Internship report

Combining three levers to regulate pests : a systemic approach in pepper agrosystem

Sarra Ayed

Supervisors
Dr Anne-Violette Lavoir
Dr Caroline Djian-Caporalino

ISA institute : MIB-IPN teams

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Abstract (355 words)

In the context of agroecological transition, reducing the use of synthetic chemical pesticides in cropping systems requires rethinking plant protection strategies. The pepper (*Capsicum annuum*), a widely cultivated species in south of Europe, is vulnerable to a variety of airborne pests such as aphids, whiteflies, thrips, and various lepidopterans, and soil borne pests (e.g. Root-knot nematodes. These pests, which can sometimes act as vectors for viruses, can lead to significant economic losses. To address these multi-pest simultaneously or in sequence during the cropping period, it's necessary to assess the effectiveness of alternative agronomic practices.

This project conducted in the field aimed to find new alternatives to the use of pesticides by studying the effects of three different pest regulate levers: a resistant variety (nematode resistance), a biocontrol plant (*Tagetes erecta*), and a biostimulant agent (mycorrhizal fungi). The effect of these levers, alone or in combination, was evaluated on the abundance of airborne pests as well as the effect on crop plant growth and the yield.

The genetic resistance lever alone has not been significantly effective in reducing pest abundance. On the other hand, the addition of *Tagetes erecta* significantly reduced the population of aerial pests ($p<0.001$) whether associated with the susceptible variety (Almuden) or the resistant variety (Criptonio). The association of mycorrhizae with *Tagetes* and the resistant variety (3 combined levers) also led to a significant reduction in pest abundance, in the same way as *Tagetes* without mycorrhizae. No competition effect between the cover plant and the peppers was observed (no significant impact of the levers on the growth of the pepper seedlings). In conclusion, the combination of the 3 levers is a good alternative to pesticides against aerial pests in terms of durability and pest reduction effect. It does however, include additional tasks to be carried out for farmers, for instance the establishment of *Tagetes* in addition to the interest crop plants of the farmer, the addition of mycorrhizae on each *Tagetes* as well as the uprooting of the *Tagetes* after their effects have been achieved. These additional tasks incur financial costs as well as additional energy costs for the farmer.

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1. Introduction

Crop plants can be attacked by a diversity of pests and pathogens in agriculture [1]. In response, farmers tend to apply various chemical pesticides, in massive quantities, which leads to several issues such as impacts on human health [2] as well as on the environment and biodiversity [3], along with the emergence of resistance in the targeted pests due to the massive application of pesticides, ultimately rendering pesticide action ineffective [4]. All these pesticide-related issues have led to the banning of a multitude of pesticides in France [5] and Europe [6], forcing farmers to find themselves with limited means to eliminate pests for the protection of their crops. This situation is not sustainable for farmers. It’s necessary to find alternative methods to pesticides and to enable farmers to manage their pest populations, thus continuing to protect their crops.

To achieve this, our team is turning to IPM (Integrated Pest Management) strategies [7][8], combining the different agroecological levers we can use for a more efficient and sustainable pest management, such as the use of resistant varieties, biocontrol plants, biostimulant agents, auxiliaries, organic cultivation methods… These levers taken separately do not have the same effectiveness as chemical pesticides and, for resistant varieties for example, they are not very sustainable over time [9]. This is mainly due to the fact that agroecological levers have a less powerful mode of action and/or are highly dependent on the environment compared to pesticides. A typical example is the use of resistant varieties, containing one or more resistance genes against specific pathogens and pests. These resistance genes can be overcome over time by the evolution and adaptation of these pests and pathogens, and then become no more functional against them.[9]. On the other hand, if we try to combine several pest regulate levers, we can obtain effects that can reduce pests below the economic threshold while being more sustainable and environmentally friendly[10][11][12].

The research project CapZeroPhyto (ANR, France) propose a combination of 3 levers to regulate soil borne pests targeting, particularly root-knot nematodes (*Meloidogyne spp.*), causing significant direct damages to a variety of crops and able to transmit viruses or creating entry points for pathogens. This combination consists of :

(i) **a resistance lever**, relying on the use of resistant varieties that express resistance genes. For instance, 2 resistance genes were identified in pepper against *Meloidogyne spp.*, *Me* and *N* [13]. They code for resistance proteins R of the NBS-LRR type (nucleotide-binding site – leucine-rich repeat). These proteins have an intracellular receptor function, able to recognize pathogen effectors (molecules produced by the nematode to manipulate the plant for example) and activating the plant's immune signaling pathways in response, leading to the synthesis of several types of defense molecules (Phytoalexins, reactive oxygen species (ROS),...) [13]. The expression of these 2 genes have the particularity of limiting the penetration of root-knot nematodes, but also disrupting the formation of giant cells at the root level, suppressing their reproduction and thus reducing the production of galls and egg masses. These phenomena are mainly due to a hypersensitive reaction at the level of the attacked roots, which allows for resistance to root-knot nematodes, specifically against: *M. incognita, M. javanica*, and *M. arenaria* [14].

(ii) **a biocontrol plant,** consisting in a plant added in the agrosystem to provide the pest regulation service. For instance, *Tagetes erecta* (Asteraceae) has several effects against nematodes, including the secretion of root exudates and the production of toxic compounds inside the root, that have demonstrated ovicidal and repellent effects or larvicidal effects for respectively [15]. In addition, the volatile organic compounds (VOC) produced by *Tagetes* can activate defense genes in the neighbour plant (tested on tomato), notably the *CSL* gene (*Cysteine sulfoxide lyase*), *EIN3 (Ethylene insensitive 3*), and *TPS (Terpene synthase)*, which have shown to be involved in the resistance against root-knot nematodes [16]. In the field, all those effects seem efficient only on the first 2 cycles of nematodes. And the production of toxic exudates against nématodes is more efficient before the appearance of the flower stage, after that, exudates are no longer produced and the effect of the *Tagetes* no longer works against nematodes [17].

(iii) **a biostimulant agent** which improves plant health, growth and defense.

The *Tagetes* have an additional feature, they are mycorrhizal plants [18]. It allows an associated crop plant to be mycorrhized much more efficiently and faster. It has been shown that peppers take on average 6 weeks to start mycorrhization from spores compared to 3 to 4 weeks for the transmission of mycorrhizae from *Tagetes* to peppers [19]. The mycorrhizal spores take longer to germinate, settle in, and then create a network of hyphae in the plant than if the hyphae were already on site and associated with the roots of the *Tagetes* and then directly transmitted to the associated plant. Yet, arbuscular mycorrhizae reduce nematode penetration by developing precisely where nematodes tend to penetrate, creating a physical barrier at the root level [19]. Mycorrhizae also have the ability to reduce nematode reproduction by decreasing the formation of galls and egg masses [20], and they also allow the plant to be more tolerant against nematodes due to the improvement of plant health and growth [20].

The hypothesis behind the combination of these 3 levers simultaneously or in sequence is an addition or multiplication of these individual effects provided by each lever in order to achieve better efficiency and a stronger effect in controlling nematodes. However, we know that in crop field, nematodes are not the only pests that can cause significant damages on crops, as some aerial pests can also do, and we might wonder if a multi-lever combination could be multi-targeted rather than targeting just one type of pest. It has already been demonstrated in the literature, as a matter of fact that our 3 levers described above have also shown effect on aerial pests.

(i) A resistance variety targeted one pest could affect other pests. For instance, in tomatoes, the resistance gene *Mi*, coding for an NBS-LRR type protein acts as an intracellular receptor. It can recognize one or more effectors produced by the nematode and provides resistance against root-knot nematodes [21]. But it has also been shown to provide resistance against certain sap-sucking insects such as the potato aphid (*Macrosiphum euphorbiae*) [22] and whiteflies (*Bemisia tabaci*) [23] due to a hypersensitive reaction at the leaf level and changes in defense hormone signaling pathways (activation of *Jasmonic acid*, *Salicylic acid and Ethylene*). These genes allow the reduction of aerial pest survival but also their reproduction rate. Since the Me and N resistance genes in pepper code for the same type of proteins as the Mi resistance gene in tomatoes, we can suppose that they act in the same way against aerial pests.

(ii) Multi-pest biocontrol plant [17], *Tagetes* plant species also have the ability to repel certain airborne pests such as whiteflies [17], aphids [24], and bugs (e.g., *Lygus hesperus*) [25] due to the emission of VOCs. They can also create a physical barrier that can hide crop plants, thereby preventing the establishment of these pests [17]. Thanks to these effects, *Tagetes* thus help to block the settlement, oviposition and the reduction of airborne pest populations on crop plants [17].

(iii) Mycorrhizae also have effects against certain aerial pests (*B. tabaci* [26], *Helicoverpa armigera, Aphis spp., Spodoptera spp*… [27]), and pathogens *(Phytophtora capsici…)* [28]) such as the stimulation of the expression of defense genes and proteins in plant host, the increase in the synthesis of plant defense compounds, the increase in the attraction of parasitoids and other predators of herbivores, and the induction of a priming immune effect. All these effects make plants less palatable and/or more toxic to aerial pests or help limit the development of pathogens [27].

2. Objectives

This context leads us to the following question : Can this combination of 3 levers, initially designed to regulate nematodes, also regulate aerial pests?

To try to answer this general question, several sub-questions are raised:

1. Taken separately, can these levers have an effect against airborne pests in field conditions?
2. Can the combination of 2 or 3 levers have additive, synergistic, or even antagonistic effects on aerial pest regulation?

3. Does this combination of 3 levers have an effect on the growth of crop plants and on the yield? (Will there be a risk of plant-plant competition?)

To test the effect of these combined levers, a farming system experiment is conducted in the field, in the tunnels of a farmer, specifically on pepper crop plants. Different modalities are set up based on these 3 levers in order to compare them alone or in combination.

3. Materials and methods

3.1 Location and characteristics of the farming system

The farming system experiment was conducted in a farm in Saint Jeannet (PACA), in the southeast of France **(Figure 1 annex)**, in spring 2025. This site benefits from a Mediterranean climate, characterized by mild winters and hot, dry summers, with an average annual temperature of 15°C and an annual rainfall of 826 mm. In this experiment, we are working on pepper plants, a Mediterranean crop, which is the main product for the farmer of our experimental field.
This site was chosen due to the strong presence of nematodes in the soil, the main pests targeted in this experiment. This high infestation is mainly due to the fact that the farmer never rotates crops in his tunnels : peppers grow from April to November every year without any other crop in between. In addition, their peppers are only treated conventionally, but the tunnels considered in this experiment were managed according to organic standards, without the use of pesticides in the experimental area.

3.2. Biological materials

3.2.1. Pepper crop varieties

For this farming system experiment, the targeted crop plant is the pepper *Capsicum annuum* (Solanaceae) and specifically 2 different varieties of peppers: Almuden, provided by Syngenta, which corresponds to a sensible variety to root-knot nematodes, and Cryptonio, provided by RijkZwaan, representing the resistant variety to root-knot nematodes. These 2 varieties of peppers have been grown from seeds to seedlings in a greenhouse from late February until mid-April, then planted around the end of April **(Figure 2 annex)** in a tunnel system consisting of a loamy soil with low phosphorus content. They were watered twice a day for a total duration of 60 minutes, by an automatic irrigation system. In total, 420 pepper plants were planted in 2 tunnels, representing 210 plants of each variety. The peppers are covered with plastic mulch at the base of each plant.

3.2.2 Biocontrol plant : Tagetes erecta

The second lever is the biocontrol plant, *Tagetes erecta* (Asteraceae), precisely the CrackerJack variety provided by Lidea. This species was selected from among many other biocontrol plants based on the results of previous experiments aimed at testing the effect of certain biocontrol plants (including *Tagetes*) on soil and aerial pests [17]. *Tagetes erecta* is non-host for *Meloidogyne spp*. and it has shown a larvicidal, ovicidal and nematode-repellent effects, which led to a reduction of nematode penetration, adult nematodes, and the number of galls [17].

Tagetes were grown for seeds to seedlings in small pots with sandy loam soil. Then planted in co-culture one : one with the peppers. This choice is based on previous experience demonstrating that a single *Tagetes erecta* is sufficient to regulate nematode population in the soil around a single pepper, for the first 2 cycles of nematodes [17]. This one-to-one co-culture also helps to reduce competition for resources, light, and space between *Tagetes* and peppers. When *Tagetes* grew faster than peppers, they were regularly topped to avoid them to be taller than pepper plants.

3.2.3 Biostimulant agent: Arbuscular mycorrhizae (AMF)

The third lever used in this study is the AMF, specifically the species *Rhizophagus irregularis* formerly known as *Glomus intraradices* (Glomeraceae). These AMF are in the form of powder containing conidiospores mixed with *Bacillus pumilus* bacteria (AGTIV® AMPLIFY™ product provided by Premier Tech). The combination of the two microorganisms allows for a healthy and expanded root system that absorbs more nutrients and water thanks to the mycorrhizae and the formation of a biofilm on the root due to the bacteria.

After *Tagetes* reached an ideal size with a well-developed root system, half of the *Tagetes* were mycorrhized with this product, about 15 days before their planting in the tunnel. In total, 264 *Tagetes* were planted in the tunnels in the associated modalities, of which 132 were mycorrhized.

The powder was diluted in water at a concentration of 6g/60ml. One ml of this solution was applied per *Tagetes* plant using pipettes, representing approximately 1 g of *R. irregularis* and 500 mg of *Bacillus pumilus*. To apply the solution, 4 small holes are made in the soil around *Tagetes* seedling beforehand, and the 1 ml is distributed evenly in these 4 holes in order to promote uniform mycorrhization. **(Figure 3 annex)**

3.3. Experimental set-up

The experiment is conducted under 2 agricultural tunnels, next to each other, each measuring 30 meters long and 3.70 meters wide. Inside these tunnels, 3 rows (A, B, and C) each containing 70 peppers planted on line are distributed into 6 different modalities **(Figure 1)**:

- Sensitive peppers alone (Almuden)
- Almuden with associated *Tagetes* (Almuden + *Tagetes*)
- Almuden + *Tagetes* and mycorrhizae (Almuden + *Tagetes* + Myco)
- Resistant peppers alone (Cryptonio)
- Cryptonio + *Tagetes* (Cryptonio + *Tagetes*)
- Cryptonio + *Tagetes* + mycorrhizae (Cryptonio + *Tagetes* + Myco)

Those 6 modalities are distributed differently in the 2 tunnels **(Figure 1)**. Each modality is applied over a distance of 4.5 m long and includes 11 peppers each. The peppers were planted every 40 cm, and for the modalities containing *Tagetes*, they were planted in the middle of 2 peppers at a distance of 20 cm apart and in a staggered pattern. At the beginning and end of each row, 2 peppers were placed as buffer peppers.

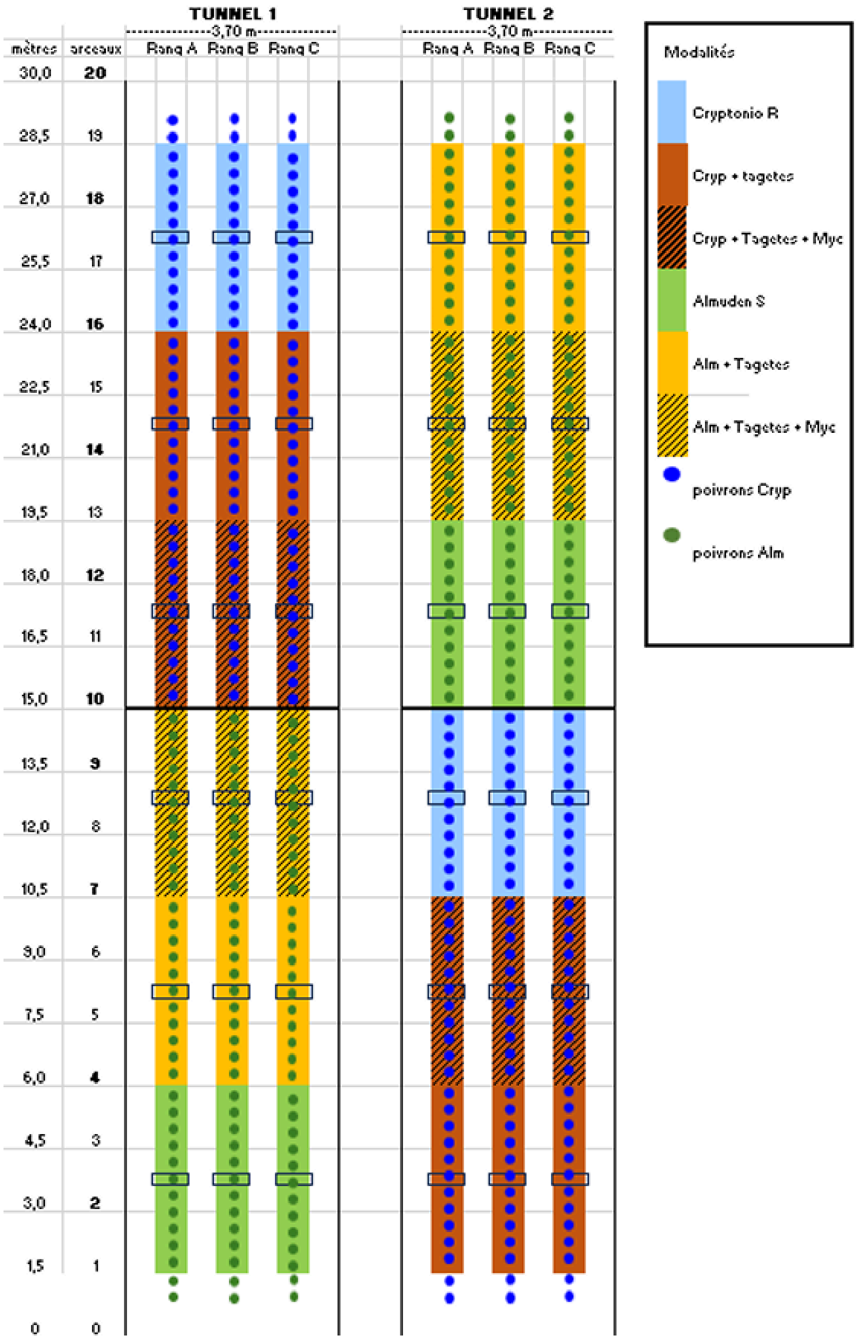

Figure 1: Scheme of the experimental setup

3.4 Data recorded

The CapZeroPhyto project targets the effects of these 3 levers on nematode infestations, pepper yield and aerial pest regulation. My work as intern in this project was to record the aerial pest and plant data. Nematode infestation was recorded by another team, they are not presented here.

3.4.1 Counting and identification of arthropods

The abundance (e.g. number of individuals) and the taxonomic richness (e.g. number of taxa) of arthropods (including aerial pests, natural enemies, and other categories) was carried out through counting made by eye in the field once every 10/15 days. The counting is specifically conducted on the 5 central pepper plants among the 11 peppers of each modality.
Arthropods were also assessed on *Tagetes* associated with peppers for the modalities that possess them. The counts are preferably carried out on clear weather to avoid influencing the dynamics of insect populations, and preferably in the morning between 7 AM and 12 AM to avoid the strong heat.
Data have been collected for 7 time sessions: May 13 and 14 (corresponding to days 134 and 135 of the year), May 20th and 21st (days 141 and 142), June 2nd and 3rd (days 154 and 155), 12th and 13th June (days 163 and 164), 24th and 25th June (days 175 and 176), 8 and 9 July (days 189 and 190) and 22nd July (day 203).

3.4.2 Plant growth and crop yield

A measurement of the height of the five central pepper plants and *Tagetes* (if present) was carried out for each modality every 10–15 days. Measurements were taken from the collar to the apical meristem of the tallest stem. For *Tagetes*, stem diameter was also measured in order to estimate biovolume, considering the plant as a cylindrical volume. Plant phenology was recorded throughout the experiment (flowering, fruiting).\
When pepper fruits reached maturity, harvests were conducted separately for each modality, following the same commercial standards applied by the farmer in the other tunnels. Fruits were counted and classified as marketable or non-marketable, and marketable fruits were weighed for each modality. These data were used to assess yield components, including the number of fruits per plant and the average weight of marketable fruits.

3.4.3 Statistical analyses of the data

To answer objective 1 and 2, we compared the abundance and taxonomic richness of pests between the 6 different modalities. For objective 3, we used the same statistical analyses with the same model, but on pepper plant growth data and the number of fruits.

The data obtained are temporal series of data, counting through a time period. All of this data will therefore be analyzed using the same type of model : GLMM TMB, an adapted model that allows for the handling of temporal data and incorporates certain disturbing effects as random effects (such as tunnels, the plant identifier and the positioning of modalities within the tunnel that may influence our data).

4. Results

4.1 Arthropods taxonomic richness

An initial bibliographic research was conducted to establish a preliminary list of arthropod species expected to be found in the field, including pepper pests. This list allows to better know and identify the main pests of peppers once on the field. We have listed 16 species belonging to 5 different orders **(Figure 4 annex)**.

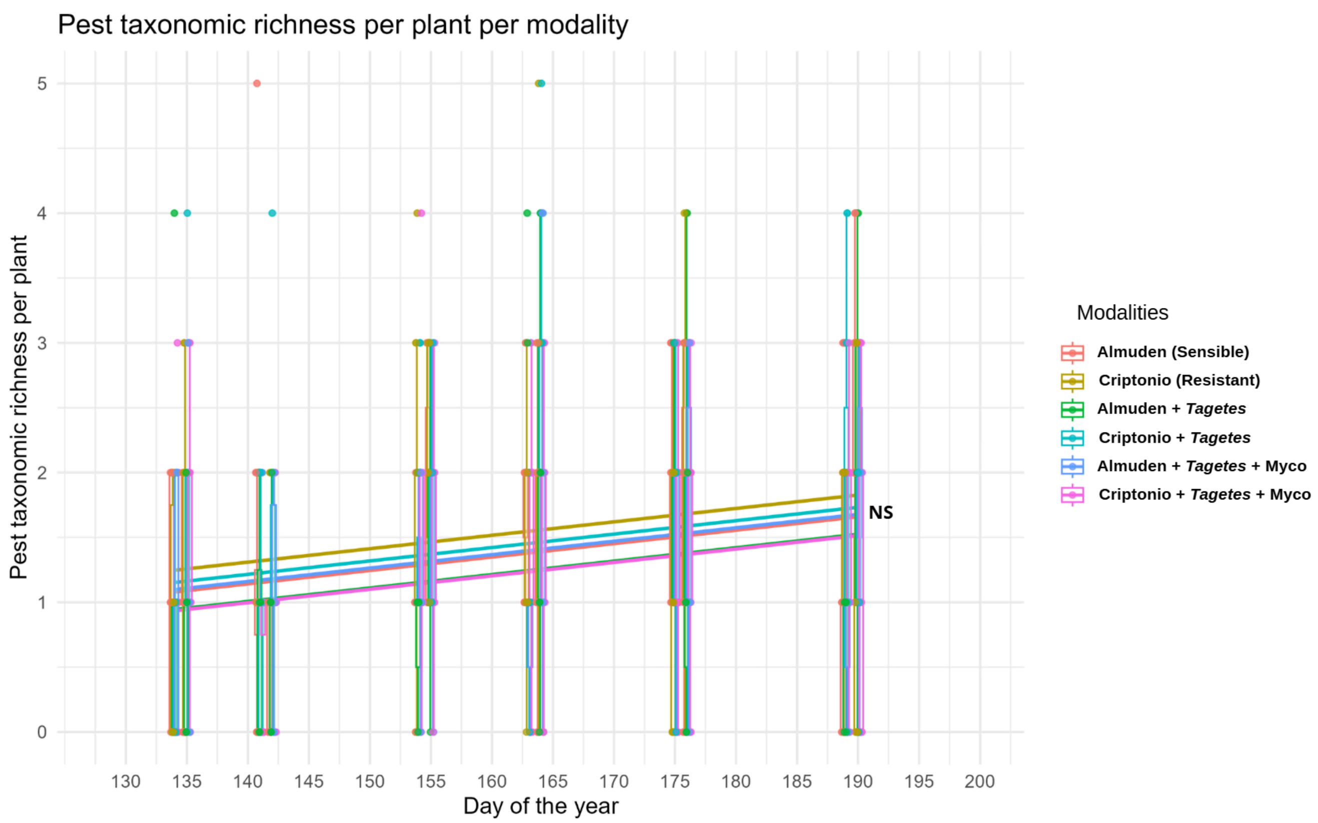

Figure 2 : Taxonomic richness of pepper pest per plant per modality

The taxonomic richness of pepper pests per plant varies slightly but not significantly ($p>0.05$) between the 6 modalities, throughout the study period. Overall, the values remain low, mostly between 0 and 3 taxa per plant. The curves corresponding to the 6 modalities significantly overlap, indicating similar dynamics of pest taxonomic richness over time regardless of the modality. But we can still observe a trend of higher taxonomic richness in the modality possessing the resistant variety Cryptonio compared to the other modalities.

4.2. Pest abundance

One of the main objectives is to see if the 3 levers, resistant variety, biocontrol plant (*Tagetes erecta*), and biostimulant agent (Mycorrhiza) alone or in combination can have effects on aerial pests. To do so, we will compare the results obtained from the GLMM TMB on pest abundance across the different modalities.

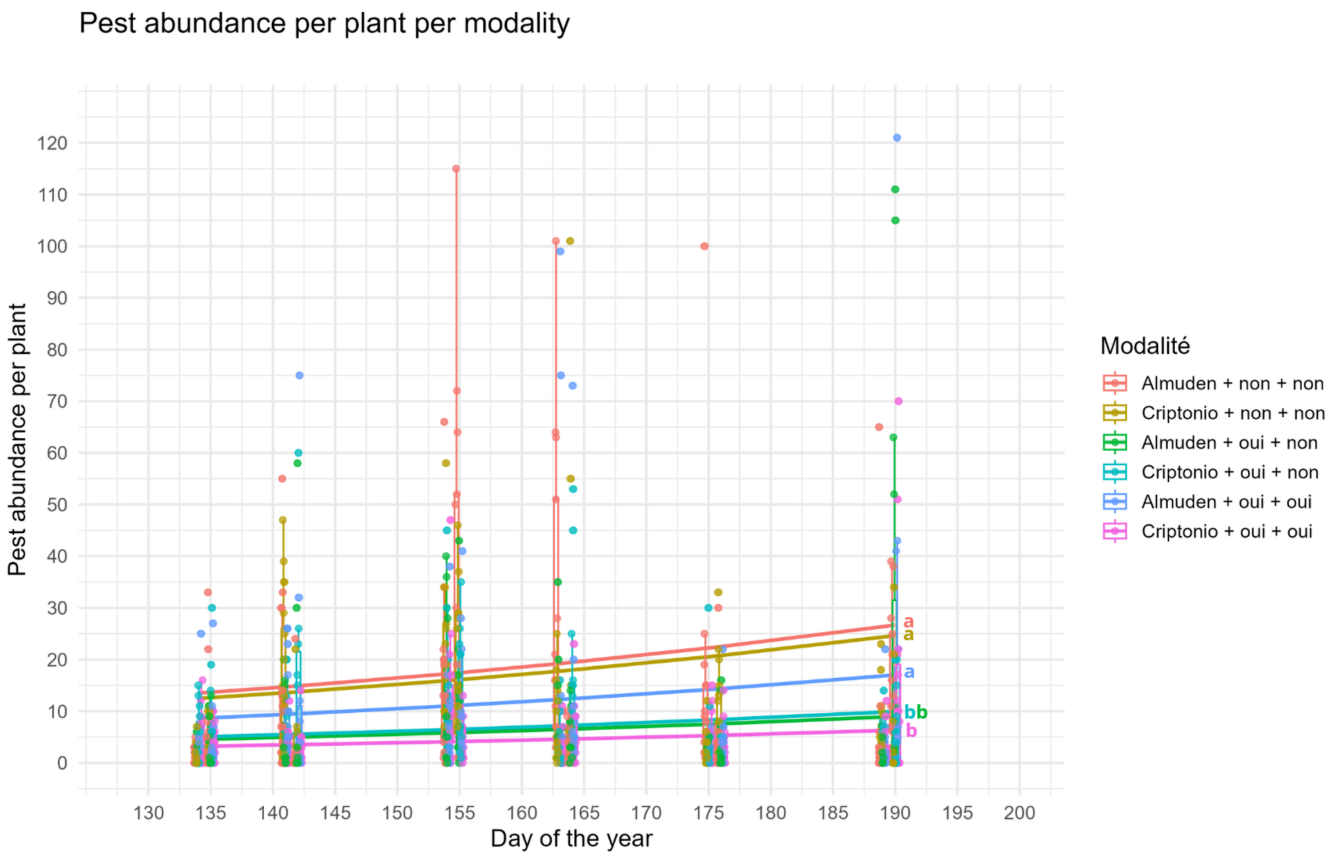

Figure 2 : Graph of pest abundance according to different modalities

For the resistance lever alone, we can observe that there is no significant difference in the pest abundance compared to the susceptible variety (Almuden) alone **(Figure 2)**. The resistant variety (Criptonio) alone is not sufficiently effective to reduce the number of aerial pests on pepper plants . But if we add *Tagetes*, we can see a significant decrease in the number of aerial pests present on pepper plants (p<0.001), going from 25 to 5 pests per plant on average. And this, whether it's associated with Almuden or with Criptonio.

When *Tagetes* are associated with Mycorhiza and placed with Almuden peppers, they decreased, but not significantly, the number of pests compared to Almuden alone, going from 25 to 17 pests per plant on average. If we compare now with Almuden + *Tagetes* without mycorrhiza, we see that mycorrhized *Tagetes* with Almuden peppers lead to a significant increase of the pests number, rising from 5 to 15 pests on average per plant.

This is not the case when mycorrhizal *Tagetes* are combined with Criptonio peppers. Compared to Criptonio peppers alone, the addition of *Tagetes* and Mycorriza with this variety lead to a significant decrease of pest number, rising from 20 to 4 pests on average per plant. But compared to Criptonio + *Tagetes*, we can observe almost the same pest abundance per plant.

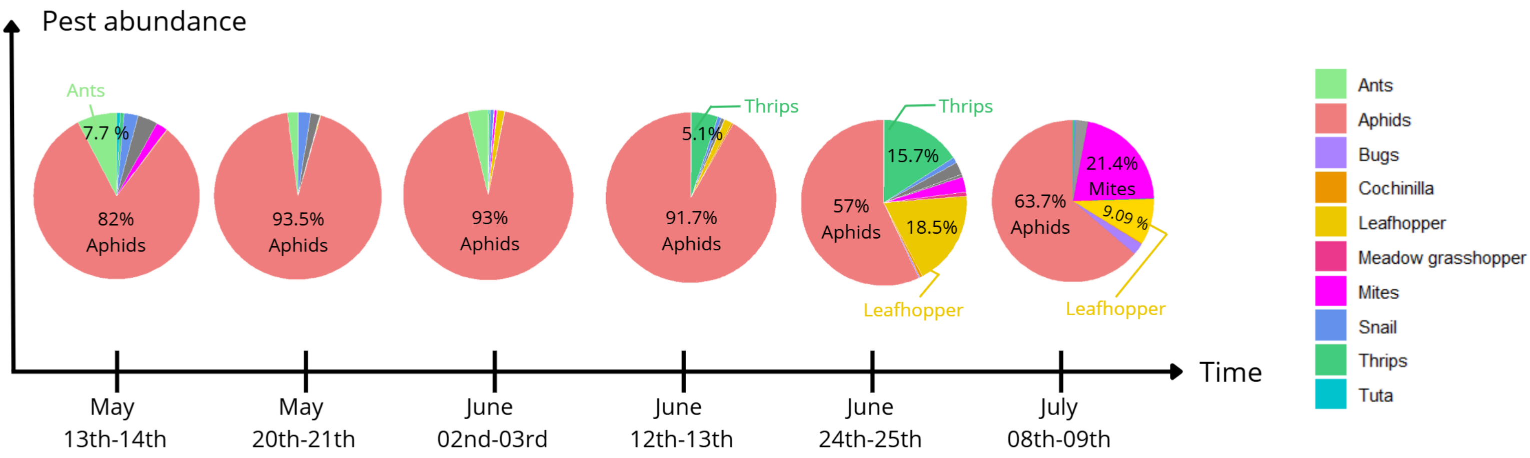

Figure 3 : Evolution of pest abundance over time

It's interesting to observe the evolution of the different types of aerial pests as well as their diversity during the counts (Figure 3). As we can see, the most predominant aerial pests found during our counts are aphids. From the second part of June, the thrips begin to appear and increase until the end of June. Then come the leafhoppers and around the beginning of July, the mites (*Tetranychus urticae*). The composition of pest populations is never the same depending on the months.

4.3. Natural enemies abundance

When peppers are alone (Almuden and Criptonio without *Tagetes* and mycorrhiza), we can find the largest number of aerial pests compared to other modalities. And there are no significant differences in the abundance of natural enemies between these 2 varieties. We can find up to 5 natural enemies per plant there **(Figure 4)**. When *Tagetes* are added to these 2 peppers varieties, we have a significant decrease of pest number compared to Almuden and Criptonio peppers alone. Between Almuden + *Tagetes* and Criptonio + *Tagetes* we can find almost the same number of natural enemies per pepper plant. When we add mycorrhiza on *Tagetes* associated with Almuden peppers, we don't find any significant increase or decrease of natural enemies compared to all the other modalities. On the other hand, Criptonio + *Tagetes* + Mycorrhiza have significantly fewer natural enemies found on pepper plants than on Criptonio or Almuden alone.

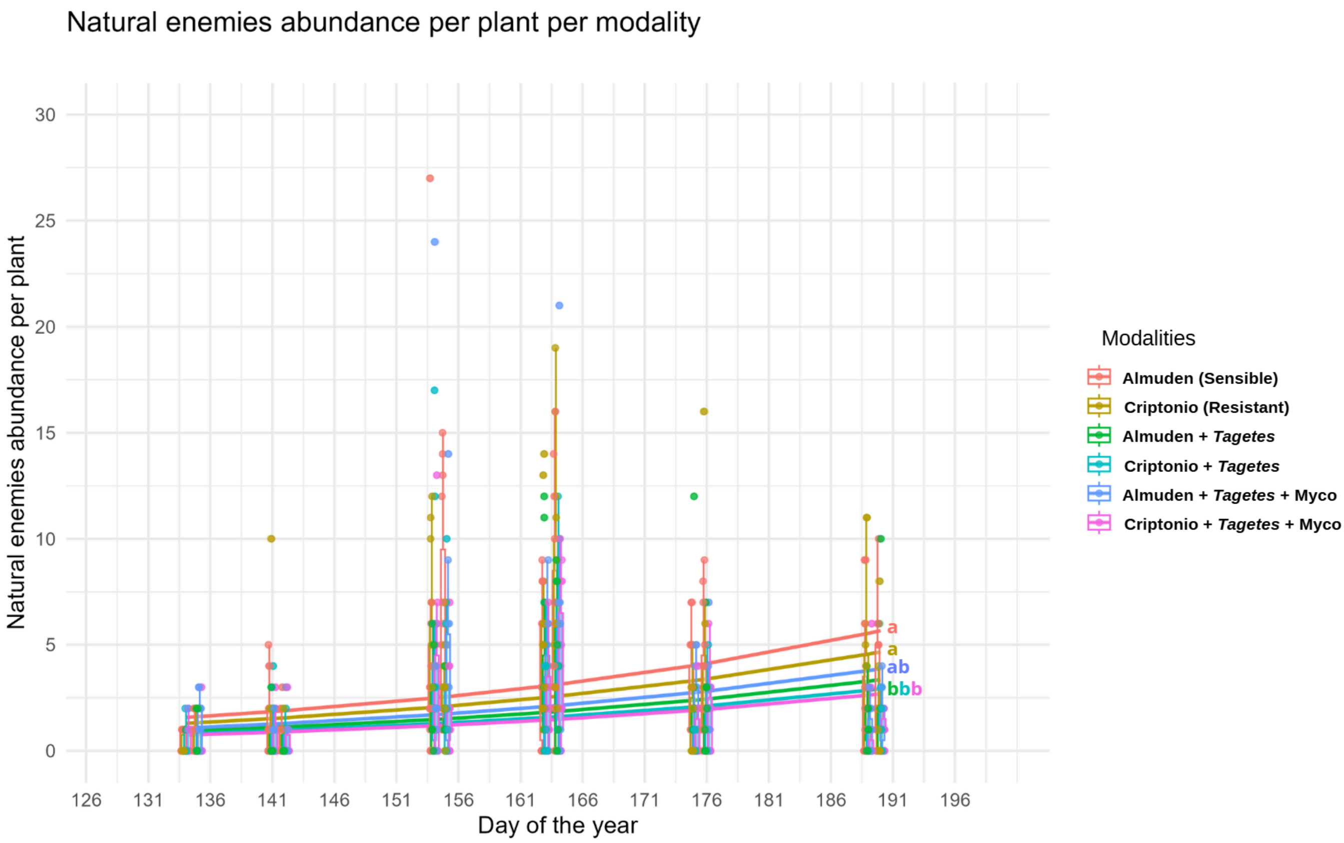

Figure 4 : Graph of natural enemies abundance according to different modalities

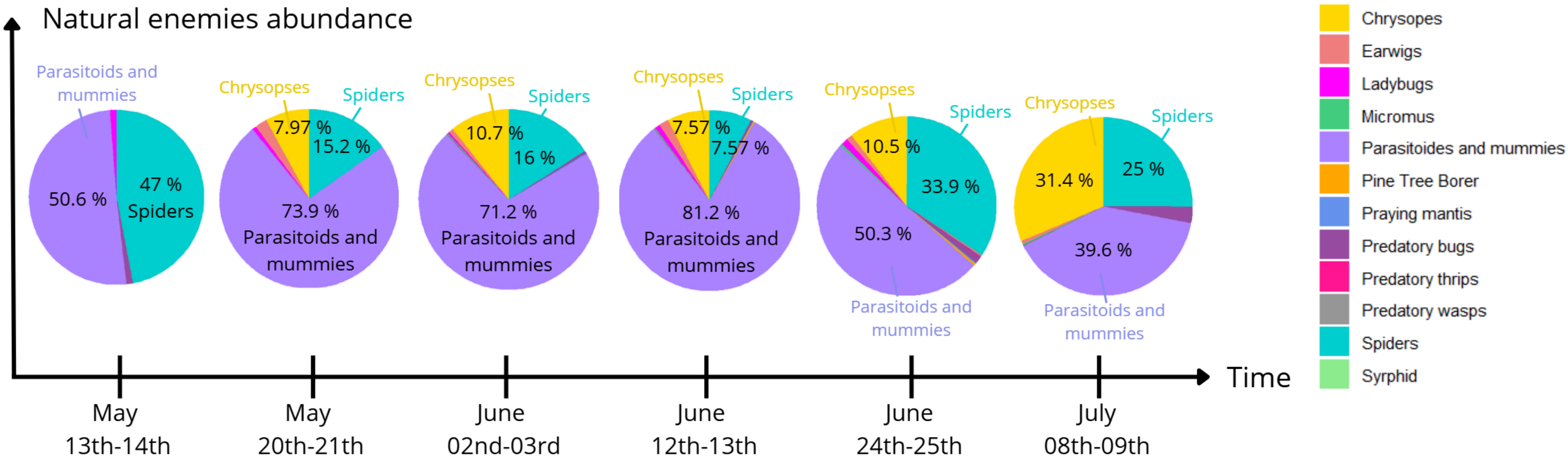

Figure 5 : Evolution of natural enemies abundance over time

By analyzing the composition of natural enemies observed during the counts, we can notice that the main natural enemies observed are mummies and parasitoids, spiders, as well as lacewings **(Figure 5)**.

4.4 Plant growth

In the field, all the modalities applied with pepper plants had no significant effect on the growth of the pepper plants **(Figure 6)**

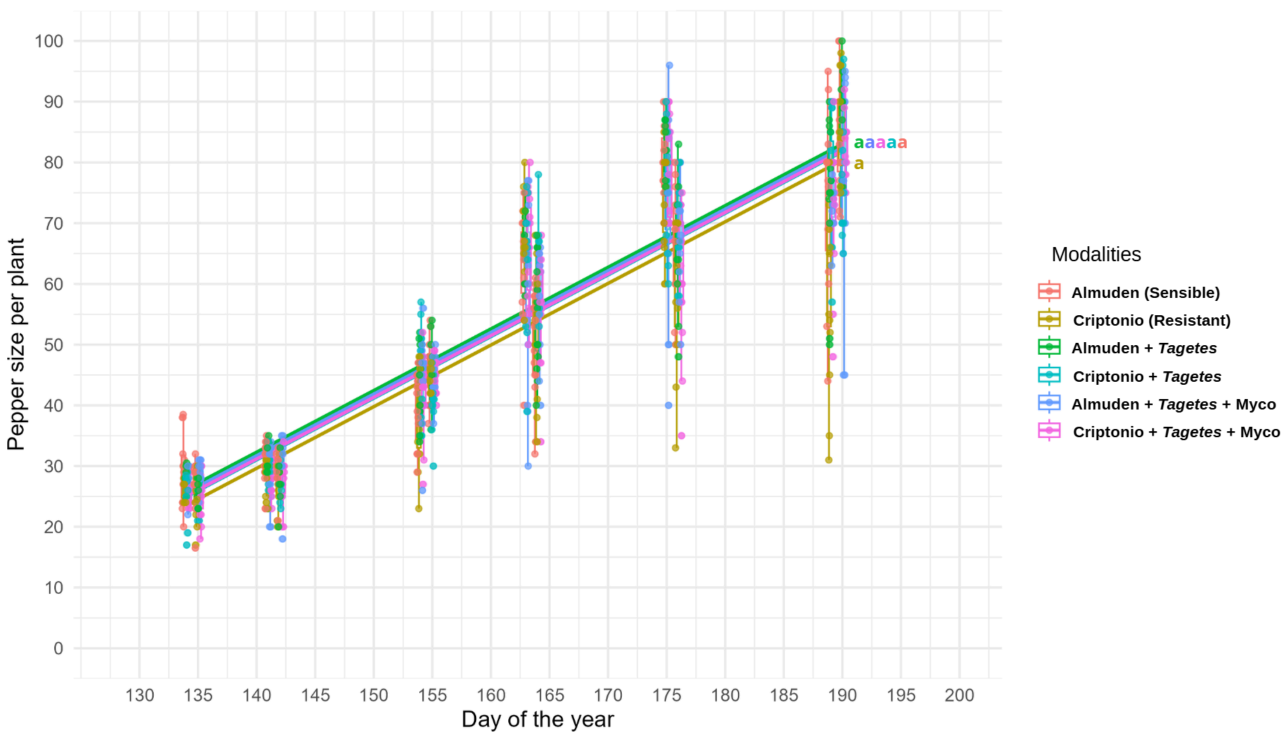

Figure 6 : Graph of pepper growth per plant per modality

To go further, we can analyse the correlation between the pepper size and that of the *Tagetes*. One of our concerns was that the *Tagetes* might enter in competition with the peppers for resources, light, and space. To find out, we conducted a correlation between the pepper size and that of the *Tagetes* **(Figure 7)**. We can see on the graph that the size of the peppers increases when the size of the *Tagetes* also increases. This indicate to us that the *Tagetes* would force the pepper plants to grow and push higher to reach or exceed the height of the *Tagetes* and thus not miss out on space or light. We can therefore highlight a competition between the *Tagetes* plants and the pepper plants.

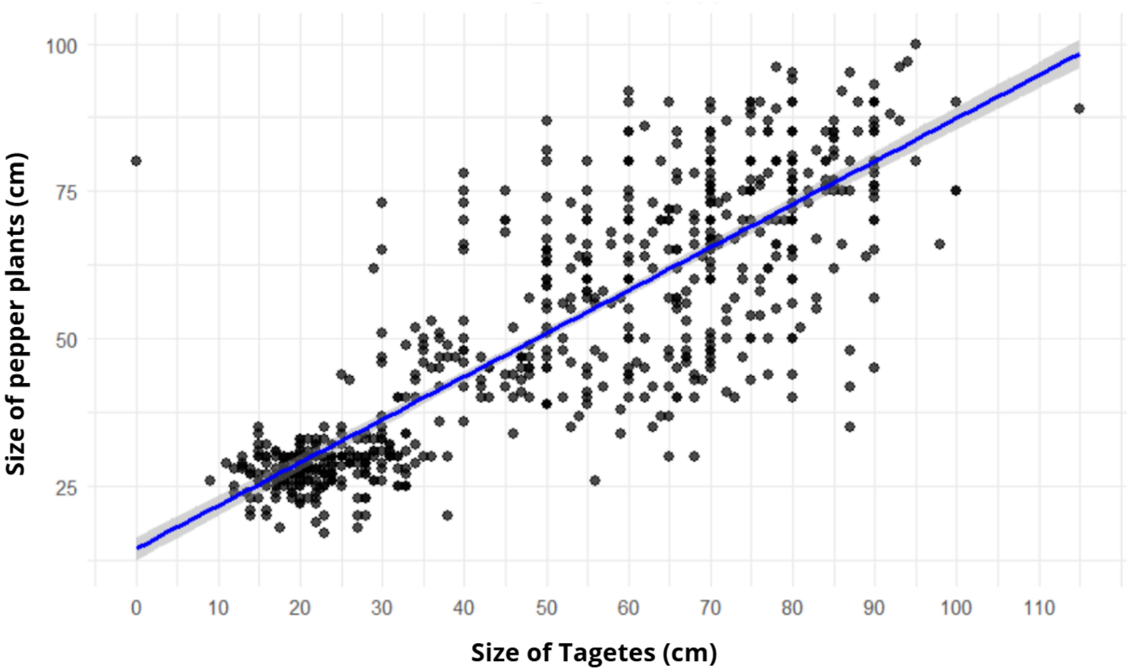

Figure 7 : Graph of the correlation between the size of Peppers and *Tagetes.*

The graph of fruits number produced per pepper plant across the 6 modalities showed a more significant fruit production under the Almuden and Criptonio modalities alone than under the modalities with mycorrhizal or non-mycorrhizal *Tagetes*, associated with pepper plants **(Figure 8)**.

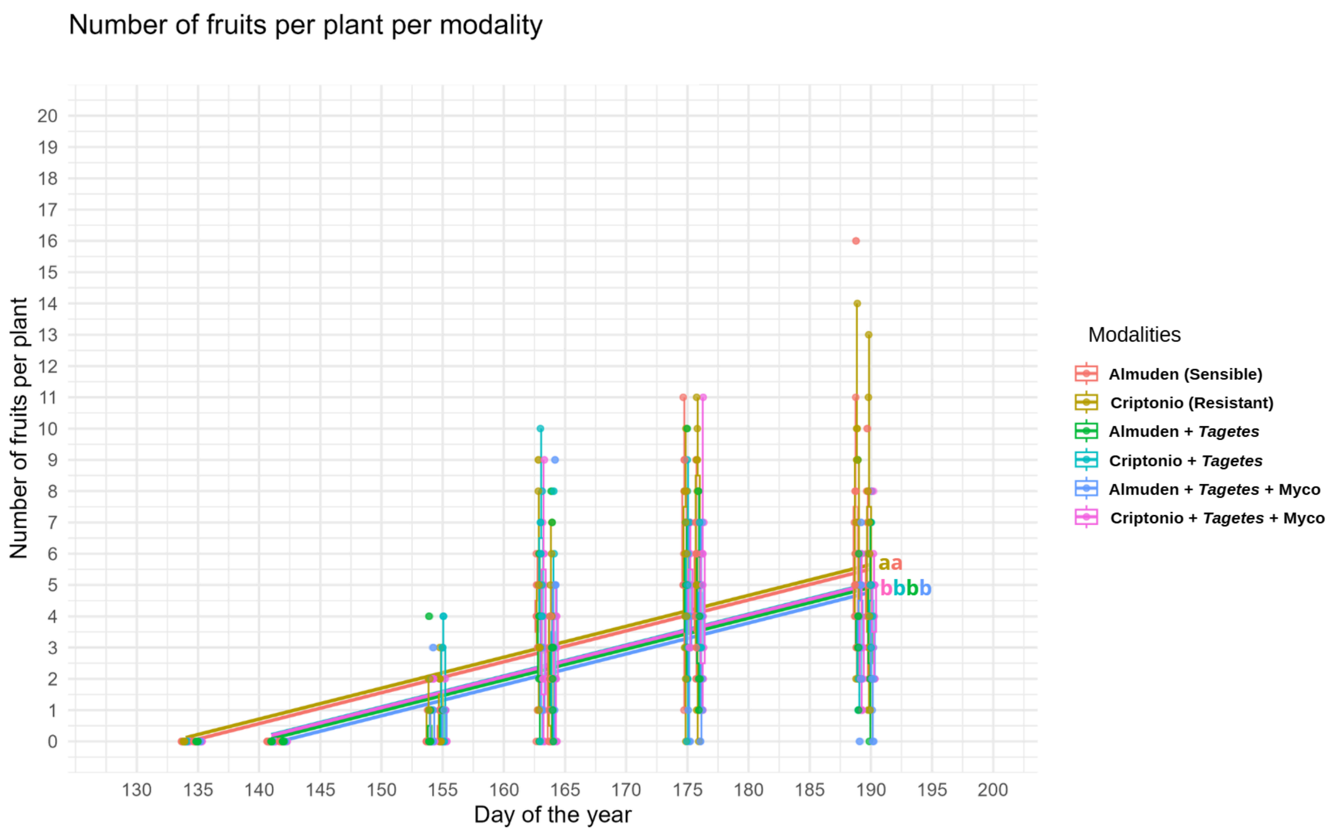

Figure 8 : Graph of the number of fruit per plant per modality

4.5 Yield assessment

Following the counts, the yield was assessed based on the number of marketable and non-marketable peppers, as well as the average weight of marketable peppers according to the modality.

The number of marketable fruits per plant varies significantly depending on the experimental conditions and over time **(Figure 9)**. Overall, pepper plants grown alone show a higher yield than those in the modalities with *Tagetes* and mycorrhization, with an average increase of about 19% in the number of fruits per plant.

Regardless of the modalities considered, a gradual decrease in the number of fruits is observed over the course of the year. This temporal trend is similar across the modalities, indicating a strong effect of time on yield, independent of the agronomic strategies tested.

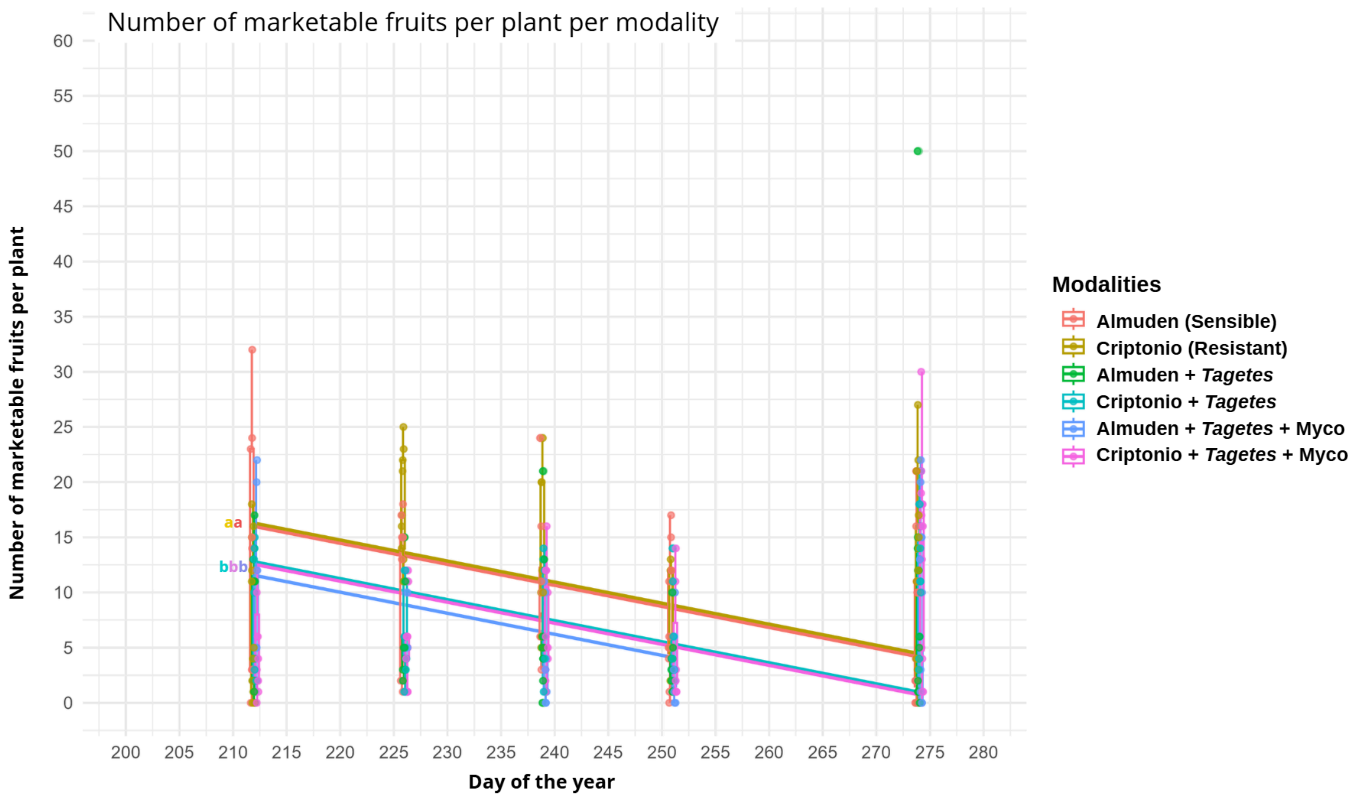

Figure 9 : Graph of the number of marketable fruits per plant per modality

The number of pepper fruits based on the modalities alone (not dependent on time) was also evaluated, as well as their obtained weight **(Figure 10)**. For pepper yield, the modalities alone produced significantly more peppers than the modalities combined with *Tagetes*. The average weight of marketable peppers per plant does not differ significantly between the studied modalities, indicating that neither the presence of *Tagetes* nor mycorrhiza affected the individual weight of the fruits produced by the pepper plants.

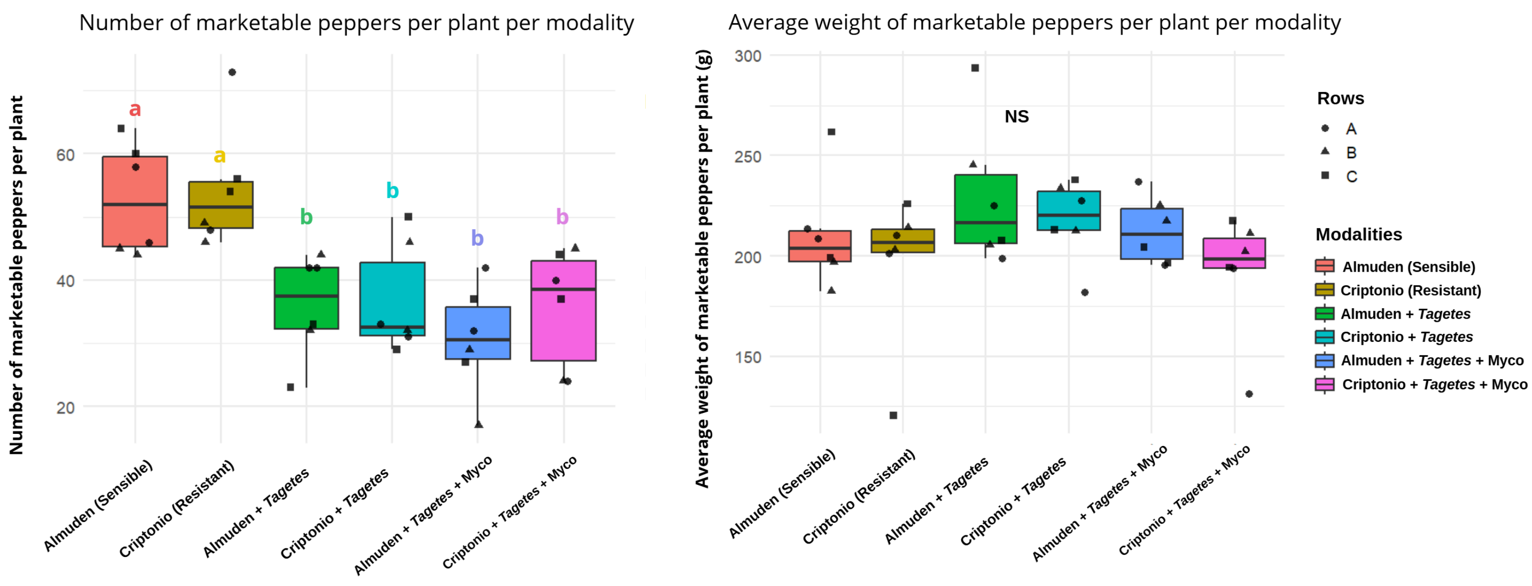

Figure 10 : Graph of the number and average weight of marketable peppers per plant per modality

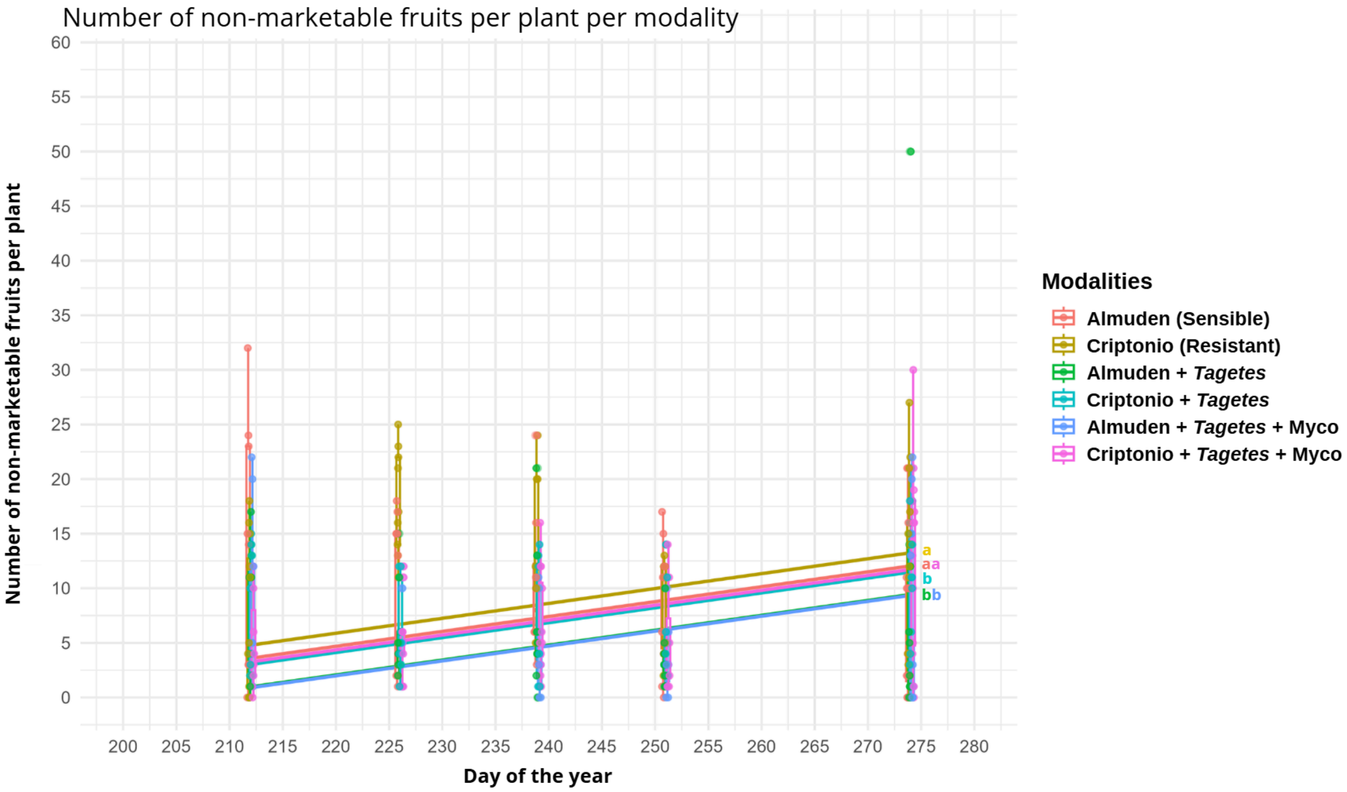

Figure 11 : Graph of the number of non-marketable fruits per plant per modality

The number of unsellable fruits per plant varies depending on the modalities and over time **(Figure 11)**. The results show that the modalities consisting solely of pepper varieties generally have a higher number of unsellable fruits compared to the modalities combining peppers with *Tagetes* and mycorrhiza, with an increase of about 23%.

An increase in the number of unsellable fruits is observed over time for all modalities. However, this increase is more pronounced in the modalities without *Tagetes* and mycorrhiza compared to modalities with*Tagetes* and mycorrhiza.

5. Discussion

The analysis of pest abundance showed that when the sensitive variety (Almuden) and the nematode-resistant variety (Criptonio) are combined with *Tagetes*, there is a significant reduction of 80% in pest abundance compared to the two varieties alone. *Tagetes* are known for their repellent effect on certain aerial pests, including aphids [17], the main pests observed during the counts, which would explain the significant decrease observed. With the addition of mycorrhizae to the roots of *Tagetes*, pest abundance for the Almuden variety significantly increased compared to Almuden + *Tagetes* alone, which is not the case for Criptonio + *Tagetes* + Myco, which has the same number of pests as Criptonio + *Tagetes* alone. Here, we can assume a variety-dependent effect of mycorrhizae, since different pepper varieties did not obtain the same result.

By looking more closely at the taxonomic richness of these pests according to the different modalities, none showed differences in this parameter.

Regarding natural enemies, we can see that the curves obtained closely resemble those obtained for pest abundance. This means that the modalities where the most pests are found are also those where the most natural enemies are present. This result seems consistent, as it is known that natural enemies are attracted to plants attacked by pests through the emission of volatile compounds (Herbivore Induced Plant Volatiles: HIPVs) and by the pests themselves through the emission of volatile compounds from their bodies and feces [30, 31]. This explains why more natural enemies are found in the modalities where the most pests are present..

We also focused on plant parameters such as growth and the number of fruits to see if these factors could affect the plant directly or indirectly, particularly in the modalities involving *Tagetes*, which can compete with associated plants. The results showed that none of the factors affected the growth of pepper plants, including those with *Tagetes*. However, the correlation test between pepper plants and *Tagetes* revealed that there is indeed competition between the two, where *Tagetes* would cause the pepper plants to grow more in order to reach the size of the *Tagetes* and avoid being dominated by them. Regarding the number of fruits, the modalities with *Tagetes* reduced the number of fruits per plant by 10% compared to the modalities without *Tagetes*. We can suppose that this is due to the mobilization of energy from the pepper plants into their growth (in order not to be overwhelmed by the *Tagetes*), rather than into fruit production. This could potentially explain the reduction in the number of fruits for peppers associated with *Tagetes*.

For the yield part, the modalities with *Tagetes* decreased the number of marketable fruits per plant. We can suggest here a competitive effect of *Tagetes* on the number of peppers. This result leads us to rethink the organization and arrangement of the *Tagetes* to limit this effect. The *Tagetes* species used here is *Tagetes erecta*, a *Tagetes* with rapid and dense growth that can create a physical barrier effect preventing certain pests from entering but also has a repellent effect on some pests that can attack pepper crops (aphids, whiteflies, etc. [17]). When co-cultivated with pepper plants, these *Tagetes* species effectively fulfilled its protective role but caused a slight competition with the pepper plants. Another species of *Tagetes* with the same characteristics as *Tagetes erecta* could be more suitable; this is *Tagetes patula*, a smaller *Tagetes* than *Tagetes erecta*, which can easily be placed in co-culture and could potentially avoid competition with pepper plants, particularly in fruit production. Therefore, *Tagetes erecta* can be placed around the tunnel or plot containing the pepper plants to maintain this barrier and repellent effect around the plot, reinforced by the presence of *Tagetes patula* in co-culture with the pepper plants.

For unsellable peppers obtained based on the different modalities, the results show that the modalities consisting solely of peppers have a higher number of unsellable fruits than those combining peppers with *Tagetes* and mycorrhizae. This trend suggests that the integration of *Tagetes* and mycorrhizae could help improve fruit quality by reducing the proportion of non-marketable fruits.

The results for the average weight of marketable peppers indicate that it does not differ significantly between the studied modalities. This suggests that neither the presence of *Tagetes* nor mycorrhizae influenced the size of the fruits produced by the pepper plants under the trial conditions. This result can be explained by the fact that fruit weight is strongly determined by genetic factors specific to the pepper varieties, as well as by general environmental conditions (water, fertilization, climate) [32], which were similar across the modalities. The tested agronomic strategies therefore appear to affect more the number or quality of the fruits (marketable or unmarketable) than their individual development.

6. Conclusion and perspectives

This study was conducted in the context of the agroecological transition, which aims to reduce reliance on synthetic pesticides while maintaining effective crop protection. The objective was to evaluate, under field conditions, whether a combination of three agronomic levers : a nematode-resistant pepper variety, a biocontrol plant (Tagetes erecta), and arbuscular mycorrhizal fungi, initially developed to manage soil-borne pests, could also contribute to the regulation of aerial pests in pepper crops.

The results show that the levers considered individually had limited effects on aerial pest regulation. In contrast, the association of pepper plants with Tagetes erecta led to a strong and significant reduction in aerial pest abundance, regardless of the pepper variety. The combination of the three levers also resulted in a significant decrease in pest abundance, confirming the relevance of multi-lever strategies for pest management, although no clear additional benefit compared to the pepper–Tagetes association alone was observed.

Arthropod community analyses indicated that differences between modalities mainly concerned pest abundance, while taxonomic richness remained similar across treatments. Variations in the abundance of natural enemies followed those of pest populations, reflecting a close trophic relationship between pests and their auxiliaries.

From an agronomic perspective, the tested modalities did not significantly affect pepper plant growth. However, the presence of Tagetes was associated with a reduction in the number of fruits per plant, while simultaneously decreasing the proportion of non-marketable fruits. Fruit weight was not affected by the treatments.

Overall, this work demonstrates that combining agroecological levers, particularly through the use of a biocontrol plant, represents a promising alternative to chemical pesticides for the regulation of aerial pests in pepper cropping systems. These results highlight the importance of systemic approaches in crop protection and provide perspectives for optimizing service plant use to improve both pest regulation and agronomic performance.

7. Acquired knowledge and expertise

During this internship, I was able to strengthen and broaden my knowledge in several key areas of agronomy, agroecosystem ecology, and integrated pest management. Firstly, I gained a better understanding of the interactions between plants, pests, and agricultural practices, particularly in the context of strategies for controlling pests that serve as alternatives to the systematic use of plant protection products. The experimentation on agronomic levers used in this internship: resistant varieties, companion plants (*Tagetes*), or even mycorrhizal inoculation, allowed me to concretely grasp the principles of applied agroecology.

Methodologically, I have acquired solid skills in setting up and monitoring an agronomic trial: experimental protocol, observation under uncontrolled conditions, being able to recognize and note bio-aggressors, managing experimental variability and also to work in a team, to discuss with the farmers…

I have also acquired the skills to implement advanced statistical analyses in R (GLMM TMB, pie charts…)

Acknowledgments

First of all, I would like to thank the GIS PICIég and CapZeroPhyto for the funding of my internship project.

I would like to thank with all my gratitude my internship supervisors, Anne-Violette Lavoir and Caroline Caporalino, with whom I learned a lot during this internship. Their wise advice and scientific expertise were essential for the success of this internship.

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Finally, I thank the MIB team at ISA for their warm welcome, logistical support, and the enriching exchanges I benefited from.

References

1. Dekebo A, Major pests and pest management strategies in the sweet pepper (*Capsicum annuum*). In: Yllano OB, ed. *Capsicum - Current Trends and Perspectives*. IntechOpen, London. pp. 1–18 (2022). Available from: https://www.intechopen.com/chapters/82890

2. Ali S, Ullah MI, Sajjad A, Shakeel Q and Hussain A, Environmental and health effects of pesticide residues. In: Inamuddin, Ahamed MI and Lichtfouse E, eds. *Sustainable Agriculture Reviews 48: Pesticide Occurrence, Analysis and Remediation. Vol 2 Analysis* [Internet]. Springer International Publishing, Cham. pp. 311–36 (2021). Available from: https://doi.org/10.1007/978-3-030-54719-6_8 [cited 2025 Jun 16].

3. Mahmood I, Imadi SR, Shazadi K, Gul A and Hakeem KR, Effects of pesticides on environment. In: Hakeem KR, Akhtar MS and Abdullah SNA, eds. *Plant, Soil and Microbes. Vol 1: Implications in Crop Science* [Internet]. Springer International Publishing, Cham. pp. 253–69 (2016). Available from: https://doi.org/10.1007/978-3-319-27455-3_13 [cited 2025 Jun 17].

4. Hawkins NJ, Bass C, Dixon A and Neve P, The evolutionary origins of pesticide resistance. *Biol Rev* 94:135–55 (2019).

5. République Française, Loi Labbé n° 2017-348 du 20 mars 2017. Ministère de l'Agriculture et de la Souveraineté alimentaire [Internet]. (2017) [cited 2025 Jun 16]. Available from: https://agriculture.gouv.fr/entree-en-vigueur-de-linterdiction-des-neonicotinoides-le-1er-septembre

6. European Parliament, Regulation (EC) No 1107/2009: Les substances chimiques et les pesticides. *Fiches thématiques sur l'Union européenne* [Internet]. (2024) [cited 2025 Jun 16]. Available from: https://www.europarl.europa.eu/factsheets/fr/sheet/78/les-substances-chimiques-et-les-pesticides

7. Meissle M, Romeis J and Bigler F, Bt maize and integrated pest management: a European perspective. *Pest Manag Sci* 67:1049–58 (2011).

8. Rose DC, Sutherland WJ, Barnes AP, Borthwick F, Ffoulkes C, Hall C, et al, Integrated farm management for sustainable agriculture: lessons for knowledge exchange and policy. *Land Use Policy* 81:834–42 (2019).

9. Collange B, Navarrete M, Peyre G, Mateille T and Tchamitchian M, Root-knot nematode (*Meloidogyne*) management in vegetable crop production: the

challenge of an agronomic system analysis. *Crop Prot* 30:1251–62 (2011).

10. Djian-Caporalino C, Mateille T, Bailly-Bechet M, Marteu N, Fazari A, Bautheac P, et al, Evaluating sorghums as green manure against root-knot nematodes. *Crop Prot* 122:142–50 (2019).

11. Martin T, Caractériser les leviers techniques permettant de développer des alternatives à l'usage des pesticides de synthèse. In: CIRAD, ed. *Chapitre 2*. CIRAD, Montpellier (année inconnue).

12. Turquet M, Amiraux C, Geny A, Ginez A, Naulleau C and Souriau R, FRAGASYST: 5 années de combinaison de leviers innovants pour gérer les bioagresseurs sur fraisier de printemps. *Innov Agron* 98:335–48 (2024).

13. Djian-Caporalino C, Fazari A, Arguel MJ, Vernie T, VandeCasteele C, Faure I, et al, Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome. *Theor Appl Genet* 114:473–86 (2007).

14. Bleve-Zacheo T, Bongiovanni M, Melillo MT and Castagnone-Sereno P, The pepper resistance genes *Me1* and *Me3* induce differential penetration rates and temporal sequences of root cell ultrastructural changes upon nematode infection. *Plant Sci* 133:79–90 (1998).

15. Njekete C, Caravel C, Massol F, Lavoir AV and Djian-Caporalino C, Screening and host suitability assessment of nematicidal plants for root-knot nematode control. *Nematology* [Internet]. (2025 May 30) [cited 2025 Jun 16]. Available from: https://brill.com/view/journals/nemy/27/5/article-p533_7.xml

16. Sadon AP and Gaucher M, Communication personnelle avec Djian-Caporalino (2025).

17. Njekete C, Multi-target biocontrol plants to manage multi-species attack in tomato agrosystems [Internet] [thesis]. Université Côte d'Azur, Nice (2025) [cited 2025 Jun 16]. Available from: https://theses.hal.science/tel-05109661

18. Lavoir A and Djian-Caporalino C, Les plantes de services vers de nouveaux agrosystèmes [Internet]. [cited 2025 Jun 16].

19. Rodriguez-Heredia M, Djian-Caporalino C, Ponchet M, Lapeyre L, Canaguier R, Fazari A, et al, Protective effects of mycorrhizal association in tomato and pepper against *Meloidogyne incognita* infection, and mycorrhizal networks for early mycorrhization of low mycotrophic plants. *Phytopathol Mediterr*

59:377–84 (2020).

20. Hol WHG and Cook R, An overview of arbuscular mycorrhizal fungi–nematode interactions. *Basic Appl Ecol* 6:489–503 (2005).

21. El-Sappah AH, Imran MM, El-Awady H, Yan S, Qi S, Liu J, et al, Tomato natural resistance genes in controlling the root-knot nematode. *Genes (Basel)* 10:925 (2019).

22. Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE and Williamson VM, The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proc Natl Acad Sci U S A* 95:9750–4 (1998).

23. Nombela G and Muñiz M, Host plant resistance for the management of *Bemisia tabaci*: a multi-crop survey with emphasis on tomato. In: Stansly PA and Naranjo SE, eds. *Bemisia: Bionomics and Management of a Global Pest* [Internet]. Springer Netherlands, Dordrecht. pp. 357–83 (2010) [cited 2025 Jun 16]. Available from: https://doi.org/10.1007/978-90-481-2460-2_14

24. Dardouri T, Gautier H, Costagliola G and Gomez L, How French marigold (*Tagetes patula* L.) volatiles can affect the performance of green peach aphid. *ResearchGate* [Internet]. [cited 2025 Jun 16]. Available from: https://www.researchgate.net/publication/323943393_How_French_marigold_Tagetes_patula_L_volatiles_can_affect_the_performance_of_green_peach_aphid

25. Fabrick JA, Yool AJ and Spurgeon DW, Insecticidal activity of marigold *Tagetes patula* plants and foliar extracts against the hemipteran pests, *Lygus hesperus* and *Bemisia tabaci*. *PLoS One* 15:e0233511 (2020).

26. Shafiei F, Shahidi-Noghabi S, Sedaghati E and Smagghe G, Arbuscular mycorrhizal fungi inducing tomato plant resistance and its role in control of *Bemisia tabaci* under greenhouse conditions. *Neotrop Entomol* 53:424–38 (2024).

27. Selvaraj A and Thangavel K, Arbuscular mycorrhizal fungi: potential plant protective agent against herbivorous insects and its importance in sustainable agriculture. In: Shrivastava N, Mahajan S and Varma A, eds. *Symbiotic Soil Microorganisms: Biology and Applications* [Internet]. Springer International Publishing, Cham. pp. 319–37 (2021) [cited 2025 Jun 16]. Available from: https://doi.org/10.1007/978-3-030-51916-2_19

28. Alejo-Iturvide F, Márquez-Lucio MA, Morales-Ramírez I, Vázquez-Garcidueñas MaS and Olalde-Portugal V, Mycorrhizal protection of chili plants challenged

by *Phytophthora capsici*. *Eur J Plant Pathol* 120:13–20 (2008).

29. Rijk Zwaan France, Graines de poivron | Demi long | CRIPTONIO RZ F1 [Internet]. [cited 2025 Jun 18]. Available from: https://www.rijkzwaan.fr/poivron/CRIPTONIO-RZ-F1-35-LR70400-prdCA10984-ctgCrops.sweet-pepper

30. Holopainen JK, Blande JD. Molecular Plant Volatile Communication. In: López-Larrea C, editor. Sensing in Nature [Internet]. New York, NY: Springer US; 2012 [cited 2026 Jan 2]. p. 17–31. (Advances in Experimental Medicine and Biology; vol. 739). Available from: http://link.springer.com/10.1007/978-1-4614-1704-0_2

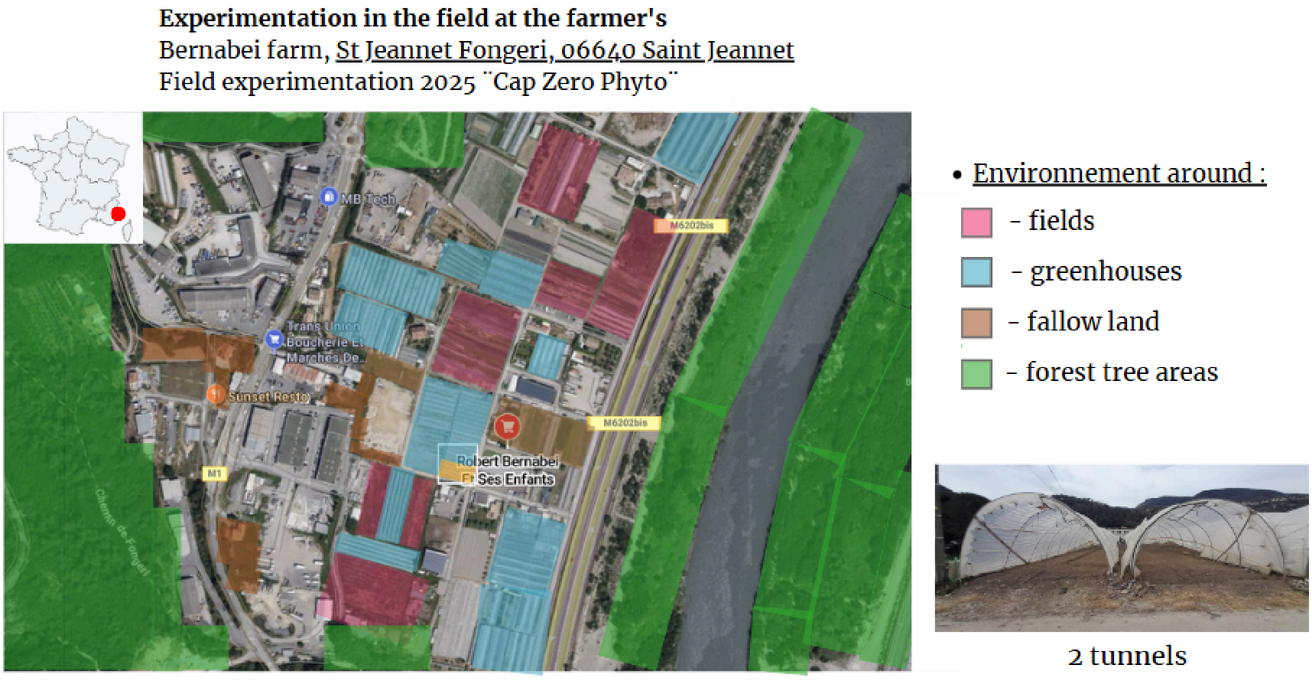
31. Li T, Holopainen JK, Kokko H, Tervahauta AI, Blande JD. Herbivore-induced aspen volatiles temporally regulate two different indirect defences in neighbouring plants. Functional Ecology. 2012;26(5):1176–85.

1.

32. Bu H, Sun X, Hu Y, Gu G, Yang Y, Yu W. Research Advances in the Regulation of Fruit Size: An Integrated Perspective of Genetic, Hormonal, Epigenetic, and Environmental Control. Biology. 2025 Dec;14(12):1643.

Annex

Figure 1 : Experiment location



The experimental site is located in close proximity to forest areas, cultivated fields, greenhouses and fallow land, which creates a diverse landscape environment.

Figure 2 : Experimental time line

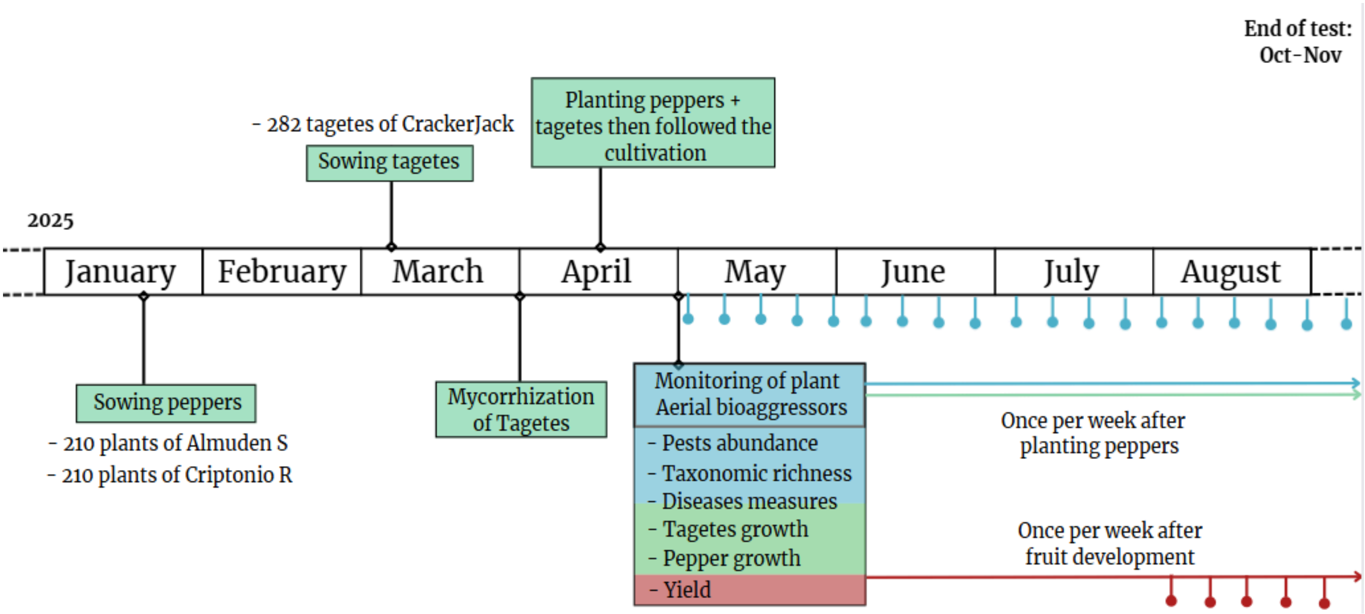


Figure 3 : Experimental protocol for *Tagetes* mycorrhization

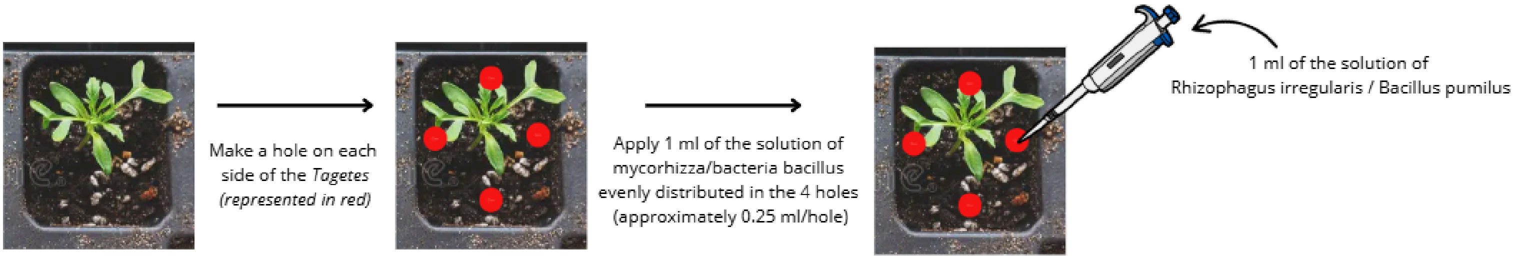
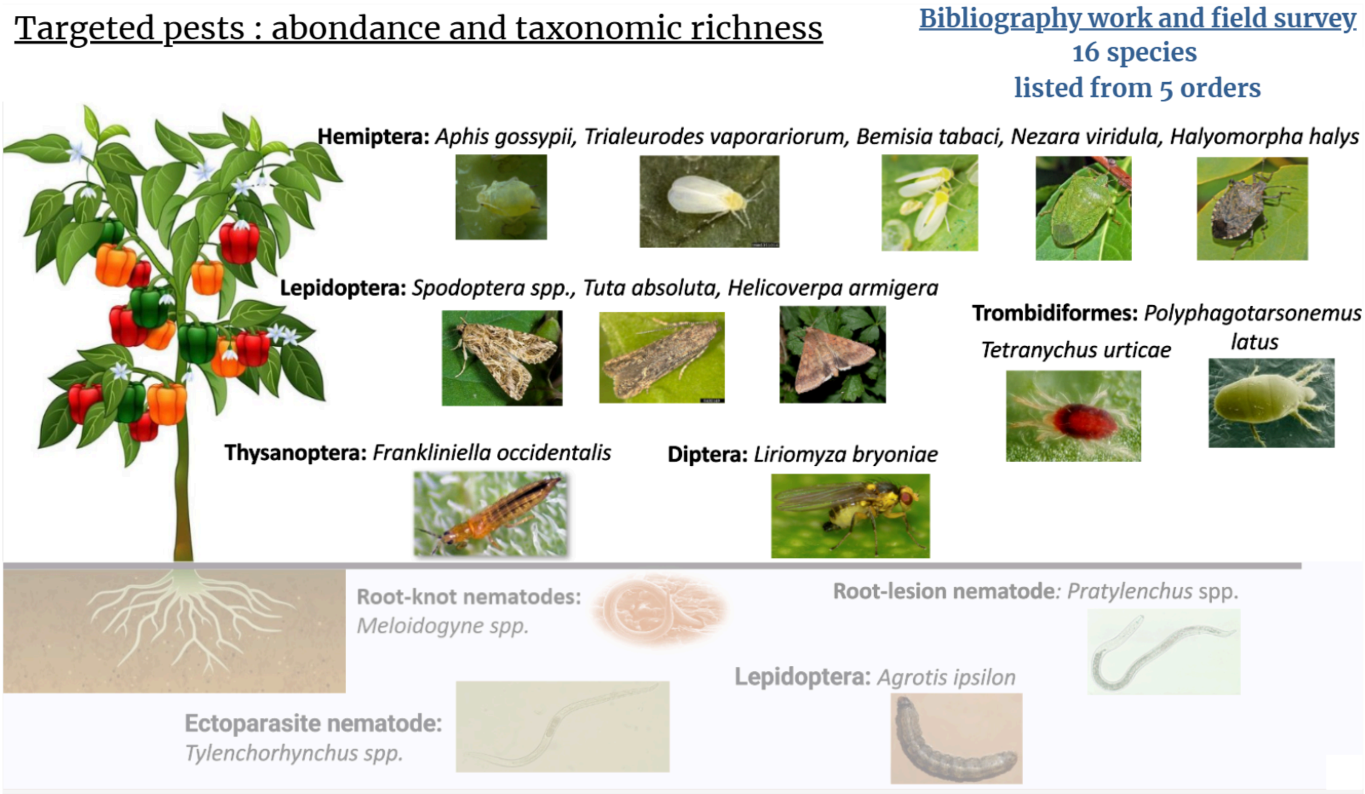


Figure 4 : Taxonomic richness of pepper aerial pests



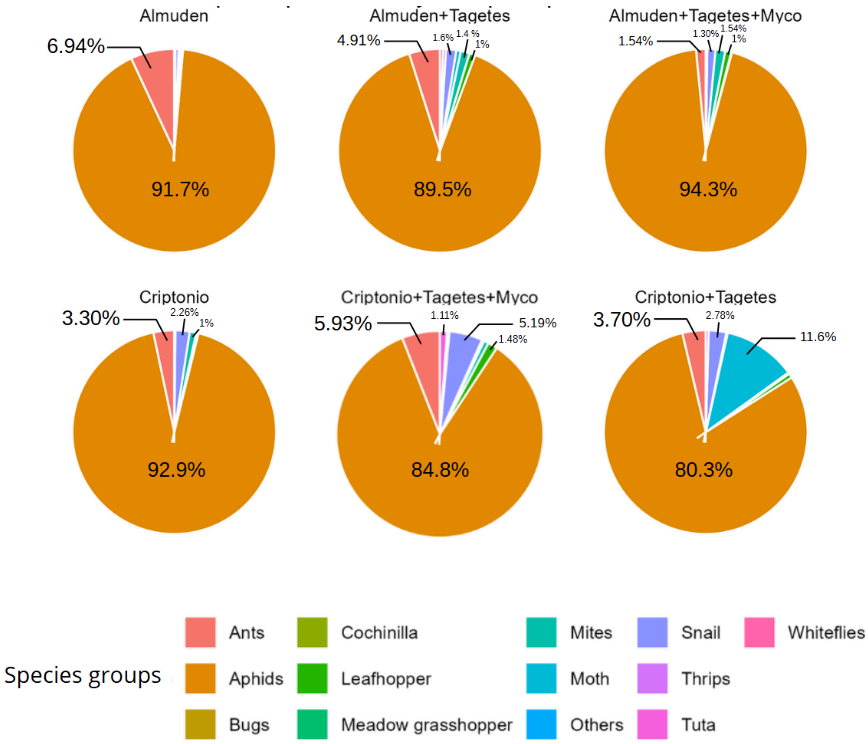


Figure 5 : Pie chart of the pepper pests distribution between the different modalities for the 3 time sessions combined.

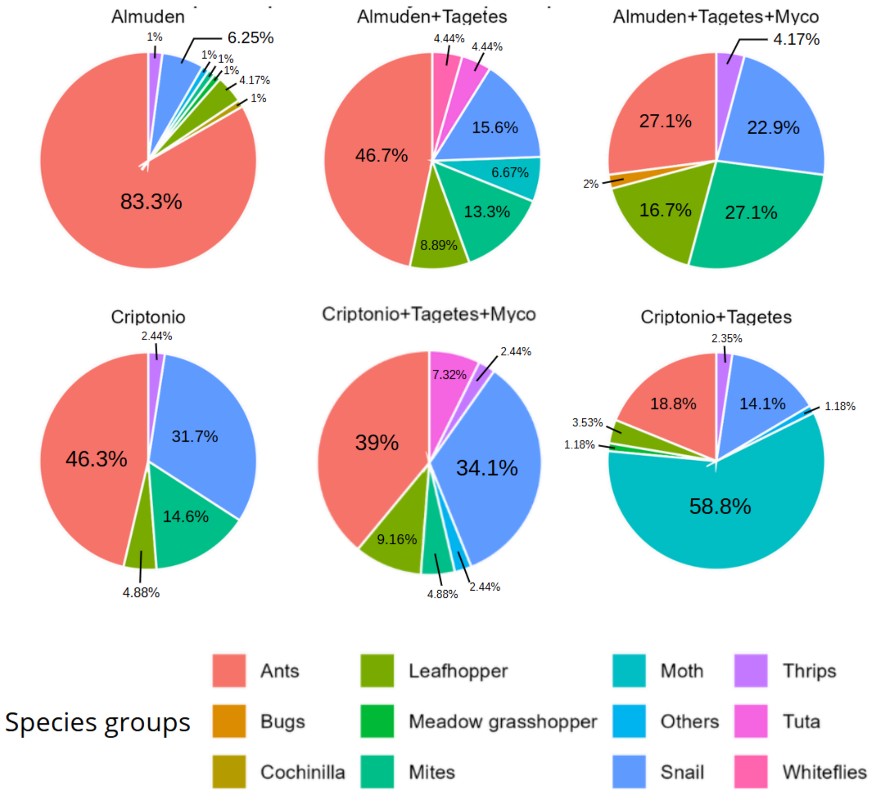
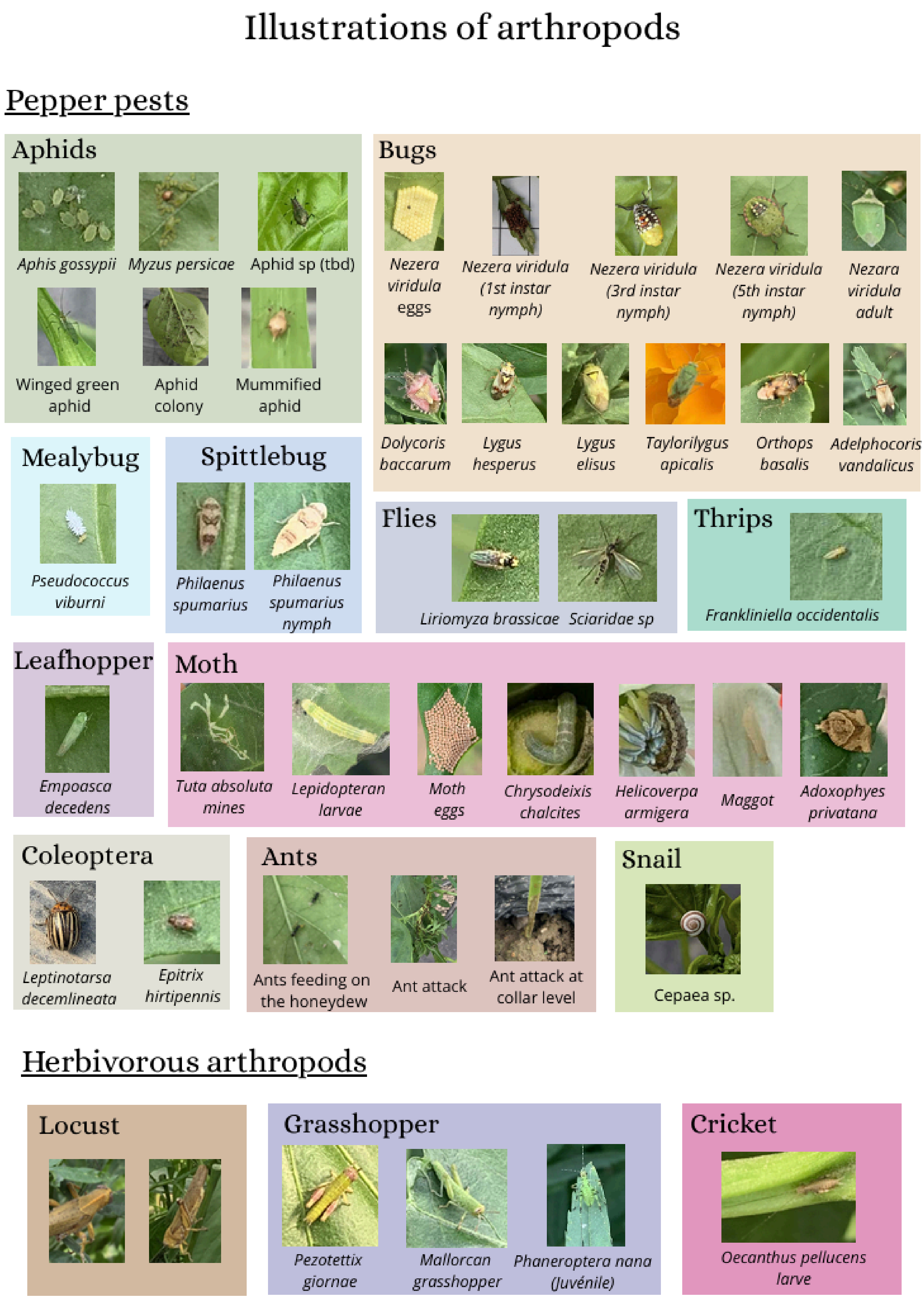


Figure 5 : Pie chart of the pepper pests distribution between the different modalities without Aphids for the 3 time sessions combined.

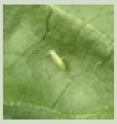
Photos of the different arthropods seen in the experimental field :

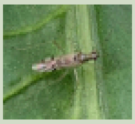


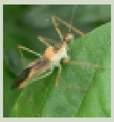
Illustrations of arthropods

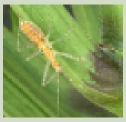
Aerial natural enemies

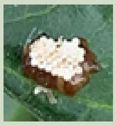
Predatory bugs

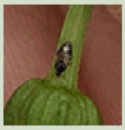

Macrolophus pygmaeus

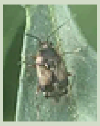

Nesidiocoris tenuis

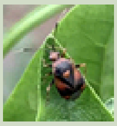

Zelus renardii adult

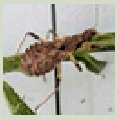

Zelus renardii Juvenile

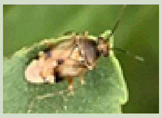

Zelus renardii eggs

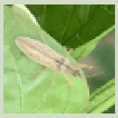

Orius insidiosus


Deraeocoris pallens

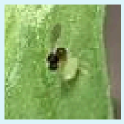

Deraeocoris ruber


Hoplistoscelis sordidus

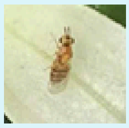

Deraeocoris serenus

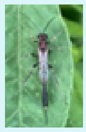

Nabis ferus

Parasitoids

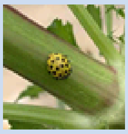

Parasitoid attacking an aphid

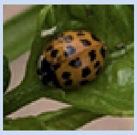

Parasitoid

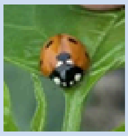

Eulophidae

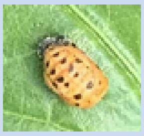

Deutéroxorides sp

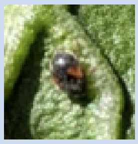
Ladybugs

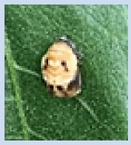

Psyllobora vigintiduopunctata

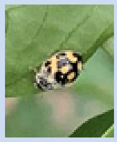

Harmonia axyridis

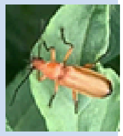

Coccinella septempunctata


Coccinella septempunctata pupa


Scymnus laetificus


Ladybugs pupa

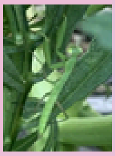

Propylea quatuor-decimpunctata


Rhagonycha fulva

Earwig


Forficula auricularia

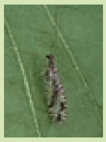
Mantodea


Mantis religiosa

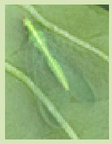
Predatory thrips


Aeolothrips fasciatus

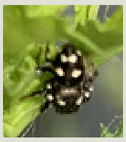
Lacewing

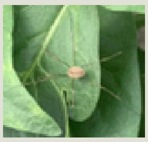

Micromus variegatus

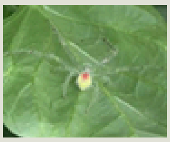

Micromus posticus

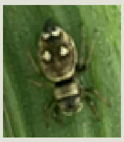

Chrysoperla carnea

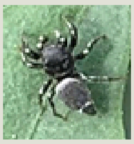
Spiders

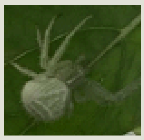

Heliophanus apiatus

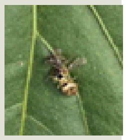

Leiobunum flavum


Heriaeus hirtus

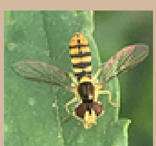

Heliophanus rufithorax






Salticid

Syrphid fly

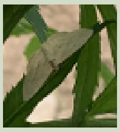

Episyrphus balteatus

Illustrations of arthropods

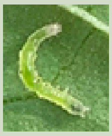
Other aerial arthropods

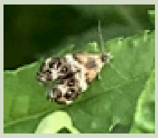
Moth

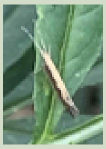

Scythris inertella


Scopula opitiva

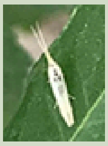

Pandemis cerasana adult

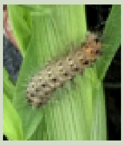

Pandemis cerasana larva

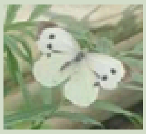

Tebenna micalis (asteraceaes pests)


Plutella xylostella


Suleima helianthana (asteraceaes pests)


Coleophora cratipennella


Pine processionary caterpillar

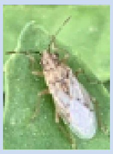

Pieris brassicae

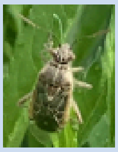
Flies

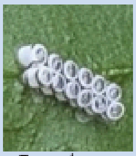
Thereva nobilitata


Nephrotoma appendiculata

Bugs

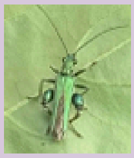

Nysius graminicola (asteraceaes pests)


Liorhyssus hyalinus (asteraceaes pests)


Eurydema eggs

Woodworm


Anobium punctatum


Oedemera nobilis

Psocodea


Graphopsocus cruciatus

R script :

```r
#Chargement des packages nécessaires
library(glmmTMB)
library(dplyr)

# Préparer les données
# Crée la colonne Modalité en combinant les leviers
compt_data <- compt_data %>%
  filter(!(Myco == "OUI" & Tagetes == "NON")) %>%  # Supprime les lignes illogiques
  mutate(Modalité = interaction(Poivrons, `Tagetes`, `Myco`, sep = "_"))

# Vérifie que les colonnes aléatoires sont des facteurs
compt_data <- compt_data %>%
  mutate(
    Tunnel = as.factor(Tunnel),
    `Positionnement de modalité` = as.factor(`Positionnement de modalité`),
    `Identifiant plante` = as.factor(`Identifiant plante`),
    Modalité = as.factor(Modalité),
    Dates = as.numeric(Dates) # déjà sous forme de jour dans l'année
  )

# Modèle GLMM (Negative Binomial)
mod_glmm_tmb <- glmmTMB(
  `Abondance ravageurs poivrons` ~ Modalité + Dates +
    (1 | Tunnel) +
    (1 | `Positionnement de modalité`) +
    (1 | `Identifiant plante`),
  data = compt_data,
  family = nbinom2()
)

summary(mod_glmm_tmb)
```