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Efficacy of pest and pathogen control, yield and quality of winter lettuce crops managed with reduced pesticide applications

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Abstract

In conventional agriculture, lettuce crops receive large amounts of pesticides to meet stringent industrial specifications and market requirements. Pesticides are used on lettuce to ensure high-yielding, attractive products free from foreign bodies and damage. Pesticide reduction is a major challenge for lettuce growers in this context. The objective of this study was to assess the risk arising from a reduction in pesticide applications by using a combination of alternative techniques for the management of pests and diseases in winter lettuce crops. Two alternative crop protection strategies (called low-input and intermediate) were designed by prototyping and then compared to a conventional lettuce protection strategy in independent trials carried out in three locations over two successive winters. The efficacy of each strategy for pest and pathogen control, as well as lettuce yield and quality, were assessed and compared. Pesticides were reduced by 32% in the intermediate crop protection strategy, and by 48% in the low-input crop protection strategy. At least 15% of lettuces were affected by pest and pathogen damage, whatever the strategy. Among possible pests or diseases, aphids were the only biotic stress which differed significantly between strategies (9.25% of lettuces infested and 2.7% of commercial losses under the low-input strategy, as compared to 0.83% of lettuces infested and 0% of commercial losses under the conventional strategy). Globally, biotic damage was less important than abiotic damage (frost and tip burn), and resulted in low commercial losses under all strategies. Similar yields and lettuce quality were recorded under the three systems. Therefore the performances of intermediate and low-input crop protection strategies were consistent with market expectations, and possible improvements are discussed. This work provides a methodology and landmarks for the design and dissemination of cropping systems targeted to leafy vegetables and less dependent on chemical control of pests and pathogens.

Keywords: *Lactuca sativa*, crop protection, prototyping, low-input, alternative techniques

Abbreviations:

IBEB : International Bremia Evaluation Board

CPS: Crop protection strategy

TFI: Treatment Frequency Index

L: Location

PACA: Provence Alpes Côte d’Azur

W: Winter

1. Introduction

Conventional farming systems use large amounts of pesticides to manage pest and pathogen populations. Pesticides are used to improve yield and visual quality of harvested products (Wilson and Tisdell, 2001). However, due to their harmful effects on the environment (Geiger et al., 2010; Goulson, 2013) and possibly on consumers' and applicators' health (Mostafalou and Abdollahi, 2013), the viability of conventional cropping systems is nowadays widely questioned. The European Union recently established a directive to reduce pesticide consumption and promote the use of non-chemical methods wherever possible (EU, 2009) .

In the past decades innovative cropping systems have been designed, aimed at reducing pesticide use, mainly for arable crops. During the last 20 years, a range of low-input cereal-based cropping systems has been experimented and assessed (Debaeke et al., 2009; Loyce et al., 2012). These systems rely on reduced sowing rates and/or nitrogen inputs, sometimes reduced tillage, and on the use of suitable, multi-resistant varieties (in mixtures or in monocrop systems). Profit margins are maintained, since yield losses associated with reduced inputs are balanced by lower costs. In temperate and Mediterranean climates, winter lettuce is usually grown under shelter, typically in high tunnels, with two or three harvests between September and April. In these systems, inputs are relatively marginal as compared to labor costs, and chemical protection represents only 3 to 6% of the total production costs. Therefore the design of innovative lettuce-based cropping systems cannot rely on the same strategy as cereal-based systems, since yield losses cannot be offset by significantly reduced input costs. Thus, any pesticide reduction in lettuce crops has to be achieved without yield reductions. Furthermore, lettuce is usually eaten raw and the aerial parts of the plant are almost entirely consumed, so market specifications for visual quality and the absence of foreign bodies are very high (Palumbo and Castle, 2009). On average, the tolerance threshold of the industry for the presence of animal foreign bodies (including pests but also beneficial insects) is no more than 10% of products infested with no more than 5 individuals per item. Pesticides are therefore considered as a means to ensure high-yielding and high-quality products. In organic agriculture, the possible yield reduction (de Ponti et al., 2012) can be balanced by a higher sale price of organic products, but many consumers are unwilling to pay these prices. Therefore, the question is how and by how much is it possible to reduce the use of pesticides in conventional lettuce crops without affecting lettuce quality or yield.

Many pests and pathogens can threaten lettuce crops, such as biotrophic or necrotrophic, soil- or air-borne fungi, viruses, bacteria as well as aphids, moths, slugs, thrips, etc. The incidence and severity of each pest and pathogen depends on growing conditions (crop type, cultivation under shelter or in open fields, season) and changes over the year. In winter lettuce crops under shelter, pathogens are predominant. The most important is probably *Bremia lactucae* (Regel), the causal agent of lettuce downy mildew, because of its rapid and devastating spread in the field. The pathogen may attack the plant throughout its life. The primary inoculum typically consists of airborne sporangia from diseased plants of the genus *Lactuca* located close to the crop, or of mycelia present on plant debris in the soil (Crute, 1992). *Sclerotinia sclerotiorum* (de Bary), *Sclerotinia minor* (Jagger), *Botrytis cinerea* (Pers.), *Rhizoctonia solani* (Kühn), and *Pythium tracheiphilum* (Matta) are other important fungal pathogens of winter lettuce crops. Collectively, these pathogens cause symptoms of basal rot, i.e. rotting of the leaves in contact with the soil surface (Van Beneden et al., 2009). *S. minor* and *S. sclerotiorum* are of major concern for the cultivation of lettuce because they may affect a wide range of plant species and their sclerotia may remain latent in the soil for more than 8 years (Bolton et al., 2006; Melzer et al., 1997). Moreover, sclerotia are often buried and dispersed by tillage (Subbarao et al., 1996). Therefore, basal rot causes long-term problems in conventional lettuce crops since sclerotia are taken back up to the soil surface at each tillage. *B. cinerea and R. solani* can also cause significant damage depending on the growing season. *B. cinerea* injury to lettuce leaves is enhanced by cool and moist conditions, while *R. solani* sclerotia and mycelia are most frequently found in the soil in summer (Van Beneden et al., 2009). The fungus *Olpidium virulentus* is not a direct threat to lettuce, but a vector of two lettuce viruses that can cause significant damage, especially in winter: ‘Mirafiori lettuce virus’, responsible for big vein disease; and ‘Lettuce big-vein associated virus’, suspected to be the agent of ring necrosis (Lot et al., 2002; Maccarone, 2013; Verbeek et al., 2013). The resting spores of *O. virulentus* can persist in the soil for many years, and viruliferous zoospores that infect lettuces are very mobile, so management of the disease is complex (Campbell, 1985; Maccarone, 2013). Several aphid species can proliferate in winter lettuce crops such as *Nasonovia ribisnigri* (Mosley), *Myzus persicae* (Sulzer), *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), and *Hyperomyzus lactucae* (L.). *N. ribisnigri* is the most damaging one because it develops preferentially in the lettuce heart (Liu, 2004). In addition to feeding damage and the loss of product quality due to their presence when the lettuce is marketed, aphids are also vectors of viruses, such as the lettuce mosaic virus. Finally, slugs (*Deroceras sp.* and *Arion sp*.) and snails can also cause feeding damage to lettuce in winter.

In conventional lettuce crops in the Mediterranean region, eight to ten pesticides are applied on average to manage pests and pathogens during the 60- to 90-day-long crop cycle. In winter, these are mainly fungicides. Due to the long time required before harvest for the elimination of active ingredients by lettuce and the lack of curative efficacy of pesticides for some pathogens such as *Bremia lactucae*, pesticides are almost exclusively applied preventively. Several alternative techniques with a partial effect on diseases and pests are currently available and might be combined to design innovative lettuce cropping systems less dependent on pesticides (Barriere et al., 2014). These alternative techniques are generally preventive and have only a partial effect on pests and diseases. They can act at different times in the pest and pathogen cycle: they can i) limit and reduce primary inoculum sources, ii) limit the development of pests and pathogens through the modification of the abiotic environment or iii) increase plant defenses, and iv) have a curative action. Techniques that reduce primary inoculum in lettuce crops are sanitation methods such as the removal of infected plants, solarization, or some biological control agents such as *Coniothyrium minitans* and *Trichoderma harzianum. C. minitans* is an efficient biocontrol agent against *Sclerotinia sclerotiorum* (Chitrampalam et al., 2008). This fungus preferentially parasitizes overwintering structures by synthesizing chitinases, glucanases, and antifungal metabolites (Zeng et al., 2012). *T. harzianum* also disturbs populations of *B. cinerea, Pythium spp*., *R. solani*, and *Sclerotinia spp*. in the soil by acting either as a competitor or as a parasite (Elad, 2000; Harman, 2006; Howell, 2003; Vinale et al., 2008). Another way to protect lettuce crops is to limit the development of pests and pathogens through the modification of the abiotic environment. Indeed, the germination of infectious forms (spores or sclerotia) of numerous pathogenic fungi depends on climatic factors such as humidity and temperature. Drip irrigation, as opposed to sprinkler irrigation which is widespread in southern France, may reduce leaf wetness duration, which is an important factor for the germination of *B. lactucae* sporangia (Scherm and Bruggen, 1994) and *B. cinerea* spores (Elad and Shtienberg, 1995). Lower plant density can also reduce foliage wetness due to better aeration, and limit soil-borne disease development by affecting the microclimate under lettuces. Alternative techniques can also modify plant susceptibility to pests and pathogens. Genetic resistance, resistance inducers and nitrogen fertilization have shown a partial effect on lettuce diseases and pests. Lettuce varieties with major resistance genes against *B. lactucae* are available and widely used. However, information about resistance is only provided by seed companies for the most common and widespread isolates (identified and denominated by The 'International Bremia Evaluation Board'- IBEB-). *B. lactucae* can rapidly develop new virulent isolates. Therefore resistance breakdown is common and leads to a rapid turnover of lettuce varieties (Michelmore and Wong, 2008). Thirty-two races of *B. lactucae* are currently registered by the IBEB. Complete resistance to the aphid *N. ribisnigri*, and partial resistance to *Myzus persicae*, are conferred by a dominant gene called Nr, which has been introduced in many European cultivars (Cid et al., 2012; Liu and McCreight, 2006). However, this resistance was recently bypassed by a new *N. ribisnigri* biotype named Nr:1 (ten Broeke et al., 2013). Apart from genes conferring complete resistance, different susceptibility levels of lettuce accessions to *S. sclerotiorum* have been reported (Elia and Piglionica, 1964; Grube and Ryder, 2004; Hayes et al., 2010). In addition to genetic resistance, some techniques can strengthen plant defense. Several compounds, such as β-amino butyric acid or potassium phosphite (K_2HPO_3), have been identified as resistance inducers of lettuce against *B. lactucae* (Pajot et al., 2001). Some of them, such as potassium phosphite, also have a direct biocide effect on oomycetes (Massoud et al., 2012). Fertilization can affect plant-pathogen and plant-pest interactions. The nitrogen content of lettuce leaves is positively correlated to damage by *B. cinerea* and *S. sclerotiorum* (Lecompte et al., 2013). Leaf nitrogen content is also positively correlated to lettuce palatability for slugs (Pakarinen et al., 1990). So low nitrogen applications could help to lower lettuce susceptibility to pests and pathogens.

The efficacy of each alternative technique to pesticides is usually assessed separately, as a stand-alone technique. Very few studies have investigated the effect of a coherent combination of alternative techniques on the control of the lettuce pest/pathogen complex (Collange et al., 2014). In this case, it is not a technique by itself that is evaluated for its performance, but a cropping system as a whole. Prototyping consists in designing, implementing and evaluating innovative cropping systems, and allows for theoretical constructs to be applied to production constraints (Lancon et al., 2007; Vereijken, 1997). In order to adapt to varying local factors, techniques are described as a set of contingent decision rules that govern practices according to biotic and abiotic constraints (Debaeke et al., 2009; Papy, 2001).

Field tests are necessary to assess the risks from reduced pesticide applications. In this study, we designed combinations of alternative techniques and reduced applications of pesticides to create alternative crop protection strategies (CPSs) by prototyping, and compared them to a reference strategy corresponding to current growers' practices. We implemented these CPSs, and we assessed their efficacy to manage pests and diseases, the yield and the quality of harvested products in three locations over two seasons to cover the contextual variation of pest and pathogen pressure.

2. Materials and methods

2.1. Designing crop protection strategies

Fifteen persons including farmers, scientists, technical advisors, suppliers and end-product distributors participated in two expert meetings held in February and June 2011 to design CPSs with reduced pesticide use. At the first meeting, the objectives and constraints of all the stakeholders were defined, and an inventory of possible alternative techniques to pesticides was made. Candidate CPSs were then designed accordingly and refined during the second meeting, during which combinations of appropriate alternative techniques were validated and a target performance of each strategy was agreed upon. A first CPS, called low-input CPS, sharply reduced inputs and was intended to investigate the technical boundaries of pesticide reduction. The second CPS was at an intermediate level of pesticide reduction (intermediate CPS) between the low-input CPS and current practices. It was intended to meet farmers' socio-economic and agronomic objectives. A conventional CPS, representative of current practices in protected winter lettuce crops, was also defined from the synthesis of four farmers' practices.

2.2. Experimental design

The three lettuce CPSs (conventional, intermediate and low-input) were used in Batavia lettuce production under high plastic tunnels in 3 locations in south-eastern France during winters 2012-2013 (W1) and 2013-2014 (W2). A total of 18 lettuce crops were monitored (3 CPSs x 3 locations x 2 years). Two locations were conventional farms located at Candillargues (L1) (43°62'N; 4°06'E; 3 m elevation) and at Villelongue de la Salanque (L2) (42°73'N; 2°98'E; 6 m elevation) in the Languedoc Roussillon region. The third location (L3) was the INRA experimental station of Avignon (43°91'N; 4°87'E; 31 m elevation) in the Provence Alpes Cote d'Azur (PACA) region. Crop rotation in each site, planting and harvesting dates are given in Table 1. Crop lifespan was very similar for all experiments, between 70 and 84 days. We called the various combinations of locations and years L1-W1, L1-W2, L2-W1, L2-W2, L3-W1 and L3-W2.

2.3. Crop protection strategies

The innovative CPSs relied on the joint implementation of alternative techniques to pesticides (Table 2). Pesticide applications and alternative techniques were managed by fixed or contingent decision rules to suit local constraints.

2.3.1. Techniques that affect several pests and pathogens

2.3.1.1. Irrigation

Except for the plot cultivated under the low-input CPS in L3, all the plots were sprinkler-irrigated throughout crop growth. Just after planting, water was applied to field capacity. The moisture content was maintained in the balls containing the plantlets by short daily irrigations until the roots started to grow into the soil. After this early growth stage, drip irrigation was used under the low-input CPS in L3 only. Irrigation was triggered according to the soil water potentials measured by 6 Watermark® sensors placed at 3 positions and 2 depths (15 cm and 35 cm), following the current guidelines in SE France. In the other sites and CPSs, sprinkler irrigation was triggered once every two or three weeks, according to potential evapotranspiration. Towards the end of the cropping cycle, short sprinkler irrigations were applied when the temperature exceeded 30°C, to allow for a rapid cooling of the lettuces.

2.3.1.2. Fertilization

A few days before planting, about fifteen 30-cm deep soil samples were randomly collected from each plot. Soil nitrate content was measured in each plot with a Nitrachek reflectometer to assess fertilizer requirements. Under the conventional and intermediate CPSs, fertilization followed farmers' practices, i.e. soil N was adjusted to 100-120 kg NO_3^- -N ha^{-1}, with mixed N-P_2O_5-K_2O fertilizers (10-20-20 or 5-7-9 depending on the site). When the nitrate content exceeded 120 kg ha^{-1} prior to planting, no fertilizer was applied.

Under the low-input CPS, nitrate fertilization was reduced and split. Before planting, soil nitrate-N content was adjusted to 40 kg ha^{-1} with mixed N-P_2O_5-K_2O fertilizers. At the 16th leaf stage, a new soil sample was collected and analyzed following the same procedure, and the soil NO_3^- -N content was adjusted to 60 kg ha^{-1} with ammonitrate. Due to significant N mineralization during early growth, the soil nitrate stock at this stage was usually not exhausted, hence low fertilizer applications. As a result, the total application of N fertilizers in the plots cultivated under low-input CPS was much lower than in the other two CPSs.

2.3.1.3. Genotype

A different cultivar was used in each CPS. Cv Notilia (Clause), used in the conventional CPS, is a fast-growing variety, has an incomplete range of resistance to *B. lactucae* (Bl 1-28, 30-32). It was chosen for its agronomic quality in situations of full chemical protection. Cv Ostralie (Rijk Zwaan), was used in the intermediate CPS. This cultivar grows slightly more slowly, has a complete range of resistance to *B. lactucae* (Bl: 1-32), is resistant against the aphid biotype Nr:0, and has a semi-upright habit. The cultivar retained in the low-input CPS, Lasydo (Syngenta seed), is a fast-growing variety with an incomplete range of resistance to *B. lactucae* (Bl 1-28, 30-32) but low susceptibility to *S. sclerotiorum*, as assessed in preliminary tests in our laboratory.

2.3.1.4. Planting density

Lettuces were planted in parallel rows, on micro- and macro- perforated black plastic mulch (Optimac®). Planting density in the low-input CPS was reduced from 14.25 to 12.75 plants per square meter.

2.3.1.5. Infected plant management

Under the low-input CPS, any lettuce showing pathogen damage rendering the plant unmarketable was carefully removed from the plot to avoid inoculum dissemination.

2.3.2. *B. lactucae* management

2.3.2.1. Fungicide applications

All fungicides were applied with a spray boom, following a calendar-based program. In plots cultivated under conventional CPS, Infinito® (Bayer CropScience) at 1.6 l ha^{-1} and Sygan® (Dupont) at 2.5 kg ha^{-1} were each applied twice against *B. lactucae* 8, 30 and 20, 40 days (± 2 days) after planting, respectively. In plots cultivated under intermediate and low-input CPSs, the number of fungicide applications was reduced: Infinito® at 1.6 l ha^{-1} and Sygan® at 2.5 kg ha^{-1} were applied 10 and 32 days (±2 days) after planting, respectively.

2.3.2.2. Resistance inducer

Under the intermediate and low-input CPSs, fungicide treatments were supplemented by applications of a plant resistance inducer (Potassium phosphite – LBG 01F34®, at 2 l ha^{-1}) applied by spraying 22 and 44 days (± 2 days) after planting.

2.3.3. Basal rot management

2.3.3.1. Biological control

The biocontrol fungus *Coniothyrium minitans* (Contans®) was applied once, before the first lettuce crop in october, at 2 kg ha^{-1} on the soil surface of each CPS to limit the development of *S. sclerotiorum* propagules. A second application was made before planting the monitored crop under the intermediate and low-input CPSs. Additionally, under the low-input CPS, the biocontrol fungus *Trichoderma harzianum* (strain T22 – Trianum P®) was applied twice on the balls, at the cotyledon stage (at 1.5 g m^{-2}) and just prior to planting (at 1 kg per 8,500 plants) for broad spectrum control of basal rot pathogens. The presence of *Trichoderma* spp. on lettuce roots was assessed 30 days after planting.

2.3.3.2. Fungicide applications

On plots cultivated under conventional CPS, Signum® (BASF Agro) at 1.5 kg ha^{-1}, Switch® (Syngenta) at 0.6 kg ha^{-1} and Rovral® (BASF Agro) at 1 kg ha^{-1} were applied 8, 20 and 30 days (± 2 days) after planting, respectively. In plots cultivated under intermediate and low-input CPSs, the number of fungicide applications was reduced. Signum® (BASF Agro) at 1.5 kg ha^{-1} and Switch® (Syngenta) at 0.6 kg ha^{-1} were applied on plots cultivated under the intermediate CPS 10 and 32 days (± 2 days) after planting, respectively. Under the low-input CPS, only Signum® (BASF Agro) at 1.5 kg ha^{-1} was applied 20 days (±2 days) after planting.

2.3.4. Aphid management

2.3.4.1. Biological control of aphids

Mixtures of parasitoid species of aphids (Basilprotect®) including *Aphidius colemani*, *Aphidius ervi*, *Aphidius matricariae*, *Aphelinus abdominalis*, *Aphedrus cerasicola* and *Praon volucre* were preventively introduced at the center of each plot cultivated under the intermediate and low-input CPSs at a rate of 1.2 individuals per m² every two weeks.

2.3.4.2. Insecticide application

Under the conventional CPS, Movento® (Bayer Cropscience) at 0.75 l ha^{-1} or Supreme® (Certis) at 0.25 kg ha^{-1} were applied every seven days as soon as aphids were spotted in the production area. Under the intermediate and low-input CPSs, insecticide treatments were triggered when the number of aphids reached species-dependent thresholds. The count was made every week on 40 randomly selected lettuces per plot. The thresholds for *N. ribisnigri* were 2% and 10% of lettuce plants infested in plots under intermediate and low-input CPSs, respectively. For other aphid species, they were 5% and 15%, respectively. Under these CPSs, pirimicarb (Pirimor G®, Syngenta) at 0.25 kg ha^{-1} was preferred to spirotetramat (Movento®) for its potential curative effect.

2.4. Performance assessment

2.4.1. Pesticide use

The Treatment Frequency Index (TFI) (Gravesen, 2003) was used to assess the amount of pesticide applied under each CPS. TFI accounts for the number of compounds, the number of treatments and the rate applied per unit area, and is calculated as follows:

$$\mathrm{TFI}_{\mathrm{CPS}} = \sum \frac{\text{Applied rate x area treated}}{\text{Authorized minimal dose x plot area}}$$

2.4.2. Monitoring of pests and pathogens

In France, pest and pathogen monitoring networks have been created since the implementation of a national plan (Ecophyto) to reduce pesticide use. They aim to assess the epidemiological risks and to broadcast agricultural warning reports for each crop in each region. Regional agricultural warning reports for lettuce, broadcast in the PACA and Languedoc Roussillon regions about every 15 days, were used in this study to determine timing of the preventive insecticide applications in the plots cultivated under the conventional CPS and to appraise pest and pathogen pressure during the experiments.

The presence of pests and pathogens in the experimental plots was assessed weekly on 40 plants selected at random at each date; 60 plants per plot were collected at harvest. The percentage of lettuces exhibiting damage was recorded.

The damage caused by each pest or pathogen was scored from 1 to 3 (Table 3). A score of 1 at the time of harvest indicates no effect on sale; a score of 2 affects lettuce quality without preventing sale, and with a score of 3 a lettuce is unsaleable.

2.4.3. Lettuce yield and quality

At harvest, 60 lettuces from each CPS were randomly sampled and used to assess production performance. The percentage of marketable lettuces (%ML), average lettuce fresh weight (LW, g), marketable weight (after the removal of unmarketable basal leaves, MLW, g), and the percentage of unmarketable basal leaves were measured. Gross yield (t fw.ha^{-1}) was calculated as:

$$\text{Gross yield} = \text{LW x planting density / 100}$$

Marketable yield (t fw.ha^{-1}) was calculated as:

$$\text{Marketable yield} = \text{MLW} \times (\%\text{ML}) \times \text{planting density} / 100$$

Ten marketable lettuces were randomly harvested from each plot for visual quality assessment based on plant appearance and absence of foreign bodies. The ratings were 0 (very poor quality), 1 (poor quality), 2 (middling quality), 3 (good quality), 4 (very good quality), 5 (excellent quality).

2.5. Data analysis

2.5.1. Analysis of CPS efficacy

The incidence of damage in a cultivated plot reflects the efficacy of the CPS, the presence of pests and pathogens and of favorable conditions for their development. To analyze the efficacy of each CPS, the structure of the damage caused by each pest or pathogen was studied. Six types of structures might be recorded (Table 4). Those structures were used to organize the 'Results' section and guide the discussion. Damage structure in each experimental plot, together with several hypotheses about pest or pathogen pressure, enabled us to draw conclusions about the efficacy of each CPS. We checked whether CPSs were efficient under all experimental conditions, or only under some conditions, or yet again simply inefficient. In the absence of pest pressure, no conclusions were drawn about CPS efficacy.

2.5.2. Statistical analyses

Considering the small size of the samples (18 values per variable), non-parametric Kruskal-Wallis rank sum tests (with a significance threshold of 0.1) were used to analyze the effect of CPS on each performance indicator. To test for the effect of CPSs on the incidence of a particular pest or pathogen, only the locations where the pest/pathogen was seen were kept for the Kruskal-Wallis rank sum test. Rank-based multiple comparison tests (De Mendiburu, 2014) were used when significant differences among CPSs, locations or winters were found. All statistical analyses were performed using R software.

3. Results

3.1. Pesticide reduction

The TFI differed significantly among CPSs (P= 0.002794) (Figure 1). On average, pesticides were reduced by 31.8% under the intermediate CPS and by 47.7% under the low-input CPS. Fungicides were reduced by 43.1% and 56.7% under the intermediate and low-input CPSs, respectively, while insecticides were reduced by 55.3% and 78.0%. Lower pesticide use was partly counter-balanced by resistance inducers, which are accounted for in TFI, and resulted in a mean increase of 1.14. No significant effect of location or year was found.

3.2. Efficacy of pest and pathogen management

3.2.1. Presence of damage in some locations, under some CPSs: Aphids

Aphids were found in 8 of the 18 experimental plots. Two species were seen: *N. ribisnigri* and *M. persicae*. Lettuces under the low-input CPS were significantly more infested than those under the intermediate and conventional CPSs (P= 0.053) (Table 5). No significant difference was found between winters or locations. Aphids caused 0, 0.9% and 2.7% of lettuces to become unmarketable at harvest in the conventional, intermediate and low-input CPS plots, respectively.

Population dynamics in the most infested plots L1-W1 and L3-W2 are reported in Figure 2. Some of the variability among sampling dates might be explained by an uneven dispersal of aphids in the fields. Nevertheless, some patterns can be distinguished. In L1-W1, the field interventions were identical in the plot cultivated under the low-input and intermediate CPSs. They consisted of preventive introductions of parasitoids, without any insecticide application. Under the conventional CPS, spirotetramat at 0.75 l ha^{-1} was applied preventively. This single application failed to provide complete control, since aphids were observed on 5% of the plants at harvest. Despite identical management under the low-input and intermediate CPSs, the percentage of lettuces infested by *M. percisae* was greater under the low-input CPS, especially at harvest (Figure 2(a)). However, severity was generally higher under the intermediate CPS, which might indicate that aphids were less mobile under that strategy. Although density was lower under the low-input CPS, the cultivar had higher growth rates, and there was more contact between leaves of adjacent plants at the end of growth, which might have favored dispersal. Under the low-input CPS, 7.8% of aphids were parasitized at harvest, while no mummified aphids were found in the plot cultivated under intermediate CPS.

N. ribisnigri was seen only in L3 during W2, and only under the intermediate and low-input CPSs (Figure 2(b)). Under the conventional CPS, three insecticides (spirotetramat on February 14th and March 7th and acetamiprid on March 14th) were applied. Under the alternative CPSs, parasitoids were introduced preventively, but the threshold for *N. ribisnigri* infestation was reached on three sampling dates under the intermediate CPS plots, and on one sampling date under the low-input CPS plots. As a result, pesticides were applied curatively each time (pirimicarb on February 14th and March 7th, and acetamiprid on March 14th under the intermediate CPS, acetamiprid on March 20th under the low-input CPS). Neither of these chemical strategies succeeded in controlling the aphids. Similarly to L1-W1, severity was higher in the intermediate CPS plots, while the percentage of infested lettuce plants increased faster in the low-input CPS plots. 3.6% and 15.4% of aphids were parasitized at harvest under the intermediate and low-input CPSs, respectively.

3.2.3. Presence of damage under all CPSs in some locations

3.2.1.1. Basal rot

Basal rot symptoms were noted under all CPSs at some locations during at least one of the two winters (Table 6). However, damage was limited: fungal rots usually developed on the lettuce collar and/or the oldest leaves, and were removed at harvest. On average, 92% of the lettuce plants with basal rot symptoms were marketable. Significant differences in basal rot incidence were noted among locations (P= 0.070) and between winters (P= 0.005), but not among CPSs (P= 0.3379). None of the CPS, location or winter modalities had a significant effect on basal rot severity.

Infected and healthy lettuce weights were compared at harvest in plots where basal rot incidence was over 15% (5 plots). The weight of the infected lettuce plants was significantly higher than the weight of the healthy lettuce plants in three of them (data not shown), indicating that bigger lettuce plants might be more affected by basal rot than smaller ones.

3.2.1.2. Slugs and snails

CPS did not have a significant impact on the incidence of slugs and snails, or on damage severity (P= 0.7605). However, a significant effect of location was found (P= 0.055). Slugs and snails were particularly numerous in L2 during W1 and in L3 (Table 7). They caused major losses, since on average 19.9% of the lettuces they attacked were unmarketable.

3.2.2. Overall pest and pathogen damage

In summary, pest or disease damage was noted under all CPSs (Figure 3(a)). Overall pest and pathogen incidence differed among CPSs; however, these differences were only significant between the low-input and intermediate CPSs (P= 0.07783). The conventional CPS showed intermediate pressure, and did provide more effective protection than the low-pesticide treatments. Overall pest and disease incidence also varied sharply among locations (P= 0.02606) (Figure 3(b)).

3.2.3. Unobserved damage

Several pests and pathogens with frequent occurrence in winter lettuce crops were not observed in this study. This is the case of *B. lactucae* and viruses transmitted by *O. brassicae*. However, *B. lactucae* was mentioned in 2/5 and 3/4 of the agricultural warning reports during W1 in the PACA and Languedoc Roussillon regions, respectively, and in half of the warning reports during W2 in both regions. Viruses transmitted by *O. brassicae* were reported only in the PACA region and in 1/5 of the reports during winters 1 and 2.

3.2.4. Other damage

Frost and tip burn (marginal necrosis due to calcium deficiency favored by incorrect water supply) damage were also repeatedly noted. Abiotic damage accounted for 64%, 41% and 26% of total damage in L1, L2 and L3, respectively. Tip burn incidence was influenced by CPS (P= 0.03177), while frost damage depended more on location (P= 0.0345). As a whole, these abiotic forms of damage caused 9% of commercial loss, i.e. more than the 5% of loss caused by pests and pathogens.

3.3. Yield and lettuce quality

The percentage of marketable lettuce plants, marketable lettuce weight, gross yield and marketable yield were not significantly influenced by CPS or by location (Table 8). Average marketable yield was 37.40 t ha^{-1} (± 12.68). The percentage of unmarketable basal leaves at harvest differed among locations ($P = 0.06477$), but not among CPSs. However, the CPS affected the quality rating ($P = 0.01131$) (Table 8). The lettuce plants with the best appearance were harvested from plots cultivated under the intermediate CPS, while the lowest visual quality was noted in the conventional CPS plots (Table 8).

4. Discussion

This study was designed to compare the performances of three lettuce crop protection strategies (CPSs) relying on different levels of chemical control. The main aim was to assess the range of responses under varied conditions (i.e. soil, climate, pest and pathogen pressure), in terms of efficacy of the CPS and of product quality and yield, and thus to assess the risk related to lettuce production with fewer pesticides. Several studies already used systemic approaches to assess this risk, mainly on cereal-based cropping systems, but to our knowledge this is the first to focus on a leafy vegetable with high esthetic requirements. The three CPSs were repeated six times (in three locations, over two winters). We demonstrated that, except for aphids, the pest-and-pathogen complex can be managed with less pesticides. Moreover, average yield and product quality were equal or higher in the CPSs with reduced pesticide applications. The higher proportion of marketable lettuce plants under the low-input CPS partially offset the lower planting density. This explains why its overall yield was similar to yields from the other two CPSs. Therefore the prototyping of coherent cropping systems with low pesticides is a feasible option with lettuce, and possibly with other leafy vegetable production systems. However, additional experiments in contexts of high pest and disease pressure are necessary to strengthen the conclusions suggested by our results. The risk of protection failure, and subsequent lower marketable yields, is a major issue in lettuce and other vegetable cropping systems. As regards aphids, basal rot, slugs and snails, but also abiotic damage, we recorded strong variability among locations and years. Contextual decision rules make it possible to adapt to these variable biotic pressures. Such adaptive strategies are required to give up preventive chemical control. However, new tools, notably epidemiological models for pathogens, are necessary to reinforce crop protection strategies with low pesticide applications.

Although the systemic experiments performed here were not conceived to thoroughly understand the complexity of the interactions between techniques, environment and pests and pathogens, but to assess the risk to produce lettuce with fewer pesticides, some conclusions on management techniques or sets of management techniques can be drawn.

Some lettuce pests were seen only in some locations and under some CPSs. For instance, aphids were seen in 44% of the plots. Aphid damage was the only biotic pressure significantly affected by CPS. However, aphids were observed in every CPS, so none of the strategies was completely effective. Under the conventional CPS, preventive insecticide applications when aphids were seen in the production area resulted in no or few aphids at harvest both for *N. ribisnigri* and *M. persicae*, so chemical control was moderately efficient. In L1-W1, the preventive introductions of parasitoids in plots cultivated under low-input and intermediate CPSs helped to maintain *M. persicae* populations below the treatment thresholds. Under the low-input CPS, a higher proportion of lettuce plants contained aphids, while the number of aphids per plant was higher under the intermediate CPS. This difference may have been mediated by the plants, as several studies indeed show different levels of susceptibility to aphids among lettuce cultivars (Dunn and Kempton, 1980; Lu et al., 2011). Furthermore, studies on *Brassica sp.* show that aphid populations increase with narrower plant spacing (Sarwar, 2008; Yamamura, 1999). In our work, planting density was greater but lettuce spacing was wider under the intermediate CPS because the cultivar displayed a moderate growth rate as compared to the cultivar of the low-input CPS. Wider lettuce spacing may have impeded aphid spreading, leading to fewer lettuce plants attacked, but to a higher number of aphids per plant.

It is commonly thought that the industrial requirements for visual quality and the absence of foreign bodies (Palumbo and Castle, 2009) cannot go together with the introduction of biological control agents in lettuce crops (Palumbo and Castle, 2009). Yet, this study shows that preventive introduction of parasitoids can help to maintain *M. persicae* populations below the threshold for chemical treatment in certain situations (5% and 15% of lettuce plants infested under the intermediate and low-input CPSs, respectively), and provide high-quality products. However, in the case of *N. ribisnigri* invasions (as in L3-W2), the preventive introduction of parasitoids failed to maintain populations below the treatment thresholds of 2% and 10% of lettuce plants infested under the intermediate and low-input CPSs, respectively. This aphid develops preferentially in the lettuce heart (Liu, 2004), which may hinder its detection by parasitoids. It is not susceptible to contact insecticides (Liu, 2004; Mackenzie and Vernon, 1988), so we used systemic insecticides . Their preventive application under the conventional CPS ensured good protection. However, the curative efficacy of the active substance chosen under the intermediate CPS was not as good, since three insecticide applications were not sufficient to suppress aphids totally. Moreover, the resistance conferred by the Nr gene did not confer protection, suggesting that biotype Nr:1 aphids were present. Some Nr:1 populations can reproduce fast in cultivars bearing the Nr gene, while other populations have a reduced reproduction rate (ten Broeke et al., 2013). Thus, although the greatest *N. ribisnigri* infestation noted under the low-input CPS in L3-W2 was probably due to the reduced number of chemical treatments we cannot rule out an effect of the cultivar. Anyhow, the relative failure of either strategy suggests that lower treatment thresholds might be required for *N. ribisnigri*. A more stringent threshold was already defined by Morales et al. (Morales et al., 2013) for *N. ribisnigri*, i.e. 0.06 and 0.07 aphids per lettuce for field-grown lettuces in central Spain in spring and autumn, respectively. Those thresholds could be tested for winter lettuce grown under shelter.

Basal rot was recorded under all CPSs in some locations. The three strategies were equivalent in terms of damage, although they relied on different control techniques. Only a few alternative techniques were added under the intermediate CPS (a biological control agent and a semi-upright lettuce habit) to reduce fungicide applications by one third. Lettuce weight sometimes influenced on basal rot incidence. This could partly reflect the effect of the microclimate under each lettuce on basal rot incidence. The slower growth rate and upright lettuce habit under the intermediate CPS may have reduced the humidity level below the plants and limited basal rot development. Under the low-input CPS, we used several techniques known topartially limit the damage caused by basal rot (biological control agents, sanitation, reduced N fertilization, cultivars with reduced susceptibility to *S. sclerotiorum*, reduced planting density and, in one case, drip irrigation). We used them to reduce the number of chemical treatments against basal rot by two-thirds. All of them had had a partial effect on at least one of the causal agents of basal rot in factorial experiments (Chitrampalam et al., 2008; Dow et al., 1988; Lecompte et al., 2013). The field experiments presented here enabled us to assess CPS efficacy but did not allow us to fully understand potential synergy or antagonism among techniques. The techniques that were most likely to interact with one another were those acting at the same time in the pathogen's life cycle. For example, we do not know how N fertilization may impact the level of resistance to *Sclerotinia sclerotiorum* noted in the cultivar used under the low-input CPS. Moreover, long-term effects or improved efficacy of some alternative techniques after several seasons of application may be expected. We assessed the incidence and severity of basal rot over two winters, which is not long enough to evaluate such effects.

Some of the main winter lettuce pathogens were not found in this study. The high pressure of *B. lactucae* reported in the production area during winters 2012-2013 and 2013-2014 suggests that all three CPSs may have provided efficient protection against this pathogen. Genetic resistance to *B. lactucae* relies on specific gene-for-gene interactions. Two of our cultivars did not cover the whole range of downy mildew races. Moreover, resistance breakdowns are frequently reported; as a consequence, genetic control of *B. lactucae* is not self-sufficient. Additional techniques, such as fungicide applications, are usually used to ensure the sustainability of genetic resistance and the efficacy of the *B. lactucae* control (Crute, 1992). Under the intermediate and low-input protection strategies against *B. lactucae,* preventive fungicide applications were partly replaced by plant resistance inducers. The combination of genetic resistance, reduced fungicide applications and resistance inducers might be an effective strategy to manage *B. lactucae*.

According to these first results, some improvements of the CPSs can be suggested, such as using a lower treatment threshold for *N. ribisnigri*. As close plant spacing appeared to favor both the development of basal rot and aphids, we also suggest planting a compact lettuce variety, *i.e.* small but heavy to maintain yield. Moreover, in this study, much of the damage was due to abiotic stresses such as frost and tip burn. The alternative CPS design was focused on potential biotic damage. One way to improve the CPS would be to take into account potential abiotic damage. For example, varietal choice should include tip burn resistance, especially when drip irrigation is used. In general, interactions between variety and cultural practices may influence lettuce quality, number of pesticide residues, lettuce weight, etc. Perhaps because of the rapid turnover of lettuce varieties, this information is seldom available, and therefore it is critical to pay particular attention to variety/practice interactions during the design process.

In conclusion, despite the stringent market requirements in terms of visual quality and the need to maintain yield, pesticides were reduced by half under the low-input CPS. This reduction is consistent with those obtained on other low-input cropping systems designed by prototyping (Clark et al., 1998; Simon et al., 2011). Marketable yield was not affected by CPS. The prototyping method makes it possible to design crop protection strategies that are compatible with market expectations and production constraints. The possible environmental gains of intermediate and low-input CPSs could be significant and should be assessed together with socio-economic performance to encourage the adoption of alternative strategies by lettuce growers.

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Table 1. Crop rotation, planting and harvesting dates in the three experimental sites L1, L2 and L3. L: lettuce; bs: bare soil; S: solarization; M: melon. The lettuce crops studied in each site are underlined.

	L1	**L2**	**L3**
Rotation	S-L-L-M-S-L-L-M	S-L-L-L-S-L-L-bs	bs-L-L-S-bs-L-L
Planting date	12/10/2012 ; 12/05/2013	12/19/2012 ; 10/29/2013	01/07/2013 ; 01/15/2014
Harvesting date	02/26/2013 ; 02/24/2014	03/05/2013 ; 01/21/2014	03/18/2013 ; 03/28/2014

Table 2. Description of the techniques used under each crop protection strategy (CPS)

Technique	Conventional CPS	Intermediate CPS	Low-input CPS
Fungicide applications	Calendar-based	Calendar-based, including resistance inducers	Calendar-based, including resistance inducers
Insecticide applications	Regional alert	Presence in plots, with threshold	Presence in plots, with threshold
N fertilization	100 < soil N content < 120 kg.ha^{-1}	100 < soil N content < 120 kg.ha^{-1}	Reduced, split applications
Irrigation	Sprinkler	Sprinkler	Sprinkler or drip
Planting density.m^{2}	14.25	14.25	12.75
Genotype	Notilia	Ostralie	Lasydo
Biological control of pathogens	*C. minitans*	*C. minitans*	*C. minitans* and *T. harzianum*
Biological control of aphids	No	Parasitoïd mix	Parasitoïd mix
Infected plant management	No	No	Removal

Table 3. Scoring for damage caused by lettuce pathogens and pests and by growing conditions.

Pest or pathogen	1: no effect	2: effect on quality	3 : unmarketable lettuce
Aphids	< 5	5 < aphids < 9	> 10
Basal rot agents	Collar mark	Symptoms on 1 or 2 basal leaves	More symptoms
B. lactucae	/	1 to 2 lesions	> 2 lesions
Slugs	Slight damage on basal leaves	Severe damage on basal leaves	More damage
Damage with abiotic cause (Tip burn, frost, etc.)	Bursting of central ribs of 1 or 2 basal leaves	Symptoms on basal leaves	Symptoms on other leaves

Table 4. Six possible types of damage structure in the experimental plots implemented in three locations (L1, L2 and L3) over two winters (W1 and W2). CIL refers to conventional, intermediate and low-input CPSs. In the examples, underlined letters indicate plots with damage.

DAMAGE STRUCTURE		EXAMPLE		
All CPSs with damage at all locations			W1	W2
		L1	$\underline{\text{C I L}}$	$\underline{\text{C I L}}$
		L2	$\underline{\text{C I L}}$	$\underline{\text{C I L}}$
		L3	$\underline{\text{C I L}}$	$\underline{\text{C I L}}$
Some CPSs with damage at some locations	Some CPSs damage-free		W1	W2
		L1	$\underline{\text{C I}}$ L	C I L
		L2	C I L	C $\underline{\text{I}}$ L
		L3	C $\underline{\text{I}}$ L	C I L
	All CPSs with damage at least at one location		W1	W2
		L1	$\underline{\text{C I}}$ L	C I L
		L2	C I $\underline{\text{L}}$	C $\underline{\text{I}}$ L
		L3	C $\underline{\text{I}}$ L	C I $\underline{\text{L}}$
All CPSs with damage at some locations			W1	W2
		L1	$\underline{\text{C I L}}$	C I L
		L2	C I L	C I L
		L3	$\underline{\text{C I L}}$	$\underline{\text{C I L}}$
Some CPSs with damage at all locations			W1	W2
		L1	C $\underline{\text{I}}$ L	C $\underline{\text{I}}$ L
		L2	C $\underline{\text{I}}$ L	C $\underline{\text{I}}$ L
		L3	C $\underline{\text{I}}$ L	C $\underline{\text{I}}$ L
All CPSs damage-free			W1	W2
		L1	C I L	C I L
		L2	C I L	C I L
		L3	C I L	C I L

Table 5. Percentages of lettuce plants infested by aphids at harvest under the three CPSs and three locations (L) over the two winters (W). Superscripts represent the aphid species, Mp: *Myzus percicae*, Nr: *Nasonovia ribisnigri*. In the “average” column, letters indicate significant differences identified by Kruskal – Wallis tests.

CPS	L1-W1	L1-W2	L2-W1	L2-W2	L3-W1	L3-W2	Average
Conventional	5.0^{Mp}	0	0	0	0	0	0.83 a
Intermediate	0	0	1.7^{Mp}	0	0	6.3^{Nr}	1.33 a
Low-input	26.6^{Mp}	1.7^{Mp}	8.3^{Mp}	0	0	15.0^{Nr}	9.25 b

Table 6. Percentages of lettuce plants showing basal rot symptoms at harvest under the three CPSs and three locations (L) over two winters (W).

CPS	L1-W1	L1-W2	L2-W1	L2-W2	L3-W1	L3-W2
Conventional	1.7	21.7	3.3	45.0	0	18.3
Intermediate	0	3.3	1.7	26.7	0	0
Low-input	0	3.3	6.7	73.3	0	11.7

Table 7. Percentages of lettuce plants showing slug or snail damage at harvest in plots cultivated under the three different CPSs and three locations (L) over two winters (W).

CPS	L1-W1	L1-W2	L2-W1	L2-W2	L3-W1	L3-W2
Conventional	0	6.7	30.0	0	11.7	21.7
Intermediate	0	1.7	28.3	0	10.0	17.5
Low-input	0	6.7	65.0	1.7	23.3	20.0

Table 8. Marketable lettuce weight (MLW), percentage of marketable lettuce (ML), marketable yield and quality rating of lettuce plants cultivated under conventional, intermediate and low-input CPSs. P-values are the result of Kruskal- Wallis rank sum tests.

	MLW (g)	ML (%)	Marketable yield ($t.ha^{-1}$)	Quality rating
Conventional	362	71.4	36.34	1.65 a
Intermediate	336	82.9	39.88	2.80 b
Low-input	359	78.3	35.99	2.00 a
P- value	ns	ns	ns	< 0.05

Figure 1. Plant resistance inducer, insecticide and fungicide contributions to the Treatment Frequency Index (TFI) under the different crop protection strategies. Results are given as the means of 3 locations and 2 years. Different letters indicate significantly different values in the multiple comparison tests.

[1-column fitting image]

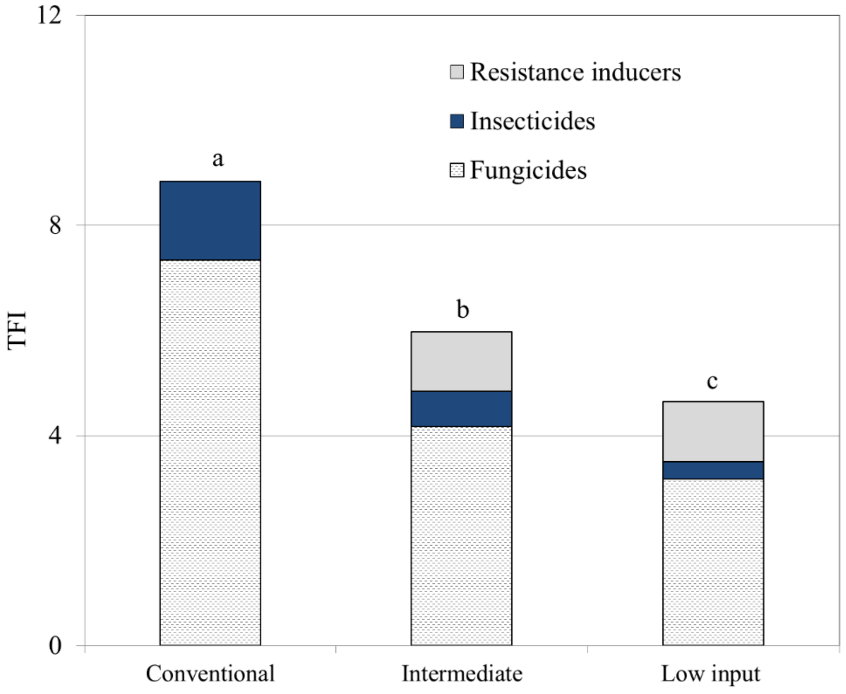


Figure 2. Dynamics of the percentage of lettuce plants infested by *M. persicae* in L1-W1 (a) and *N. ribisnigri* in L3-W2 (b) in plots cultivated under conventional, intermediate or low-input CPSs. Numbers next to symbols indicate mean severity scores. ***[2-column fitting image]***

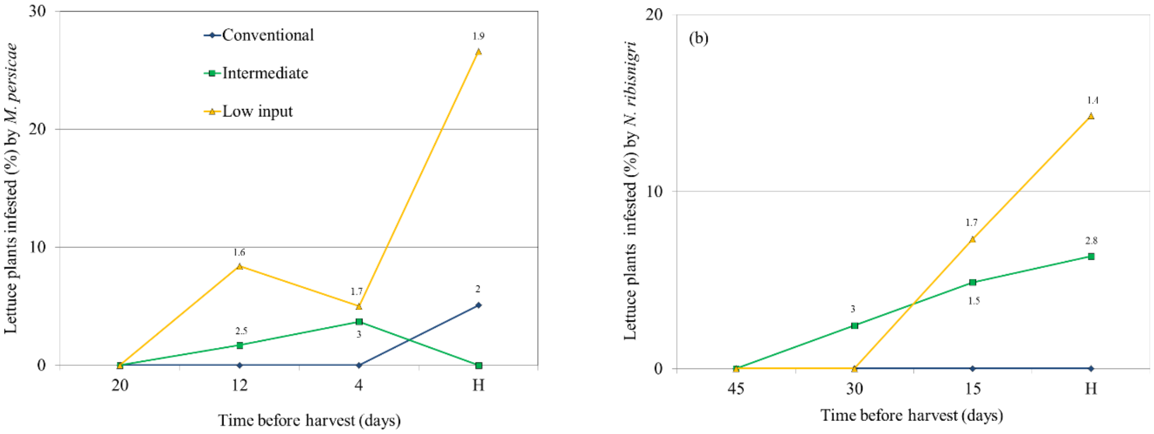
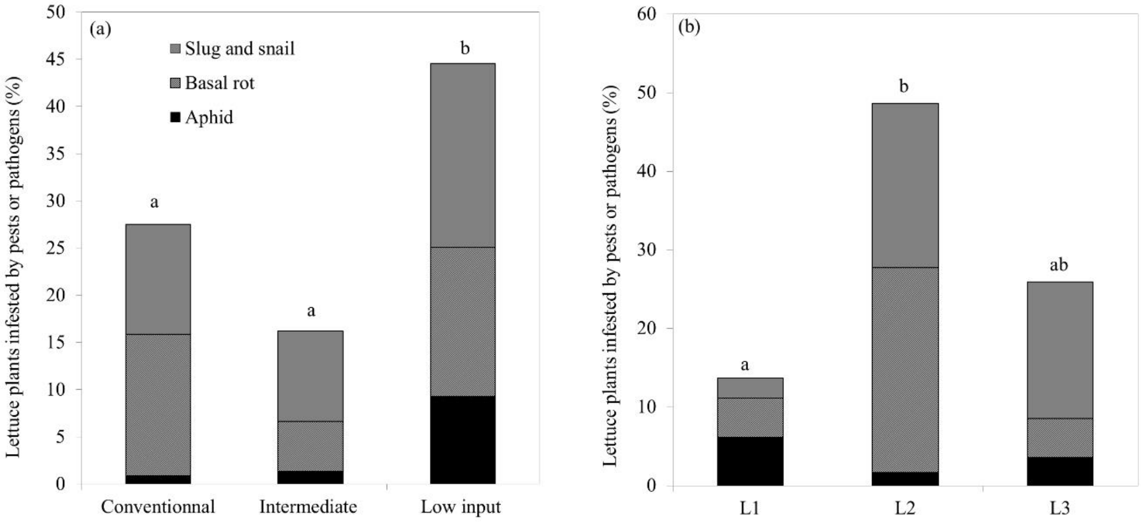


Figure 3. Percentages of lettuce plants (%) attacked by aphids, basal rot causal agents or slugs and snails under conventional, intermediate or low-input CPSs (a), and in locations L1, L2 and L3 (b). Letters indicate the results of Kruskal – Wallis tests. ***[2-column fitting image]***



714