI on *C. pomonella* (estimate: -0.452; NS).

2.2) *D. plantaginea* colony ecosystem: population dynamics of *D. plantaginea*, abundances of predators and ants, and interactions between taxa

The evolution of *D. plantaginea* individuals within the colonies, as well as that of the relative growth rate of colonies are presented in figure 16. Representation with the treatments aims at rendering it more readable (compared to a representation with the NCI). The last date was not represented for the relative growth rate (no sense to calculate it). Evolutions for both parameters were slightly similar between treatments, with a great variability. There was first an increase in the number of individuals and then a decrease that followed with a delay the observation of first alate (May 6th). Relative growth rate of *D. plantaginea* followed the same dynamic with a decrease in all treatments since first alate observations, becoming negative the last dates.

In total, 1,953 arthropods were observed in aphid colonies over the whole monitoring, among which there were 1,463 ants and 490 active predators (*ie* eggs not considered). Syrphidae larvae were the most abundant taxa of predators in aphid colonies over all the period (figure 17). There were fewer individuals of other taxa of predators (figure 17). The last two weeks, there was an increase in abundance of other predators, especially with Coccinellidae larvae and the appearing of Cecidomyidae larvae (figure 17). This second fortnight of May, Cecidomyidae larvae became the second most abundant taxa (figure 17).

Table 6: Abundance of D. plantaginea, *predators in D. plantaginea colonies on all the period.*
Estimates of the effect of NCI and significance using GLMM. For D. plantaginea, *proxy of abundance was the AUC, while for the other arthropods proxy was the sum across the season.*
*NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*

Variable	Estimate (significance)
logAUC *D. plantaginea*	0.723 (**)
Sum of ants	0.409 (***)
Sum of total predators	-0.0438 (NS)
Sum of hoverfly larvae	0.0956 (NS)
Sum of ladybirds	-0.985 (*)
Sum of midge larvae	-0.165 (NS)
Sum of earwig	-0.470 (NS)
Sum of spider	0.165 (NS)

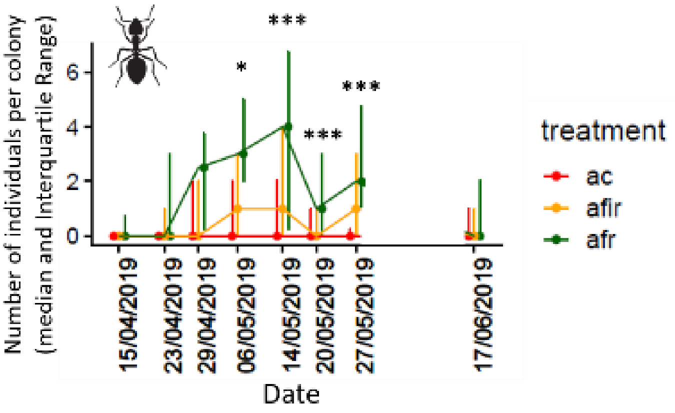


*Figure 18: Evolution of the number of ants per colony of D. plantaginea (median and interquartile range by treatment). Monitoring had to be stopped between May 27th and June 17th. Results of significance using GLMMs date per date are provided. *: <0.05; **: <0.01; ***: <0.001*

Table 7: Evolution of the number of ants per colony of D. plantaginea
*Estimate of the effect of the NCI and significance using GLMM. In blue: Date for which medians equal 0 are hardly interpretable. NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*

Date	Estimate (significance)
15/04/2019	-0.610 (NS)
23/04/2019	0.169 (NS)
29/04/2019	0.0726 (NS)
06/05/2019	0.458*
14/05/2019	0.721***
20/05/2019	0.775***
27/05/2019	0.840***

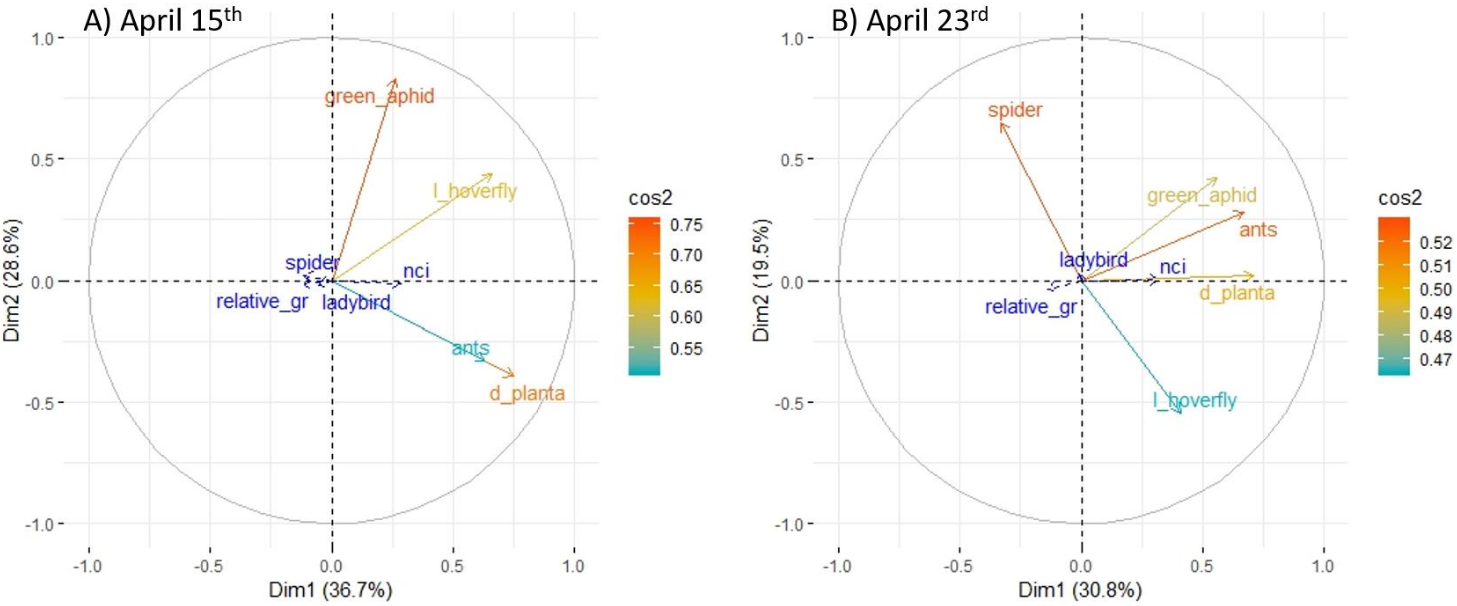


Figure 19: Correlation between D. plantaginea relative growth rate, NCI and arthropod abundances by taxon.
PCA plots date per date of the variables on the taxa abundance within the colonies. Variables are coloured according to their cos². In blue dotted arrows: illustrative quantitative variables. See table at the end of the figure for the layout of the name of the variables.

2.2.1) *D. plantaginea*, predators’ and ants’ abundance within the colonies

The abundance of *D. plantaginea* within the colonies approximated by logAUC was positively correlated with the NCI (table 6).

For ants and predators, the inclusion of *D. plantaginea* abundances in the model allowed to focus on the individual effect of the NCI: indeed, abundances of each taxon were correlated to these effectives (not represented). Ants were significantly more abundant in the colonies when NCI increased (table 6, appendix X). The sum of the total number of predators (eggs excluded) was not significantly correlated to the NCI (table 6, appendix X). Globally, NCI did not significantly correlated to the sum of the different taxa of predators, except Coccinellidae (adults and larvae) - which were negatively linked to NCI (table 6, appendix X).

2.2.2) Phenology of ants

Impact of AF context on the phenology of arthropods could only be done for ants (not enough individuals observed in the other taxa). Evolutions seemed to follow similar patterns between the two treatments (increase followed by a decrease), with variability (figure 18). Positive correlation with the NCI appeared in May, coinciding with the first observation of alate *D. plantaginea* (figure 18, table 7).

2.2.3) Interactions of predation and myrmecophily

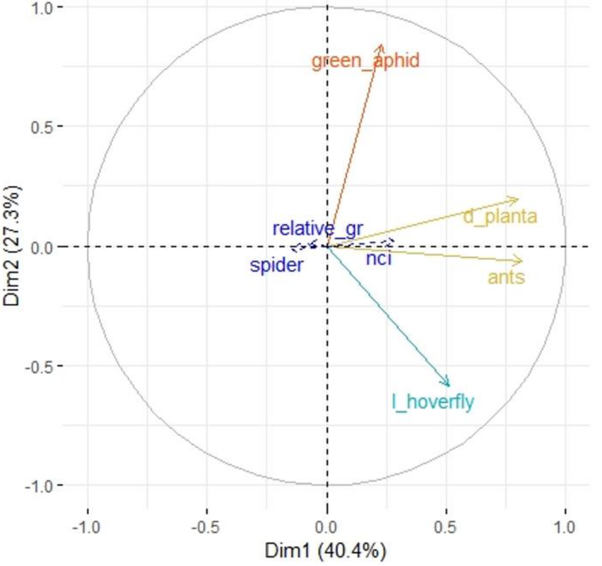
One may wonder whether these arthropods led to an enhancement (ants) or an efficient regulation (predators) of *D. plantaginea* colonies in the FT-AFS studied.

To study this question, a first representation date per date by PCA is described. For these PCA, only the two first dimensions were represented, accounting for the maximum of variance explained (above 30% for the sum of the two axes) (appendix XI). The increase in abundance of predators’ taxa the last two dates allowed to include them as active variables for these dates.

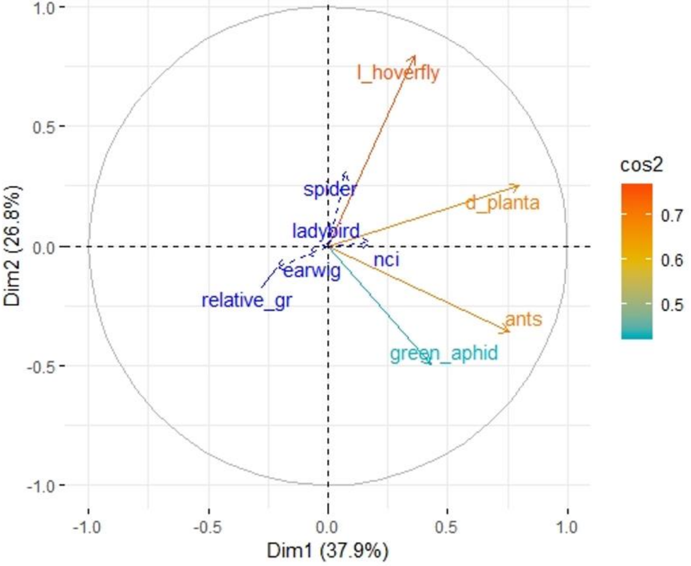
On the PCA, relative growth rate was neither correlated with the first two dimensions (appendix XI) nor well represented on these dimensions (figure 19 and continuation), except on May 6th – where it negatively correlated with all the other variables on dimension 1 (particularly ants, Syrphidae larvae and *D. plantaginea*), with a correlation between relative growth rate and axis 1 having a p-value below 0.05 threshold (appendix XI). Furthermore, on May 27th, it was positively correlated with most of the other variables on axis 1, with a p-value below 0.01 threshold (correlation between axis 1 and relative growth rate (appendix XI)).

Globally, regardless the date, dimension 1 showed an arthropod abundance effect, *ie* an opposition of points with several arthropods to those with less arthropods, meaning there was not antagonisms between them on this dimension. Dimension 2 tended to oppose ants with predators, especially Syrphidae larvae, except on April 29th (figure 19 C, appendix XI). Also, association between ants and aphids, more especially *D. plantaginea* could be observed (figure 19, appendix XI).

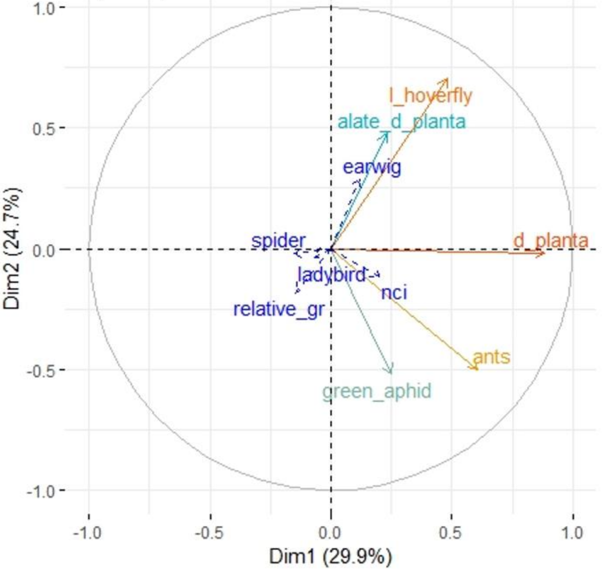
C) April 29th



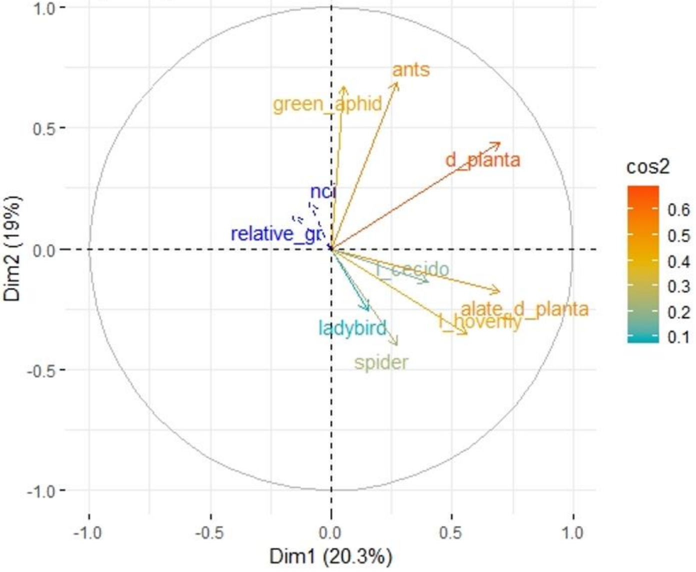
D) May 6th



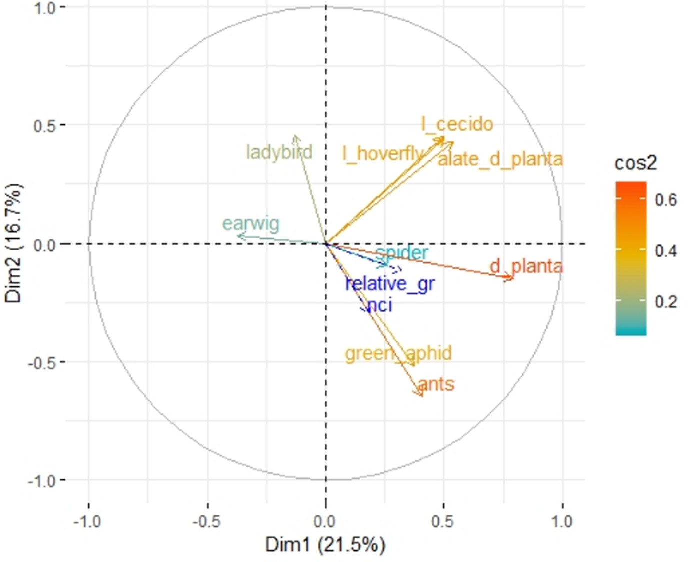
E) May 13th



F) May 20th



G) May 27th



Name of the variables	**Variable**
d_planta	D. plantaginea number
relative_gr	Relative growth rate of D. plantaginea
green_aphid	Aphis sp number
ants	Ants number
l_hoverfly	Syrphidae larvae number
ladybird	Coccinellidae (larvae and adult) number
l_cecido	Cecidomyidae number
earwig	Earwig number
spider	Spider number
nci	Neighbourhood Competition Index

Figure 19 (continuation): Correlation between D. plantaginea relative growth rate, NCI and arthropod abundances by taxon. PCA plots date per date of the variables on the taxa abundance within the colonies. Variables are coloured according to their cos². In blue dotted arrows: illustrative quantitative variables. See table at the end of the figure for the layout of the name of the variables.

In addition, from April 15th to April 29th NCI was positively correlated with Syrphidae larvae, ants and aphids on dimension 1 (correlation between axis 1 and NCI with a p-value below either 0.05 or 0.01 thresholds) (figure 19 A to C, appendix XI). Then, it appeared not correlated to first dimensions, except on May 27th, date for which it appeared positively correlated to ants, with a p-value below 0.01 threshold (correlation between NCI and axis 2, and ants and axis 2) (figure 19 G, appendix XI).

More precisely, one can wonder if relations of predations and myrmecophily could also be disentangled thanks to regression analysis (table 8).

Until the two last weeks, correlations between relative growth rate of *D. plantaginea* and other predators than Syrphidae larvae are not discussed, as they occur less than 5 times over the total number of colonies followed at each date. The model showed significant negative correlation between Syrphidae larvae and the relative growth rate of RAA only for two dates (May 14th and 20th), after the beginning of *D. plantaginea* migration. No significant correlation was observed the two last dates with other predators. In addition, ants were never correlated with *D. plantaginea* relative growth rate.

3) Discussion

3.1) Impact of AF on pests, predators and biological regulations

The inclusion of apple tree as a global random factor in most of the analyses allowed to evaluate dependencies among various measurements within a same tree. It thus permitted to focus on the potential correlations between AF context and the different arthropods and processes of regulation and ants-tending. The effect of AF context seems, for the fourth year of the plantation, limited.

3.1.1) Incidence of *D. plantaginea* and *Aphis* sp

The existence of an effect of AF on aphid incidence depended on the variable and the aphid species. The hypothesis that the AF context affects apple aphid incidence (H1, figure 4) was partially validated, for *Aphis* sp, regarding on the proportion of infested shoots. It was not possible to determine if it was due to direct (microclimate) or indirect effects (through bottom-up processes) of AF context.

The probability of a tree to be infested by either of the two aphids was not significantly linked to AF context. The natural tendency to crowding of aphids and their reported random distribution (Borges et al., 2006) is proposed as an explanation. *D. plantaginea* is known to have a stable aggregated pattern of distribution determined by surrounding habitat (orchard edges and groundcover) while *Aphis* sp tends to have an aggregated, random distribution (Kozár et al., 1994). This tendency could also explain partially what is observed regarding the proportion of infested shoots.

Regarding the proportion of infested shoots, results differed according to the aphid species. NCI was not significantly correlated to the proportion of infested shoots by *D. plantaginea*. For *Aphis* sp, there was a positive correlation with the NCI. Most aphid species are considered as shade-loving insects (Schroth et al., 2000). As *Aphis* sp is more mobile during Spring infestation thanks to winged morphs that are produced to colonize the orchard (Trillot et al., 2002), there might be an effect of microclimate

Table 8: D. plantaginea colonies relative growth rate correlation with ants' and predators' abundances by taxon. Estimate (significance) using LM and GLMM of the different variables explaining this growth rate between t and t+1 with the number arthropods observed at t by taxon. Changes in the sample of colony monitored between the first (only one colony followed per tree) and the following dates (several colonies followed per tree) implied to change statistical model, from LM to GLMM. In grey: not discussed (less than 5 occurrences of the taxa over 84 colonies). NA: Not possible to extract an estimate (zero observations of the taxa).

Date	**Type of model**	**Ants**	**Hoverfly larvae**	**Ladybird (adult and larvae)**	**Midge larvae**	**Earwig**	**Spider**
15/04/2019	**LM**	-0.0283 (NS)	-0.189 (NS)	-0.384 (NS)	NA	NA	-0.384 (NS)
23/04/2019	**GLMM**	-0.00178 (NS)	0.0758 (NS)	-0.165 (NS)	NA	NA	0.249 (NS)
29/04/2019	**GLMM**	0.00348 (NS)	-0.0620 (NS)	NA	NA	NA	-0.419 (NS)
06/05/2019	**GLMM**	-0.00260 (NS)	-0.0782 (NS)	-0.146 (NS)	NA	-0.241 (NS)	0.147 (NS)
14/05/2019	**GLMM**	0.000169 (NS)	-0.0536*	-0.0835 (NS)	NA	-0.0934 (NS)	-0.0105 (NS)
20/05/2019	**GLMM**	0.000319 (NS)	-0.0217*	-0.0667 (NS)	-0.000317 (NS)	NA	0.0119 (NS)
27/05/2019	**GLMM**	0.00197 (NS)	0.00330 (NS)	0.0101 (NS)	-0.00199 (NS)	-0.0680 (NS)	0.00603 (NS)

on the choice of shoots/trees by *Aphis* sp. This effect might not be observed for RAA that expand during this period mainly by walking (Simon et al., 2012) or thanks to ants' dissemination (Collins and Leather, 2002). Regarding microclimate, no difference of temperature was observed between treatments (appendix VIII). Though, there was a real shading effect of AF: radiations were mitigated under AF context compared to full sun conditions (appendix VIII). Thus, it might be expected that aphid desiccation was decreased under AF context, leading to these higher proportions of infested shoots for *Aphis* sp. Yet, the AF effect of microclimate alteration fully expressed after May onset, as walnut buds break at this period (appendix VIII).

The other factors considered (azimuth and shoot type) may play a role in determining the proportion of infested shoots. Azimuth had only a significant effect on *Aphis* sp, with shoots on the North side of the apple tree more infested. As this aphid spreads in an orchard as winged individuals thanks to wind (Trillot et al., 2002), it could have been expected that dominant winds (from the North) may have led to an accumulation on the leeward side of the apple tree (South) (Pasek, 1988). The contrary was observed: one may argue that this might not be so straightforward: trees are relatively porous, small, and wind patterns are more complex. Regardless the date and the aphid species, vegetative shoots were less infested in proportion. This is consistent with Simon et al. (2012) and with the well-documented literature on source-sink processes (resource hypothesis). Sap-nutrient quality induced by these relationships may lead to a prevalence of aphids on fruiting shoots (Larson and Whitham, 1997; Fischer et al., 2013). In other words, fruiting shoots, acting as carbohydrate and water sinks, might be more palatable for aphids - either pedestrian or winged - and identified as better hosts. Also, it cannot be discarded that, as trees did not have the same proportions of floral shoots over the total number of shoots - some of the trees having few floral shoots, especially in AFR -, floral shoots may be relatively more infested.

We propose to review some of the factors that were not integrated in our analysis and that may play a key role in driving the process of infestation. For example, the apple tree vigour is determinant for aphid infestations, though complementary mechanisms exist. Grechi et al. (2008) conclude that *Myzus persicae* attacks more severely peach trees with higher proportions of growing shoots. *Aphis pomi* population positively correlates with shoot growth and length (Stoeckli et al., 2008). On the other hand, competition-stressed plants can be assumed to be less tolerant/resistant to pests (Schroth et al., 2000). It may have been interesting to integrate a more precise and dynamic description of each shoots within apple trees (*eg* number of growing leaves). Yet, this was time-requiring and could have been delicate to integrate in the models since most vigour variables at tree scale (total number of shoots, cumulated leaf surface) tightly correlates with the NCI. Besides, spatial effects at plot scale were not considered in our study though they must not be overlooked. For instance, a tree with infested neighbours might be more prone to have high proportions of infested shoots and *vice-versa*. Also, landscape can drive spatial patterns in aphid within-plot distribution (hedges distance…) (Albert, 2017), which was not included.

Another question that may have been explored is the growth rate of the number of RAA colonies

within a tree according to AF context. We could have related it to the number of colonies with ants (Collins and Leather, 2002) and Coccinellidae (Dixon and Agarwala, 1999), as they are assumed to lead to colonies' dispersal respectively due to attendance or predation.

Eventually, some limits of our study are associated to these uncovered explanatory factors, but also to the study itself (see section 3.2).

3.1.2) Incidence of *C. pomonella*

It is hard to conclude on the effect of AF context, and check our hypothesis that walnut trees could act as barrier for *C. pomonella*, due to the low level of incidence whatever the treatment (mean from 0.7 to 5%). Consequently, the absence of significant correlation found between *C. pomonella* incidence and the NCI is hardly reliable. Inoculum of *C. pomonella* was very low as: 1) there were not apple tree on the Domaine since 40 years, 2) it was the first year fruits were left on trees in the orchard, 3) only the first generation *C. pomonella* was considered. The low number of fruits (< 5 fruits per tree) on several trees (21,4% over 117 trees) amplified observation bias. Codling moth incidence may have been underestimated in our survey, since counting was done after manual fruitlet thinning: several damaged fruits may have been removed by this cultural operation, even if workers were encouraged not to remove fruits based on their aspect. Furthermore, there are less trees with high NCI, and consequently less observations for high values of NCI. Also, damages from the next generations were not considered. Significance of NCI effect might have been more reliably disentangled considering these generations (higher incidence proportions). In addition, other statistical models might be best suited to our dataset (see section 3.2.2).

3.1.3) Colonies of *D. plantaginea*: *D. plantaginea* dynamics, ants and predators

3.1.3.1) Global abundances of predators and their sequential arrival

A limit in our study is that we did not consider parasitism within RAA colonies, though parasitoids can exert some regulation on these populations (Dib, 2010). Mummies were very rare and could be visible by the end of *D. plantaginea* migration, suggesting a low abundance of parasitoids.

Except Syrphidae – the dominant taxon as in previous studies (Dib, 2010; Albert, 2017) – other predator taxa presented low abundances. By the end of the monitoring, Cecidomyidae larvae were the second most abundant taxon, contrarily to Miñarro et al. (2005), Dib et al. (2010) and Albert (2017) observations where Coccinellidae are the second most abundant taxon. Though, Coccinellidae were observed in greater numbers at the peak of RAA migration (B. Pitchers, personal observation), after field colonization, during the period when monitoring had to be stopped. Reported sequential arrival of NE (Miñarro et al., 2005; Dib, 2010) was observed: Syrphidae were the most precocious, then Coccinellidae followed by earwigs.

Furthermore, other monitoring done in apple tree canopies – beating, sentinel-prey cards and strap strips – outlined respectively a low abundance of NE at the beginning of RAA migration (May14th), a low activity of predation particularly before RAA migration onset (April 29th) and a low number of spiders and earwigs (June 17th) (appendices V to VII).

Low abundances of predators were noticed maybe because the system was still young and planted in an area far from orchards: predators populations need more time to build up than that of their prey.

3.1.3.2) Main effect of AF on the different taxa

The hypothesis that AF context will positively correlate with arthropod abundances (hypothesis H1, figure 4) was only verified for *D. plantaginea* densities and ants. For Coccinellidae, the contrary was observed. Other predators appeared not significantly impacted, but since there were few observations, it is hard to reliably conclude.

It was not possible to check whether differences in phenology (hypothesis H1'', figure 4) could be attributed to the NCI for the different taxa since abundance by taxon was low.

NCI was positively correlated with *D. plantaginea* AUC, the sum of ants per colony and negatively correlated to the sum of Coccinellidae per colony. Greater RAA densities in colonies might be explained by an indirect effect of AF *via* ants' favoring (see section 3.1.3.3), or *via* bottom-up processes (competition stressed plants are assumed to be less resistant to pests; Schroth et al., 2000). Direct effect of AF on RAA through microclimate alteration could also act, as this latter is hypothesized as an important factor impacting insect pests – particularly aphids – in AFS (Schroth et al., 2000; Altieri and Nicholls, 2004). In our context, temperatures seemed unaffected by AF context, though radiation intensity were decreased (appendix VIII), which is expected to limit desiccation of aphid pest and thus to favor it. Ants' enhancement in AF context is consistent with previous studies demonstrating that ants are favored by hedges (Albert, 2017) and woody habitats (Stamps, Linit, 1998). Ligneous vegetation and the associated groundcover flora might provide niches and alternative resources to this taxon.

Coccinellidae were found to be negatively correlated to NCI: Albert (2017) also highlights a negative effect of hedges on Coccinellidae. However, the number of observations of Coccinellidae was low, questioning the conclusions of our analysis.

3.1.3.3) *D. plantaginea* regulations and ants-attendance

Analysis date per date, with either the PCA or the GLMM aimed to assess the dynamics and changes in the interactions of regulations (top-down processes, figure 4) and attendance within RAA colonies. For the PCA, NCI was also integrated to determine if AF context correlated with arthropod variables. In the GLMM performed, we considered all predators and ants as influencing the relative growth rate of RAA proportionally to RAA abundance. Specialist predators were thus not distinguished from generalist predators. However, the former could be expected to eat a constant number of aphids, needed for their development, while the latter might be expected to eat a number of aphids that differ according to prey availability and the probability of meeting between the prey and predator.

The fact to have changed the colonies sample size (to increase the number of colonies followed) between the first and second date can be criticized, as it impacts the results.

In opposition to Albert (2017) work, our study did not show any clear regulation by predators or effects of ants-tending thanks to PCA analysis performed with RAA colony relative growth rate (proxy of

population dynamics). Except for two dates (May 6th and 27th), it neither correlated nor was well represented on the first two dimensions constructed with predators, ants and RAA abundances. For May 6th, the negative correlation between the relative growth rate and the abundance of Syrphidae larvae might highlight some regulation. The positive correlation on May 27th between this growth rate and the abundances of predators might translate the fact that evolutions of *D. plantaginea* populations were linked to migration, and that regulation was not playing a role in these evolutions (Hemptinne and Magro, 2002).

Similarly, NCI was poorly represented, though it correlated with ants, Syrphidae and RAA abundances at the beginning, and with ants abundances at the end, consistently with results discussed in section 3.1.3.2.

According to the GLMM performed date per date, Syrphidae larvae were the unique predator having a significant negative effect on RAA at two dates (14th and 20th of May), interestingly at the beginning of RAA migration. Predation by Syrphidae is already outlined in previous studies yet observed earlier in the pest lifecycle on apple tree (Dib, 2010; Albert, 2017). Larvae of Syrphidae, having low mobility, are considered as performing a great regulation activity on RAA colonies (Ricard et al., 2012). However, RAA colonies evolutions may have been more linked to the migration (Hemptinne and Magro, 2002) regardless the abundances of NE. Interestingly, this predation effect was proportional to the number of RAA in the colonies, contrarily to the assumption that specialist predators need to eat a constant number of aphids to fulfill their development. Besides, it would have been interesting to determine if AF context lead to differences in Syrphidae control efficiency.

Coccinellidae were not found as having a significant predation activity on RAA colonies in opposition to previous findings (Dib, 2010; Albert, 2017), maybe because they were not abundant and desynchronized with their preys (they became more abundant at the peak of RAA migration). The low abundances of other predators observed and the desynchronization with RAA lifecycle might explain the difficulty to disentangle their effective regulations of RAA.

The greater *D. plantaginea* AUC in AF context could be linked to the fact that there were greater numbers of ants, especially at the beginning of RAA migration, due to ant-attendance (Stewart-Jones et al., 2008). Ants-tending benefit to aphids in different ways: it entails higher reproductive rates of aphids (El-Ziady, 1960) and higher aphid individual sizes (Stadler and Dixon, 1999); it improves protection against NE (Stadler and Dixon, 1999; Stewart-Jones et al., 2008) and betters colony hygiene (El-Ziady and Kennedy, 1956). This results also in greater aphid densities in colonies and colonies' sizes (Collins and Leather, 2002), and delayed dispersal in crowded colonies (El-Ziady and Kennedy, 1956). This attendance can also be associated to costs for some aphid species (Stadler and Dixon, 1999), though to our knowledge this was not studied for apple aphids. It would have been expected to see at the beginning of the infestation either a positive correlation (colonies-tending and defense from NE; Stewart-Jones et al., 2008) or a negative correlation (colonies splitting; Collins and Leather, 2002) between ants abundance and RAA relative growth rate (PCA or GLMM). By the middle of the infestation period on apple trees, ants might have been supposed

to be negatively associated to relative growth rate, as their attendance might lead to reach more rapidly the peak in RAA population that triggers migration (Albert, 2017).

Also, as ants are assumed to split aphid colonies (Collins and Leather, 2002), it could be expected that the proportion of infested shoots increase with higher NCI. However, this effect of ants-attendance was not observed, with neither the monitoring of incidence, PCA nor GLMM with the relative growth rate of *D. plantaginea*, maybe because of small sample size and/or due to the fact that ants' abundances may vary greatly according to the moment of the observation (high mobility of these arthropods). Reported ants abundances in this study might not represent the real abundances of ants in the colonies.

On the other hand, it would have been interesting to determine whether there was an antagonism between ants and NE in our study, as ants were reported to attack NE (Stewart-Jones et al., 2008).

3.2) Limits of the methods used: well suited to disentangle a walnut tree effect?

The effect of the AF in the context of the system experiment studied seems limited: the system design itself and the methods used might partly explain why.

3.2.1) System design and age

The system design itself can be discussed, concerning the potential to disentangle significant AF effects on pests and NE dynamics.

First, the AFS studied is relatively small (150 apple trees) with contiguous treatments, and one can argue that it might not recreate an orchard environment for pests and NE, impeding comparisons with a traditional orchard system. Although from the tree point of view the NCI makes sense, from the arthropod point of view we may hypothesize that the NCI or treatments would have shown effects on a greater area and in an isolated site far from forests. Given the mobility of adults NE, and the fact that their occurrence is driven by prey presence, it might seem consistent not to see an effect of AF context on these latter if their preys are also unaffected by it.

Also, surrounding environment may interfere with the assessment of AF context effect (Marshall et al., 2003; Holland et al., 2017): flora is complex and diverse within and at the edges of the plot (see part 2), providing NE with various niches and alternative resources (pollen, nectar, alternative preys) (Landis et al., 2000). Potentially, it can divert NE from the pests on apple trees (Kozár et al., 1994; Koss, Snyder, 2005) or increase intraguild predation (Straub et al., 2008); explaining partially why low abundances of NE were observed. As no sampling (*eg* beating) was made within surrounding AEI, it is not possible to check this hypothesis (*ie* if NE were more abundant and diverse in the hedges and/or Short Rotation Coppices).

In addition, being a young system (fourth year after the plantation), it might require time for arthropod populations build up, especially NE (higher trophic levels). Therefore, it may be normal to see few NE in abundance and diversity. Studies ought to be continued during the following years.

3.2.2) NCI variable or treatment?

One can wonder whether NCI variable is suited to disentangle potential effects of AF on arthropods. As it tightly correlates with ecophysiological and architectural responses of apple trees (B. Pitchers,

unpublished results), it was initially thought as also impacting directly and indirectly pests and diseases through bottom-up processes (Sileshi et al., 2008). However, it was impossible to determine with our study (focus on correlations), when NCI effect was highlighted, if it was due to direct or indirect effect of AF.

In addition, trees are unevenly scattered on the gradient of NCI: there are less trees with NCI above 2,000 cm²/m.

It might be suggested to perform all our analysis on the treatment also, and check if results are consistent between both NCI and treatment.

3.2.3) Methods used for pest survey

First, observation bias occurred during the monitoring, leading to over- or under-estimation of the variables collected (*ie* visual counting of the number of shoots infested and of arthropods in *D. plantaginea* colonies).

Relatively small sample sizes (*eg* 45 trees for aphid incidence and 84 colonies for *D. plantaginea* dynamics) appeared as a great limitation in our study. For incidence, it might be suggested to increase the sample of trees browsed (for instance, browsing trees not on all their height). I also suggest a higher sample size of RAA colonies followed, to potentially increase the number of NE and ants' observations.

Given their nature, ants and predators are highly mobile. Yet, their abundances were measured in several colonies during the same day: the potential evolution of their presence in function of the hour of the day was not considered in our study.

Then, by focusing on NE present in RAA colonies, one is more prone to miss NE that are more mobile, though reported as exerting a significant regulation activity, *eg* spiders, true bugs, Cantharidae, earwigs (Dib, 2010; Albert, 2017). Other samplings can also be proposed *eg* regular beating twice a month in apple tree canopy and strap strips, and not only conducted exploratively (appendices V to VII).

To finish, when studying biological regulations, it is crucial to integrate yield and crop damages (Simon et al., 2010; Cahenzli et al., 2019). Assessment of *C. pomonella* first generation damages was a first approximation of damages, however, the internship ended before apple harvest.

3.2.4) Validity of the statistical models used

Models that were used for data analyses can be discussed.

First, interpretation of significant/non-significant distinction is criticized in the literature, regarding on the p-value threshold of 0.05, especially its consideration as the basis of all this distinction, and the fact that a non-significant effect does not mean that there is no effect (Amrhein et al., 2019).

Then, it cannot be discarded that our use of models were not always valid: validation diagnosis is essential either for GLM or GLMM (Bolker et al., 2009), though it was delicate to master it within the time of the internship – especially for GLMM (Zuur et al., 2009; J.B. Durand, personal comm.). For GLM, the validation made was to test in routine the goodness of fit of the model (ratio residual deviance/degree of freedom). For GLMM, to the best of our knowledge, no such test is currently validated by the statistician community (J.B. Durand, personal comm.).

Furthermore, the probability distribution used (either Betabinomial, Negative Binomial or Gaussian) might not adjust to our data.

For GLMM, it was assumed that random effect of the apple tree was a constant (intercept) and that it was not modifying the effects of explanatory variables themselves (*ie* NCI). It might have been interesting to have time to compare both type of model structures (random effect on intercept and/or slope), with the question of model selection which appears in this case.

In addition, if we had more time, we would have checked the within plot spatial autocorrelation, testing if each model led to a spatially uniform residual error or an over/underestimation in some plot areas.

To finish, other models may suit better to the data analysed, though it was challenging to master them within the internship duration (*eg* test zero-inflated models instead of betabinomial for example; Zuur et al., 2009). Maybe nonparametric tests would have been best suited to our data.

3.3) Interest of multi-strata AT-AFS with walnut trees for improving pest control

Regarding pest incidence, AF context correlated significantly positivley only with *Aphis* sp, positively. Effect on arthropod abundance was also limited to greater densities of RAA within the colonies, enhancement of ants, and negative impact on Coccinellidae.

This is in opposition with theories and results found in the literature. Increase in habitat complexity in the plantation studied - associated to walnut trees - might be expected to favour arthropods (in abundance and diversity) (Peng et al., 1993), especially NE (Pumariño et al., 2015) and in turn to favour pest regulation (Chaplin-Kramer et al., 2011). Yet, this link between plant diversity, pests, NE increase and biological control enhancement is not always verified (Schroth et al., 2000). AF is generally considered as a lever for increasing pest control (Jose, 2009). Though, effects can differ according to the system (spatio-temporal arrangement), the environmental context (climate, soil, surrounding vegetation…) and species identities (crop, associated plants, pest, NE) (Rao et al., 2000). In warm regions, AF is expected to favour sap-sucking insects as aphids, providing them a suitable microclimate (shade, temperature and humidity conditions that decrease desiccation) (Rao et al., 2000). Microclimate effects can be expected for NE, in addition to the provision of complementary and supplementary resources for them (Rao et al., 2000). Contrarily to Pumariño et al. (2015) meta-analysis, there were no increase in NE abundances and diversity in our AF context. Yet, they did not provide information on the area and climate in which studies were done, and how the effect of AF was determined in the studies (*ie* comparison between full sun and AF fields, or internal control). Our results are consistent with Smits et al. (2012), who demonstrates in adjacent plots to our experiment that wheat aphids and their NE are not impacted by AF context, maybe due to a blurring effect from surrounding landscape with high floral diversity (forest and fallow patches) (Smits et al., 2012). A similar conclusion can be proposed for our study, besides the fact that plantation was young and contiguous.

In addition, interest of walnut trees themselves in terms of biological control can be discussed. Indeed, pest lifecycle and walnut tree effects maybe be desynchronized. Walnut trees effect on microclimate is limited at the beginning of the season: their buds break in May while aphid eggs hatch by the end of the Winter. For RAA, one may wonder if walnut tree shade affects migration return on apple tree in the Autumn.

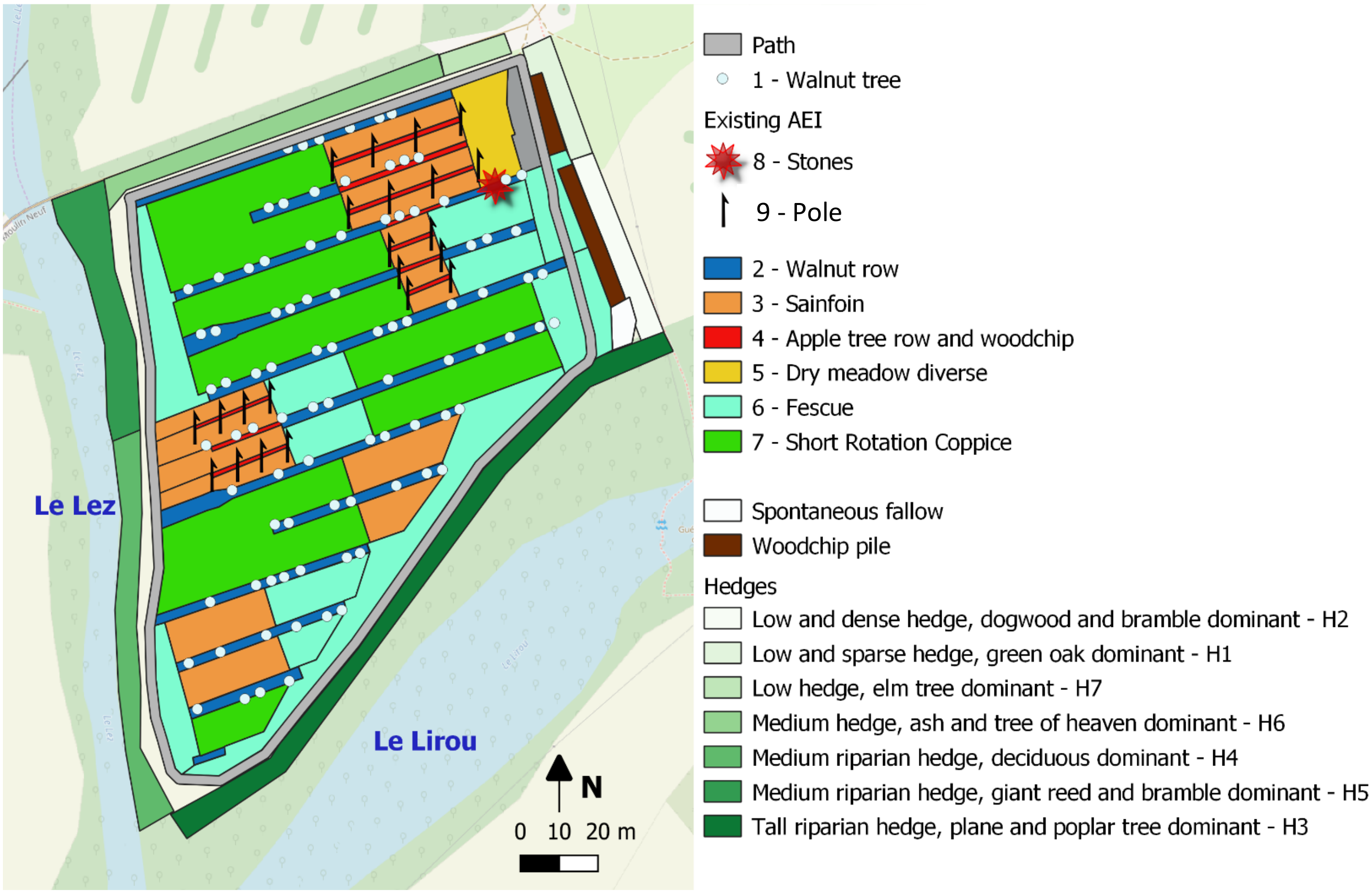


Figure 19: Map of the plot with the existing Agroecological Infrastructures

Concerning NE, interest of hybrid walnut trees is restricted (Ricard et al., 2012): we can suppose it mostly acts as a shelter. Associated flora on walnut tree row might be more interesting for NE, as well as surrounding hedgerows, fallows and forest patches.

Besides, AF context may lead to enhance some diseases, *ie* apple scab, due to microclimate alteration (increased leaf wetness, lower UV radiation exposure, temperature buffering). In our context, used cultivars are scab tolerant, but this question is raised for susceptible cultivars.

If in the present context the AF context seemed to have only few impacts on the biological regulation service, effect on other ES might be also crucial to consider, especially production service.

Indeed, according to B. Pitchers (unpublished results), competition induced by AF context led for instance to more frequent and longer shoots' growth cessation, penalizing growth and possibly cropping. On the other hand, sunburn damages on fruits were suppressed in AF context.

Walnut tree effect seems limited in the studied context with relatively low abundances of NE, that arrived too late to efficiently regulate *D. plantaginea*. Evolutions in the system, thanks to AEI implementation, can be proposed in order to increase pest regulation while enhancing NE.

Part 2: Diagnosis and proposal of Agroecological Infrastructures (AEI)

1) Context and objective of the proposal

In the context of the ALTO project, the current organic low-input apple tree-based Agroforestry system (AT-AFS) in Restinclières is encouraged to evolve by integrating new AEI, in order to improve the resilience of the system regarding pests and thus decrease the reliance on insecticides. Here, the AEI aim at increasing the diversity of habitats and niches at plot scale, which is expected in turn to promote the diversity and abundance of auxiliaries including NE, and consequently to enhance pest control (Altieri et al., 1984; Simon et al., 2010; Ricard et al., 2012). Integration of livestock or vegetables in the inter-row is promising (Lauri et al., 2019), though it is not compatible with current research objectives conducted at UMR SYSTEM. Therefore, it will not be investigated in this report.

After the presentation of the method used for the first diagnosis of the existing AEI in the current system, results of this diagnosis are presented as map and table. Then, improvements of this existing context and integration of new AEI are proposed.

2) Materials and Methods

The diagnosis of the existing context was made in two times: first in April for the AEI within the plot, and second in July for the AEI surrounding the plot (hedges). For hedges, a list of tree, shrub and liana species was made to determine whether they can present an interest for auxiliaries (Ricard et al., 2012; Chambre d'Agriculture France, 2017).

The proposal of new AEI, and their spatial arrangement was made based on scientific literature and the site context. First, it seemed important to maintain the existing AEI and it was not possible to settle AEI

Table 8: List of the existing Agroecological Infrastructures, their expected benefits and limits, and propositions of improvements

Main AEI existing (pictures in annex 1)		Element on the map on figure 19	Interest	Limits	Proposition of improvements
Walnut rows	**Hybrid walnut trees**	**1**	Undisturbed habitat, tree continuity with the hedges	Low potential for auxiliaries promotion, potential alternate host for Zeuzera (Ricard et al., 2012)	Thinning or pruning operation to increase light availability for apple trees
	Walnut row cover	**2**	Undisturbed habitat, spontaneous vegetation providing floral resources to auxiliaries (Projet SEBIOGREF, 2017)	Potential source of adventice	No modification
Alley cover of Sainfoin		**3**	Apple trees inter-row cover that does not compete with apple tree for N	Covering by sainfoin is scattered	**Modification proposed (part 3.2)**
Woodchip on apple tree row		**4**	Avoid weed development, habitat for arthropods (spiders, carabids…) (Ricard et al., 2012)	Require more N input to avoid N deficiency and maintenance (weeding, regular input of woodchip…); can increase the risk of voles (Hucbourg et al., 2015) and promote ants	No modification. Permits to avoid soil tillage, and harbours various arthropods. Also, roots of apple trees have developed superficially in this woodchip so it may be better to keep it rather than remove it.
Hedges		**H1 to H7 (see annex 1 and 2)**	Multistrata, large, diverse and connected hedges. Presence of several species having interest for auxiliaries (Ricard et al., 2012). Riparian hedges: Source of water, beneficial for vertebrate and invertebrate fauna (Ricard et al., 2012; M. Jay, personal comm.)	H1: Hedge with 2 holes letting pass dominant wind from the North	**Modification proposed (part 3.2)**
				H2: One apple tree is present and can be a source of pollen and seems unaffected by apple pests	No modification
				H7: Sparse hedge with low diversity, trees having a little interest for auxiliaries	**Modification proposed (part 3.2)**
Dry meadow diverse		**5**	Spontaneous vegetation rich in Asteraceae, flowering between March and May, favorable for precocious auxiliaries (Projet SEBIOGREF, 2017)	Rapidly during the growing season (since May onset), the meadow dries and stops providing floral resources	No modification. It seems important to keep this patch of spontaneous vegetation for its early flowering. Later in the season, the role of floral resources provisioning can be completed by other AEI.
Fescue and sainfoin blocks		**6 (fescue) and 3 (sainfoin)**	Sown cover, provide diversity in the habitat	May disappear if the aim is to expand the orchard.	
Short Rotation Coppice	**Poplar and alder tree**	**7**	Relatively undisturbed habitat, provide a shrub stratum within the plot	Poplar trees are hosts of Zeuzera (Chambre d'Agriculture Occitanie, 2018) – Tree harvesting for wood chips (each three-four years) generates an important perturbation of the fauna	Modification possible if the aim is to expand the orchard, but in this case, it would be important to integrate shrubs and hedges in the new design.
	Spontaneous groundcover		Relatively undisturbed habitat, with spontaneous vegetation providing auxiliaries with floral resources (Projet SEBIOGREF, 2017)		
Stones		**8**	Habitat for arthropods and reptiles, relay of the riparian habitat (Ricard et al., 2012). Can be interesting for voles control		No modification
Poles for apple tree trellising		**9**	Can serve as posts for hawks, interesting for voles control (Hucbourg et al., 2015)		No modification
Strap strips in cardboard at apple tree basis		**-**	Act as a shelter for arthropods (spiders and earwigs) (Ricard et al., 2012)	Must be changed each year	No modification

on the path that surrounds the plot (public Domaine). It is important to be consistent with the existing context (soil, light availability) and to keep in mind the feasibility in terms of management. It was assumed that water was not limiting.

3) Diagnosis and proposal

3.1) Existing AEI within and around the plot

Current AT-AFS in Restinclières is more diversified than a conventional orchard though it does not integrate companion plant species known to enhance pest regulation. It benefits from the surrounding environment, mosaic of diverse habitats (figure 19 and table 8), which is expected to harbour and favour a diversity of organisms. The dry meadow and walnut tree rows harbour a spontaneous vegetation, rich in forbs that were identified as interesting for auxiliaries (*eg* Asteraceae). Pictures of the different AEI are provided in appendix XII. A list of the species in the hedges is available in appendix XIII.

3.2) Proposal of improvements and new AEI

Based on the existing context, some improvements and new AEI can be proposed, in a new design (figure 20 and table 9). For us, flower strips are an inescapable AEI to set in the plot, as they are recognized as having great potential for the enhancement of pest regulation (Herz et al., 2019). Relatively easily AEI to implement are the passerine nesting boxes, the branch piles, and the *Fraxinus* sp setting in the hedge H1, though there is no urgency for that. Risks of damages to apple fruits and need for regular management (check for occupation, cleaning) represent some limits of passerine nesting boxes. Some other AEI might be more delicate to set, as aromatic strips and shrubs (end of each apple tree rows and enrichment of hedges the hedges), due to the necessity to find them in volume. Aromatic strips are expected to be the most expensive.

It is important to underline that making this proposal was challenging. Literature is well developed on the principles of AEI and Conservation Biological Control. Yet, resulting pest regulation was seldom quantified (Simon et al., 2010; Albert, 2017; Cahenzli et al., 2017). Not only the choice of AEI and its adaptation to local context, but also the spatial arrangement and the management are crucial (Simon et al., 2016). AEI design must be combined with other levers to achieve pest regulation objective (choice of the plant material, apple tree training and pruning, management of the plantation…).

If in the following years the objective is to extend the orchard, I propose not to expand necessarily with apple trees: other species can be interesting, either fruit trees (*Prunus, Pyrus, Ficus, Diospiros kaki*), bushes (*Ribes nigrum*, *Ribes rubrum*, *Rubus occidentalis*, *Vacinium myrtillus*) or nut trees (*Corylus avellana*, *Pistacia vera*). These latter could be planted to create a spatial barrier for apple trees. Though, one can argue that these species may not perform well under high levels of competitions for nutrients, water or shade with the walnut upper stratum (as apple trees that are affected by high levels of competition). Thus, it appears crucial to carefully think the expansion of the orchard regarding on the choice of the species, the distance to walnut trees and walnut tree management as well.

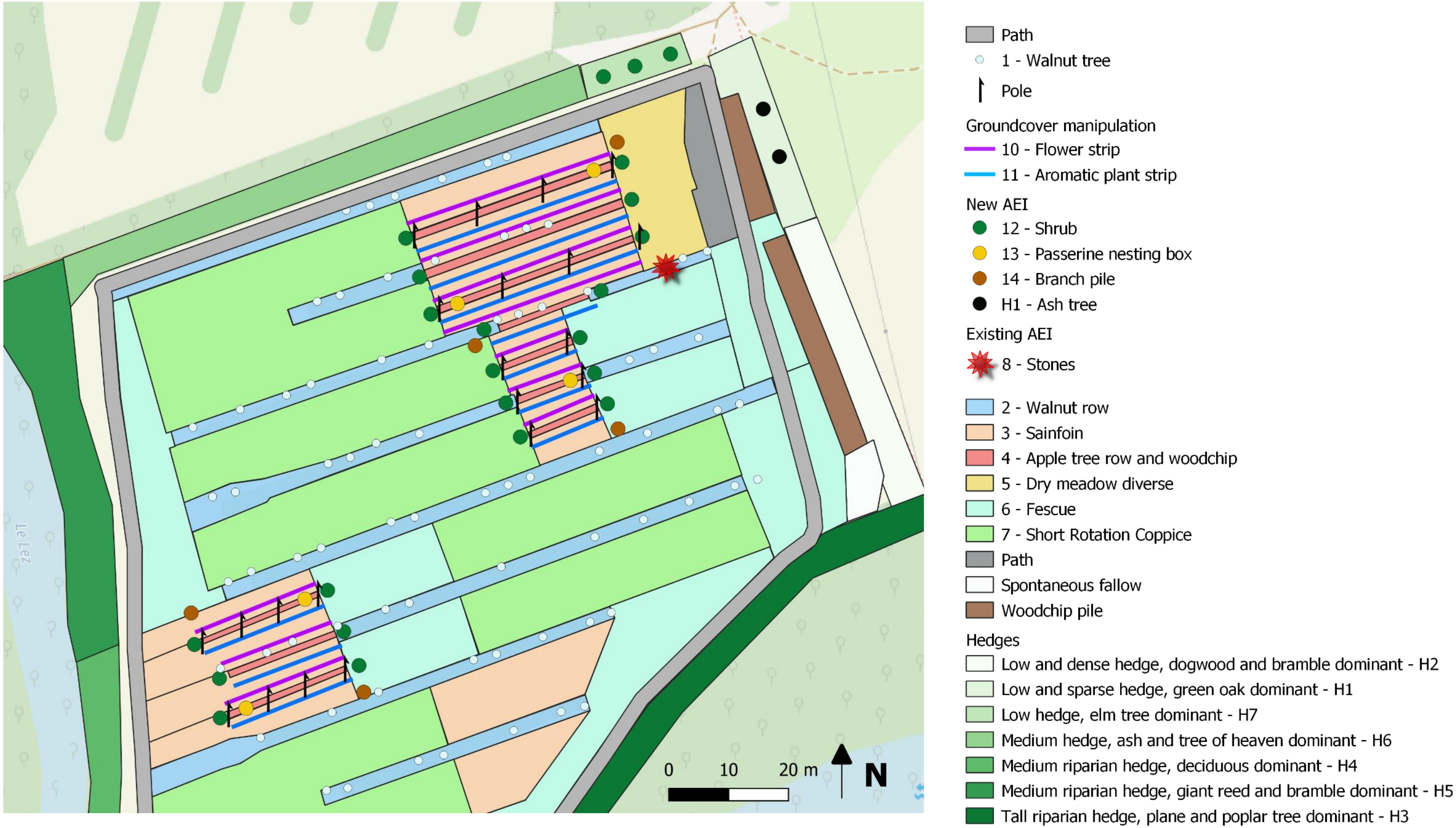


Figure 20: Map of the plot with the new Agroecological Infrastructures

Conclusion

Effect of AF context on biological regulations was tested in a multi-strata apple tree based AFS in Montpellier, South of France. This system corresponds to alley-cropping AF, with walnut trees (upper stratum) covering apple trees (intermediate stratum) and sainfoin (lower stratum). Main hypothesis tested regarding the impact of AF context on arthropods is that AF will directly enhance arthropod abundances (microclimate alteration, increase in diversity in the system and provision of a more stable habitat). To analyse AF effect more accurately and account for the heterogeneity in AF environment within the studied plot, the Neighbourhood Crowding Index (NCI) was introduced. It allowed to characterise AF context for each apple tree individually. To test our hypothesis, main apple pest incidence (*D. plantaginea*, *Aphis* sp dynamics and *C. pomonella* for the first generation) was followed. Then, NE were sampled within apple tree canopy (exploratory work in appendices). Finally, population dynamics of *D. plantaginea* colonies was monitored, along with ants' and predators' abundances by taxon.

The main results obtained - in our specific context, for Spring 2019 - are the following.

Hypothesis was partially validated regarding pest incidence. The probability of a tree to be infested by both types of aphids was not significantly correlated to the NCI. Then, the NCI was not significantly correlated to the proportion of infested shoots in the case of *D. plantaginea*, while it was positively associated in the case of *Aphis* sp. Low levels of infestation by *C. pomonella* impeded further conclusion.

Similarly, hypothesis was partially validated for arthropods within *D. plantaginea* colonies. AF context positively correlated with abundances of ants and *D. plantaginea* and negatively correlated with that of Coccinellidae. No significant correlation was found between AF context and predators (in total or by taxon), invalidating the hypothesis of NE enhancement in AF. Eventually, interactions of regulation or ants-tending within *D. plantaginea* colonies were hardly disentangled.

Limited effects of AF observed in our context may be related to several factors, especially the system itself (young, relatively small plantation and limited interest of walnut trees for pest regulation's promotion, blurry effect from surrounding vegetation diversity), the methods used (arthropod surveys, statistical models used). Also, for NE, low abundances observed might explain this absence of differences observation.

To enhance NE population and promote pest regulations, integration of new AEI in the system was proposed.

Further research should be carried on during the following years, continuing the work undertaken, accounting for the spatial heterogeneity in AF context within treatments, and including also the potential impact of surrounding AEI.

Table 9: Proposal of improvement of existing Agroecological Infrastructures and new Agroecological Infrastructures

New AEI	Location	Type/Composition	Aim/Intended effect	Setting	Management
Improve-ment of existing hedges	**H1**	Enrichment with *Fraxinus* sp (ash tree)	Buffer dominant wind, keep some porosity, and favour auxiliaries (ash harbour alternative preys for predatory bugs) (Ricard et al., 2012), with a deciduous tree complementary to green oak that can grow in the plot	Transplant in each hole in the hedge one seedling of ash tree taken from other hedges (especially H3)	Extensive (pruning not all the years)
	H7	Enrichment with *Virbunum tinus* (Laurestine), *Rahmnus alaternus* (buckthorn), *Fraxinus* sp (ash tree)	Use shrubs with persistent foliage that are known for their interest for auxiliaries (Ricard et al., 2012), and adapted to the context	Plant in the holes in the hedge seedling of the shrubs	Extensive (pruning not all the years)
10 - Flower strips	**North side of apple tree rows**	Mix tested in EcoOrchard project (Cahenzli et al., 2019)	Provide auxiliaries with floral resources all year round, by using a mix of biennials and perennials which was associated to efficient pest control and was viable in other contexts (Albert, 2017; Cahenzli et al., 2019). Potentially divert ants from aphid colonies (Albert, 2017)	At 1.5 m from the apple tree row, on a strip of 1 m width. For additional information regarding the setting of the strips, see Pfiffner et al. (2018). Setting is proposed on the North side to be complementary with the decision made for companion plants .	For the specific management of the flower strip, see Pfiffner et al. (2018)
11 - Companion plant strips	**South side of apple tree rows**	**Population varieties:** *Rosmarinus officinalis* (repellent effect on green peach aphid (Dardouri, 2018) and RAA (Dieudonné, 2017)), *Mentha piperrata* (repellent effect on RAA (Dieudonné, 2017)), *Thymus vulgaris* and *Satureja hortensis* (attractivity for NE, G. Fremondière, personal comm.)	Companion plants considered as having great potential for pest regulation, thanks to Volatile Organic Compounds (VOC) production that might repel pest and attract NE (Dardouri, 2018; Frémondière, personal comm.). For the moment, effects are complex to assess: no evidence exists of direct effect of these plants on aphids in orchard (repellency and/or perturbation effect demonstrated in the lab) (Dardouri, 2018). These effects are dependent on plant genotype, existence of a synchrony between VOC production – pests/NE growing cycles, the year and the management practices. Population varieties are proposed to make sure that the plants are going to settle well. At least, it is expected to favour NE, and potentially we increase the chance to have individuals that negatively impact aphid pests (sampling effect).	At 1.5 m from the apple tree row on the South side, make a soil tillage on a strip of 1 m width and plant seedlings of the companion plants. Setting is proposed on the South side as it is expected that companion plants will receive on this side less shade from apple trees in the Agricultural control. Yet, this can be meaningless for the areas under walnut tree shade.	Cut the companion plants in autumn. Some operations (cutting…) can be made to promote VOC production at tipping points in pest lifecycle
12 - Shrubs	**End of each apple tree rows**	*Viburnum tinus, Virbunum lantana* (wayfarer), *Cornus sanguinea* (dogwood), *Corylus avellana* (hazelnut)	Settle auxiliaries within the plot, by providing shelter, overwintering sites, early bloom (Ricard et al., 2012)	Put 1 shrub at each end of the apple tree row, at 1 m from the post	Extensive (pruning not all the years)
13 - Passerine nesting boxes	**Uniformly settled in the plot**	-	Settle passerines (especially tits) in the plot, to benefit from the control they can exert on Lepidoptera and aphids (García et al., 2018; Le Maire, 2018)	Density adviced is between 3 and 5 nesting boxes/ha (Hucbourg, 2015)	Check for occupation and clean the boxes in autumn.
14 - Branch piles		Pruning branches (from the walnut trees or the hedges)	Provide an undisturbed shelter for invertebrates and vertebrates within the plot, potentially auxiliaries (Hucbourg, 2015)	Make a pile with the pruning residuals	Do not disturb the pile once it is put in place
Ants exclusion	**At all the apple tree**	Glu strap	Hamper ants to attend aphid colonies (Albert et al., 2017). Yet, this can also hinder spiders’ and earwigs’	Pose the glu strap during the Spring phase of *D.plantaginea* on apple	

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Appendices

Appendix I: Biology and lifecycle of *Cydia pomonella*

Considered as a major pest of apple tree, it causes direct damage to fruit, making it drop before maturation.

1) Lifecycle

The main features of *C. pomonella* lifecycle are summed up in figure 21.

Larvae overwinter in apple tree bark, or in a shelter near the soil (Trillot et al., 2002). Depending on the regions, adults of the first offspring appear from the beginning of April to June (Trillot et al., 2002). In general, one to two successive offsprings occur according to the region and the year, even three in South of France: the first offspring gives birth either to the second offspring of the year or to the first offspring of the following year (Trillot et al., 2002).

Adults mate at dawn when temperature is above 15°C. Female lays 30 to 50 eggs.

Larval development lasts 20 to 30 days (Ephytia, 2015). The first offspring hatch generally by the end of May. Few days (2-5 days) after hatching, larvae enter the fruit, eat the apple grain, then exit the fruit and pupates in soil or under apple tree bark (Ricard et al., 2012).

Codling moth are sensitive to temperature (Howell and Neven, 2000), and microclimatic variables trigger different behaviour of adults, according to the stage and the sex (Kuhrt et al., 2006b ; 2006a).

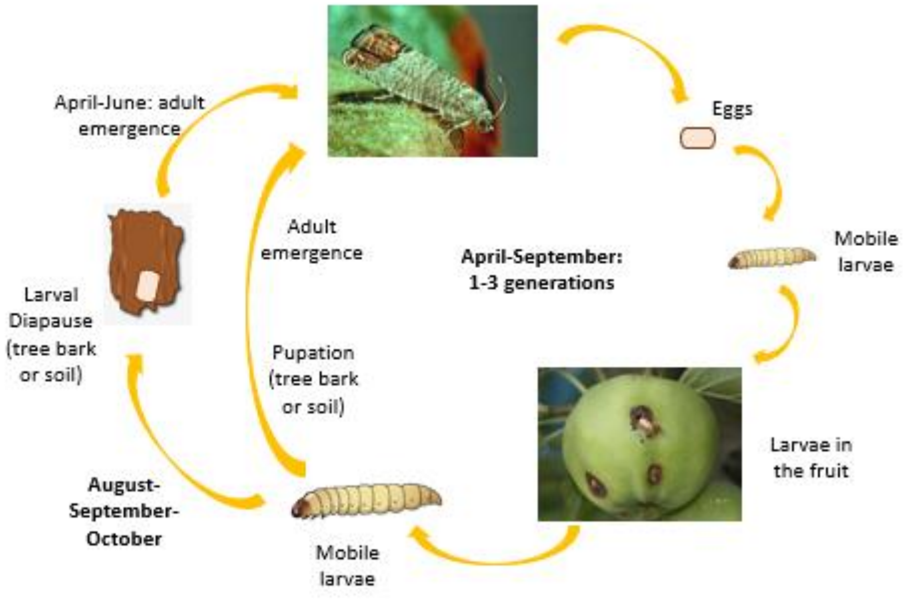


Figure 21 : Life cycle of C. pomonella (adapted from Ricard et al., 2012) (Picture: © Ephytia and Washington State University)

2) Location of the host plant and the fruit

Codling moth adults and larvae uses apple volatiles to locate their host plant and the fruit: these volatiles stimulate female activity (pheromone release, oviposition, upwind orientation) (Yan et al., 1999).

2.1) Host plants

Various trees can host *C. pomonella* : apricot (*Prunus armeniaca*), quince (*Cydonia oblonga*), nut (*Juglans* spp.), pear, apple (*Malus domestica*), peach (*Prunus persica*), plum (Ephytia, 2015).

2.2) Predators and parasites

Eggs, larvae and chrysalides can be parasitized by different microorganisms (Beauvaria bassiana, virus) (Ephytia, 2015).

Most of the predators of Codling moth are generalist. In spring, during adult emergence, soil arthropods and more especially spiders can eat Codling moth. Vertebrates, such as birds or bats, are the main predators of the adults (Ricard et al., 2012).

Parasitoids can efficiently regulate the population at egg stage, provided that they are abundant and in synchrony with Codling moth cycle (Ricard et al., 2012).

By the end of the summer-autumn, before diapause, generalist predators have been reported as exerting codling moth regulation, more especially spiders (Lefebvre, 2016) and some species of carabid beetles and staphylinids (Garcin et al., 2016).

Appendix II: Biology and lifecycle of *Aphis pomi*

1) Biology

This species is holocyclic, with a unique sexual reproduction in autumn, and monoecious (apple tree is its unique host).

2) Lifecycle (INRA HYPPZ)

The lifecycle of *A. pomi* is represented in figure 22.

Eggs overwinter on apple tree. They hatch after bud-burst, giving birth to fundatrices – apterous parthenogenetic viviparous females that produce a generation of viviparous parthenogenetic females. One fundatrix requires about 3 weeks to develop and gives birth to 60 females. 10 to 15 generations successive generations occur from spring to autumn.

Winged females appear from April onwards, migrating to other trees, to form other colonies. Thanks to wind-dispersion, they can cover up to several dozens of kilometres.

In Summer, the elevated temperature hampers their multiplication. In October and November, winged oviparous females and apterous males appear and mate. Then, female lay eggs on apple twigs, at the apical position on shoots. Eggs are layed by group, unlike other aphid pests.

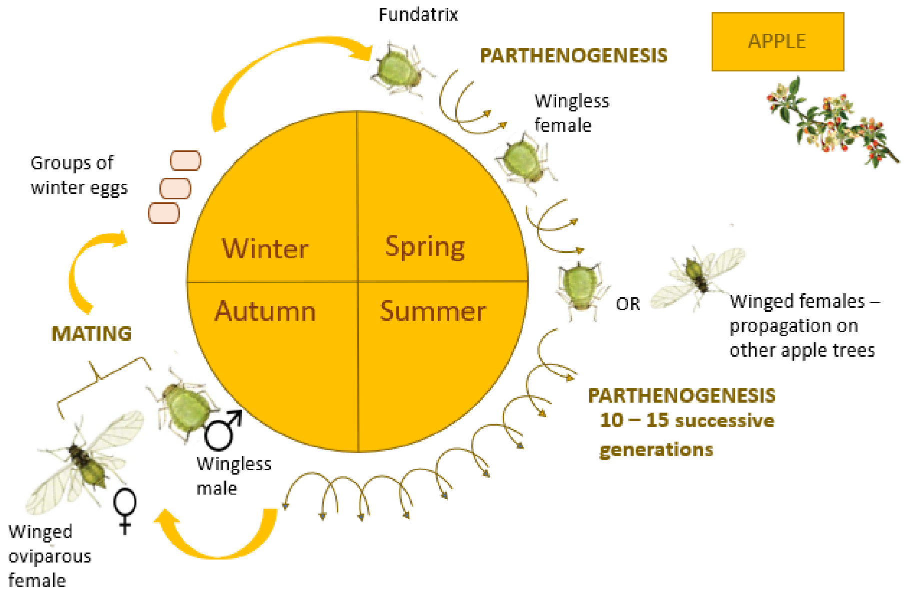


Figure 22: Life cycle of Aphis pomi (adapted from INRA HYPPZ)

Appendix III: Biology and lifecycle of *Aphis spiraecola*

3.1) Biology

This species is anholocyclic in Europe and dioecious. It is polyphagous and has a wide range of potential hosts.

3.2) Lifecycle

In southern Italy – where its cycle is continuous – more than 40 generations a year occur (Hyppz INRA).

Usually it overwinters on its primary hosts (Spirea or Citrus) and then emigrates on apple tree. Yet, *A. spiraecola* development on apple tree without primary host have been reported (Andreev et al., 2009).

The case of alternation between 2 hosts is described here, adapted from Chambre d'Agriculture Occitanie (2012)Female overwinter on Spirea and Citrus. In Spring, several successive generations are produced by parthenogenesis. In Summer, winged female appear and emigrate on other trees, including apple trees. In Autumn, apterous females give birth to individuals overwintering.

Appendix IV: Biology and lifecycle of *Dysaphis plantaginea*

1) Biology

This species is holocyclic and dioecious. Host alternation with Plantago spp is considered as a strategy to escape from their natural enemies (Bonnemaison, 1959).

D. plantaginea is characterized by a low developmental threshold of 4.5°C in spring, compared to the other aphids (5.9°C for *A. pomi* and 5.5°C for *R. insertum*) (Graf et al., 1985). Eggs hatch consequently on apple tree early at the beginning of the bud burst, and colonies build up on the abaxial side of leaves.

2) Lifecycle (Bonnemaison, 1959)

Figure 23 provides a synthesis of *D. plantaginea* lifecycle.

A unique sexual reproduction occurs in Autumn, producing winter eggs that are laid at the basis of apple buds or under bark. They hatch in Spring at bud burst, giving rise to fundatrices, viviparous apterous females. Fundatrices give birth by parthenogenesis to about 70 apterous fundatrigens. Then, 3 to 6 successive generations occur and develop in colonies on inner side of apple leaves.

By the end of Spring or onset of the Summer, elevated densities in colonies and evolution of apple phenology trigger the appearance of winged fundatrigens that emigrates on plantain, its secondary host. 3 to 8 generations occur on this host. By autumn onset, photoperiod changes trigger the production of sexuparae. This latter gives birth to winged gynoparae (females specialized in the production of females with sexual reproduction) and winged males, that emigrate to apple tree (return migration). On apple trees, gynoparae produce sexed female oviparous, that mate with winged males. These females lay winter eggs.

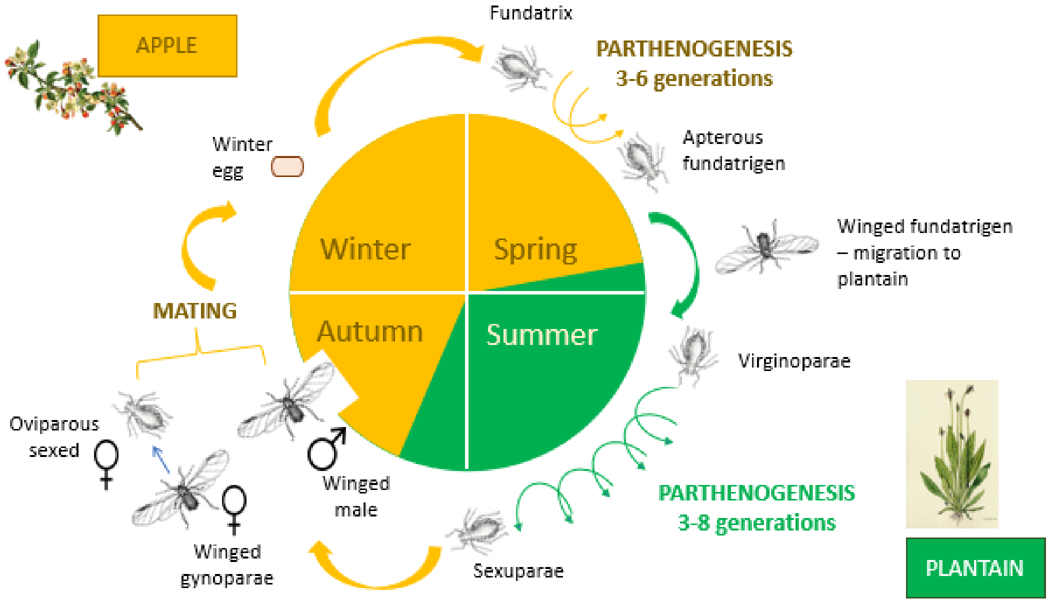


Figure 23: Lifecycle of D. plantaginea (adapted from Bonnemaison, 1959)

Appendix V: Beating tray at the beginning of *D. plantaginea* migration

1) Material and methods

1.1) Beating operation

At the beginning of *D. plantaginea* migration – on May 13th, one beating was made on 60 apple trees, including the 45 trees followed for the incidence: 20 trees per treatment were selected. The idea was to have an overview of the arthropod community present in apple tree canopy (either phytophagous, natural enemies (NE), or “neutral” for apple trees).

Beating was non-destructive: one branch per tree was beaten, and arthropods were visually identified and counted by order, and family when possible.

1.2) Statistical analysis

Except for aphids, low abundance of arthropods was observed for each taxa (17 taxa, cf results). Clustering was made in 5 groups: aphids, other phytophagous (not considered as main pests on apple tree), ants, NE and other (considered as “neutral” for apple tree).

A PCA was performed (FactoMineR package; Husson et al., 2019) with these clustering variables as active variables, and Neighborhood Crowding Index (NCI) as supplementary variable. Dimensions explaining >20% of the variance (Dimension 1 to 3), and only variables with a cos² > 0.1 on the dimension were considered.

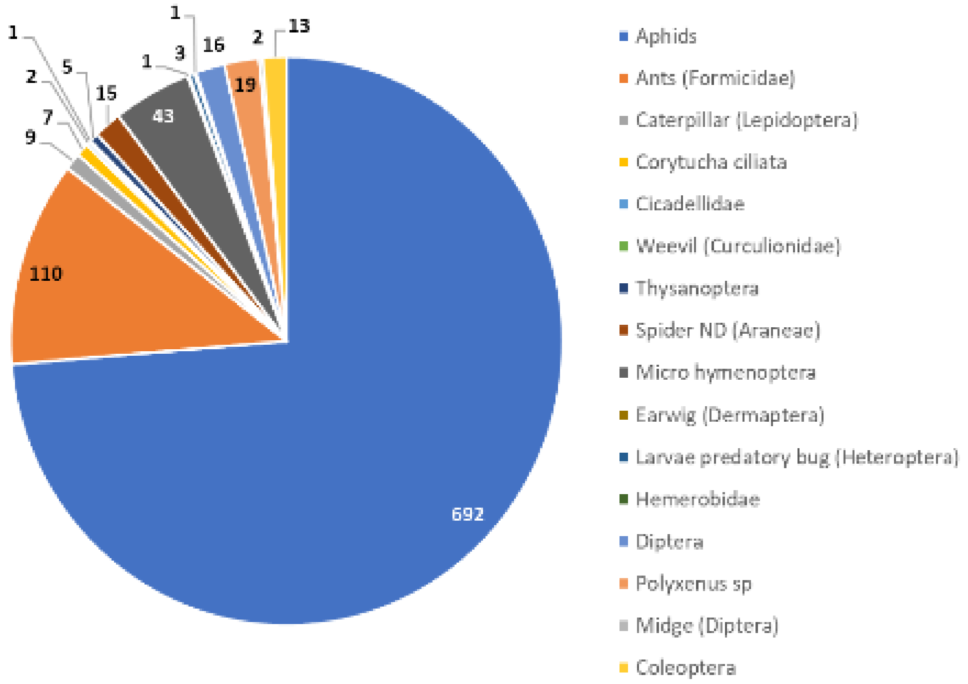


Figure 24: Total number of arthropods by taxa observed during the beating. All the trees pooled, n = 60 trees)

2) Results

On all the trees pooled, 973 arthropods from 17 taxa were observed: aphids being dominant (692 individuals), and only 62 individuals categorized as NE (figure 24). Also, the level of identification was not homogenous (*ie* either order, family or species). We thus clustered the 17 taxa in 5 groups (table 10 and figure 25).

Table 10: List of taxa observed during the beating and their aggregation cluster

Taxa	Aggregation cluster
D. plantaginea	Aphids
Aphis spp	
Caterpillar (Lepidoptera)	Other phytophagous
Cicadellidae	
Corytucha ciliata	
Weevil (Curculionidae)	
Thysanoptera	
Ant (Formicidae)	Ants
Earwig (Dermaptera)	Natural enemies (NE)
Micro hymenoptera	
Larvae predatory bug (Heteroptera)	
Spider (Araneae)	
Coleoptera	Other
Diptera	
Midge (Diptera)	
Polyxenus sp	
Other (not identified)	

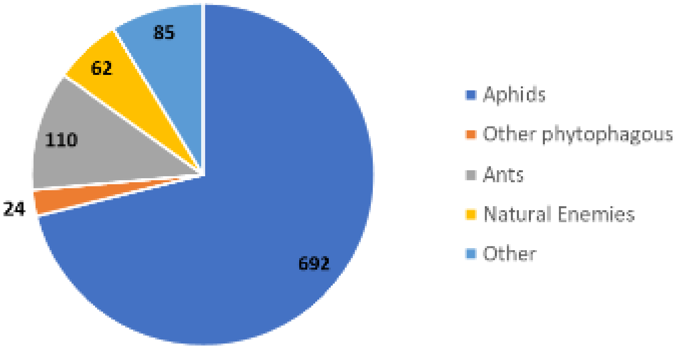


Figure 25: Abundance within each regrouping group, observed during the beating. All the trees pooled, n = 60

On the PCA, except NCI, all the variables were correlated to Dimension 1. This dimension opposed points with several ants, aphids, other phytophagous and “neutral” individuals to that with NE (figure 26, table 11).

Aphids and “neutral” group were not correlated to Dimension 2 (table 11). NCI was only correlated with dimension 2, positively correlated with ants and negatively to NE. NE and other phytophagous were opposed to ants on this dimension (figure 26, table 11).

Dimension 3 was only constructed with other phytophagous and “neutral” (table 2).

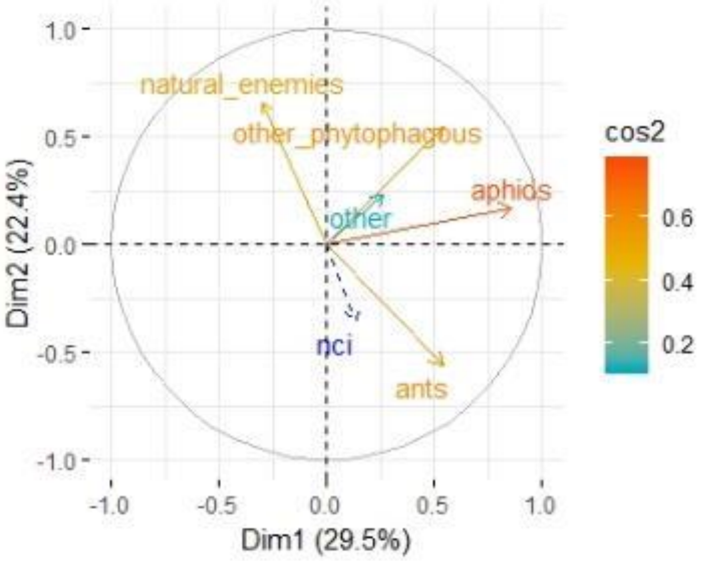


Figure 26: Beating operation in apple tree canopy. Correlation between variables on dimension 1 and 2. Variables are colored according to their cos²

*Table 11: Beating operation in apple tree canopy. Results of correlation and significance for the three first dimensions, using PCA analysis on the clustering variables. NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*

Variable	Dim 1	Dim 2	Dim 3
Aphids	0.860(***)	NS	NS
Other phytophagous	0.541(***)	0.540(***)	0.890(***)
Ants	0.536(***)	-0.561(***)	NS
Natural enemies	-0.299(*)	0.658(***)	NS
Other	0.260(*)	NS	-0.468(***)
NCI	NS	-0.359(**)	NS

3) Discussion

Though this kind of representation can be considered as trivial (few active variables), it highlighted the positive links between aphids and ants, and the opposition to NE as reported in the literature (Stewart-Jones et al., 2008; Miñarro et al., 2010).

Ants seem to be associated to higher NCI, and thus favoured by AF context, while it is the contrary for NE. Yet, other observations (especially in *D. plantaginea* colonies) may provide more reliable information on this potential link.

Interestingly, NE seem negatively correlated to aphids: it could be expected that observations of aphids are associated to observations of NE, which is not the case. Yet, fewer NE were observed during this monitoring, perhaps because beating was made too early in the season (2 weeks later, an increase in NE abundance and diversity was observed).

To finish, the results here presented can be discussed:

- only one beating operation was made, few individuals were observed, making impossible further conclusions.
- furthermore, ideally a same volume of branch should be beaten to make results comparable. In our case, depending on the apple tree with a decrease of branch volume with the increase of NCI, branches did not have the same amounts of shoots and leaves, making comparisons between them difficult.

Appendix VI: Sentinel-prey cards in apple tree canopy

1) Material and Methods

1.1) Cards' fabrication and exposure

To approximate the potential for natural regulation in apple tree canopy, sentinel-prey card method was tested – based on the SEBIOPAG project protocol (Sebiopag, 2013). The aphid *Acyrthosiphon pisum* and *C. pomonella* eggs were used as sentinel preys, to approximate the presence of entomophagous and oophagous insects, respectively.

Preys were stuck on cards of sandpaper, with a dimension of 5 x 5cm and 2 x 2cm for aphids and *C. pomonella*, respectively (figure 27). Then, cards were frozen, and exposed in the experiment at 1,000 and 1,800 degree-days (daily mean temperature). 45 cards of each prey were exposed, on the 45 apple trees selected by Benjamin Pitchers (one card per apple tree). For that, each card was stapled on the abaxial side of an apple leaf. In a tree, cards of *C. pomonella* eggs and aphids were separated by 1m. Aphid cards were left 1 day, and *C. pomonella* ones 4 days. After exposure, the number of predated aphids (absent) and *C. pomonella* eggs (absent and empty eggs) were counted.

1.2) Statistical analysis

Figure 27: Sentinel-prey cards with A. pisum, before exposition in the orchard

GLM betabinomial (glmmTMB package; Magnusson et al., 2019) was performed on the number of preys (aphids or eggs) predated over the number remaining on each card (see equation). Analysis was only possible at the second session, due to very low predation in the first session. NCI covariable was scaled, as recommended by Schielzeth (2010). Betabinomial distribution was used to tackle overdispersion (Zuur et al., 2009).

GLM equation:

Cbind (nb of prey predated, nb of prey remaining) ~ NCI (scaled), family = betabinomial (link = logit)

2) Results

There were more observations for NCI from 0 to 2,000 cm²/m than for NCI > 4,000 cm²/m.

2.1) Sentinel-prey cards with aphids

Mean predation rate of aphids was, for the plot and per card, about 14.7% and 46.3% on the first and second sessions respectively (not represented). Analysis was only made the second session (enough predation). It does not seem that there was a correlation between the NCI and the predation rate of aphids during this session (figure 28), which was confirmed by the GLM analysis (Estimate = 0.453 with a Not Significant (NS) p-value).

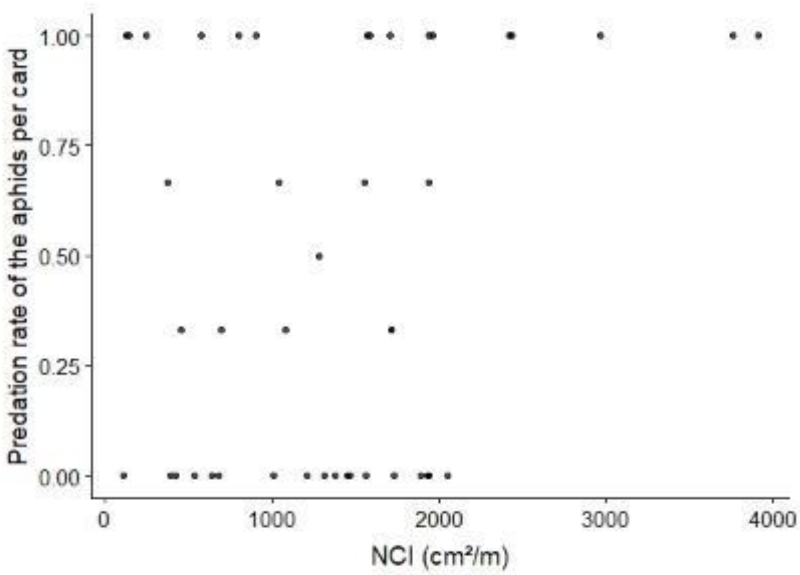


Figure 28: Predation rate per sentinel-prey card of A. pisum aphid according to the NCI, second session. n = 45 cards (one card per apple tree and 3 aphids per card)

2.2) Sentinel-prey cards with C. pomonella eggs

Mean predation rate of *C. pomonella* eggs was, for the plot and per card, about 1.55% and 16.3% on the first and second sessions respectively (not represented). Analysis of the effect of AF on this variable was only made for the latter session. It does not seem that NCI correlates with predation rate of codling moth eggs (figure 29), as the GLM analysis validates it (Estimate = -0.126, not significant p-value).

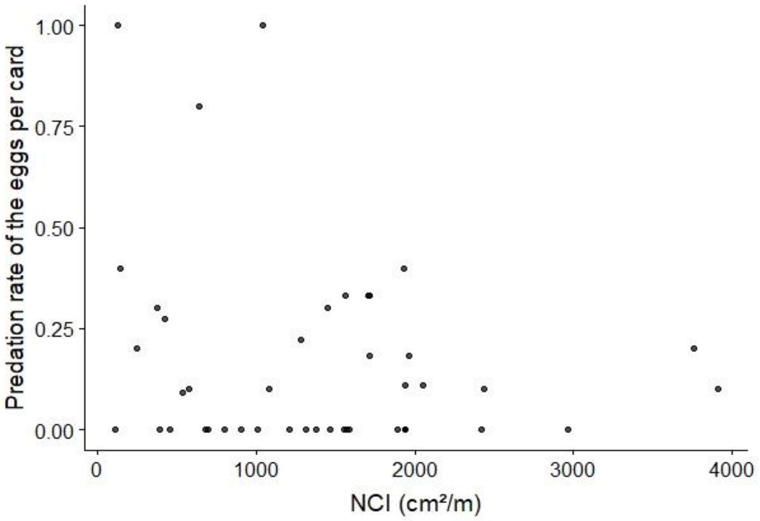


Figure 29: Predation rate per sentinel-prey card of C. pomonella eggs according to the NCI, second session. n = 45 cards (one card per apple tree and 7 to 11 eggs of C. pomonella *per card)*

3) Discussion

AF context did not appear to influence the activity of predation in apple tree canopy approximated by sentinel prey-card technique (either for aphids or for codling moth eggs).

Sentinel-prey card technique use is controversial, regarding the methodology developed and potential reliability as a proxy of predation activity (Lövei and Ferrante, 2017; A. Gardarin, personal comm.). According to them, no standardized method exists for the moment. Results rarely represent the entire predation pressure and arthropods attacking the preys do not necessarily consume this targeted pest in routine (scavengers). Other proxies might be more reliable and consistent (*eg* growth rate of the population of the investigated prey, along with natural enemies' presence).

Appendix VII: Earwig and spider monitoring through strap strips sampling

1) Material and Methods

1.1) Arthropod sampling

In June, strap strips of corrugated cardboard were sampled for earwigs and apple tree canopy spiders monitoring. These strips were placed at the basis of apple tree trunks (10 to 30 cm from the soil) in September 2019. 45 strips on 45 trees were sampled in total, on the same sample trees than those used by B. Pitchers for his thesis. For each strap strips, the number of earwigs was reported, as well as the number of spiders. Spiders were put in alcohol 70°C in order to identify until the family and even species when possible.

1.2) Statistical analysis

GLM Poisson or Negative Binomial (glmmTMB package; Magnusson et al., 2019) was performed (see Equation below). Negative binomial distribution was used to tackle overdispersion that was diagnosed for earwigs. This analysis was only exploratory, as low abundances for each taxa impeded further conclusions.

GLM equation:

Abundance of earwigs or spiders per strap strip ~ NCI (Scaled), family = Poisson or Negative Binomial (link = log)

2) Results

There were more observations for NCI in 0-2,000 cm²/m class than in 2,000-4,000 cm²/m class (*eg* figure 30 and 31).

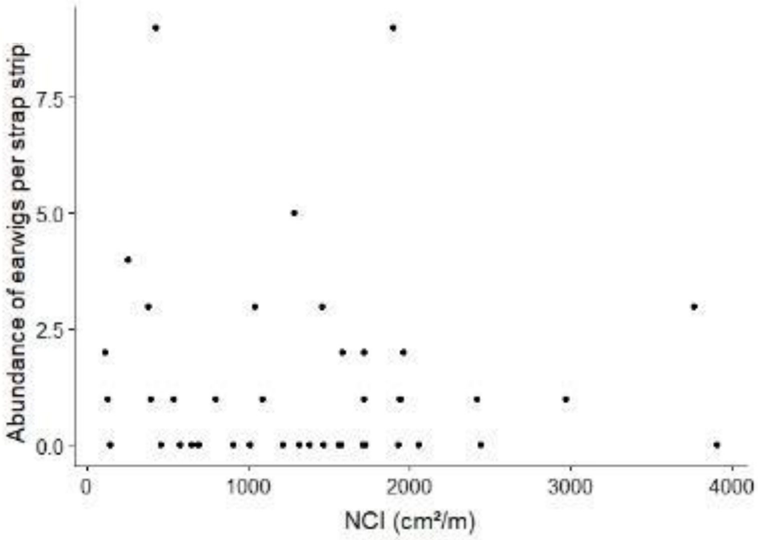


Figure 30: Abundance of earwigs per strap strip according to the NCI. n = 45 strap strips (one strap strip per tree).

2.1) Abundance of earwigs

Over the 24 strips with earwigs, 59 individuals were observed. Abundance of earwigs seemed not correlated to NCI (figure 30). This was confirmed by the model, though we collected a little number of individuals on which base this analysis (estimate = - 0.0985, p-value not significant).

2.2) Abundance of spiders

45 spiders were present in 23 strap strips over the 45 sampled. Abundance of spiders did not seem to correlate to NCI (figure 31). Though, the model outlined a slight negative effect (estimate = - 0.400, p-value below 0.05 threshold).

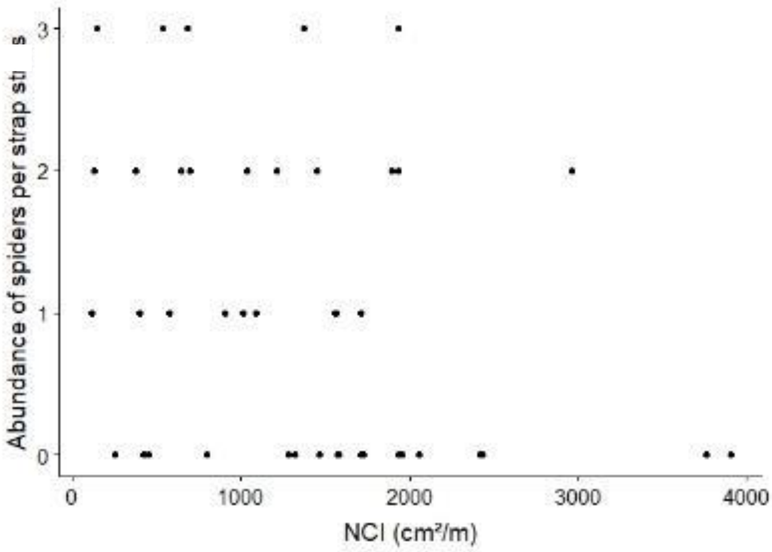


Figure 31: Abundance of spiders per strap strip according to the NCI. n = 45 strap strips (one strap strip per tree)

2.3) Taxa of spiders

Over the 12 taxa observed, dominant taxa in the sampling (57 % of the individuals) was a juvenile from Gnaphosidae family, which could not be identified further (figure 32). Then, Salticidae Heliophanus sp was the second most abundant (22 %).

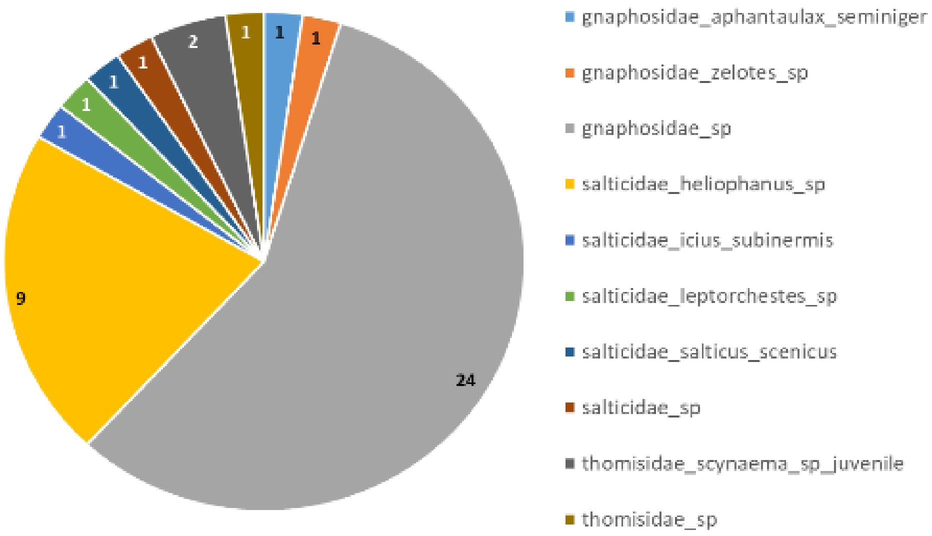


Figure 32: Number of individuals in each taxon on the whole sample of strap strips. n = 45 strap strips

3) Discussion

We performed GLM to test the effect of NCI, yet the low abundances observed – either for spiders or earwigs – impeded to further conclude based on the outputs of the model.

For spiders, dominant taxa belonged to those commonly found in orchards in South of France (Lefebvre, 2016). It would have been interesting to assess whether AF context was associated to the enhancement of some taxa of spiders, but the data itself was not suited for that (low abundances).

It is difficult to draw reliable conclusions, due to some methodological limits, especially for spiders:

- Relatively low sample of strap strips (n = 45), with fewer observations for high values of NCI
- Some strap strips were touching the soil, which may have led to sample not only the spiders from apple tree canopy but also those in soil community
- The sampling was only made at one date, whereas spiders might be more abundant and diverse at other periods (especially in autumn and winter) (Ricard et al., 2012)
- The use of NCI might not be consistent to disentangle AF context effect on these arthropods

Also, this method of sampling can be discussed, as the targeted arthropods (earwigs and spiders) may prefer to shelter in the diverse niches present in the plot rather than in the cardboard.

Appendix VIII: Microclimate in the plot during the monitoring period

Daily Mean, Maximal and Minimal Temperatures (figure 33 to 35) were not different between treatments. This may be linked to the manner temperatures are measured, which does not represent the contribution of radiation to temperature variations. Yet, there seemed to be variations between treatments as attested by the sunburn damages on fruits that were only present on apple trees in Agricultural Control. Furthermore, one can argue that one sensor per treatment is not enough to account for the bias that can occur: depending on the place where the sensor is, results may greatly differ, as microclimate changes rapidly.

As expected according to walnut tree shading effect, global radiation tended to be the highest in AC, and the lowest in AFR, AFIR being intermediate (figure 36). The differences became more contrasted since walnut tree buds break (beginning of May).

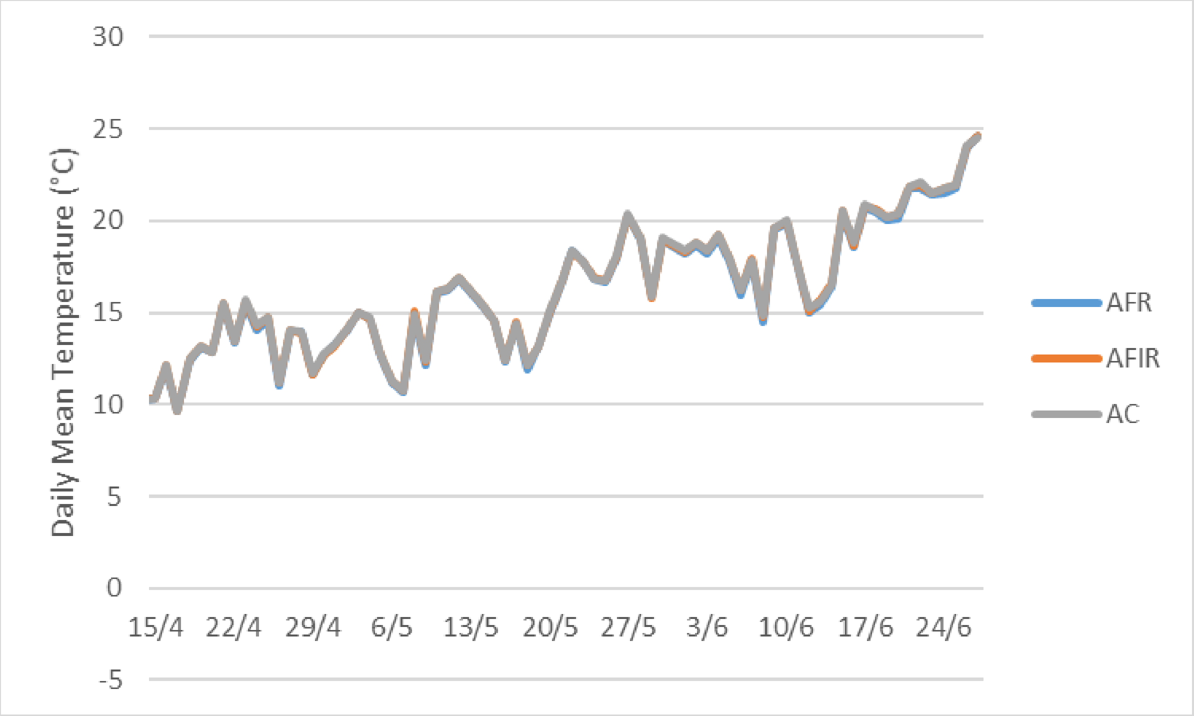


Figure 33: Daily Mean Temperature in the plot in the different treatments (AC: Agricultural Control, AFIR: Agroforestry Inter-Row; AFR: Agroforestry Row)

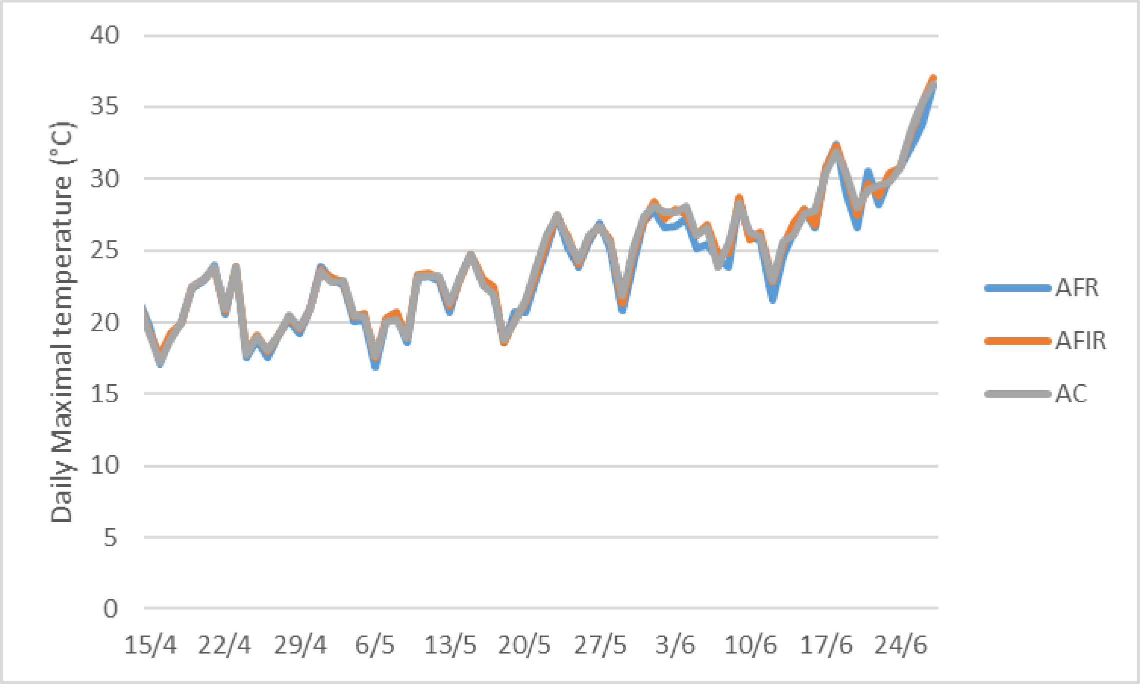


Figure 34: Daily Maximal Temperature in the plot in the different treatments (AC: Agricultural Control, AFIR: Agroforestry Inter-Row; AFR: Agroforestry Row)

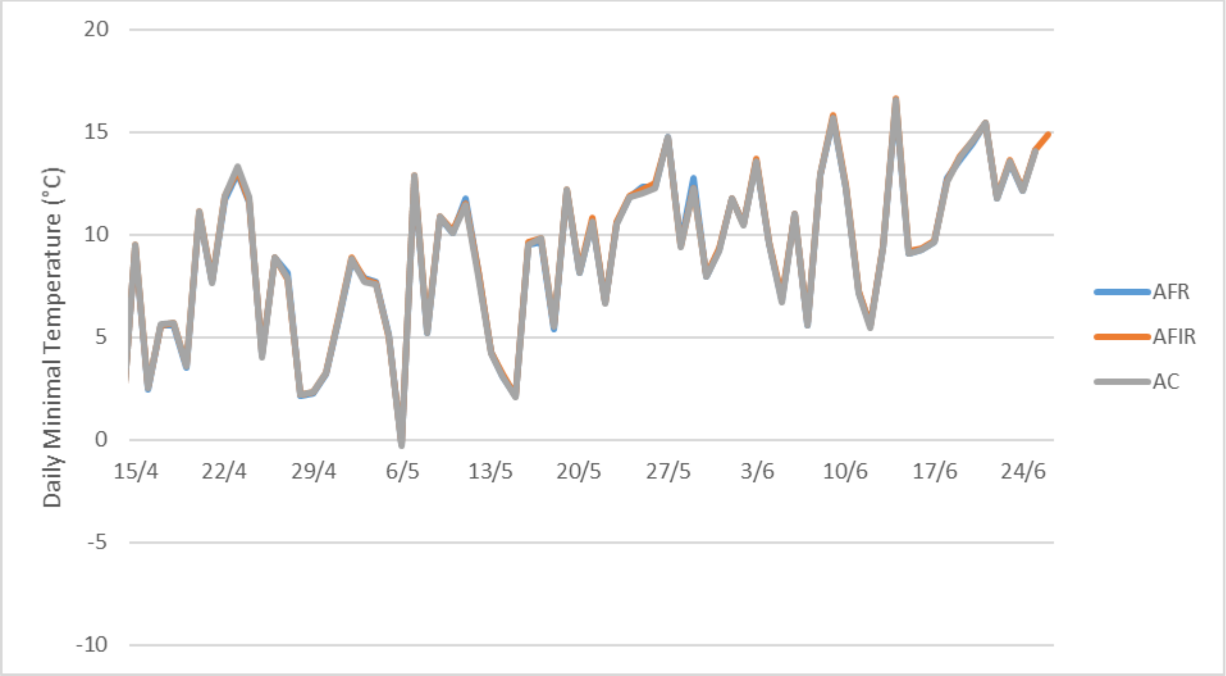


Figure 15: Daily Minimal Temperature in the plot in the different treatments (AC: Agricultural Control, AFIR: Agroforestry Inter-Row; AFR: Agroforestry Row)

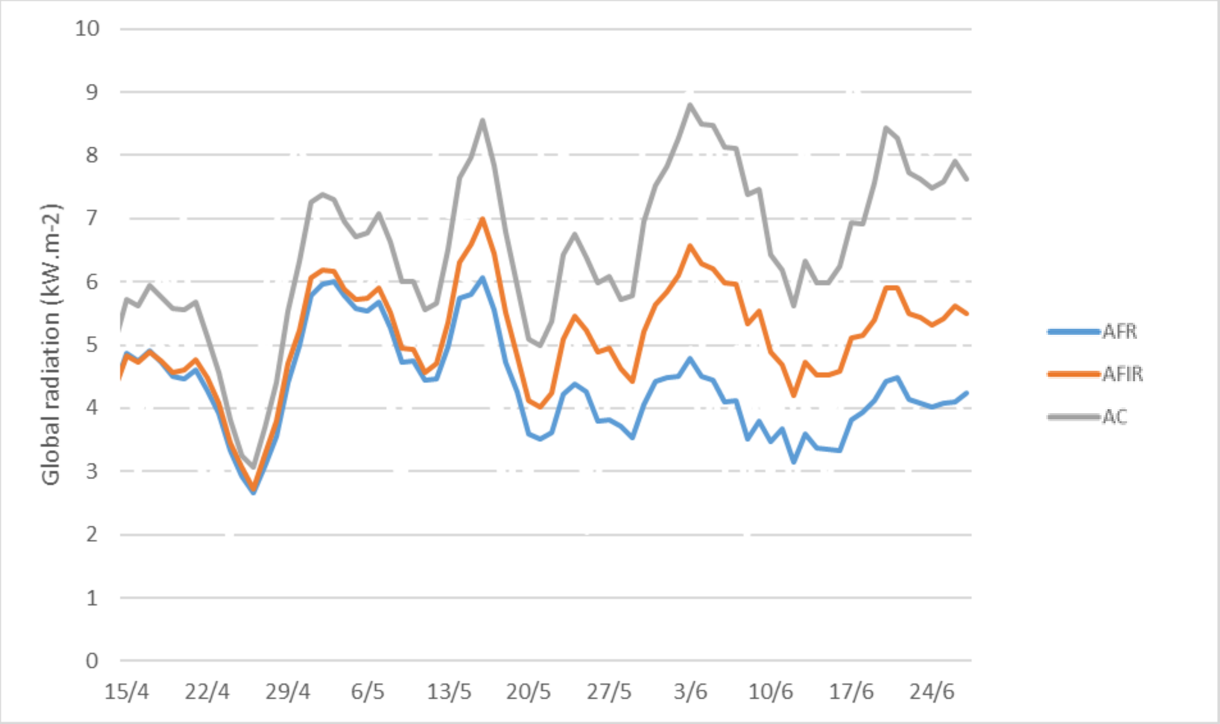
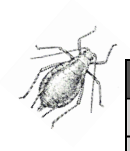


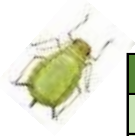
Figure 36: Daily sum of the Global radiation in the plot in the different treatments. Moving average over 5 days are represented. (AC: Agricultural Control, AFIR: Agroforestry Inter-Row; AFR: Agroforestry Row)

Appendix IX: Evolution of the incidence at tree scale of *D. plantaginea* and *Aphis* sp. Estimates for the NCI of the GLM model

Table 12: Evolution of the incidence at tree scale of D. plantaginea (left) and Aphis *sp. (right). Estimate of NCI effect on the probability of a tree to be infested, with significance using GLMs. NS: Not Significant; *: <0.05; **: <0.01; ***: <0.001*



Date	Estimate (significance)
10/04/2019	-0.740 (NS)
18/04/2019	0.440 (NS)
24/04/2019	0.279 (NS)
02/05/2019	-0.277 (NS)
09/05/2019	0.667 (NS)
16/05/2019	0.343 (NS)
23/05/2019	0.367 (NS)



Date	Estimate (significance)
10/04/2019	0.217 (NS)
18/04/2019	0.745 (NS)
20/06/2019	0.700 (NS)
27/06/2019	0.342 (NS)

Appendix X: Sum of the number of ants and of natural enemies according to the NCI

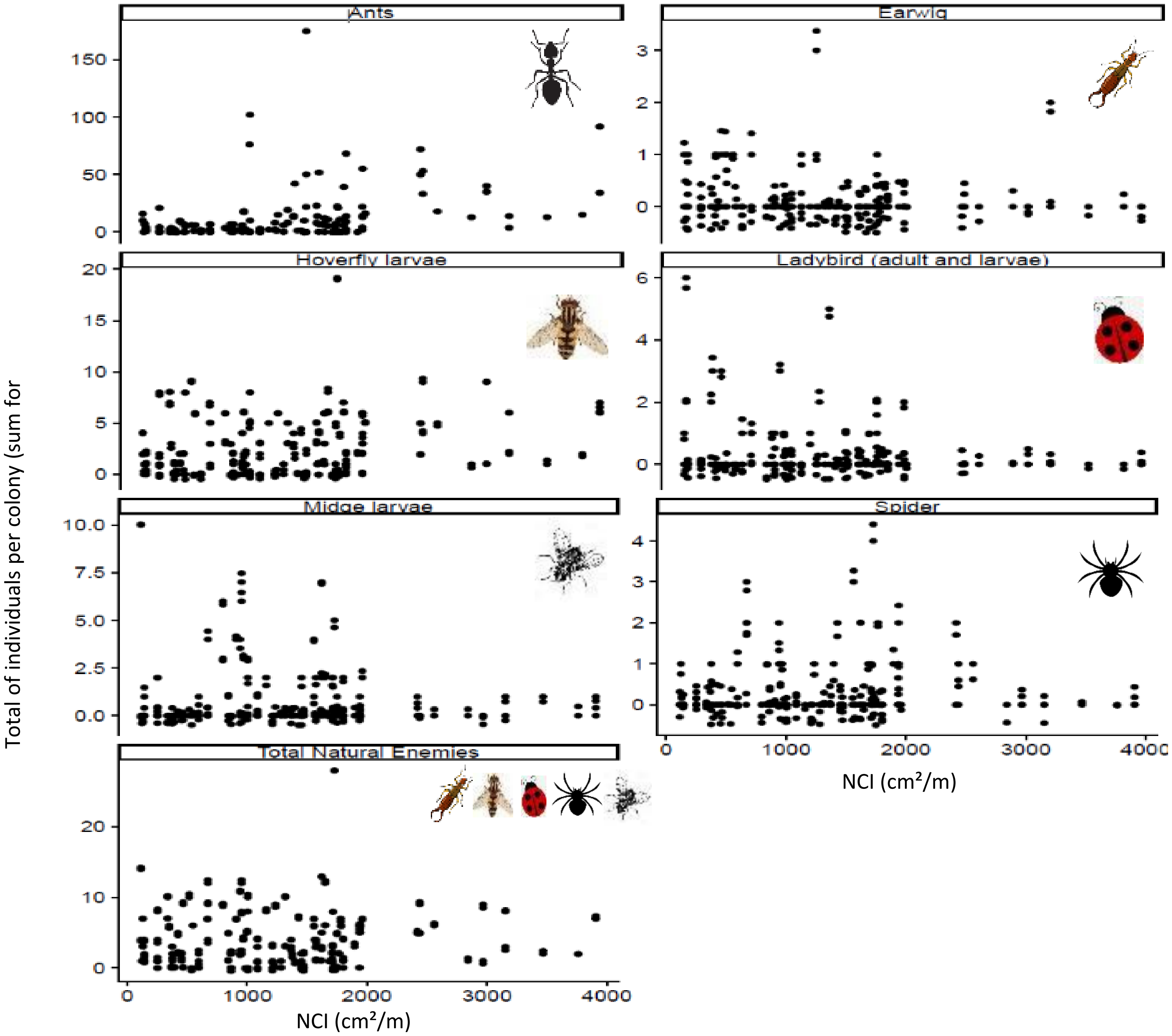


Figure 37: Total number of individuals of ants and Natural enemies in D. plantaginea colonies for all the period (sum for all the dates in each colony), according to the NCI

Appendix XI: Explained variances, correlations and their significance between variables and axes for the PCA designed to study interactions within *D. plantaginea* colonies

A) April 15th

	Dim 1	Dim 2
% of explained variance	36.7	28.6

		Correlation	
	Variable	**Dim 1**	**Dim 2**
Active	**d_planta**	0.749(***)	-0.394(**)
	ants	0.633(***)	-0.331(**)
	l_hoverfly	0.660(***)	0.437(***)
	green_aphid	0.262(*)	0.828(***)
Illustrative	***NCI***	0.290(*)	NS
	spider	NS	NS
	ladybird	NS	NS
	Relative_gr	NS	NS

B) April 23rd

	Dim 1	Dim 2
% of explained variance	30.8	19.5

		Correlation	
	Variable	**Dim 1**	**Dim 2**
Active	**d_planta**	0.709(***)	NS
	ants	0.668(***)	0.281(**)
	l_hoverfly	0.411(***)	-0.544(***)
	green_aphid	0.559(***)	0.421(***)
Illustrative	***NCI***	0.322(**)	NS
	spider	-0.330(**)	0.648(***)
	ladybird	NS	NS
	Relative_gr	NS	NS

C) April 29th

	Dim 1	Dim 3
% of explained variance	40.4	27.3

		Correlation	
	Variable	**Dim 1**	**Dim 3**
Active	**d_planta**	0.797(***)	NS
	ants	0.817(***)	NS
	l_hoverfly	0.512(***)	-0.583(***)
	green_aphid	0.230(*)	0.842(***)
Illustrative	***NCI***	0.283(**)	NS
	spider	NS	NS
	Relative_gr	NS	NS

D) May 6th

	Dim 1	Dim 2
% of explained variance	37.9	26.8

		Correlation	
	Variable	**Dim 1**	**Dim 2**
Active	**d_planta**	0.797(***)	0.250(*)
	ants	0.755(***)	-0.361(***)
	l_hoverfly	0.360(***)	0.795(***)
	green_aphid	0.428(***)	-0.497(***)
Illustrative	***NCI***	NS	NS
	spider	NS	-0.262(**)
	ladybird	NS	NS
	earwig	NS	NS
	Relative_gr	-0.216(*)	NS

E) May 13th

	Dim 1	Dim 2
% of explained variance	29.9	24.7

		Correlation	
	Variable	**Dim 1**	**Dim 2**
Active	**d_planta**	0.884(***)	NS
	alate_d_planta	0.230(*)	0.476(***)
	ants	0.606(***)	-0.498(***)
	l_hoverfly	0.482(***)	0.702(***)
	green_aphid	NS	-0.516(***)
Illustrative	***NCI***	NS	NS
	spider	NS	NS
	ladybird	NS	NS
	earwig	NS	0.288(**)
	Relative_gr	NS	NS

F)May 20th

	Dim 1	Dim 2
% of explained variance	20.9	19

		Correlation	
	Variable	**Dim 1**	**Dim 2**
Active	**d_planta**	0.698(***)	0.437(***)
	alate_d_planta	0.698(***)	NS
	ants	0.270(**)	0.689(***)
	l_hoverfly	0.561(***)	NS
	green_aphid	NS	0.671(***)
	l_cecido	0.400(***)	NS
	spider	0.273(**)	-0.400(***)
	ladybird	NS	NS
Illustrative	***NCI***	NS	NS
	Relative_gr	NS	NS

Layout: NS: Not significant; *: <0.05%; **: <0.01%; ***: <0.001%

G) May 27th

	Dim 1	Dim 2
% of explained variance	21.5	16.7

		Correlation	
	Variable	**Dim 1**	**Dim 2**
Active	**d_planta**	0.793(***)	NS
	alate_d_planta	0.537(***)	0.425(***)
	ants	0.407(***)	-0.646(***)
	l_hoverfly	0.498(***)	0.438(***)
	green_aphid	0.372(***)	-0.519(***)
	l_cecido	0.497(***)	0.450(***)
	spider	0.258(*)	NS
	ladybird	NS	0.455(***)
	earwig	-0.371(***)	NS
Illustrative	***NCI***	NS	-0.297(**)
	Relative_gr	0.324(**)	NS

Appendix XII: Picture of the different AEI present in and around the plot


5 - Dry meadow in April (left) and in July (right)


6 - Fescue


3 - Sainfoin


8 - Stones


9 - Pole


7 - Short Rotation Coppice (here, poplar tree)

H1 – Low and sparse hedge, green oak dominant

H2 – Low and dense and sparse hedge, dogwood and brambles dominant

H3 – Tall riparian hedge, plane and poplar tree dominant

H4 – Medium riparian hedge, deciduous dominant

H5 – Medium riparian hedge, giant reed and bramble dominant

H6 – Medium hedge, ash and tree of heaven dominant

Appendix XIII: List of the shrub and tree species in the hedges surrounding the plot

Hedges typology was qualitative, based on their height, their porosity, the dominant species or type of bushes/trees (deciduous or persistent foliage), as these aspects are expected to drive the functions fulfilled by the hedges. For each species, we considered its relative abundance in terms of number of individuals, its flowering period (determinant for auxiliaries) and its known specific interest for auxiliaries' enhancement.

The dominance of a type of vegetation was determined in terms of number of individuals and volume occupied:

+++: dominant species; ++: second dominant species; +: species occupying less volume; presence: presence of one individual; ND: No information found in the literature regarding on the specific interest for NE

Abundance auxiliary fauna, and comments associated to each species are based on Ricard et al. (2012) and Chambre d'Agriculture France (2017).

1) H1: Low and sparse hedge, green oak dominant (7 species)

Species	Relative abundance	Abundance auxiliary fauna	Flowering period	Specific interest and/or risk associated
Quercus ilex – Evergreen oak	+++	***	Spring	Rich and diverse fauna. Hosts phytophagous bugs
Euonymus europaeus - Spindle	+	**	May	Diverse fauna, persistent fruits in winter. Hosts *Myzus persicae* and *Aphis fabae*
Pyrus communis – Pear tree	+		April	Can act as a reservoir for pests and diseases of pear tree
Ulmus minor – Field elm	+	*/***	March	Hosts phytoseiidae
Clematis vitalba – Old man's beard	presence	*	July-September	Hosts phytoseiidae
Phillyrea latifolia – Green olive tree	presence	ND	Spring	ND
Quercus pubescens – Downy oak	presence	***	April-May	

2) H2: Low and dense hedge, dogwood and bramble dominant (7 species)

Species	Relative abundance	Abundance of auxiliary fauna	Flowering period	Specific interest and/or risk associated
Cornus sp - Dogwood	+++	**	Spring	

Rubus sp - Bramble	+++	*/***	Summer	Diverse fauna including phytoseiidae. Host *D.suzuki*
Phillyrea latifolia – Green olive tree	+	ND	Spring	ND
Quercus ilex – Evergreen oak	+		Spring	Rich and diverse fauna. Hosts phytophagous bugs
Crataegus monogyna – Common hawthorn	presence	**	May	Diverse fauna, sensible to fire blight
Malus sp – Apple tree	presence		April	Host for pests and diseases of apple tree
Morus alba – White mulberry	presence	ND	April, May, August, September	ND

3) H3: Tall riparian hedge, plane and poplar trees dominant (17 species)

Species	Relative abundance	Abundance of auxiliary fauna	Flowering period	Specific interest and/or risk associated
Platanus xhispanica – Hybrid plane	+++	ND	Spring	ND
Populus alba – Silver poplar	+++	*	Spring	Low interest for auxiliaries
Hedera helix – Common ivy	++	****	Autumn	Supplement NE with food at the end of the growing season, overwintering site. Hosts *Aphis hedera*
Quercus pubescens – Downy oak	++	***	April-May	
Rubus sp - Bramble	++	*/***	Summer	Diverse fauna including phytoseiidae. Hosts *D.suzuki*
Acer campestre – Field maple	+	**	May	Aphids in April attracting Nevroptera, Staphylinidae, Coccinellidae and Miridae
Acer platanoides – Norway maple	+	ND	April	ND
Cornus sp - Dogwood	+	**	Spring	
Crataegus monogyna – Common hawthorn	+	**	May	Diverse fauna, sensible to fire blight
Euonymus europaeus - Spindle	+	**	May	Diverse fauna, persistent fruits in winter. Hosts *Myzus persicae* and *Aphis fabae*
Fraxinus sp - Ash	+	**	April-May	Potential host of psylla and midge pests, attracting Anthocoridae
Morus alba – White mulberry	+	ND	April, May, August, September	ND

Phillyrea latifolia – Green olive tree	+	ND	Spring	ND
Prunus sp – plum tree	+		March-April	Potential host of pests and diseases of crops from Prunus genus
Ulmus minor – Field elm	+	*/***	March	Hosts phytoseiidae
Juglans sp (hybrid ?) – Walnut tree	presence	Very limited	April	*Juglans regia* favors hoverflies, hosts *C. pomonella* and *Zeuzera pyrina*
Viburnum tinus - Laurestine	presence	****	January-April	Diverse fauna thanks to winter bloom and persistent foliage

4) H4: Medium riparian hedge, deciduous dominant (19 species)

Species	Relative abundance	Abundance of auxiliary fauna	Flowering period	Specific interest and/or risk associated
Fraxinus sp - Ash	++	**	April-May	Potential host of psylla and midge pests, attracting Anthocoridae
Hedera helix – Common ivy	++	****	Autumn	Supplement NE with food at the end of the growing season, overwintering site. Hosts *Aphis hedera*
Morus alba – White mulberry	++	ND	April, May, August, September	ND
Prunus domestica – plum tree	++	ND	March-April	ND
Rubus sp - Bramble	++	***/*****	Summer	Diverse fauna including phytoseiidae. Hosts *D.suzuki*
Acer campestre – Field maple	+	**	May	Aphids in April attracting Nevroptera, Staphylinidae, Coccinellidae and Miridae
Acer platanoides – Norway maple	+	ND	April	ND
Clematis vitalba – Old man's beard	+		July-September	Hosts phytoseiidae
Coriaria myrtifolia - Redoul	+	ND	March-October	ND
Cornus sp - Dogwood	+	**	Spring	
Crataegus monogyna – Common hawthorn	+	**	May	Diverse fauna, sensible to fire blight
Euonymus europaeus - Spindle	+	**	May	Diverse fauna, persistent fruits in winter. Hosts *Myzus persicae* and *Aphis fabae*

Ligustrum angustifolium – Common privet	+	ND	Summer	ND
Platanus xhispanica – Hybrid plane	+	ND	Spring	ND
Prunus domestica – plum tree	+		March-April	Potential host for pests and diseases of crops from Prunus genus
Quercus pubescens – Downy oak	+	***	April-May	
Ulmus minor – Field elm	+	*/***	March	Hosts phytoseiidae
Viburnum tinus - Laurestine	+	****	January-April	Diverse fauna thanks to winter bloom and persistent foliage
Vitis vinifera – Grape vine	presence	ND	Spring	ND

5) H5: Medium riparian hedge, giant reed and bramble dominant (5 species)

Species	**Relative abundance**	**Abundance of auxiliary fauna**	**Flowering period**	**Specific interest and/or risk associated**
Arundo donax – Giant reed	+++	ND	All the year	ND
Rubus sp - Bramble	++	***/*****	Summer	Diverse fauna including phytoseiidae. Hosts *D.suzuki*
Clematis vitalba – Old man's beard	++	*****	July-September	Hosts phytoseiidae
Prunus domestica – plum tree	presence		March-April	Potential host of pests and diseases of crops from Prunus genus
Rosa sp - Rose	presence	ND	ND	ND

6) Medium hedge, ash and tree of heaven dominant (9 species)

Species	**Relative abundance**	**Abundance of auxiliary fauna**	**Flowering period**	**Specific interest and/or risk associated**
Ailanthus altissima – Tree of heaven	+++	ND	July-August	ND
Fraxinus sp - Ash	+++	**	April-May	Potential host of psylla and midge pests, attracting Anthocoridae.
Hedera helix – Common ivy	++	****	Autumn	Supplement auxiliaries with food at the end of the growing season, overwintering site. Hosts *Aphis hedera*
Rubus sp - Bramble	++	***/*****	Summer	Diverse fauna including phytoseiidae. Hosts *D.suzuki*

Clematis vitalba – Old man's beard	++	*	July-September	Hosts phytoseiidae
Cornus sp - Dogwood	+	**	Spring	
Euonymus europaeus - Spindle	+	**	May	Diverse fauna, persistent fruits in winter. Hosts *Myzus persicae* and *Aphis fabae*
Prunus domestica – plum tree	+		March-April	Potential host for pests and diseases of crops from Prunus genus
Ulmus minor – Field elm	+	*/***	March	Hosts phytoseiidae

7) Low hedge, elm tree dominant (2 species)

Species	**Relative abundance**	**Abundance of auxiliary fauna**	**Flowering period**	**Specific interest and/or risk associated**
Ulmus minor – Field elm	+++	*/***	March	Hosts phytoseiidae
Crataegus monogyna – Common hawthorn	+	**	May	Diverse fauna, sensible to fire blight

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Titre français : Incidence des ravageurs et régulations biologiques dans un système agroforestier à base de pommiers

Titre anglais : Pest incidence and biological regulations in an apple tree-based agroforestry system

Résumé :

L'agroforesterie peut être un levier pour favoriser les services écosystémiques, tels que le contrôle biologique. Un système agroforestier (AF) innovant a été mis en place, avec des pommiers cultivés sous noyers hybrides. Les effets d’un tel design sur les ravageurs du pommier, les ennemis naturels (NE) et les régulations biologiques ont été traités pour la première fois dans le cadre de ce stage. Un indice de voisinage (NCI) a été utilisé comme variable explicative continue caractérisant le contexte AF pour chaque pommier, un indice élevé traduisant un fort effet de l’agroforesterie. Au printemps, l'incidence de *Dysaphis plantaginea*, *Aphis* sp et de la première génération de *Cydia pomonella* ont été suivis chaque semaine. Des colonies marquées de *D. plantaginea* ont été suivies chaque semaine pour évaluer la dynamique de *D. plantaginea* en lien avec l’abondance des fourmis et des NE par taxon. L'incidence de *D. plantaginea* n'était pas significativement corrélée au NCI, contrairement à celle d'*Aphis* sp (corrélation positive). La faible incidence de *C. pomonella* a empêché de conclure. Dans les colonies de *D. plantaginea*, la densité de pucerons corrélait positivement au NCI, ce qui pourrait être lié à l'abondance accrue des fourmis. Parmi les NE, seule l’abondance des Coccinellidae corrélait négativement avec le NCI. Aucun effet des fourmis ou des NE sur *D. plantaginea*, à l'exception des larves de Syrphidae, n’a été décelé. Le noyer semblant avoir un effet limité sur les régulations biologiques, de nouvelles infrastructures visant à favoriser le contrôle biologique ont été proposées.

Abstract:

Agroforestry (AF) is considered as a lever to enhance ecosystem services, especially pest control. An innovative Apple tree-based AF system was set, with apple trees grown under hybrid walnut trees, in an alley-cropping design. The impact on apple tree pest dynamics, Natural Enemies (NE) and biological regulations of such design has been investigated for the first time. The Neighbourhood Crowding Index (NCI), a continuous variable, was used as explanatory variable characterizing the AF context for each apple tree: the higher this index, the stronger the agroforestry effect. In Spring 2019, *Dysaphis plantaginea* and *Aphis* sp incidence were followed weekly, as well as the incidence of the first generation of *C. pomonella*. Marked colonies of *D. plantaginea* were followed weekly to assess *D. plantaginea* dynamic, together with ants and NE abundances by taxon. *D. plantaginea* incidence was not significantly correlated to NCI, contrarily to *Aphis* sp (positive correlation). Low levels of *C. pomonella* incidence did not permit satisfactory conclusion. Within *D. plantaginea* colonies, aphid densities were positively correlated to NCI, potentially linked with the greater abundance of ants observed in AF context. No correlation was found between NCI and NE abundances, with however a negative correlation with Coccinellidae. No effects of ants-tending or predation on *D. plantaginea* (except for Syrphidae larvae) could be shown. As walnut tree seems to have limited effect on biological regulations, the proposal of new agroecological infrastructures was made to increase pest control.

Mots-clés : Agroforesterie multi-strate ; *Dysaphis plantaginea* ; *Aphis* sp ; *Cydia pomonella* ; ennemis naturels, Lutte biologique par conservation

Key Words: Multi-strata agroforestry; *Dysaphis plantaginea*; *Aphis* sp; *Cydia pomonella*; natural enemies; conservation biological control

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