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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

76 and by how much is it possible to reduce the use of pesticides in conventional lettuce crops without

77 affecting lettuce quality or yield.

78 Many pests and pathogens can threaten lettuce crops, such as biotrophic or necrotrophic, soil- or air-

79 borne fungi, viruses, bacteria as well as aphids, moths, slugs, thrips, etc. The incidence and severity of

80 each pest and pathogen depends on growing conditions (crop type, cultivation under shelter or in open

81 fields, season) and changes over the year. In winter lettuce crops under shelter, pathogens are

82 predominant. The most important is probably *Bremia lactucae* (Regel), the causal agent of lettuce downy

83 mildew, because of its rapid and devastating spread in the field. The pathogen may attack the plant

84 throughout its life. The primary inoculum typically consists of airborne sporangia from diseased plants

85 of the genus *Lactuca* located close to the crop, or of mycelia present on plant debris in the soil (Crute,

86 1992). *Sclerotinia sclerotiorum* (de Bary), *Sclerotinia minor* (Jagger), *Botrytis cinerea* (Pers.),

87 *Rhizoctonia solani* (Kühn), and *Pythium tracheiphilum* (Matta) are other important fungal pathogens of

88 winter lettuce crops. Collectively, these pathogens cause symptoms of basal rot, i.e. rotting of the leaves

89 in contact with the soil surface (Van Beneden et al., 2009). *S. minor* and *S. sclerotiorum* are of major

90 concern for the cultivation of lettuce because they may affect a wide range of plant species and their

91 sclerotia may remain latent in the soil for more than 8 years (Bolton et al., 2006; Melzer et al., 1997).

92 Moreover, sclerotia are often buried and dispersed by tillage (Subbarao et al., 1996). Therefore, basal

93 rot causes long-term problems in conventional lettuce crops since sclerotia are taken back up to the soil

94 surface at each tillage. *B. cinerea and R. solani* can also cause significant damage depending on the

95 growing season. *B. cinerea* injury to lettuce leaves is enhanced by cool and moist conditions, while *R.*

96 *solani* sclerotia and mycelia are most frequently found in the soil in summer (Van Beneden et al., 2009).

97 The fungus *Olpidium virulentus* is not a direct threat to lettuce, but a vector of two lettuce viruses that

98 can cause significant damage, especially in winter: 'Mirafiori lettuce virus', responsible for big vein

99 disease; and 'Lettuce big-vein associated virus', suspected to be the agent of ring necrosis (Lot et al.,

100 2002; Maccarone, 2013; Verbeek et al., 2013). The resting spores of *O. virulentus* can persist in the soil

101 for many years, and viruliferous zoospores that infect lettuces are very mobile, so management of the

102 disease is complex (Campbell, 1985; Maccarone, 2013). Several aphid species can proliferate in winter

103   lettuce crops such as *Nasonovia ribisnigri* (Mosley), *Myzus persicae* (Sulzer), *Aulacorthum solani*

104   (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), and *Hyperomyzus lactucae* (L.). *N. ribisnigri* is the

105   most damaging one because it develops preferentially in the lettuce heart (Liu, 2004). In addition to

106   feeding damage and the loss of product quality due to their presence when the lettuce is marketed, aphids

107   are also vectors of viruses, such as the lettuce mosaic virus. Finally, slugs (*Deroceras sp.* and *Arion sp*.)

108   and snails can also cause feeding damage to lettuce in winter.

109   In conventional lettuce crops in the Mediterranean region, eight to ten pesticides are applied on average

110   to manage pests and pathogens during the 60- to 90-day-long crop cycle. In winter, these are mainly

111   fungicides. Due to the long time required before harvest for the elimination of active ingredients by

112   lettuce and the lack of curative efficacy of pesticides for some pathogens such as *Bremia lactucae*,

113   pesticides are almost exclusively applied preventively. Several alternative techniques with a partial

114   effect on diseases and pests are currently available and might be combined to design innovative lettuce

115   cropping systems less dependent on pesticides (Barriere et al., 2014). These alternative techniques are

116   generally preventive and have only a partial effect on pests and diseases. They can act at different times

117   in the pest and pathogen cycle: they can i) limit and reduce primary inoculum sources, ii) limit the

118   development of pests and pathogens through the modification of the abiotic environment or iii) increase

119   plant defenses, and iv) have a curative action. Techniques that reduce primary inoculum in lettuce crops

120   are sanitation methods such as the removal of infected plants, solarization, or some biological control

121   agents such as *Coniothyrium minitans* and *Trichoderma harzianum. C. minitans* is an efficient

122   biocontrol agent against *Sclerotinia sclerotiorum* (Chitrampalam et al., 2008). This fungus preferentially

123   parasitizes overwintering structures by synthesizing chitinases, glucanases, and antifungal metabolites

124   (Zeng et al., 2012). *T. harzianum* also disturbs populations of *B. cinerea*, *Pythium spp*., *R. solani*, and

125   *Sclerotinia spp*. in the soil by acting either as a competitor or as a parasite (Elad, 2000; Harman, 2006;

126   Howell, 2003; Vinale et al., 2008). Another way to protect lettuce crops is to limit the development of

127   pests and pathogens through the modification of the abiotic environment. Indeed, the germination of

128   infectious forms (spores or sclerotia) of numerous pathogenic fungi depends on climatic factors such as

129   humidity and temperature. Drip irrigation, as opposed to sprinkler irrigation which is widespread in

130   southern France, may reduce leaf wetness duration, which is an important factor for the germination of

131   *B. lactucae* sporangia (Scherm and Bruggen, 1994) and *B. cinerea* spores (Elad and Shtienberg, 1995).

132   Lower plant density can also reduce foliage wetness due to better aeration, and limit soil-borne disease

133   development by affecting the microclimate under lettuces. Alternative techniques can also modify plant

134   susceptibility to pests and pathogens. Genetic resistance, resistance inducers and nitrogen fertilization

135   have shown a partial effect on lettuce diseases and pests. Lettuce varieties with major resistance genes

136   against *B. lactucae* are available and widely used. However, information about resistance is only

137   provided by seed companies for the most common and widespread isolates (identified and denominated

138   by The 'International Bremia Evaluation Board'- IBEB-). *B. lactucae* can rapidly develop new virulent

139   isolates. Therefore resistance breakdown is common and leads to a rapid turnover of lettuce varieties

140   (Michelmore and Wong, 2008). Thirty-two races of *B. lactucae* are currently registered by the IBEB.

141   Complete resistance to the aphid *N. ribisnigri*, and partial resistance to *Myzus persicae*, are conferred

142   by a dominant gene called Nr, which has been introduced in many European cultivars (Cid et al., 2012;

143   Liu and McCreight, 2006). However, this resistance was recently bypassed by a new *N. ribisnigri*

144   biotype named Nr:1 (ten Broeke et al., 2013). Apart from genes conferring complete resistance, different

145   susceptibility levels of lettuce accessions to *S. sclerotiorum* have been reported (Elia and Piglionica,

146   1964; Grube and Ryder, 2004; Hayes et al., 2010). In addition to genetic resistance, some techniques

147   can strengthen plant defense. Several compounds, such as β-amino butyric acid or potassium phosphite

148   (K_2HPO_3), have been identified as resistance inducers of lettuce against *B. lactucae* (Pajot et al., 2001).

149   Some of them, such as potassium phosphite, also have a direct biocide effect on oomycetes (Massoud

150   et al., 2012). Fertilization can affect plant-pathogen and plant-pest interactions. The nitrogen content of

151   lettuce leaves is positively correlated to damage by *B. cinerea* and *S. sclerotiorum* (Lecompte et al.,

152   2013). Leaf nitrogen content is also positively correlated to lettuce palatability for slugs (Pakarinen et

153   al., 1990). So low nitrogen applications could help to lower lettuce susceptibility to pests and pathogens.

154   The efficacy of each alternative technique to pesticides is usually assessed separately, as a stand-alone

155   technique. Very few studies have investigated the effect of a coherent combination of alternative

156   techniques on the control of the lettuce pest/pathogen complex (Collange et al., 2014). In this case, it is

157   not a technique by itself that is evaluated for its performance, but a cropping system as a whole.

158   Prototyping consists in designing, implementing and evaluating innovative cropping systems, and

159   allows for theoretical constructs to be applied to production constraints (Lancon et al., 2007; Vereijken,

160   1997). In order to adapt to varying local factors, techniques are described as a set of contingent decision

161   rules that govern practices according to biotic and abiotic constraints (Debaeke et al., 2009; Papy, 2001).

162   Field tests are necessary to assess the risks from reduced pesticide applications. In this study, we

163   designed combinations of alternative techniques and reduced applications of pesticides to create

164   alternative crop protection strategies (CPSs) by prototyping, and compared them to a reference strategy

165   corresponding to current growers' practices. We implemented these CPSs, and we assessed their

166   efficacy to manage pests and diseases, the yield and the quality of harvested products in three locations

167   over two seasons to cover the contextual variation of pest and pathogen pressure.

168   **2. Materials and methods**

169   2.1. Designing crop protection strategies

170   Fifteen persons including farmers, scientists, technical advisors, suppliers and end-product distributors

171   participated in two expert meetings held in February and June 2011 to design CPSs with reduced

172   pesticide use. At the first meeting, the objectives and constraints of all the stakeholders were defined,

173   and an inventory of possible alternative techniques to pesticides was made. Candidate CPSs were then

174   designed accordingly and refined during the second meeting, during which combinations of appropriate

175   alternative techniques were validated and a target performance of each strategy was agreed upon. A first

176   CPS, called low-input CPS, sharply reduced inputs and was intended to investigate the technical

177   boundaries of pesticide reduction. The second CPS was at an intermediate level of pesticide reduction

178   (intermediate CPS) between the low-input CPS and current practices. It was intended to meet farmers'

179   socio-economic and agronomic objectives. A conventional CPS, representative of current practices in

180   protected winter lettuce crops, was also defined from the synthesis of four farmers' practices.

181   2.2. Experimental design

182   The three lettuce CPSs (conventional, intermediate and low-input) were used in Batavia lettuce

183   production under high plastic tunnels in 3 locations in south-eastern France during winters 2012-2013

184   (W1) and 2013-2014 (W2). A total of 18 lettuce crops were monitored (3 CPSs x 3 locations x 2 years).

185   Two locations were conventional farms located at Candillargues (L1) (43°62'N; 4°06'E; 3 m elevation)

186   and at Villelongue de la Salanque (L2) (42°73'N; 2°98'E; 6 m elevation) in the Languedoc Roussillon

187   region. The third location (L3) was the INRA experimental station of Avignon (43°91'N; 4°87'E; 31 m

188   elevation) in the Provence Alpes Cote d'Azur (PACA) region. Crop rotation in each site, planting and

189   harvesting dates are given in Table 1. Crop lifespan was very similar for all experiments, between 70

190   and 84 days. We called the various combinations of locations and years L1-W1, L1-W2, L2-W1, L2-

191   W2, L3-W1 and L3-W2.

192   2.3. Crop protection strategies

193   The innovative CPSs relied on the joint implementation of alternative techniques to pesticides (Table

194   2). Pesticide applications and alternative techniques were managed by fixed or contingent decision rules

195   to suit local constraints.

196   2.3.1. Techniques that affect several pests and pathogens

197   2.3.1.1.   Irrigation

198   Except for the plot cultivated under the low-input CPS in L3, all the plots were sprinkler-irrigated

199   throughout crop growth. Just after planting, water was applied to field capacity. The moisture content

200   was maintained in the balls containing the plantlets by short daily irrigations until the roots started to

201   grow into the soil. After this early growth stage, drip irrigation was used under the low-input CPS in L3

202   only. Irrigation was triggered according to the soil water potentials measured by 6 Watermark® sensors

203   placed at 3 positions and 2 depths (15 cm and 35 cm), following the current guidelines in SE France. In

204   the other sites and CPSs, sprinkler irrigation was triggered once every two or three weeks, according to

205   potential evapotranspiration. Towards the end of the cropping cycle, short sprinkler irrigations were

206   applied when the temperature exceeded 30°C, to allow for a rapid cooling of the lettuces.

207   2.3.1.2.   Fertilization

208 A few days before planting, about fifteen 30-cm deep soil samples were randomly collected from each

209 plot. Soil nitrate content was measured in each plot with a Nitrachek reflectometer to assess fertilizer

210 requirements. Under the conventional and intermediate CPSs, fertilization followed farmers' practices,

211 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-

212 7-9 depending on the site). When the nitrate content exceeded 120 kg ha^{-1} prior to planting, no fertilizer

213 was applied.

214 Under the low-input CPS, nitrate fertilization was reduced and split. Before planting, soil nitrate-N

215 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

216 sample was collected and analyzed following the same procedure, and the soil NO_3^- -N content was

217 adjusted to 60 kg ha^{-1} with ammonitrate. Due to significant N mineralization during early growth, the

218 soil nitrate stock at this stage was usually not exhausted, hence low fertilizer applications. As a result,

219 the total application of N fertilizers in the plots cultivated under low-input CPS was much lower than in

220 the other two CPSs.

221   2.3.1.3.   Genotype

222 A different cultivar was used in each CPS. Cv Notilia (Clause), used in the conventional CPS, is a fast-

223 growing variety, has an incomplete range of resistance to *B. lactucae* (Bl 1-28, 30-32). It was chosen

224 for its agronomic quality in situations of full chemical protection. Cv Ostralie (Rijk Zwaan), was used

225 in the intermediate CPS. This cultivar grows slightly more slowly, has a complete range of resistance to

226 *B. lactucae* (Bl: 1-32), is resistant against the aphid biotype Nr:0, and has a semi-upright habit. The

227 cultivar retained in the low-input CPS, Lasydo (Syngenta seed), is a fast-growing variety with an

228 incomplete range of resistance to *B. lactucae* (Bl 1-28, 30-32) but low susceptibility to *S. sclerotiorum*,

229 as assessed in preliminary tests in our laboratory.

230   2.3.1.4.   Planting density

231 Lettuces were planted in parallel rows, on micro- and macro- perforated black plastic mulch

232 (Optimac®). Planting density in the low-input CPS was reduced from 14.25 to 12.75 plants per square

233 meter.

234 ### 2.3.1.5.   Infected plant management

235 Under the low-input CPS, any lettuce showing pathogen damage rendering the plant unmarketable was

236 carefully removed from the plot to avoid inoculum dissemination.

237 ### 2.3.2. *B. lactucae* management

238 ### 2.3.2.1.   Fungicide applications

239 All fungicides were applied with a spray boom, following a calendar-based program. In plots cultivated

240 under conventional CPS, Infinito® (Bayer CropScience) at 1.6 l ha^{-1} and Sygan® (Dupont) at 2.5 kg ha^{-}

241 ^{1} were each applied twice against *B. lactucae* 8, 30 and 20, 40 days ($\pm$ 2 days) after planting, respectively.

242 In plots cultivated under intermediate and low-input CPSs, the number of fungicide applications was

243 reduced: Infinito® at 1.6 l ha^{-1} and Sygan® at 2.5 kg ha^{-1} were applied 10 and 32 days ($\pm$2 days) after

244 planting, respectively.

245 ### 2.3.2.2.   Resistance inducer

246 Under the intermediate and low-input CPSs, fungicide treatments were supplemented by applications of

247 a plant resistance inducer (Potassium phosphite – LBG 01F34®, at 2 l ha^{-1}) applied by spraying 22 and

248 44 days ($\pm$ 2 days) after planting.

249 ### 2.3.3. Basal rot management

250 ### 2.3.3.1.   Biological control

251 The biocontrol fungus *Coniothyrium minitans* (Contans®) was applied once, before the first lettuce

252 crop in october, at 2 kg ha^{-1} on the soil surface of each CPS to limit the development of *S. sclerotiorum*

253 propagules. A second application was made before planting the monitored crop under the intermediate

254 and low-input CPSs. Additionally, under the low-input CPS, the biocontrol fungus *Trichoderma*

255 *harzianum* (strain T22 – Trianum P®) was applied twice on the balls, at the cotyledon stage (at 1.5 g

256 m^{-2}) and just prior to planting (at 1 kg per 8,500 plants) for broad spectrum control of basal rot pathogens.

257 The presence of *Trichoderma* spp. on lettuce roots was assessed 30 days after planting.

258 ### 2.3.3.2.   Fungicide applications

259 On plots cultivated under conventional CPS, Signum® (BASF Agro) at 1.5 kg ha^{-1}, Switch® (Syngenta)

260 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

261 planting, respectively. In plots cultivated under intermediate and low-input CPSs, the number of

262 fungicide applications was reduced. Signum® (BASF Agro) at 1.5 kg ha^{-1} and Switch® (Syngenta) at

263 0.6 kg ha^{-1} were applied on plots cultivated under the intermediate CPS 10 and 32 days (± 2 days) after

264 planting, respectively. Under the low-input CPS, only Signum® (BASF Agro) at 1.5 kg ha^{-1} was applied

265 20 days (±2 days) after planting.

266   2.3.4. Aphid management

267   2.3.4.1.   Biological control of aphids

268 Mixtures of parasitoid species of aphids (Basilprotect®) including *Aphidius colemani*, *Aphidius ervi*,

269 *Aphidius matricariae*, *Aphelinus abdominalis*, *Aphedrus cerasicola* and *Praon volucre* were

270 preventively introduced at the center of each plot cultivated under the intermediate and low-input CPSs

271 at a rate of 1.2 individuals per m² every two weeks.

272   2.3.4.2.   Insecticide application

273 Under the conventional CPS, Movento® (Bayer Cropscience) at 0.75 l ha^{-1} or Supreme® (Certis) at 0.25

274 kg ha^{-1} were applied every seven days as soon as aphids were spotted in the production area. Under the

275 intermediate and low-input CPSs, insecticide treatments were triggered when the number of aphids

276 reached species-dependent thresholds. The count was made every week on 40 randomly selected lettuces

277 per plot. The thresholds for *N. ribisnigri* were 2% and 10% of lettuce plants infested in plots under

278 intermediate and low-input CPSs, respectively. For other aphid species, they were 5% and 15%,

279 respectively. Under these CPSs, pirimicarb (Pirimor G®, Syngenta) at 0.25 kg ha^{-1} was preferred to

280 spirotetramat (Movento®) for its potential curative effect.

281   2.4. Performance assessment

282   2.4.1. Pesticide use

283 The Treatment Frequency Index (TFI) (Gravesen, 2003) was used to assess the amount of pesticide

284 applied under each CPS. TFI accounts for the number of compounds, the number of treatments and the

285 rate applied per unit area, and is calculated as follows:

286
$$\text{TFI}_{\text{CPS}} = \sum \frac{\text{Applied rate x area treated}}{\text{Authorized minimal dose x plot area}}$$

287   2.4.2. Monitoring of pests and pathogens

288 In France, pest and pathogen monitoring networks have been created since the implementation of a

289 national plan (Ecophyto) to reduce pesticide use. They aim to assess the epidemiological risks and to

290 broadcast agricultural warning reports for each crop in each region. Regional agricultural warning

291 reports for lettuce, broadcast in the PACA and Languedoc Roussillon regions about every 15 days, were

292 used in this study to determine timing of the preventive insecticide applications in the plots cultivated

293 under the conventional CPS and to appraise pest and pathogen pressure during the experiments.

294 The presence of pests and pathogens in the experimental plots was assessed weekly on 40 plants selected

295 at random at each date; 60 plants per plot were collected at harvest. The percentage of lettuces exhibiting

296 damage was recorded.

297 The damage caused by each pest or pathogen was scored from 1 to 3 (Table 3). A score of 1 at the time

298 of harvest indicates no effect on sale; a score of 2 affects lettuce quality without preventing sale, and

299 with a score of 3 a lettuce is unsaleable.

300   2.4.3. Lettuce yield and quality

301 At harvest, 60 lettuces from each CPS were randomly sampled and used to assess production

302 performance. The percentage of marketable lettuces (%ML), average lettuce fresh weight (LW, g),

303 marketable weight (after the removal of unmarketable basal leaves, MLW, g), and the percentage of

304 unmarketable basal leaves were measured. Gross yield (t fw.ha^{-1}) was calculated as:

305   Gross yield= LW x planting density / 100

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

307   Marketable yield= MLW x (%ML) x planting density / 100

308   Ten marketable lettuces were randomly harvested from each plot for visual quality assessment based on

309   plant appearance and absence of foreign bodies. The ratings were 0 (very poor quality), 1 (poor quality),

310   2 (middling quality), 3 (good quality), 4 (very good quality), 5 (excellent quality).

311   2.5. Data analysis

312   2.5.1. Analysis of CPS efficacy

313   The incidence of damage in a cultivated plot reflects the efficacy of the CPS, the presence of pests and

314   pathogens and of favorable conditions for their development. To analyze the efficacy of each CPS, the

315   structure of the damage caused by each pest or pathogen was studied. Six types of structures might be

316   recorded (Table 4). Those structures were used to organize the 'Results' section and guide the

317   discussion. Damage structure in each experimental plot, together with several hypotheses about pest or

318   pathogen pressure, enabled us to draw conclusions about the efficacy of each CPS. We checked whether

319   CPSs were efficient under all experimental conditions, or only under some conditions, or yet again

320   simply inefficient. In the absence of pest pressure, no conclusions were drawn about CPS efficacy.

321   2.5.2.Statistical analyses

322   Considering the small size of the samples (18 values per variable), non-parametric Kruskal-Wallis rank

323   sum tests (with a significance threshold of 0.1) were used to analyze the effect of CPS on each

324   performance indicator. To test for the effect of CPSs on the incidence of a particular pest or pathogen,

325   only the locations where the pest/pathogen was seen were kept for the Kruskal-Wallis rank sum test.

326   Rank-based multiple comparison tests (De Mendiburu, 2014) were used when significant differences

327   among CPSs, locations or winters were found. All statistical analyses were performed using R software.

328   **3.   Results**

329   3.1. Pesticide reduction

330   The TFI differed significantly among CPSs (P= 0.002794) (Figure 1). On average, pesticides were

331   reduced by 31.8% under the intermediate CPS and by 47.7% under the low-input CPS. Fungicides were

332   reduced by 43.1% and 56.7% under the intermediate and low-input CPSs, respectively, while

333   insecticides were reduced by 55.3% and 78.0%. Lower pesticide use was partly counter-balanced by

334   resistance inducers, which are accounted for in TFI, and resulted in a mean increase of 1.14. No

335   significant effect of location or year was found.

336   3.2. Efficacy of pest and pathogen management

337   3.2.1.Presence of damage in some locations, under some CPSs: Aphids

338   Aphids were found in 8 of the 18 experimental plots. Two species were seen: *N. ribisnigri* and *M.*

339   *persicae*. Lettuces under the low-input CPS were significantly more infested than those under the

340   intermediate and conventional CPSs (P= 0.053) (Table 5). No significant difference was found between

341   winters or locations. Aphids caused 0, 0.9% and 2.7% of lettuces to become unmarketable at harvest in

342   the conventional, intermediate and low-input CPS plots, respectively.

343   Population dynamics in the most infested plots L1-W1 and L3-W2 are reported in Figure 2. Some of the

344   variability among sampling dates might be explained by an uneven dispersal of aphids in the fields.

345   Nevertheless, some patterns can be distinguished. In L1-W1, the field interventions were identical in the

346   plot cultivated under the low-input and intermediate CPSs. They consisted of preventive introductions

347   of parasitoids, without any insecticide application. Under the conventional CPS, spirotetramat at 0.75 l

348   ha^{-1} was applied preventively. This single application failed to provide complete control, since aphids

349   were observed on 5% of the plants at harvest. Despite identical management under the low-input and

350   intermediate CPSs, the percentage of lettuces infested by *M. percisae* was greater under the low-input

351   CPS, especially at harvest (Figure 2(a)). However, severity was generally higher under the intermediate

352   CPS, which might indicate that aphids were less mobile under that strategy. Although density was lower

353   under the low-input CPS, the cultivar had higher growth rates, and there was more contact between

354   leaves of adjacent plants at the end of growth, which might have favored dispersal. Under the low-input

355   CPS, 7.8% of aphids were parasitized at harvest, while no mummified aphids were found in the plot

356   cultivated under intermediate CPS.

357 *N. ribisnigri* was seen only in L3 during W2, and only under the intermediate and low-input CPSs

358 (Figure 2(b)). Under the conventional CPS, three insecticides (spirotetramat on February 14th and March

359 7th and acetamiprid on March 14th) were applied. Under the alternative CPSs, parasitoids were

360 introduced preventively, but the threshold for *N. ribisnigri* infestation was reached on three sampling

361 dates under the intermediate CPS plots, and on one sampling date under the low-input CPS plots. As a

362 result, pesticides were applied curatively each time (pirimicarb on February 14th and March 7th, and

363 acetamiprid on March 14th under the intermediate CPS, acetamiprid on March 20th under the low-input

364 CPS). Neither of these chemical strategies succeeded in controlling the aphids. Similarly to L1-W1,

365 severity was higher in the intermediate CPS plots, while the percentage of infested lettuce plants

366 increased faster in the low-input CPS plots. 3.6% and 15.4% of aphids were parasitized at harvest under

367 the intermediate and low-input CPSs, respectively.

368 3.2.3. Presence of damage under all CPSs in some locations

369   3.2.1.1.   Basal rot

370 Basal rot symptoms were noted under all CPSs at some locations during at least one of the two winters

371 (Table 6). However, damage was limited: fungal rots usually developed on the lettuce collar and/or the

372 oldest leaves, and were removed at harvest. On average, 92% of the lettuce plants with basal rot

373 symptoms were marketable. Significant differences in basal rot incidence were noted among locations

374 (P= 0.070) and between winters (P= 0.005), but not among CPSs (P= 0.3379). None of the CPS, location

375 or winter modalities had a significant effect on basal rot severity.

376 Infected and healthy lettuce weights were compared at harvest in plots where basal rot incidence was

377 over 15% (5 plots). The weight of the infected lettuce plants was significantly higher than the weight of

378 the healthy lettuce plants in three of them (data not shown), indicating that bigger lettuce plants might

379 be more affected by basal rot than smaller ones.

380   3.2.1.2. Slugs and snails

381   CPS did not have a significant impact on the incidence of slugs and snails, or on damage severity (P=

382   0.7605). However, a significant effect of location was found (P= 0.055). Slugs and snails were

383   particularly numerous in L2 during W1 and in L3 (Table 7). They caused major losses, since on average

384   19.9% of the lettuces they attacked were unmarketable.

385   3.2.2. Overall pest and pathogen damage

386   In summary, pest or disease damage was noted under all CPSs (Figure 3(a)). Overall pest and pathogen

387   incidence differed among CPSs; however, these differences were only significant between the low-input

388   and intermediate CPSs (P= 0.07783). The conventional CPS showed intermediate pressure, and did

389   provide more effective protection than the low-pesticide treatments. Overall pest and disease incidence

390   also varied sharply among locations (P= 0.02606) (Figure 3(b)).

391   3.2.3. Unobserved damage

392   Several pests and pathogens with frequent occurrence in winter lettuce crops were not observed in this

393   study. This is the case of *B. lactucae* and viruses transmitted by *O. brassicae*. However, *B. lactucae* was

394   mentioned in 2/5 and 3/4 of the agricultural warning reports during W1 in the PACA and Languedoc

395   Roussillon regions, respectively, and in half of the warning reports during W2 in both regions. Viruses

396   transmitted by *O. brassicae* were reported only in the PACA region and in 1/5 of the reports during

397   winters 1 and 2.

398   3.2.4. Other damage

399   Frost and tip burn (marginal necrosis due to calcium deficiency favored by incorrect water supply)

400   damage were also repeatedly noted. Abiotic damage accounted for 64%, 41% and 26% of total damage

401   in L1, L2 and L3, respectively. Tip burn incidence was influenced by CPS (P= 0.03177), while frost

402   damage depended more on location (P= 0.0345). As a whole, these abiotic forms of damage caused 9%

403   of commercial loss, i.e. more than the 5% of loss caused by pests and pathogens.

404   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

431   epidemiological models for pathogens, are necessary to reinforce crop protection strategies with low

432   pesticide applications.

433   Although the systemic experiments performed here were not conceived to thoroughly understand the

434   complexity of the interactions between techniques, environment and pests and pathogens, but to assess

435   the risk to produce lettuce with fewer pesticides, some conclusions on management techniques or sets

436   of management techniques can be drawn.

437   Some lettuce pests were seen only in some locations and under some CPSs. For instance, aphids were

438   seen in 44% of the plots. Aphid damage was the only biotic pressure significantly affected by CPS.

439   However, aphids were observed in every CPS, so none of the strategies was completely effective. Under

440   the conventional CPS, preventive insecticide applications when aphids were seen in the production area

441   resulted in no or few aphids at harvest both for *N. ribisnigri* and *M. persicae*, so chemical control was

442   moderately efficient. In L1-W1, the preventive introductions of parasitoids in plots cultivated under low-

443   input and intermediate CPSs helped to maintain *M. persicae* populations below the treatment thresholds.

444   Under the low-input CPS, a higher proportion of lettuce plants contained aphids, while the number of

445   aphids per plant was higher under the intermediate CPS. This difference may have been mediated by the

446   plants, as several studies indeed show different levels of susceptibility to aphids among lettuce cultivars

447   (Dunn and Kempton, 1980; Lu et al., 2011). Furthermore, studies on *Brassica sp.* show that aphid

448   populations increase with narrower plant spacing (Sarwar, 2008; Yamamura, 1999). In our work,

449   planting density was greater but lettuce spacing was wider under the intermediate CPS because the

450   cultivar displayed a moderate growth rate as compared to the cultivar of the low-input CPS. Wider

451   lettuce spacing may have impeded aphid spreading, leading to fewer lettuce plants attacked, but to a

452   higher number of aphids per plant.

453   It is commonly thought that the industrial requirements for visual quality and the absence of foreign

454   bodies (Palumbo and Castle, 2009) cannot go together with the introduction of biological control agents

455   in lettuce crops (Palumbo and Castle, 2009). Yet, this study shows that preventive introduction of

456   parasitoids can help to maintain *M. persicae* populations below the threshold for chemical treatment in

457   certain situations (5% and 15% of lettuce plants infested under the intermediate and low-input CPSs,

458   respectively), and provide high-quality products. However, in the case of *N. ribisnigri* invasions (as in

459   L3-W2), the preventive introduction of parasitoids failed to maintain populations below the treatment

460   thresholds of 2% and 10% of lettuce plants infested under the intermediate and low-input CPSs,

461   respectively. This aphid develops preferentially in the lettuce heart (Liu, 2004), which may hinder its

462   detection by parasitoids. It is not susceptible to contact insecticides (Liu, 2004; Mackenzie and Vernon,

463   1988), so we used systemic insecticides . Their preventive application under the conventional CPS

464   ensured good protection. However, the curative efficacy of the active substance chosen under the

465   intermediate CPS was not as good, since three insecticide applications were not sufficient to suppress

466   aphids totally. Moreover, the resistance conferred by the Nr gene did not confer protection, suggesting

467   that biotype Nr:1 aphids were present. Some Nr:1 populations can reproduce fast in cultivars bearing

468   the Nr gene, while other populations have a reduced reproduction rate (ten Broeke et al., 2013). Thus,

469   although the greatest *N. ribisnigri* infestation noted under the low-input CPS in L3-W2 was probably

470   due to the reduced number of chemical treatments we cannot rule out an effect of the cultivar. Anyhow,

471   the relative failure of either strategy suggests that lower treatment thresholds might be required for *N.*

472   *ribisnigri*. A more stringent threshold was already defined by Morales et al. (Morales et al., 2013) for

473   *N. ribisnigri*, i.e. 0.06 and 0.07 aphids per lettuce for field-grown lettuces in central Spain in spring and

474   autumn, respectively. Those thresholds could be tested for winter lettuce grown under shelter.

475   Basal rot was recorded under all CPSs in some locations. The three strategies were equivalent in terms

476   of damage, although they relied on different control techniques. Only a few alternative techniques were

477   added under the intermediate CPS (a biological control agent and a semi-upright lettuce habit) to reduce

478   fungicide applications by one third. Lettuce weight sometimes influenced on basal rot incidence. This

479   could partly reflect the effect of the microclimate under each lettuce on basal rot incidence. The slower

480   growth rate and upright lettuce habit under the intermediate CPS may have reduced the humidity level

481   below the plants and limited basal rot development. Under the low-input CPS, we used several

482   techniques known topartially limit the damage caused by basal rot (biological control agents, sanitation,

483   reduced N fertilization, cultivars with reduced susceptibility to *S. sclerotiorum*, reduced planting density

484   and, in one case, drip irrigation). We used them to reduce the number of chemical treatments against

485   basal rot by two-thirds. All of them had had a partial effect on at least one of the causal agents of basal

486   rot in factorial experiments (Chitrampalam et al., 2008; Dow et al., 1988; Lecompte et al., 2013). The

487   field experiments presented here enabled us to assess CPS efficacy but did not allow us to fully

488   understand potential synergy or antagonism among techniques. The techniques that were most likely to

489   interact with one another were those acting at the same time in the pathogen's life cycle. For example,

490   we do not know how N fertilization may impact the level of resistance to *Sclerotinia sclerotiorum* noted

491   in the cultivar used under the low-input CPS. Moreover, long-term effects or improved efficacy of some

492   alternative techniques after several seasons of application may be expected. We assessed the incidence

493   and severity of basal rot over two winters, which is not long enough to evaluate such effects.

494   Some of the main winter lettuce pathogens were not found in this study. The high pressure of *B. lactucae*

495   reported in the production area during winters 2012-2013 and 2013-2014 suggests that all three CPSs

496   may have provided efficient protection against this pathogen. Genetic resistance to *B. lactucae* relies on

497   specific gene-for-gene interactions. Two of our cultivars did not cover the whole range of downy mildew

498   races. Moreover, resistance breakdowns are frequently reported; as a consequence, genetic control of *B.*

499   *lactucae* is not self-sufficient. Additional techniques, such as fungicide applications, are usually used to

500   ensure the sustainability of genetic resistance and the efficacy of the *B. lactucae* control (Crute, 1992).

501   Under the intermediate and low-input protection strategies against *B. lactucae,* preventive fungicide

502   applications were partly replaced by plant resistance inducers. The combination of genetic resistance,

503   reduced fungicide applications and resistance inducers might be an effective strategy to manage *B.*

504   *lactucae*.

505   According to these first results, some improvements of the CPSs can be suggested, such as using a lower

506   treatment threshold for *N. ribisnigri.* As close plant spacing appeared to favor both the development of

507   basal rot and aphids, we also suggest planting a compact lettuce variety, *i.e.* small but heavy to maintain

508   yield. Moreover, in this study, much of the damage was due to abiotic stresses such as frost and tip burn.

509   The alternative CPS design was focused on potential biotic damage. One way to improve the CPS would

510   be to take into account potential abiotic damage. For example, varietal choice should include tip burn

511   resistance, especially when drip irrigation is used. In general, interactions between variety and cultural

512   practices may influence lettuce quality, number of pesticide residues, lettuce weight, etc. Perhaps

513   because of the rapid turnover of lettuce varieties, this information is seldom available, and therefore it

514   is critical to pay particular attention to variety/practice interactions during the design process.

515   In conclusion, despite the stringent market requirements in terms of visual quality and the need to

516   maintain yield, pesticides were reduced by half under the low-input CPS. This reduction is consistent

517   with those obtained on other low-input cropping systems designed by prototyping (Clark et al., 1998;

518   Simon et al., 2011). Marketable yield was not affected by CPS. The prototyping method makes it

519   possible to design crop protection strategies that are compatible with market expectations and production

520   constraints. The possible environmental gains of intermediate and low-input CPSs could be significant

521   and should be assessed together with socio-economic performance to encourage the adoption of

522   alternative strategies by lettuce growers.

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666   Table 1. Crop rotation, planting and harvesting dates in the three experimental sites L1, L2 and L3. L:

667   lettuce; bs: bare soil; S: solarization; M: melon. The lettuce crops studied in each site are underlined.

	L1	**L2**	**L3**
Rotation	S-L-<u>L</u>-M-S-L-<u>L</u>-M	S-L-<u>L</u>-L-S-<u>L</u>-L-bs	bs-<u>L</u>-L-S-bs-<u>L</u>-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

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670   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

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673   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

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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** L1 C I L L2 C I L L3 C I L *(all underlined)*	**W2** L1 C I L L2 C I L L3 C I L *(all underlined)*
Some CPSs with damage at some locations	Some CPSs damage-free	W1 L1 <u>C I</u> L L2 C I L L3 C <u>I</u> L	W2 L1 C I L L2 C <u>I</u> L L3 C I L
	All CPSs with damage at least at one location	W1 L1 <u>C I</u> L L2 C I <u>L</u> L3 C <u>I L</u>	W2 L1 C <u>I</u> L L2 C <u>I</u> L L3 C I <u>L</u>
All CPSs with damage at some locations		W1 L1 <u>C I</u> L L2 C I L L3 <u>C I L</u>	W2 L1 C I L L2 C I L L3 <u>C I L</u>
Some CPSs with damage at all locations		W1 L1 C <u>I</u> L L2 C <u>I</u> L L3 C <u>I</u> L	W2 L1 C <u>I</u> L L2 C <u>I</u> L L3 C <u>I</u> L
All CPSs damage-free		W1 L1 C I L L2 C I L L3 C I L	W2 L1 C I L L2 C I L L3 C I L

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681   Table 5. Percentages of lettuce plants infested by aphids at harvest under the three CPSs and three

682   locations (L) over the two winters (W). Superscripts represent the aphid species, Mp: *Myzus percicae*,

683   Nr: *Nasonovia ribisnigri.* In the "average" column, letters indicate significant differences identified by

684   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

685

686

687 Table 6. Percentages of lettuce plants showing basal rot symptoms at harvest under the three CPSs and

688 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

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691   Table 7. Percentages of lettuce plants showing slug or snail damage at harvest in plots cultivated under

692   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

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695   Table 8. Marketable lettuce weight (MLW), percentage of marketable lettuce (ML), marketable yield

696   and quality rating of lettuce plants cultivated under conventional, intermediate and low-input CPSs. P-

697   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

698

699   Figure 1. Plant resistance inducer, insecticide and fungicide contributions to the Treatment Frequency

700   Index (TFI) under the different crop protection strategies. Results are given as the means of 3 locations

701   and 2 years. Different letters indicate significantly different values in the multiple comparison tests.
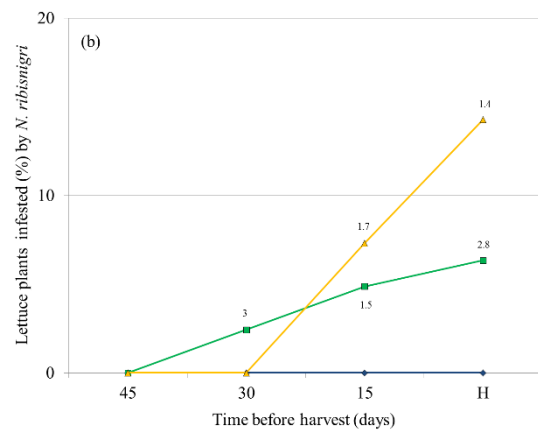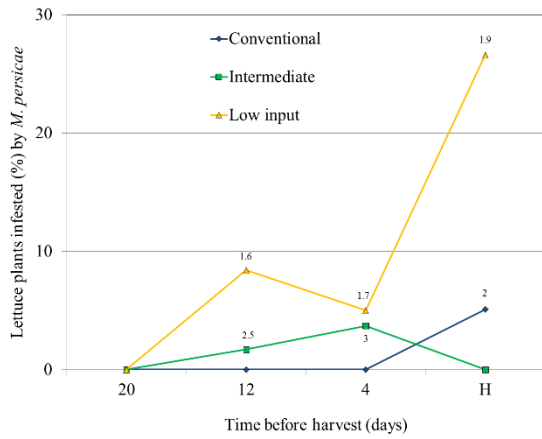
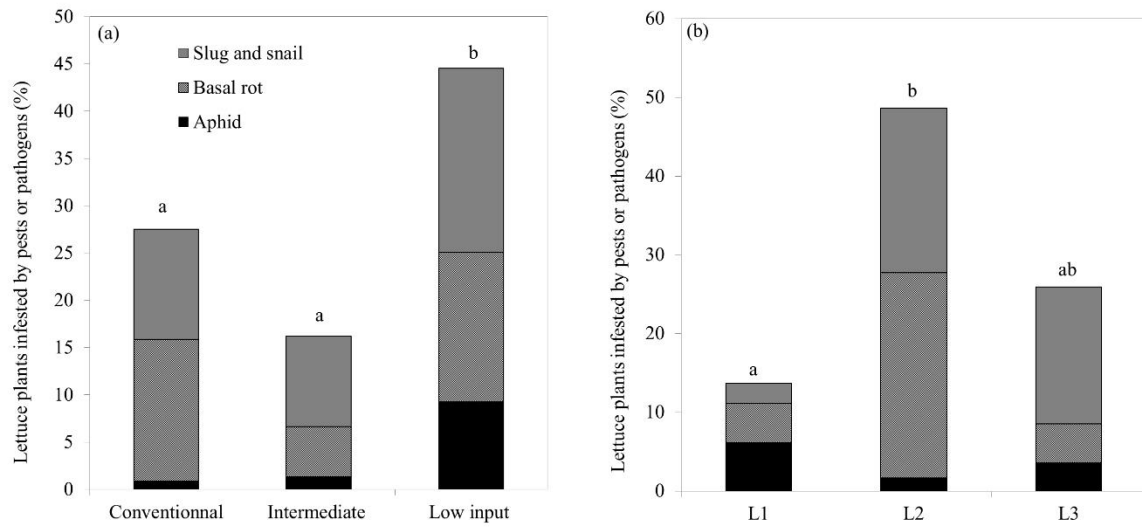702   *[1-column fitting image]*
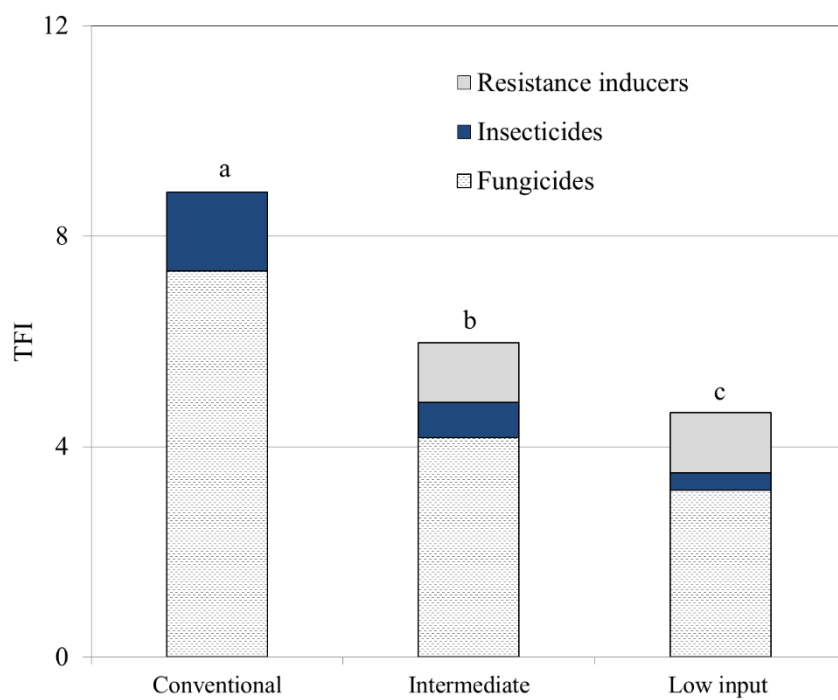
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704

705   Figure 2. Dynamics of the percentage of lettuce plants infested by *M. persicae* in L1-W1 (a) and *N.*

706   *ribisnigri* in L3-W2 (b) in plots cultivated under conventional, intermediate or low-input CPSs. Numbers

707   next to symbols indicate mean severity scores. *[2-column fitting image]*

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710   Figure 3. Percentages of lettuce plants (%) attacked by aphids, basal rot causal agents or slugs and

711   snails under conventional, intermediate or low-input CPSs (a), and in locations L1, L2 and L3 (b).

712   Letters indicate the results of Kruskal – Wallis tests. *[2-column fitting image]*

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