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INVESTIGATION OF INTERCROPPING OF LEEK (*ALLIUM PORRUM* Linnaeus) AND BELL PEPPER (*CAPSICUM ANNUUM* Linnaeus) AS A ZERO-SPRAYING METHOD AGAINST LEAF APHID (*MYZUS PERSICAE* Sulzer)

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EVALUATION DE L'UTILISATION DU POIREAU (*ALLIUM PORRUM* Linnaeus) EN CULTURE INTERCALAIRE AVEC LE POIVRON (*CAPSICUM ANNUUM* Linnaeus) COMME MÉTHODE ZÉRO-PHYTO CONTRE LE PUCERON VERT DU PÊCHER (*MYZUS PERSICAE* Sulzer)

RÉSUMÉ

Pour déterminer si la culture du poireau (*Allium porrum*) en association avec le poivron (*Capsicum annuum*) peut contribuer à la lutte contre le puceron vert du pêcher (*Myzus persicae*), des cultures de poivron sous tunnel ont été réalisées (monoculture traditionnelle et système de culture en association avec le poireau). Pour stimuler la libération de composés organiques volatils, les poireaux ont été étêtés deux fois pendant la culture. L'impact de ces deux systèmes de culture a été évalué sur les nombres de pucerons et d'insectes auxiliaires ainsi que sur les rendements. La culture en association avec les poireaux n'a pas semblé impacter la dynamique des pucerons sur poivrons. Comparé à la monoculture, le système en association a conduit à un rendement commercialisable plus élevé, mais aussi à un rendement non commercialisable plus élevé avec l'apparition de pourritures. Sur la base de cet essai en plein champ, la culture intercalaire de poivron avec du poireau pour lutter contre les pucerons n'apparaît pas comme une stratégie prometteuse pour réduire l'utilisation de pesticides. Cependant, cet essai démontre clairement la dynamique de l'équilibre biologique entre insectes ravageurs et insectes auxiliaires.

Mots-clés : Culture intercalaire ; zéro-phyto ; *Myzus persicae* ; poireau ; poivron

ABSTRACT

To determine if intercropping with leek (*Allium porrum*) can contribute to the control of green peach aphids (*Myzus persicae*) in bell pepper (*Capsicum annuum*), pepper was planted in a traditional monoculture and in an intercropping system with leek in tunnel cultivation. To trigger the release of volatile organic compounds, the leeks were topped twice during the cultivation. The number of aphids, beneficial insects and yield were recorded. The intercropping with leek plants did not seem to affect the aphid development on peppers. A higher marketable yield, but also a higher non-marketable yield and occurrence of nose rot, were reached in the intercropping system compared to the monoculture. Based on this field trial, intercropping of sweet bell pepper with leek for control of aphids in bell pepper is no promising strategy to reduce the use of pesticides. However, the trial does clearly demonstrate the dynamics of the biological balance between pests and beneficial insects.

Keywords: Intercropping ; Zero-spraying method ; *Myzus persicae* ; leek ; pepper

Introduction

The main objective of the Interreg France-Wallonia-Flanders project « ZERO-PH(F)YTO F&L(G) » is the exploration of growing fruits and vegetables without spraying crop protection agents, even organic ones. In protected bell pepper cultivation, aphid problems can appear early on in the crop and may escalate into an epidemic. The green peach aphid (*Myzus persicae* Sulzer) is often the main culprit. The control of aphids in peppers relies heavily on the use of pesticides. The use of companion plants (CPs) is being assessed to reduce the performance of green peach aphids on host plants (Ben Issa *et al.*, 2016). Intercropping with plants from the Alliaceae family is regarded as a promising alternative control strategy, since several studies have shown that volatile organic compounds (VOCs) of *Allium* spp. may have repellent effects on insects (Thibout et Auger, 1997; Dugravot *et al.*, 2004). Field experiments performed by Zhou *et al.* (2013) showed that intercropping wheat (*Triticum aestivum* L.) with garlic (*Allium sativum* L.) lead to a lower infestation by *Sitobion avenae* Fabricius, and better yields due to this association. Amarawardana *et al.* (2007) demonstrated in laboratory experiments the masking and repellent properties of leek (*Allium porrum* L.) VOCs towards *M. persicae* on bell pepper plants (*Capsicum annuum* L.).

Because of these positive properties of the VOCs from leek, intercropping with leek may contribute to the sustainable control of the green peach aphid population in bell pepper. A field trial was performed to investigate if intercropping with leek has potential to protect bell pepper against aphids in tunnel cultivation and consequently, if leek can be used as a CP as a strategy to minimize pesticide use. Therefore, bell pepper was planted in a traditional monoculture and in an intercropping system in foil tunnels and the aphid population was monitored. The effect of release of VOCs through topping of the leek was analysed. The setup of the experiment allowed to determine a critical distance between leek and bell pepper, below which a clear protection would be offered. The yield was also recorded to establish if a positive synergetic effect on pepper yield manifests when intercropping with leeks is applied.

Material and methods

Plants

The trial was set up in foil tunnels in Kruishoutem, Belgium. Two treatments were applied. In the first treatment, the control treatment, bell pepper was planted in a traditional monoculture in tunnel cultivation (50 cm plant distance). The second treatment consisted of an intercropping cultivation system (IC), in which a 1:5 replacement of bell pepper – leek was applied. The variety of bell pepper was Sprinter with Scarface as rootstock (Vitalis, Voorst, Nederland), planted on 12/05/2020. The variety of leek was Oslo, planted on 12/06/2020 with a plant distance of 15 cm in the row. Maintenance of the bell pepper, including pruning, trimming and watering was performed weekly.

Treatments

The trial was designed as a split-plot trial with four parallels (Figure 1). Plots (4 rows x 12.5 m) were assigned throughout the cultivation in a structured way. The experiment was performed in two tunnels each with four parallels. In order to specifically test the effect of VOCs of the leeks on the aphids, the leeks were topped (leaf cut 5 cm from the apex) twice during the test, on the 9th and 30th of July. Topping triggers a stress reaction in the plant, which causes, among others, an increased release of these compounds (Thibout et Auger, 1997).

Figure 1 : Design of the trial. The trial was executed in two tunnels. The plants were cultivated in a traditional system (Ctrl) or in an intercropping system with leek (IC). Each plot consisted of four rows. Within a row, the treatments were separated by several plants.

(Dispositif expérimental de l'essai. L'essai a été réalisé dans deux tunnels. Les plantes ont été cultivées dans un système traditionnel (Ctrl) ou dans un système de culture intercalaire avec du poireau (IC). Chaque parcelle était composée de quatre rangées. Dans une rangée, les traitements étaient séparés par plusieurs plantes.)

	Row 1		Row 2		Row 3		Row 4		Row 5		Row 6		Row 7		Row 8	
Tunnel A																
	IC	Plot 2	IC	Plot 2	IC	Plot 2	IC	Plot 2	Ctrl	Plot 2	Ctrl	Plot 2	Ctrl	Plot 2	Ctrl	Plot 2
	Ctrl	Plot 1	Ctrl	Plot 1	Ctrl	Plot 1	Ctrl	Plot 1	IC	Plot 1	IC	Plot 1	IC	Plot 1	IC	Plot 1
Tunnel B																
	Ctrl	Plot 4	Ctrl	Plot 4	Ctrl	Plot 4	Ctrl	Plot 4	IC	Plot 4	IC	Plot 4	IC	Plot 4	IC	Plot 4
	IC	Plot 3	IC	Plot 3	IC	Plot 3	IC	Plot 3	Ctrl	Plot 3	Ctrl	Plot 3	Ctrl	Plot 3	Ctrl	Plot 3

Evaluation

Per plot, six plants were randomly chosen for evaluation of aphid infestation. To account for the distance effect from the intercropping and determine a critical distance, below which a clear protection is offered, it was ensured that three pepper plants were positioned immediately next to the leek, and the other three plants somewhat further away (i.e. with another pepper plant between the chosen plant and the leek). Weekly, the number of wingless *M. persicae*, parasitised aphids and beneficial insects were counted on a leaf at the base, in the centre and at the head of each of these plants, which means that eighteen leaves were counted per plot.

The harvest was also evaluated for each of the eight plots. The weight, number of units and a distribution according to class were assessed. The peppers were harvested green or red, depending on the marketing availability at that moment.

Statistics

The data were checked for normality and homoscedasticity using the Levene's and Shapiro-Wilk tests. If normally distributed, an ANOVA followed by a Tukey test was performed.

Results

Aphid population

The number of wingless and parasitised aphids was monitored throughout the cultivation (Figure 2). Differences in number of aphids seemed to be associated with the plots rather than the cultivation system. A natural infestation of *M. persicae* was already observed one month after planting (Figure 2A). Remarkably, this infestation started at a particular location in the tunnel, being the entrance of the left tunnel (IC-plot 3). From July onwards, aphids multiplied rapidly everywhere, with an absolute peak of almost 500 aphids per three leaves in plot 3 of the IC system. However, during the cultivation, the number of wingless *M. persicae* was found to stabilize or decrease (Figure 2A). When wingless *M. persicae* could be observed in the tunnels, parasitised *M. persicae* were found as well (Figure 2B). The counts of parasitised aphids show the presence of parasitic wasps. The parasitic wasps could also be retrieved from the yellow sticky traps which were used during the cultivation. Moreover, one or more other types of beneficial insects were found to be present at the same time (Figure 3). The beneficial insects monitored were gall midges (*Aphidoletes aphidimyza*), lacewings (*Chrysoperla carnea*) and ladybirds.

Figure 2 : Average number of *M. persicae* per bell pepper plant, cultured in a traditional cultivation system (Ctrl) or an intercropping system with leek (IC). The number of wingless (A) and parasitised (B)

aphids was counted weekly on three leaves per plant. Per plot, the same six plants were used for evaluation.

(Nombre moyen de *M. persicae* par plant de poivron soit en système de culture traditionnel (Ctrl) ou en système de culture intercalaire avec du poireau (IC). Le nombre de pucerons aptères (A) et parasités (B) a été compté chaque semaine sur trois feuilles par plante. Par parcelle, les six mêmes plantes étaient utilisées pour l'évaluation.)

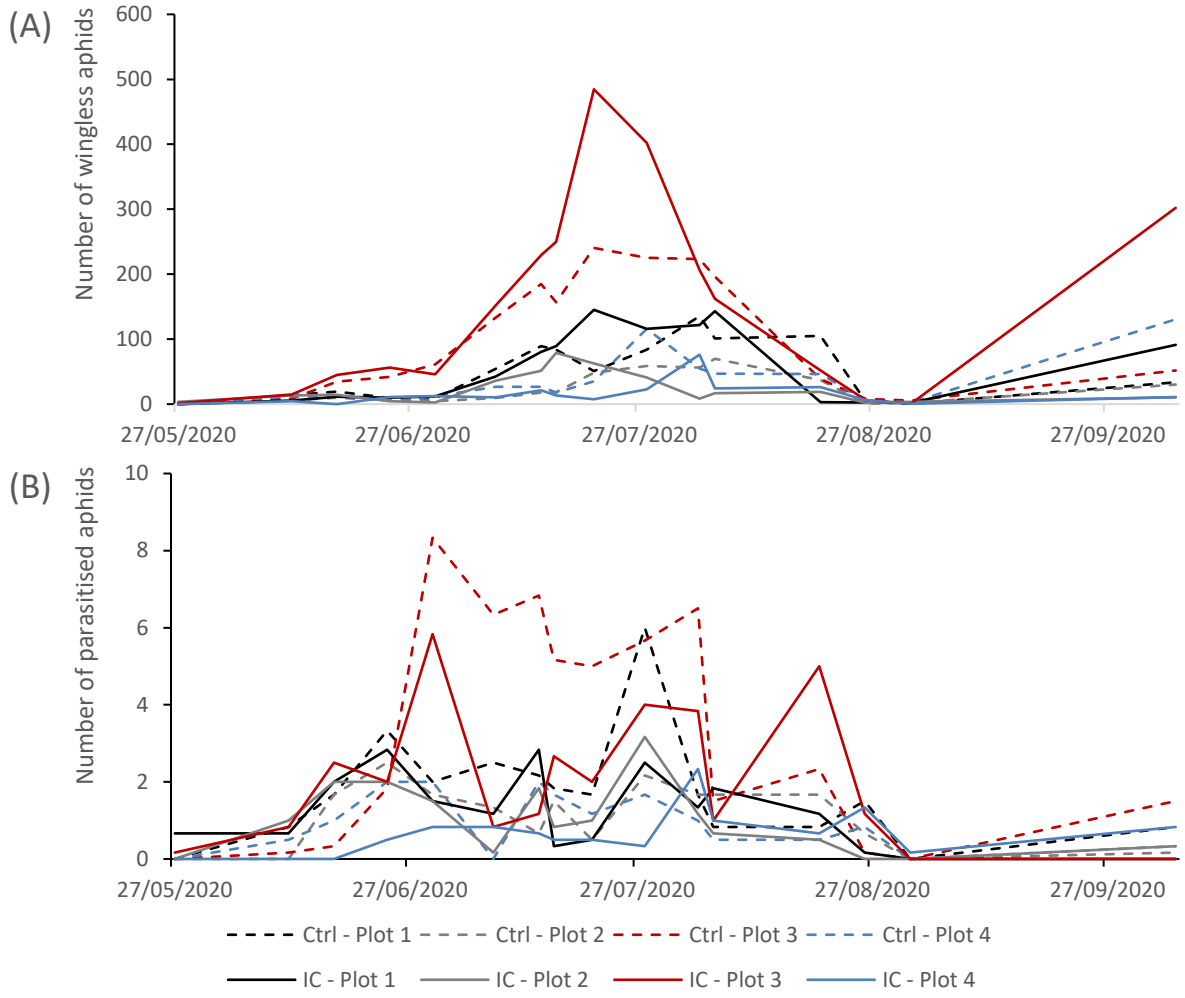
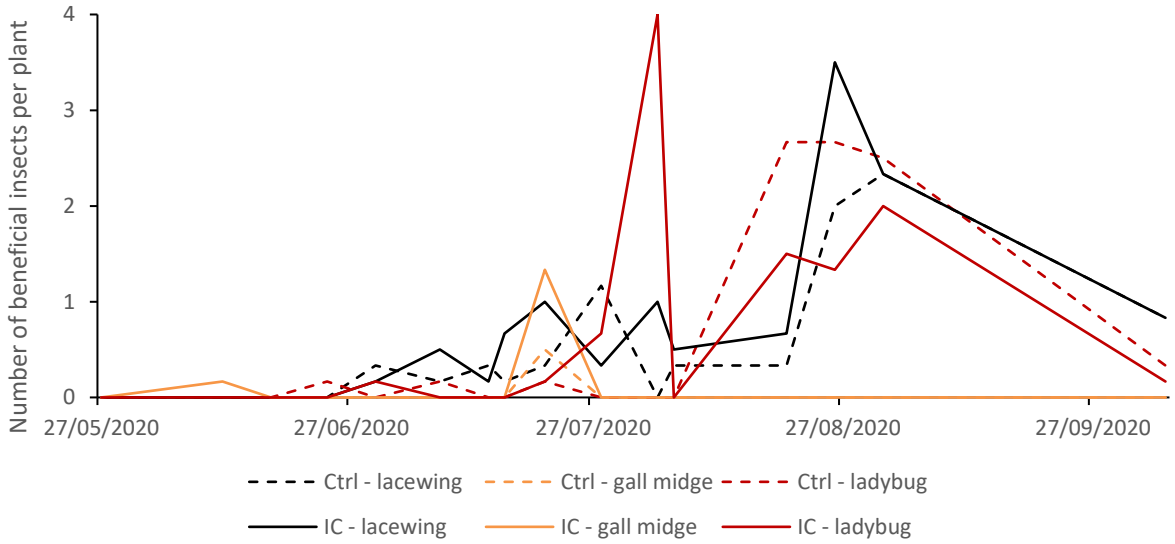


Figure 3 : Average number of beneficial insects per bell pepper plant, cultured in a traditional cultivation system (Ctrl) or an intercropping system with leek (IC). The numbers of gall midges (*Aphidoletes aphidimyza*), lacewings (*Chrysoperla carnea*) and ladybirds were counted weekly per plot. Per plot, the same six plants were used for evaluation.

(Nombre moyen d'insectes auxiliaires par plant de poivron soit en système de culture traditionnel (Ctrl) soit en système de culture intercalaire avec du poireau (IC). Le nombre de cécidomyies (*Aphidoletes aphidimyza*), de chrysopes (*Chrysoperla carnea*) et de coccinelles a été compté chaque semaine par parcelle. Par parcelle, les six mêmes plantes étaient utilisées pour l'évaluation.)



In order to release VOCs of the leeks, the leeks were topped twice during the test, on the 9th and 30th of July. Table I demonstrates the daily growth rate of the aphid population during this time. The topping of the leeks did not show to induce a clear inhibition of the growth of the aphid population (Table I).

Table I : Daily growth rate of the aphid population on bell pepper plants cultured in a traditional cultivation system (Ctrl) or an intercropping system with leek (IC). The leeks were topped on 09/07/2020 and 30/07/2020 to release volatile organic compounds. The number of aphids was counted weekly on three leaves per plant. Per plot, the same six plants were used for evaluation. The daily growth rate was calculated as the difference in number of aphids between two observations, divided by the number of days between two observations.

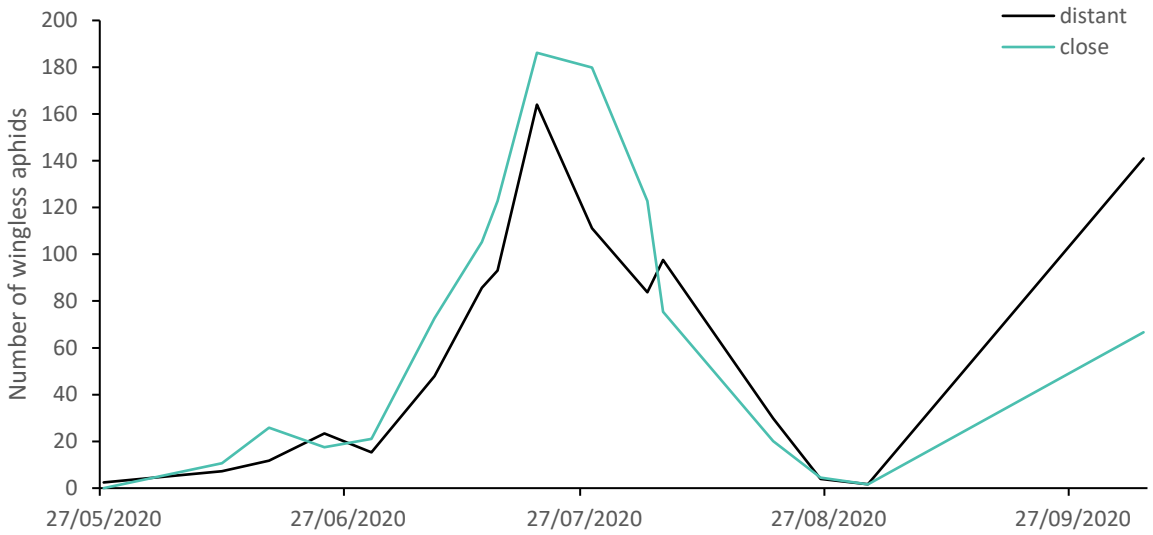
(Taux de croissance quotidien de la population de pucerons sur des plants de poivrons en système de culture traditionnel (Ctrl) ou en système de culture intercalaire avec des poireaux (IC). Les poireaux ont été étêtés le 09/07/2020 et le 30/07/2020 pour libérer les composés organiques volatils. Le nombre de pucerons a été compté chaque semaine sur trois feuilles par plante. Par parcelle, les six mêmes plantes étaient utilisées pour l'évaluation. Le taux de croissance quotidien a été calculé comme la différence du nombre de pucerons entre deux observations, divisée par le nombre de jours entre deux observations.)

Treatment	8-14 July	14-16 July	16-21 July	28 July - 4 Aug	4-6 Aug
Ctrl	32.7%	48.2%	54.4%	4.3%	40.4%
IC	44.5%	42.3%	66.6%	2.4%	43.2%

In order to evaluate the distance effect of leek intercropping (distance between a leak and a pepper plant), aphid counts of the pepper plants closer (within 37.5 cm) to the leek were compared to those further away (within 87.5 cm) (Figure 4). However, distance to leek plants was not found to affect number of aphids on pepper plants.

Figure 4 : Average number of wingless aphids (*M. persicae*) per pepper plant in relation to distance to the leek plants in an intercropping system. The number of wingless aphids was counted weekly on three leaves per plant. Per plot, the same three plants were used for evaluation.

(Nombre moyen de pucerons aptères (*M. persicae*) par plant de poivron en fonction de la distance aux plants de poireau en système de culture intercalaire. Le nombre de pucerons aptères a été compté chaque semaine sur trois feuilles par plante. Par parcelle, les trois mêmes plantes étaient utilisées pour l'évaluation.)



Yield

The harvest was evaluated as the marketable and non-marketable weight and number of fruits per plot. The cumulative yield at the end of the trial is shown in Table II. Since one fifth of the pepper plants was replaced by leek in the IC, the IC yield was recalculated towards an equal number of plants. The total marketable and non-marketable yield, both in net weight and number of fruits, was significantly higher in the IC system compared to the monoculture. Significantly more fruits of insufficient quality were harvested in the IC system. This manifested itself mainly in the form of nose rot of the fruit.

Table II : Cumulative yield of green and red bell peppers, and their total, at the end of the trial. The fruits were evaluated as marketable or non-marketable, before being weighed and counted. The bell peppers were cultured in a traditional cultivation system (Ctrl) or an intercropping system with leek (IC). The weight and number of fruits in the IC system were recalculated to account for the lower number of pepper plants per m². Significant differences between both treatments are indicated with an asterisk (ANOVA followed by Tukey test).

(Rendement total, à la fin de l'essai, et cumulé des poivrons verts et rouges. Les fruits ont été évalués comme commercialisables ou non commercialisables, avant d'être pesés et comptés. Les poivrons ont été cultivés en système de culture traditionnel (Ctrl) ou en système de culture intercalaire avec du poireau (IC). Le poids et le nombre de fruits dans le système IC ont été recalculés pour tenir compte du nombre inférieur de plants de poivrons par m². Les différences significatives entre les deux traitements sont indiquées par un astérisque {ANOVA suivie du test de Tukey}.)

Treatment	Pepper	Marketable yield		Non-marketable yield	
		Weight (kg/m²)	Number (/m²)	Weight (kg/m²)	Number (/m²)
Ctrl	green	164.69	712	34.905	212
IC	green	172.45	746	43.494	263 *
Ctrl	red	68.77	279	13.69	64
IC	red	85.1	286	20.438	100 *
Ctrl	total	233.46	991	48.595	276
IC	total	257.55 *	1031	63.931 *	363 *

Discussion

A trial was set up to evaluate how intercropping with leek affects the aphid population on bell pepper. Bell pepper was planted in a traditional way, as a control treatment, or in an IC system (1:5 replacement of bell pepper – leek). The aphid infestation, the number of beneficial insects and the yield were recorded. The number of aphids seemed to be associated with the plot rather than the cultivation system. However, in plot 3, the infestation of the intercropped peppers reached higher numbers than the peppers in monoculture. Since aphid infestations occurred in the IC plots as well, we can conclude that this trial did not lead to efficient aphid control by intercropping pepper bell crops with leek in foil tunnels. Recent lab work (Baudry *et al.*, 2021) revealed that leek as a CP had a negative effect on aphid feeding behaviour, by disturbing the balance between phloem and xylem sap ingestion, but had no influence on aphid settlement. Surprisingly, leek as a CP triggered some unexpected probiotic effects on certain life history traits, such as aphid survival, biomass, and fecundity. The latter could explain the inefficiency to control aphid by intercropping pepper bell crops with leek. It may also explain why the aphids multiplied faster in plot 3 of the IC than in plot 3 of the control.

The aphid counts of the pepper plants closer to the leek compared to those further away were analysed to evaluate if the distance between both crops can affect the aphid population. Moreover, the leeks were topped twice to evaluate if the VOCs released could affect the aphid population growth. Topping triggers a stress reaction in the plant, which causes, among others, an increased release of these compounds (Thibout et Auger, 1997). However, the aphid population showed no differences related to distance to leek plants or leek topping. Since no overall or distance effect was found, we can conclude that the intercropping with leek did not contribute to the control of aphids in bell pepper. The VOCs from leek did not show a positive effect on the aphid population on pepper, but this does not exclude other VOCs to contribute to its control. Rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*) and *Tagetes patula* cv. Nana have recently been identified as promising repellent CP species towards *M. persicae* (Ben Issa *et al.*, 2016, Dardouri *et al.*, 2019).

Aphids were present in all plots, but their population size stabilized. The control of the aphid population may be due to the presence and alternation of parasitic wasps and other beneficial insects that could naturally enter the tunnels. The gall midge was present at the time with the highest infestation rate. Indeed, gall midges are attracted by the scent of honeydew that aphid colonies spread. They lay their eggs near the colonies, giving the larvae an immediate food source. The almost complete reduction in the aphid population in early September could be linked to the presence of lacewings and ladybirds.

From this trial, we cannot conclude that pesticide use can be reduced by planting leeks as CP for bell red pepper. The timely and sufficient control of aphids in this trial was achieved by the cooperation and alternation of the different aphid predators. In this regard, an active mixed population of predators seems to be the best guarantee for successful cultivation when aphids are present.

While IC did not seem to contribute to the control of the aphid population, this cultivation system did show to increase the total marketable yield. Both the net weight and number of fruits were significantly higher in the IC system compared to the monoculture. However, the IC system also yielded significantly more fruits of insufficient quality due to nose rot of the fruit. A possible explanation for the occurrence of nose rot could be that the intercropping interrupted the build-up of a favourable, humid microclimate in the crop. Consequently, the foliage of the peppers next to the leeks would have evaporated more than in the monoculture, resulting in less calcium reaching the fruit via the sap flow, with nose rot as a consequence. Nevertheless, the IC system also resulted in a higher marketable yield, compared to the monoculture, indicating that intercropping may simultaneously provide a slightly

better generative control of the pepper crop. This may also be due to the breaks in the build-up of a humid microclimate in the crop.

Conclusion

From this field trial, it can be concluded that intercropping of sweet bell pepper with leek for control of aphids in bell pepper is not an interesting strategy to reduce the use of pesticides. The distance to leek plants did not seem to affect the aphid development on peppers. Moreover, no positive effect of VOCs from leek was observed, neither in terms of introduction of aphids nor inhibition of infestation, but the use of other promising repellent CP species for control of aphids should be explored.

In terms of yield, the results suggest that intercropping with leek leads to a more generative control of the crop. On the other hand, the IC system showed relatively more non-marketable yield, which was mainly due to a higher share of fruits with nose rot compared to the monoculture. A possible explanation for these two phenomena could be that the intercropping caused breaks in the build-up of a favourable, humid microclimate in the crop.

Although this experiment did not show a positive effect of intercropping with leek on the control of aphids in pepper, the trial does clearly demonstrate the dynamics of the biological balance between pest and beneficial insects. A good estimation of the mutual relationship is key to give the beneficial insects every chance to turn the tide in the right direction, also at critical moments. Since this is a tunnel crop, the beneficial insects in this trial had various opportunities to be introduced.

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