



UFR Sciences  
2, Bd Lavoisier  
49045 ANGERS Cedex 01



AGROCAMPUS OUEST  
65 rue de St Briec, CS 84 215,  
BP 35042 - RENNES Cedex  
Université de Rennes I  
1, 2 rue du Thabor, CS 46510 -  
35065 RENNES Cedex



EPLEFPA  
Angers le Fresne - Segré  
38, chemin du Fresne,  
49130 Sainte Gemmes sur  
Loire  
BP 43627  
49036 ANGERS Cedex 01

## Mémoire de Fin d'Etudes

**Master 2 Sciences Technologie Santé  
Mention Biologie et Technologie du Végétal  
Spécialité : Production et Technologie du Végétal (ProTeV)**

Parcours : I Productions Végétales Spécialisées / Option : Filières de l'horticulture et végétal urbain

Année universitaire 2016-2017

**Evaluation of the of the agro-ecological infrastructures (grass strips, flower strips) for the leafhopper control in thyme crop**

**Evaluation d'aménagements agro-écologiques (bandes fleuries, bandes enherbées) en culture de thym, dédiés à la régulation des cicadelles**

Par : **Ivana BILKOVÁ**

Soutenu à Angers le 18 septembre 2017

Maître de stage : Yann TRICAULT

Co-encadrant de stage : Mélissa LELOUP, Éric DUCLAUD





UFR Sciences  
2, Bd Lavoisier  
49045 ANGERS Cedex 01



AGROCAMPUS OUEST  
65 rue de St Briec, CS 84 215,  
BP 35042 - RENNES Cedex  
Université de Rennes I  
1, 2 rue du Thabor, CS 46510 -  
35065 RENNES Cedex



EPLEFPA  
Angers le Fresne - Segré  
38, chemin du Fresne,  
49130 Sainte Gemmes sur  
Loire  
BP 43627  
49036 ANGERS Cedex 01

Mémoire de Fin d'Etudes

**Master 2 Sciences Technologie Santé  
Mention Biologie et Technologie du Végétal  
Spécialité : Production et Technologie du Végétal (ProTeV)**

Parcours : I Productions Végétales Spécialisées / Option : Filières de l'horticulture et végétal urbain

Année universitaire 2016-2017

**Evaluation of the of the agro-ecological infrastructures (grass strips, flower strips) for the leafhopper control in thyme crop**

**Evaluation d'aménagements agro-écologiques (bandes fleuries, bandes enherbées) en culture de thym, dédiés à la régulation des cicadelles**

Par : Ivana BILKOVÁ



Soutenu à Angers le 18 septembre 2017

Maître de stage : Yann TRICAULT

Co-encadrant de stage : Mélissa LELOUP, Éric DUCLAUD





## **ENGAGEMENT DE NON PLAGIAT**

**M2 ProTeV**

**2016-2017**

Je, soussigné (e) : Ivana BILKOVÁ

déclare être pleinement consciente que le plagiat de documents ou d'une partie d'un document publiés sur toutes formes de support, y compris l'internet, constitue une violation des droits d'auteur ainsi qu'une fraude caractérisée.

En conséquence, je m'engage à citer toutes les sources que j'ai utilisées pour ce rapport, rédigé au cours de mon master 2 ProTeV.

Je m'engage également à respecter les consignes données pour la rédaction de ce rapport.

A : Angers

Le : 4 septembre 2017

Signature :



## Acknowledgement

---

I want to express my gratitude towards each and every person I had the chance to meet and work with during my internship; to Yann Tricault, my supervisor for guidance, freedom and kindness, to Eric Duclaud and Mélissa Leloup my co-supervisors, to Ferréol Braud and Estelle Chenu, the two technicians-magicians of IGEPP and to Anna Pollier for her help with botany records. For sharing her time and knowledge on how to grow fungi I thank to Muriel Marchi, and for putting us in contact and identifying the fungus, to Thomas Guillemette. Thanks also to all employees and interns of IGEPP Agrocampus Ouest Angers, as well as to the intern of ITEIPMAI (Clémence) and to the interns of Lycée Le Fresne (Valentin, Camille and Charlotte), to Cyril Farsy for a report and experimental design from the previous year. I thank to my family and to Rémi for his many forms of support, and to all whom I may have forgotten to name.





## List of abbreviations

---

AREXHOR	Agence régionale pour l'expérimentation horticole Pays de la Loire (Regional experimentation station in horticulture)
BIOFH'HORMA	BIOdiversité Fonctionnelle en HORTiculture et culture de plantes Médicinales et Aromatiques (Functional biodiversity in horticultural and medicinal and aromatic crops)
CA	Chambre d'Agriculture (Chamber of Agriculture/ Agriculture Council)
CFPPA	Centre de Formation Professionnelle et de Promotion Agricoles (Vocational agricultural training centre)
EPLEFPA	L'Établissement Public Local d'Enseignement et de Formation Professionnelle Agricoles (Local state centre for agricultural vocational education and training)
GEVES	Groupe d'Etude et de Contrôle des Variétés et des Semences
HVE	Haute Valeur Environnementale (High Environmental value)
INRA	Institut National de Recherche Agronomique
IRHS	Institut de Recherche en Horticulture et Semence
ITEIPMAI	Institut Technique Interprofessionnel des Plantes à Parfum Médicinales et Aromatiques (Inter-branch technical institute for PAMPs)
LEGTA	Lycée d'Enseignement Général et Technologique Agricole (Secondary agricultural school)
MLO	Mycoplasma-Like Organism
PAC (CAP)	Politique Agricole Commune (Common Agricultural Policy)
PAMP(s)	Perfume Aromatic and Medicinal Plant(s)
PDA	Potato Dextrose Agar
RBA	Rapid Biodiversity Assessment
SNES	Station Nationale d'Essais de Semences de GEVES



## List of illustrations

---

Figure 1. Logo of the BIOF'HORMA project.

Figure 2. Logos of the partners of BIOF'HORMA project.

Figure 3. 'Le Fresne', thyme field 'Noelle' and the main school building in the background.

Figure 4. Harvested thyme to be distilled and distillation platform at "Le Fresne".

Figure 5. Production surface (ha) for aromatic herbs in France, based on producer organization declarations 2014.

Figure 6. Production surface (ha) and number of producers of organic PAMPs in France, based on producer organization declarations 2014.

Figure 7. Biosynthetic pathways of the six monoterpenes in *Thymus vulgaris* L.

Figure 8. Illustration of ecosystem services

Figure 9. Illustration of the hypotheses a-g..

Figure 10. *Typhlocybinæ* leafhopper species illustration.

Figure 11. Stippling on the Leaves of thyme.

Figure 12. Stolbur on lavender and its vector *Hyalesthes obsoletus*

Figure 13. Favescence dorée (*Candidatus Phytoplasma vitis*) and its vector *Scaphoideus titanus*

Figure 14. The five instars of *Eupteryx urticae*.

Figure 15. The fifth instar larva of *Eupteryx decemnotata*, *Eupteryx atropunctata*, *Eupteryx aurata*, and photos of larva observed during the experiment

Figure 16. *Anagrus* sp.

Figure 17. *Anagrus atomus* female.

Figure 18. *Aphelopus* illustration.

Figure 19. *Dryinidae* female with chelae. *Anteon buntini* Olmi (*Anteoninae*)

Figure 20. *Aphelopus atratus* illustration.

Figure 21. *Chalarus spurius* photo.

Figure 22. Overview of the thyme fields at the "Le Fresne" horticultural farm and layout of the experimental treatments.

Figure 23. Layout of a the experimental plot (20m long) and a scheme of leaves harvest path.

Figure 24. Photos of traps.

Figure 25. Position of traps within the field margin (strip) in the bare soil control of the "Noëlle" field.

Figure 26. Number of species, total vegetation cover and flower index of Flower strips and grass strips

Figure 27. PCA of plant species composition (presence/absence) and plant species composition (cover %) in grass strips and flower strips.



Figure 28. PCA of plant species cover (%) and flowering plants (%) of the sown species in flower strips.

Figure 29. Evolution of leaf damage (%).

Figure 30. Evolution of leaf damage (%) in each plot over time.

Figure 31. Evolution of *E. decemnotata* and *Anagrus sp.* abundance (Nb trapped) average and standard deviation, for the week 1, 3 and 5

Figure 32. Correlations between *E. decemnotata* and *Anagrus sp.* abundance (nb trapped) and between *E. decemnotata* and leaf damage for the week 1, 3 and 5.

Figure 33. Evolution of leafhopper population and % leaf damage in 2016.

Figure 34. Evolution of leafhopper population and *Anagrus sp.* population in 2016.

Figure 35. Evolution of the average day and night temperature (°C) and cumulated radiation (w/m<sup>2</sup>) during the experimental season 2016 (a) and 2017(b) at 'Le Fresne'.

Figure 36. Precipitation (mm) during the experimental season 2016 and 2017 at "Le Fresne".

Table I. List of some of the (sub)-hypotheses, a-g. Comparison against the control.

Table II. List of species sown in the flower strip with scientific, common French and common English names.

Table III. List of species sown in the flower strip with scientific name, botanical family, flowering period and colour.

Table IV. Leaf damage categories.

Table V. Overview of the experiment calendar.

Table VI. Results for the leaf damage model test.

Table VII. Results of multiple comparison test (method Tukey) between the three treatments for the number of *E. decemnotata* and *Anagrus sp.* (average per plot).



## List of appendices

---

- Appendix I. How to distinguish the (typhlocybinae) leafhopper species and parasitoids present in the thyme at “Le Fresne “ fields, Angers, France.
- Appendix II. Layout of the experimental plot (14m long).
- Appendix III. List of plant species recorded in flower strips and grass strips 2016.
- Appendix IV. Flower strips and grass strips.
- Appendix V. Other activities carried out during the internship.





## Table of contents

---

1	Introduction .....	1
1.1	BIOF'HORMA project .....	1
1.2	Centre for agricultural vocational education and training Le Fresne .....	1
1.3	Production of PAMPs and thyme in France .....	2
1.4	Thyme and its use .....	3
1.5	Ecosystem services, biological control and agro-ecological infrastructures .....	4
2	The objectives and research questions/hypotheses .....	5
3	Literature review.....	6
3.1	Leafhoppers of thyme crop.....	6
3.1.1	Distribution of typhlocybine leafhopper species .....	7
3.1.2	Life cycle of the Ligurian leafhopper, <i>E. decemnotata</i> .....	8
3.2	Controlling the leafhoppers in the <i>Lamiaceae</i> crops .....	9
3.2.1	Conventional pest control.....	9
3.2.2	Vibrations and physical removal (suction) .....	10
3.3	Natural enemies-parasitoids.....	10
3.3.1	<i>Anagrus</i> Haliday ( <i>Hymenoptera: Mymaridae</i> ).....	10
3.3.2	<i>Aphelopus</i> Dalman ( <i>Hymenoptera: Dryinidae</i> ) .....	12
3.3.3	<i>Chalarus</i> Walker ( <i>Diptera:Pipunculidea</i> ).....	13
3.4	Natural enemies – Predators.....	14
4	The experiment .....	15
4.1	Materials and Methods .....	15
	The experimental design .....	15
4.1.1	The experimental field management .....	16
4.1.2	Botanical survey of the strips.....	16
4.1.3	Leaf damage estimation .....	17
4.1.4	Leafhopper population abundance and parasitoid identification .....	17



4.1.5	Arthropod diversity and natural enemies functional diversity .....	18
4.2	Data analysis.....	19
	Botanical composition of AEI.....	19
4.2.1	Leaf damage estimation .....	20
4.2.2	Leafhopper population abundance and parasitoid identification .....	20
4.2.3	Arthropod diversity and natural enemies functional diversity .....	20
4.3	Results.....	21
4.3.1	AEIs development and their botanical composition .....	21
4.3.2	Leaf damage estimation .....	22
4.3.3	Leafhopper and parasitoid population abundance .....	22
4.3.4	Arthropod diversity and natural enemies functional diversity .....	23
4.4	Discussion.....	23
4.4.1	Botanical composition of AEI.....	24
4.4.2	Leafhopper and parasitoid populations.....	24
4.4.3	Leaf damage .....	25
4.5	Conclusion .....	27
4.6	Recommendations .....	27
5	List of references.....	29
6	Online sources .....	32

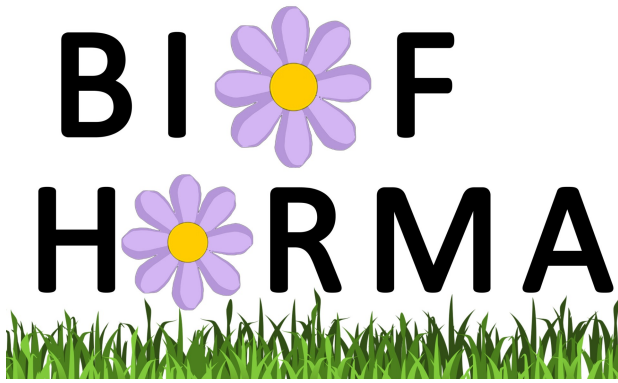


Figure 1. Logo of the BIOF'HORMA project.



Agence régionale pour l'expérimentation horticole  
Station de l'Institut technique de l'horticulture

Figure 2. Logos of the partners of BIOF'HORMA project.



Figure 3. 'Le Fresne', thyme field 'Noelle' and the main school building in the background (Photo by Rémi Dufлот).

# 1 Introduction

---

## 1.1 BIOF'HORMA project

BIOF'HORMA is a regional project of the Pays de la Loire region (2015-2017), carried out by four cooperating institutions; AGROCAMPUS OUEST (Angers), AREXHOR Pays de la Loire (Les Ponts-de-Cé), ITEIPMAI (Chemillé-en-Anjou) and (the project leader) EPLEFPA Angers le Fresne - Segré (Sainte-Gemmes-sur-Loire) (logos in **fig. 1. and fig.2.**). The main objective is to develop alternative practices in crop protection by using AEI in PAMP field crops and service plants in under cover (tunnels) PAMPs production. The three experimental axes are; (1) Mulching, service plants and repulsive plants against thrips on chives (tunnel), (2) study on parasitoid of aphids of parsley and (3) evaluation of AEI in thyme and lemon balm crop for the leafhopper control (field). This document represents a literature review, methods and preliminary results of the third axis.

## 1.2 Centre for agricultural vocational education and training Le Fresne

The centre was founded in 1967 and today it comprises three parts; (1) LEGTA – a high school with general and vocational delivering diplomas from A-levels to Bachelor (2<sup>nd</sup> –Licence Pro) in the domains of horticultural production, management of horticultural companies and landscaping, (2) CFPPA - centre for vocational training through continuing education and apprenticeship in the domains of horticultural production, landscaping and garden landscaping, delivering diplomas from vocational certificate to Bachelor (CAP – Bac Pro) and (3) horticultural farm, with green spaces, horticultural production in fields, tunnels and greenhouses. The farm production is used for local consumption, sale and educational and research purposes (**fig 3**).

The centre has several finished and ongoing research projects beside BIOF'HORMA; regional (AGREABLE, IBCUS, ENAUCS...) and CASDAR projects (FLOREGUL, MUSCARI, PLACHOB).

Efforts have been made to reduce the environmental impact within the school farm and green spaces. In 2007 the farm was equipped with Phytobac®, where leftovers of pesticides, for example from tool washing/rinsing are processed in a closed system. The green spaces and gardens on the school terrain were awarded "EcoJardin" label. The horticultural farm and its production were labelled 'Plante Bleue' level 2, an inter-branch label for ecologically responsible horticultural production, as well as HVE level 3, an official national label for environmentally responsible agricultural production.



Figure 4. Harvested thyme to be distilled and distillation platform at “Le Fresne”.

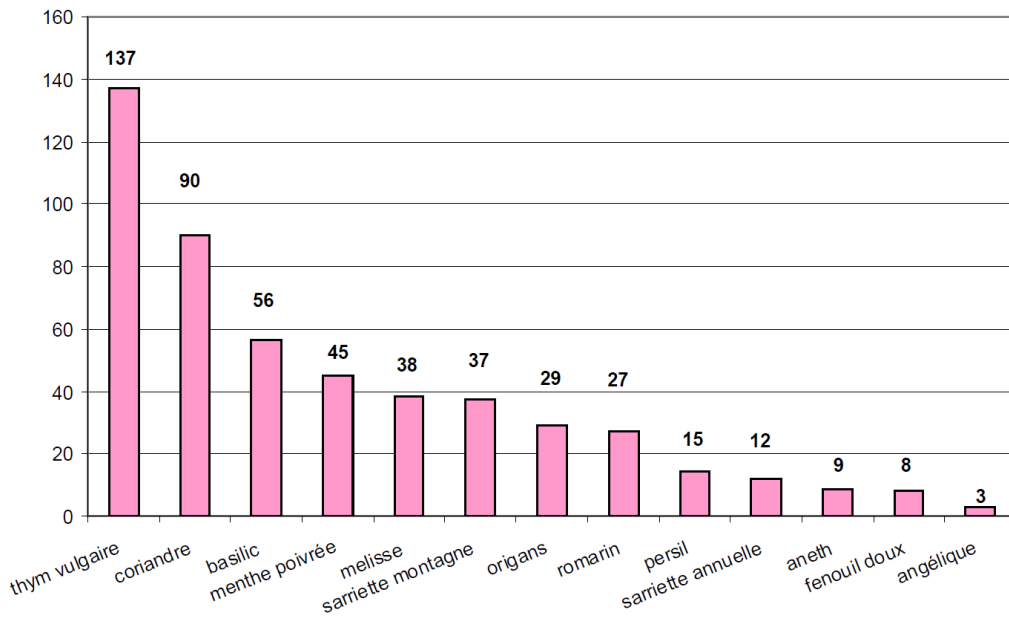


Figure 5. Production surface (ha) for aromatic herbs in France, based on producer organization declarations 2014, with Common thyme on the first position (Agrimer, 2016).

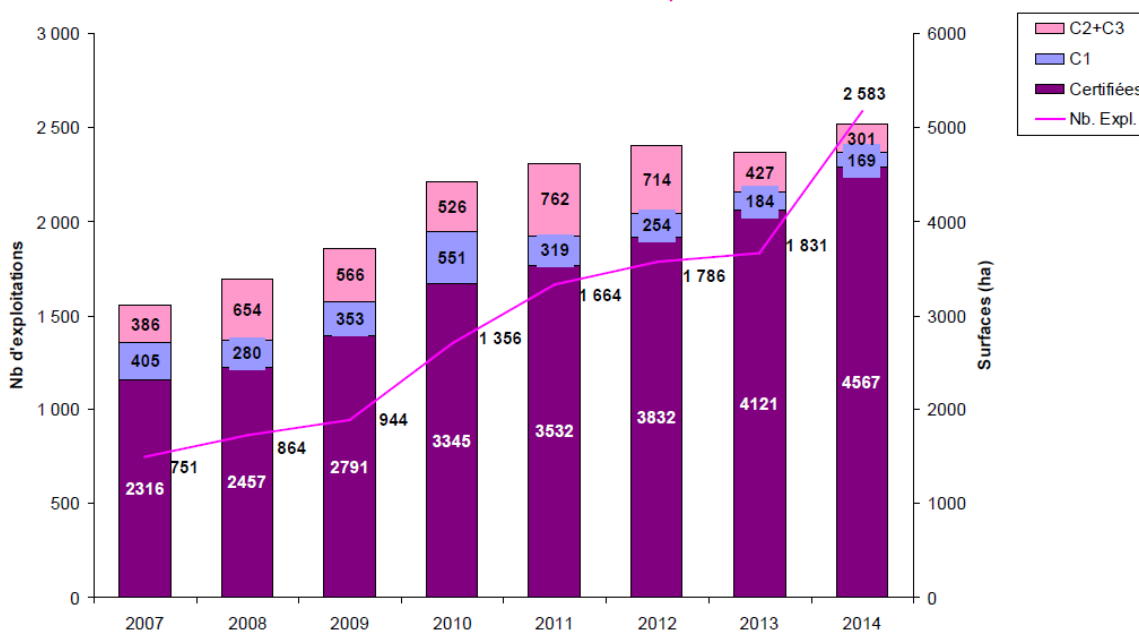


Figure 6. Production surface (ha) and number of producers of organic PAMPs in France, based on producer organization declarations 2014 (Agrimer, 2016).

The horticultural farm extends on 26.5 ha of which 2.5 ha off-ground nurseries, 0.6 ha greenhouses and around 2 ha fields of medicinal and aromatic plant production (thyme). The whole thyme production is used for essential oil extraction (on site, **fig. 4.**).

### 1.3 Production of PAMPs and thyme in France

According to CAP declarations, in 2014, 3649 French farmers cultivated perfume, aromatic and medicinal plants (PAMPs) for a total area of 42 076 ha (Agrimer, 2016). These two statistics are steadily increasing, at a rate of circa 2% per year since 2000. In 2014, nearly half (47%) of the total area was cropped in southern France and devoted to lavender and lavandin for the perfumery business. If not accounting for these 2 productions, the Maine-et-Loire department ranked sixth based on PAMPs area, with 1080 ha in 2014 and fourth in number of producers (142 in 2014) (Agrimer, 2016). The department is known mostly for the medicinal plants production that has been well-established for more than 150 years in the region of Chemillé.

The thyme production falls into two categories – aromatic (fresh/ dried/ frozen herb) and medicinal (dried/ essential oil). For the aromatic herb production, thyme ranked first at the national scale with 77.5 t of dried thyme produced from the 137 ha cultivated in 2014 (**fig. 5.**). The insufficient production compared to consumption (733.5 t per year) calls for massive imports from foreign countries, notably from Poland, representing alone 50% of the imported volumes in last ten years. The areas and yields of thyme cultivated for medicinal purposes are unknown. The total production of thyme essential oil 5 (all types) was estimated at about 1.5 t in 2010 (Fernandez *et al.*, 2012). The sales of common thyme reached € 742 000 in France in 2014, representing 12% of the total sales value generated by all aromatic and medicinal plants (about € 6 million) (Agrimer, 2016).

In 2014, the organic production of PAMPs covered 12.1 % of cultivated PAMPs areas (5037 ha) (**fig. 6.**), of which 204 ha were located in the Pays de la Loire region (Agrimer, 2016). More specifically, thyme production represented 3% of organic PAMPs acreage with 137 ha accounting for 1/3 of total thyme area. This ratio has remained stable since 2012 and is very similar to those observed for lavender and lavandin.

Of the total essential oil production in France in 2014, only 3% were organic, but because of their higher prices they represent 6% of total value (Agrimer, 2016). The demand is higher than offer for the organic thuyanol and linalool, the two out of six chemotypes of the essential oil of common thyme in France. In 2014, the sales of aromatherapy products in pharmacies and drugstores reached nearly € 180 million (increase of 16% compared to previous year), not accounting for other types of sales (direct, online, etc.) (Agrimer, 2016).

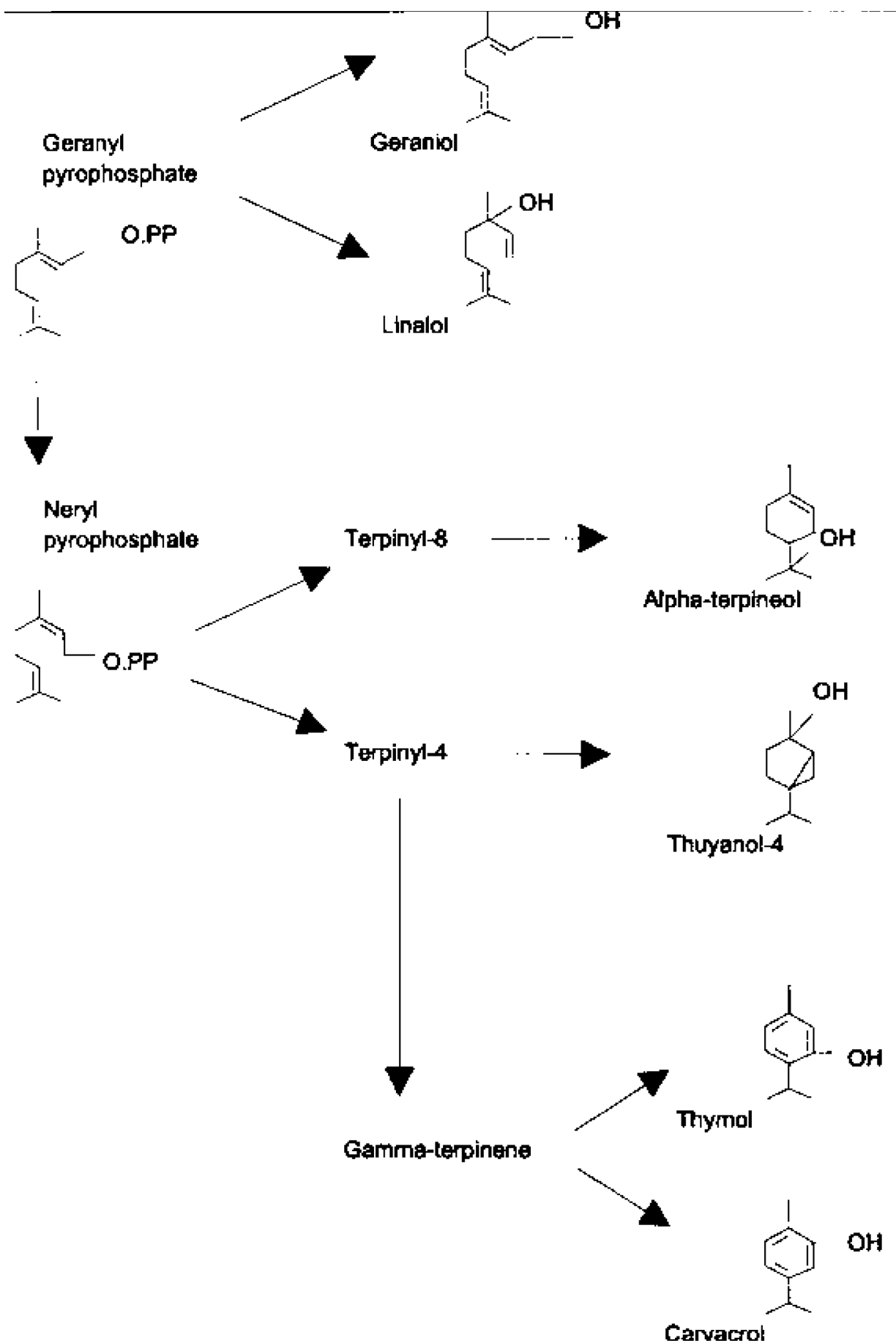


Figure 7. Biosynthetic pathways of the six monoterpenes in *Thymus vulgaris* L. from Thompson *et al.* (1998).



## 1.4 Thyme and its use

*Thymus* L. is a genus of *Lamiaceae* family, regrouping around 350 species (Stahl-Biskup and Sáez, 2003), though as many as 928 are cited by (Nabavi *et al.*, 2015).

The origins of the name *Thymus* is not clear, but may be attributed to Greek *thumos / thuos* meaning 'to perfume', referring to a wood which when burned spreads a nice perfume; or to *thuô / thuein* meaning 'to offer a sacrifice to gods' (Goust, 1999; Stahl-Biskup and Sáez, 2003), Google book genus *Thymus*). In Old French, the word can be traced to the 13th century *tym / thym* (Stahl-Biskup and Sáez, 2003). In the South of France, thyme is known as 'farigoule'.

*Thymus vulgaris* is an evergreen shrub, originating from Mediterranean area. It is adapted to dry conditions and calcareous and /or rocky soils. Too humid soils should be voided. As a crop, thyme needs little to no irrigation, based on variety and local conditions, some fertilisation and regular weeding. The pests and diseases of thyme, beside leafhoppers, are: moth caterpillars, aphids, leafbugs (*Chrysomelidae*) and soil-borne fungi (*Pythium*, *Fusarium*). The crop can last up to nine years, but the average is six years, while first year is without harvest in essential oil production (CA Aude, 2014). The optimal period for the harvest is before flowering, for dried thyme (November-May) and at the beginning of flowering for the essential oil production (from May onwards) (CA Aude, 2014; CA Rhône-Alpes, 2012). The yield increases with crop age, and for well installed thyme (4-7 year old) the average yields are: 20 kg (thymol).14 kg (linalol) and 12 kg (thuyanol) of oil per ha (CA Aude, 2014).

The thyme used for essential oil production is sorted into chemotypes, based on the composition of the essential oil distilled. In France, the common thyme exist in six major chemotypes (**fig. 7.**) and based on the production of the dominant monoterpene they are;  $\alpha$ -carvacrol, (A), thuyanol-4 (U), geraniol (G), linalol (L), carvacrol (C) and thymol (T), the two latter ones being phenolic monoterpenes (Thompson *et al.*, 1998). The production of the dominant monoterpene is controlled by an epistatic series of five biosynthetic loci :: G>A>U>L>C>T. For example, the thymol chemotype is expressed only if all the other preceding loci are all homozygous recessive (Vernet *et al.*, 1986).It is possible to distinguish geraniol chemotype easily "on the field" as it has a lemony smell and linalool chemotype reminds of lavender (Thompson *et al.*, 1998). In South-western Spain the dominant chemotypes are cineol, borneol, and camphene (Thompson *et al.*, 1998). It seems that the phenolic chemotypes are distributed more in the hot and dry climates while the non-phenolic ones are favoured in moister and cooler areas. The chemotype that is most adapted to the wet conditions is the thuyanol chemotype, and it deters slugs the most (Thompson *et al.*, 1998).

# WHAT DO WE GET FROM **ECOSYSTEMS**?

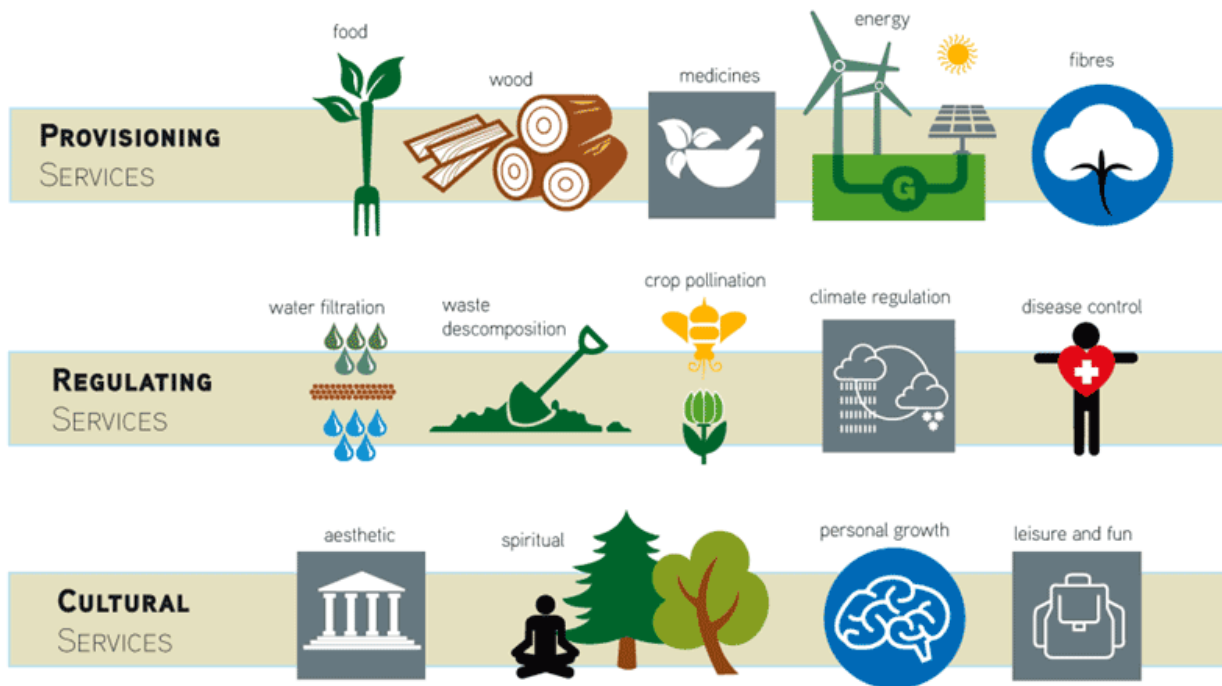


Figure 8. Illustration of ecosystem services (1).

We may trace the use of thyme in pharmacopoeia to as early as 3 000 BC in Mesopotamia (Teall, 2014). Today the thyme is used as culinary fresh or dried herb, mostly in savoury dishes and as a dried herb or an essential oil in phytotherapy/naturopathy and aromatherapy pharmacopoeia. In aromatherapy the linalol and thuyanol essential oils are considered 'the softest' and safest to use, as opposed to 'strong' thymol chemotype (Tu-Saint Girons and Saint Girons, 2014). The essential oils of common thyme are, in general, antiseptic, antibacterial antifungal, antiparasitic, and antioxidant. In traditional medicine, thyme is used in cases of respiratory infections, dyspepsia, acne and many others (Nabavi *et al.*, 2015).

## **1.5 Ecosystem services, biological control and agro-ecological infrastructures**

Ecosystem services can be defined as all the benefits from ecosystems for humankind. The ecosystem services are a result of ecosystem processes. The Millenium Ecosystems Assessment defines four major groups of ecosystem services: supporting, provisioning, regulating, and cultural. The pest and disease control falls under the 'regulating' ecosystem services (**fig. 8.**). In agricultural production, the ecosystem approach translates as the agro-ecological approach. Agro-ecology considers the agricultural production systems as ecosystems, or rather agro-ecosystems.

The intensification of agriculture is associated with negative impact on the environment, such as, decrease of biodiversity due to reduced habitat for the concerned species. The local diversity may decline and so the ecosystem services that may be provided, for example towards the pest control. Taking conscious of harmful effects of the practices of intensive agriculture, such as use of pesticides on human health and environment, the agro-ecological approach aims towards ecological intensification – through exploitation of the ecosystem services. In the pest control, this translates by using the regulating ecosystem services. They are provided by relationships that exist in the ecosystems, such as between the pests and their natural enemies. Two main groups of natural enemies are; the predators, who feed on other organisms (destructively), and parasitoids, which use their hosts for at least a part of their biological cycle and thus kill their hosts.

Exploiting the natural enemies for pest control is done in the biological control. Based on which method is used we distinguish; importation – the natural enemy is imported from the place of origin of the pest, inundation – produce and release natural enemies in high quantities and conservation – providing the 'wild' natural enemies with shelter, food and habitat.

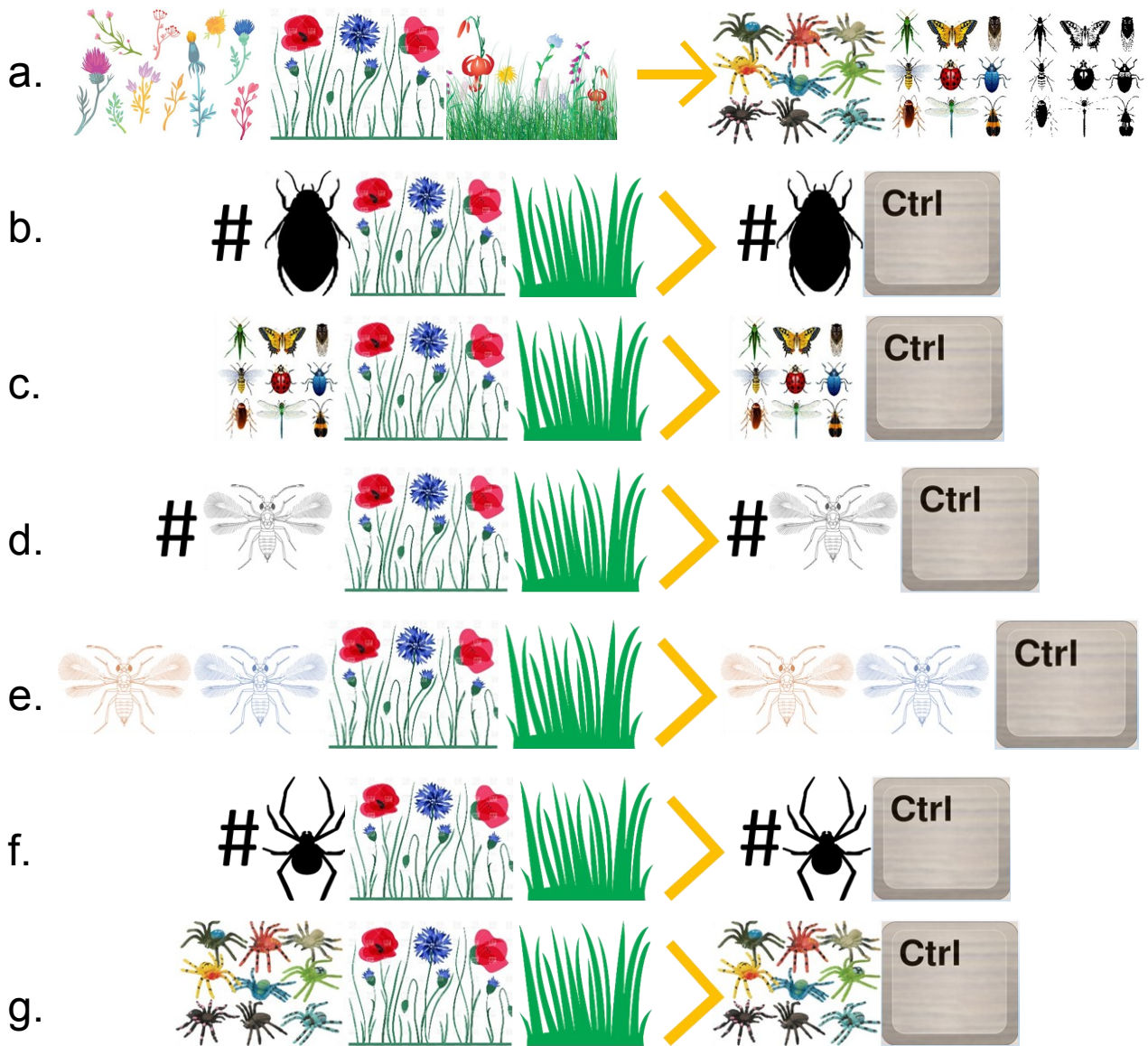


Figure 9. Illustration of the hypotheses a-g. Images used;(2-10).

Table I. List of some of the (sub)-hypotheses, a-g. Comparison against the control.

a,	Higher botanical diversity (flower strips) will translate into higher arthropod diversity
b,	Higher arthropod abundance in AEI
c,	Higher arthropod richness in AEI
d,	Higher abundance of potential parasitoids of leafhoppers in the flower strips
e,	Higher (morphotype) richness of potential parasitoids of leafhoppers in the flower strips
f,	Higher abundance of (suspected) predators of leafhoppers in AEI
g,	Higher (morphotype) richness of (suspected) predators of leafhoppers in AEI

In our study, the idea is to exploit the conservation method, through employment of agro-ecological infrastructures (AEI), providing necessary resources for the natural enemies. According to (Sarhou, 2016)(free partial translation from French), AIE can be defined as:

“Any type of habitat in the agroecosystem, within which or around which a spontaneous vegetation develops, and is composed of annual and perennial species, or composed of sown species, known as service plants, and which are intentionally not harvested. It is a semi-natural habitat that can have different forms; linear (alleys, hedges, field margins, etc.) or of irregular surface (inundation grasslands, groves, orchards, flood-meadows, wetlands etc.) or isolated and point-like (springs, ponds, lone trees, rocks, etc.).The AEI is a place where all kinds of organisms may live, reproduce, feed, find shelter or hibernate / estivate. The AEI contributes actively towards biodiversity conservation and towards the water, carbon and nitrogen cycle, thus supporting sustainably the production function of the agriculture.”

Among the examples of efficient AEI, is the use of flower strips (six species) next to wheat fields in Switzerland. Their use reduced the population of cereal leaf beetle, compared to wheat strip control, to an acceptable economic threshold (Tschumi *et al.*, 2015)

The conservation approach is under study for main crops, but information is missing for many minor crops, such as PAMPs. In leafhopper pest control, there are a few studies regarding use of AEI for the attraction of natural enemies of the leafhoppers, for example causing damage in the vineyards (Daane *et al.*, 1998; English-Loeb *et al.*, 2003).

## 2 The objectives and research questions/hypotheses

---

The main objective of this experimental study is to evaluate the potential of the AEI for the leafhopper control in thyme crop. The core of this experimentation lies in the processes described above; providing the resources (food, shelter, and habitat) to arthropods in general near/within the crop in form of grass or flower strips. Thus, higher number of arthropods and higher richness is expected in the AEIs and crop neighbouring them, as compared to control strips (bare soil). It is expected to be true also for the natural enemies of leafhoppers. We expect to find higher abundance and/or richness of natural enemies. The floral resources - nectar and pollen - in the flower strips may be important resource for the nectari- and polleni- vorous parasitoids. The hypothesis is that more of these parasitoids would be present in the flower strips and crop neighbouring them. For the two types of AEI used in this study we tested their attractiveness for the predators, providing shelter, habitat and food. If this is so, we should find more individuals and more diverse (suspected) predators of leafhoppers in the AEIs than in the control.

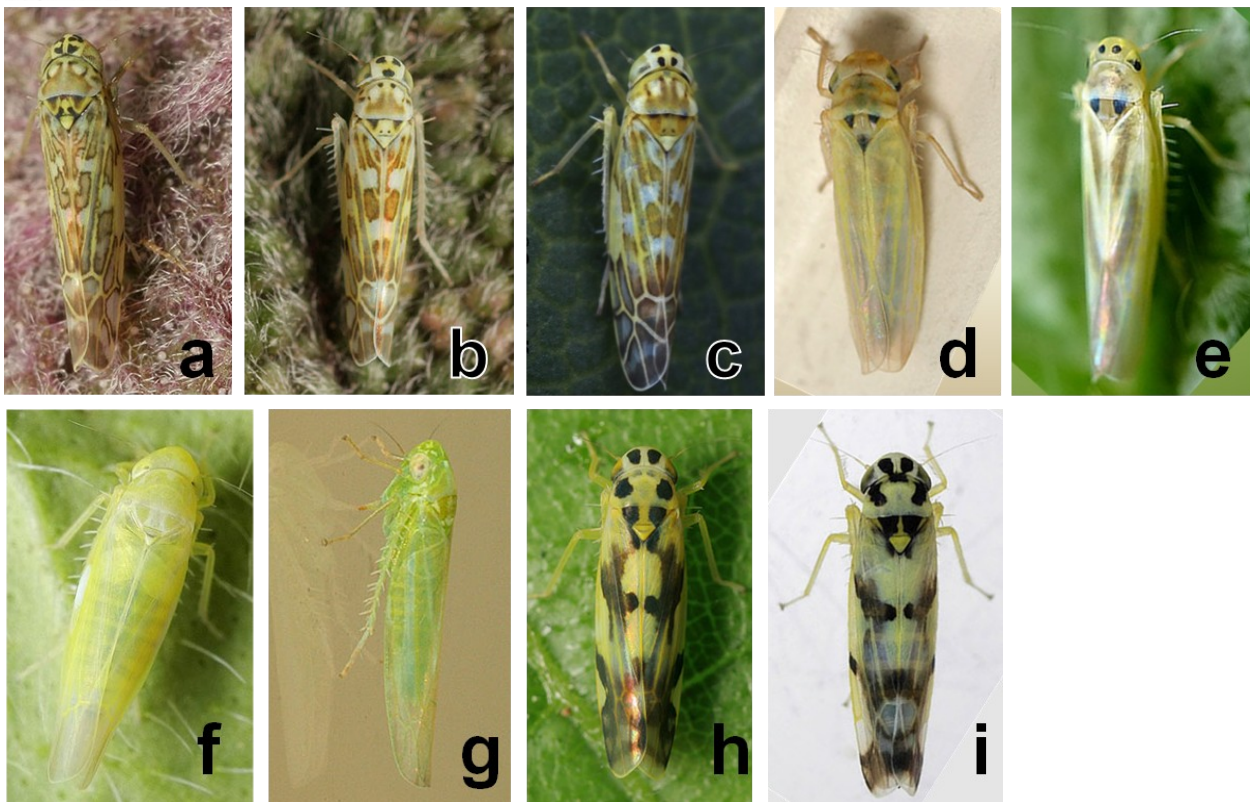
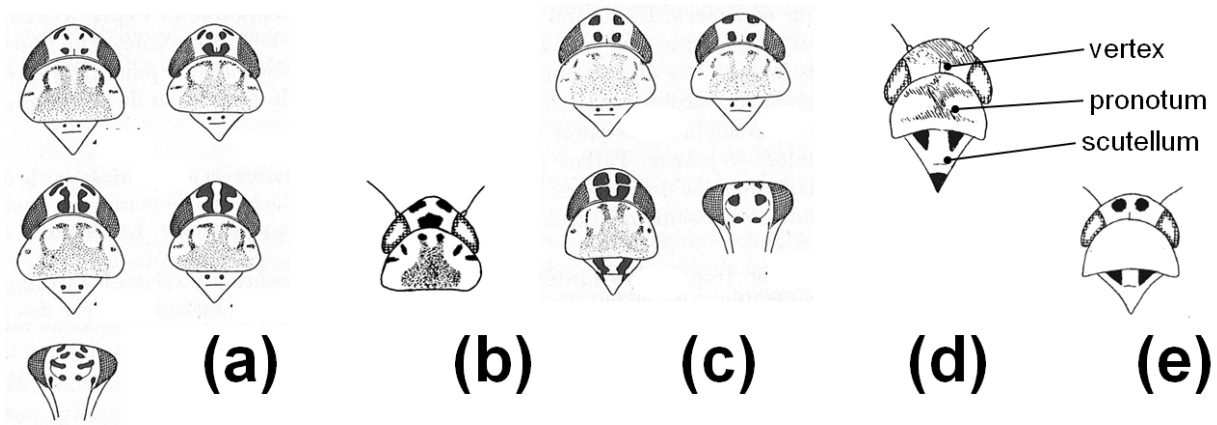


Figure 10. **a, (a)** - *Eupteryx decemnotata* Rey (2-3mm) **b, (b)** - *Eupteryx melissae* Curtis (3mm), **c, (c)** - *Eupteryx zelleri* Kirschbaum (2-3mm), **d, (d)** - *Zyginidia scutellaris* (Herrich-Schaffer) (2-2.5mm), **e, (e)** - *Hauptidia maroccana* (Melichar) (3-3.5mm), **f** - *Emelyanoviana mollicula* (Boheman) (3.2-3.6 mm), **g** - *Empoasca vitis* (Gothe) (3-4 mm), **h** - *Eupteryx aurata* (L.) (3.5-4mm), **i** - *Eupteryx atropunctata* (Goeze)(3-3.5mm).

**a, b, d, e, f, g, h** - by Tristan Bantock, **i** - by Alby Oakshott (11)  
**c**-by Gernot Kunz (12)

**(a), (c)** - from Ribault (1986) **(b), (d), (e)** (adapted) - from Le Quesne and Payne (1981).

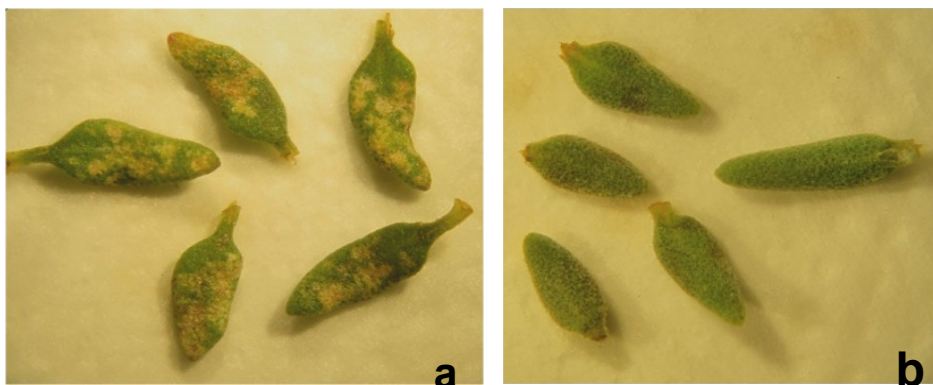


Figure 11. Leaves of thyme (*Thymus vulgaris*) **a** -with stippling **b**- healthy.

Nevertheless, even if the natural enemies are present in the AElS in higher numbers than in control and they do ‘travel’ to crop, this does not mean they will be efficient in pest control. The only measure we have to evaluate the efficiency of the natural predators is the abundance of the pest leafhoppers and the leaf damage they cause. It may well be that higher abundance of the natural enemies is related to higher abundance of the pest. In the previous year (2016), more natural enemies were trapped in the AElS and the adjacent crop, but without any reduction of leafhopper population and of leaf damage. For simplification, we will not distinguish the effect of parasitoids and predators (only sum ‘effect’ of natural enemies) on the leafhopper population and leaf damage in the following hypotheses (see **fig. 9.** and **tab. I.** for illustration of some of the sub-hypotheses).

**Major research questions: Can the AElS contribute towards leafhopper regulation in thyme?**

**Do the AElS used in the study (grass strip and flowers trips) attract natural enemies of the pest *Typhlocybinæ* leafhoppers? Do they “spill’ over” to the neighbouring crop?**

**Are they efficient in controlling the pest and the damage caused?**

### 3 Literature review

---

#### 3.1 Leafhoppers of thyme crop

Several leafhopper species (HEMIPTERA : CICADELLIDAE) belonging to the *Typhlocybinæ* subfamily cause damage on PAMPs plants of the *Lamiaceae* or mint family, such as Rosemary (*Rosmarinus officinalis* L.), Oregano (*Origanum vulgare* L.), Lemon balm (*Melissa officinalis* L.), and of course mint (*Mentha* sp. L.) and thyme (*Thymus* sp. L.). Wing venation, body parts proportions, forms, and body colour are used to distinguish the species, though precise taxonomic identification relied on genitalia shape. The *Typhlocybinæ* leafhopper species found in *Lamiaceae* crops in 2016 and 2017 (Biof’horma project) and their distinctive characteristics are illustrated on the **fig. 10.** Further description for identification can be found in **appendix I.** *Eupteryx melissae* Curtis, *E. decemnotata* Rey and *E. zelleri* Kirschbaum have all a blue-green-yellow iridescent wing coloration, simplified visual distinction is based on the number and placement of dark spots on vertex, pronotum and scutellum (**fig. 10. (d)**).

Typhlocybinae leafhoppers are mesophyll-feeders, both at larval and adult stages. They insert their stylet beneath the leaf surface into the palisade and spongy parenchyma (Pollard, 1968). The cell contents are emptied, left to be filled with air, causing whitish chlorotic leaf decolouration. These feeding marks are also described as “stippling” (Chaieb *et al.*, 2012) (**fig. 11.**). According to Nusillard, (2001), in case of heavy presence of leafhoppers, the crop may suffer due to reduced



Figure 12. **a** - Stolbur on lavender and its vector **b** - *Hyalesthes obsoletus* Signoret  
a - foto by CRIEPPAM (13), b-photo by Gernot Kunz (14)



Figure 13. **a** - Fawescence dorée (*Candidatus Phytoplasma vitis*) and its vector **b** - *Scaphoideus titanus* Ball  
a - foto by Josef Klement (15), b-photo by Yerpo, Fondazione Edmund Mach (16)



photosynthesis and loss of water. The latter is more important in dry periods. The author observed that the leafhoppers prefer irrigated plants, especially during dry period. Another negative impact of leafhopper infestation is the decreased market value of the produce, being a problem for the fresh herb producers. Chlorotic spots make it impossible to sell the produce as freshly-cut herbs and it must be directed towards dried and frozen herb use (considered secondary). The visual aspect is not important in the crop intended for the essential oil, but yield loss may occur due to stress in cases of heavy infestation.

Leafhoppers may be vectors of economically important disease, it is so for the stolbur phytoplasma of lavender and lavandin, transmitted by *Hyalesthes obsoletus* Signoret (HEMIPTERA: CIXIIDAE), causing much damage in Southern France (**fig. 12.**) and for flavescence dorée (*Candidatus Phytoplasma vitis*) transmitted by *Scaphoideus titanus* Ball (HEMIPTERA: CICADELLIDAE) causing damage in vineyards (**fig. 13.**). The Typhlocybina leafhoppers of thyme are not known as vectors of viruses, MLO or other diseases. Nusillard (2001) hypothesized that the leafhopper feeding may facilitate the entry of diseases, such as phoma on oregano and anthracnose on lemon balm.

The prevalent typhlocybina leafhopper species found during the first year (2016) of the Bioforma project, at Lycée Le Fresne (near Angers, France), was identified as the Ligurian leafhopper, *Eupteryx decemnotata* Rey (Farcy, 2016). It is assumed that this is the species responsible for the majority of the leaf damage (white/yellow chlorosis) observed in thyme field experiments. Based on naked eye and yellow sticky traps observations a strong presence of this species was detected in the thyme fields from April 2017 onwards through the season.

### 3.1.1 Distribution of typhlocybina leafhopper species

The Ligurian leafhopper is named after the Ligurian sea region (North-western Italy), where it was first recorded in 1920 (Mancini 1935 in Nickel and Holzinger, 2006). It seems to be common in Southern Europe but has been reported as new or newly recorded species in several countries in the last 20-30 years; Poland (in greenhouse on rosemary, mint, basil and lemon balm) in 2014 (Lubiarz and Musik, 2015) and Tunisia in 2009 on sage and rosemary (Chaieb *et al.*, 2012) are recent examples. As predicted by Nickel and Holzinger, (2006) the Ligurian leafhopper has spread northwards and eastwards and was found in Czech Republic in 2008 and 2010. The species was probably present prior 2010, but unnoticed (Malenovský and Lauterer, 2010). Similarly, it was recorded as a new species in Britain (Maczey and Wilson, 2004), in Denmark in 2007, in Finland (Söderman *et al.*, 2009) and Sweden in 2008 (Gillerfors, 2009). It has been “exported” as far as the United States of America (Rung *et al.*, 2009). Closely related species, such as, *Eupteryx atropunctata* (Goeze), *Eupteryx zelleri* Kirschbaum, *Eupteryx melissae* Curtis, *Emelyanoviana*

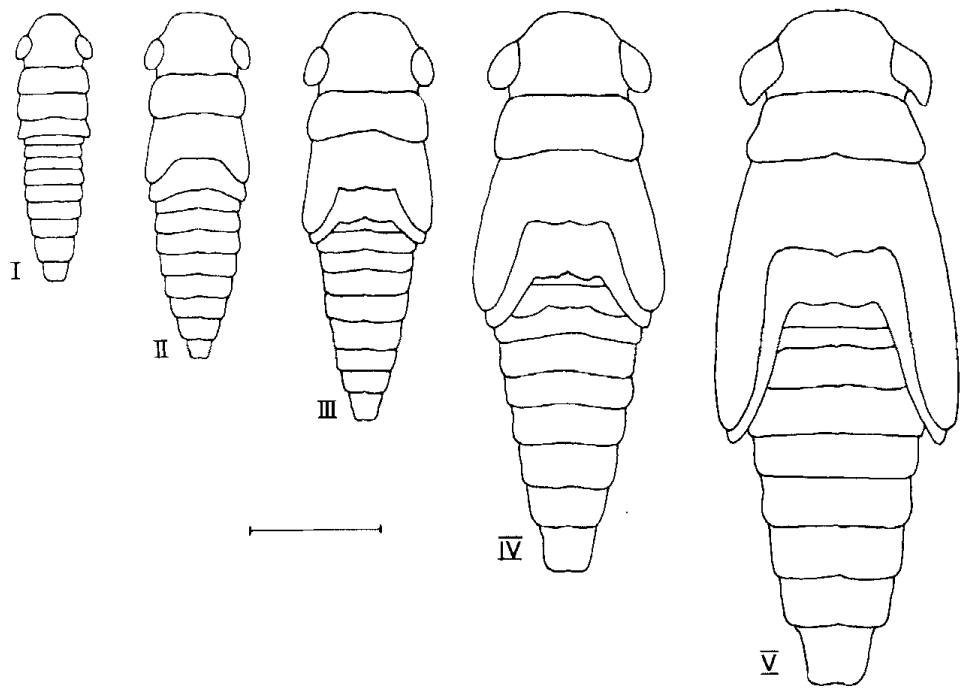


Figure 14. The five instars of *Eupteryx urticae*. Scale line = 0.5 mm, from **Stewart (1986)**.

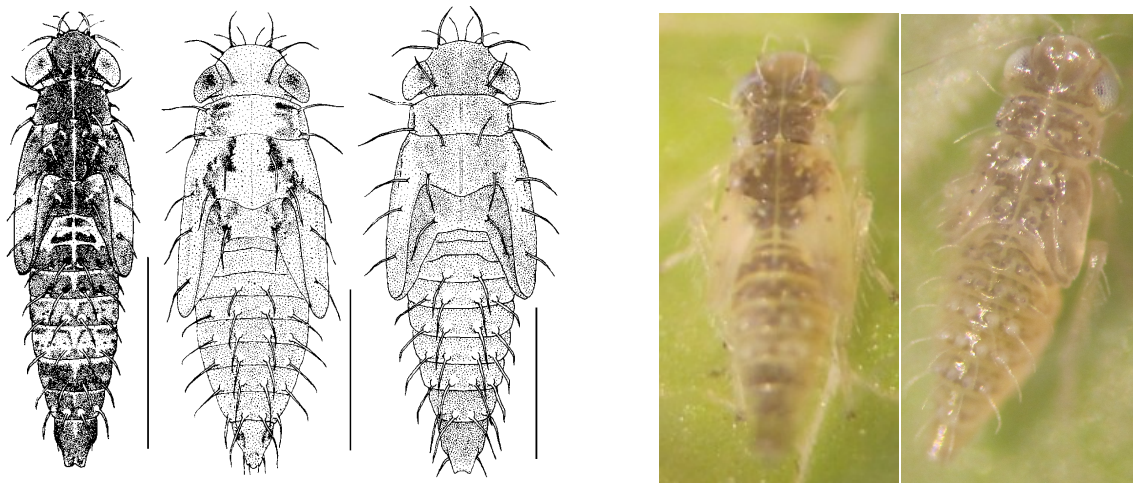


Figure 15. The fifth instar larva of *Eupteryx decemnotata*, *Eupteryx atropunctata*, *Eupteryx aurata*, from **Blum et al. (2011)** and photos of larva observed during the experiment 2017.

*mollicula* (Boheman) are often found in medicinal and aromatic herb crops in association with *E. decemnotata*. During observations done by Bouillant *et al.* (2004) in Switzerland, *E. decemnotata* was nearly the only species of leafhopper captured in 2000 in sage, lemon balm, thyme rosemary and other PAMP crops. This species was “supplemented” by *E. atropunctata* and *Em. mollicula* in 2001, one of the two becoming even the dominant leafhopper species in certain fields. The accompanying species are equally spreading across the world. *E. aurata* was also recorded as a new species in Finland in 2008 (Söderman *et al.*, 2009). *E. atropunctata* is present in whole Europe, as far as European part of Russia and Georgia, in North Africa (Algeria), and was also introduced in Canada (Dimitriev, 2017) *Em. mollicula* is reported in most of the European countries, and as far as Altaï Mountains region and *E. melissae* is now present in USA and New Zealand (Dimitriev, 2017).

Nickel and Holzinger (2006) suggest that distributional range expansion of typhlocybines leafhoppers may result from the commercial trade of their host plants. Plants on German markets often show typical marks of stippling. Furthermore, living individuals were reported on commercialized sage plants on markets and in shops in Germany.

### 3.1.2 Life cycle of the Ligurian leafhopper, *E. decemnotata*

The Ligurian leafhopper overwinters at the egg stage (Bouillant *et al.*, 2004). Because of the under-epidermal oviposition and because of their minute size, eggs are nearly invisible to the naked eye. In southern regions of Europe, adult stages may overwinter as well (Bouillant *et al.*, 2004; Mazzoni and Conti, 2006; Nusillard, 2001). According to (Mazzoni and Conti, 2006) eggs hatch between 20-26 days (21.48 +/- 1.26 days) after oviposition in laboratory conditions ( 20°C; RG 50%, 18:6 L/D photoperiod), and the five larval stages (**fig. 14., 15.**) took altogether  $19.5 \pm 1.83$  days. Each larval stage takes between three and five days and the last, 5<sup>th</sup> instar takes the longest (5.53 +/- 0.57 days).

According to preliminary study by Mazzoni and Conti (2006), the female Ligurian leafhopper can oviposit two to four eggs per day for more than a three-week period. This could result in several generations that overlap during the season. One to a few major peaks of population abundance are usually recorded when the species colonizes the crop. In mountainous regions of Switzerland in 2000 and 2001, two generations were observed Bouillant *et al.* (2004). As many as three generations are possible in Central and Southern Europe. The data from 2016 in Angers confirmed two peaks.

The cycle of leafhoppers causing damage in aromatic herb crops is greatly disturbed by the harvest cuts (Bouillant *et al.*, 2004). The male and female leafhoppers do not use pheromone attraction but



they communicate through a tymbal, a vibration-producing organ, although not audible for human ear. It was described in *Typhlocybinæ* leafhoppers by several authors, but first “heard” by Frej Ossianilsson in 1949 (Hill, 2014).

The Loire valley around Angers city and neighbouring regions represent the major horticultural production area in France, with a relatively dense network of greenhouse installations. We hypothesise that one of the inoculum source of *E. decemnotata* in the early spring, beside the overwintering eggs, might be the greenhouse-based plant nurseries of the aromatic herbs. Indeed, the “Lycée Le Fresne” possesses its own tunnels, nurseries and greenhouses. We observed stippling on the leaves of the nursery thyme plants in the early April 2017. The nursery plants are produced from the cuttings collected on the nearby infested thyme fields.

## **3.2 Controlling the leafhoppers in the *Lamiaceae* crops**

### **3.2.1 Conventional pest control**

The use of insecticides may be possible on the larval and adult stages of leafhoppers. However, these stages may partially escape the treatment by seeking shelter within the crop (under the leaves). Eggs are laid under the leaf epidermis leaving systemic or translaminar insecticides as the only options for pre emergence chemical treatments.

Seed extract of *Azadirachta indica* A. Juss (NeemAzal®) was efficient on sage under greenhouse conditions (dose 1.5 l/ha in 3-4 applications) against leafhoppers (Grohs, 2013) Its effect was smaller on lemon balm. Bark extract of *Quassia amara* L. (*Quassia nativ*®, *Quassia-MD*®, *Quassan*®) was also effective in these two crops, but not for oregano and rosemary.

(Nusillard, 2001) tried “Crésus” pesticide (deltaméthryine+ chlorpyrphos-méthyl, 0.5l/ha), used against vine leafhopper (*Empoasca vitis*, FR: Cicadelle verte, Cicadelle des grillures de la vigne) against a group of species (*E. alticola*, *E. aurata*, *Em. mollicula*, *Empoasca pteridis*, *Zyginidia scutellaris*).The treatment was not efficient in regulating leafhopper population and phoma development. The only usefulness of pesticide (and/or fungicide) use in this study was in connection with protective anti-insect textile cover of the crop. (Grohs, 2013) lists similar results - the protective nets are effective, but not in the crop already infested with leafhoppers. These results suggest that the culture can be appropriately protected only by using the anti-insect net and therefore preventing the physical contact between crop and leafhoppers.

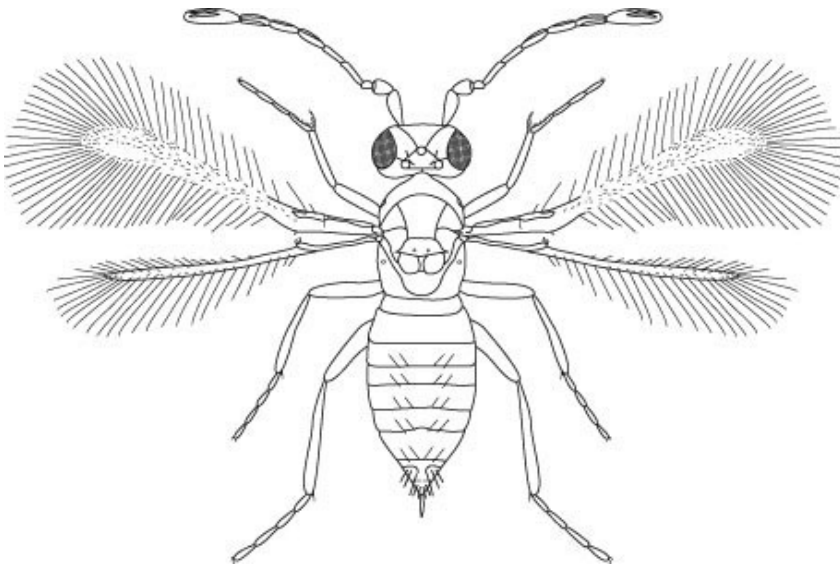


Figure 16. *Anagrus* sp. from **Chiappini (2008)**.

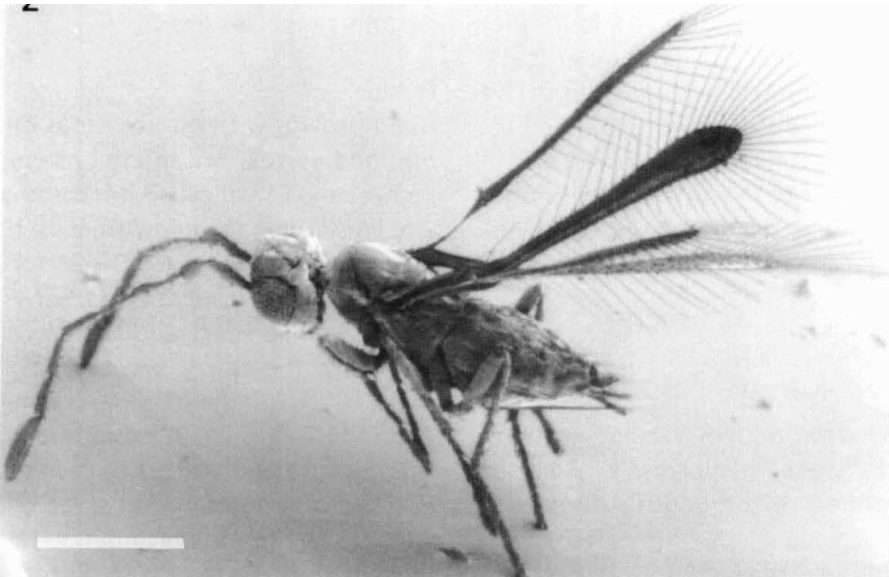


Figure 17. *Anagrus atomus* female from **Baquero and Jorciana (1999)**, scale = 200  $\mu$ m.

Among the stimulators of natural plant defence, none was efficient, only some decrease in leafhopper abundance was observed for extracts of garlic, extract of *Linnaria* sp (*Scrophulariaceae*), mineral powder and silica (Grohs, 2013)

Baroffio and Lenne (2013) tested a push-pull method for leafhopper control. They found that *E. decemnotata* preferred sage and rosemary over oregano while *Em. mollicula* preferred sage and oregano over rosemary. They propose using mint as a trap plant and chives as repulsive plant, in combination with other control methods.

### 3.2.2 Vibrations and physical removal (suction)

Vibrational confusion was tested in semi-field conditions on grapevine leafhopper, *Scaphoideus titanus* a vector of the Flavescence dorée. Emitting a recording of disruptive vibrational signal, that is naturally produced by rival males resulted in drop of copulation. The mating frequency decreased to 9% and 4% in the semi-field and mature vineyards respectively, up to distance of 940 cm from the signal (Eriksson *et al.*, 2012).

Bennison *et al.* (2009) tested physical removal of the leafhopper pest by suction machine on thyme and mint. The treated plots had significantly lower leafhopper abundance, but a large number (75-85%) of non-target arthropods were removed from the crop. There was no effect on leaf damage, probably due to dispersal of the leafhoppers from other fields or by 'flushing' effect from the passage of the machine within the field.

## 3.3 Natural enemies-parasitoids

### 3.3.1 Anagrus Haliday (Hymenoptera: Mymaridae)

*Mymaridae* is a family of parasitic wasps, in general less than 3 mm long and usually less than 1.5 mm (Goulet and Huber, 1993). The smallest insect in the world is a *Mymaridae* species. Twenty fairy fly genera are recorded in Europe (Pricop, 2013). *Mymaridae* are all parasitoids of egg insects mostly in concealed situations, for example in plant tissues. The hosts of *Mymaridae* are mainly *Homoptera* and *Hemiptera*, and less often *Psocoptera*, *Coleoptera*, *Orthoptera* and *Diptera*. (Goulet and Huber, 1993).

Considered as the major parasitoid of the *Typhlocybinae* leafhoppers of *Lamiaceae*, *Anagrus atomus* (fig. 16., fig.17.) was not detected by Nusillard (2001) in south-East France, and neither was "our" predominant species of leafhopper, *E. decemnotata*. The prevalent species were *E. alticola*, *E. aurata*, *Em. mollicula*, *Empoasca pteridis*, *Zyginidia scutellaris*, on *Lamiaceae* field crops of basil, lemon balm, oregano, rosemary, thyme sage and hysope.





Agboka *et al.* (2003) confirmed that females of *A. atomus* distinguish infested and non-infested bean leaves, as they spent significantly less time searching and ovipositioning on the latter ones.

### Life cycle

Agboka *et al.* (2004) found that the egg-adult developmental time of *Anagrus atomus* took 22.6 days (at 20°C) in average. In general, 263.2 degree-days were necessary. At 26°C, *Anagrus atomus* egg hatches within the host egg about 2-3 days after oviposition, goes through two larval stages, the first - sacciform and immobile lasts 1-2 days, the second - mobile, actively feeding lasts about seven days. The larvae undergoes prepupal stage (about 1 day) and enters into pupal stage lasting 5-6 days, ending in adult emergence (Hesami *et al.*, 2004) The temperature affected the developmental time, only 13.3 days were necessary at 28°C, (Agboka *et al.*, 2004) 17 days for at 26°C (Hesami *et al.*, 2004), but 33.6 days at 16°C ,(Agboka *et al.*, 2004).

The temperature also affects the longevity, sex ratio and number of eggs oviposited per emerged female (Agboka *et al.*, 2004). With increasing temperature, sex ratio swayed more in favour of males, but longevity and ovipositioning period of females decreased. The females reared at 16°C lived longest (15 days) and oviposited longest too, though not significantly. The most offspring (30) was reached at 24°C per female.

### Food resources

Food resources could prolong life in laboratory studies. There is a strong suspicion that *Anagrus* is nectarivorous. Some *Anagrus* species feed on honey under laboratory conditions (English-Loeb *et al.*, 2003; Krugner *et al.*, 2009). Also, life of the adults *Anagrus epos* was five-fold when provided with honey and water, and was three-fold with only honey, compared to no food treatment (Krugner *et al.*, 2009). Similarly, English-Loeb *et al.* (2003) found that access to carbohydrates had prolonged the life of *Anagrus* adults found in vineyards (parasitoids of Typhlocybina leafhoppers *Erythroneura* sp.). At the same time they suggest that not all *Anagrus* are necessary nectarivorous. They did not distinguish the *Anagrus* species in their study - there may be as many as six different species - and they have seen a variation in the data. Additionally, other *Mymaridae* were confirmed visiting flowers; *Gonatocerus* sp. on *Achillea millifolium* L. and *Convolvulus arvensis* L. and *Anaphes* sp. on *Ranunculus repens* L.(Jervis *et al.*, 1993).

### *A. atomus* and leafhopper control

*A. atomus* is a confirmed egg parasitoid of *Empoasca decipiens* Paoli (Agboka *et al.*, 2003), one of the major leafhopper pests in European greenhouses, as well as of *Arboridia kermanshah* Dlabola, a grape leafhopper (Hesami *et al.*, 2004) and other leafhopper species. Seven *Mymaridae*

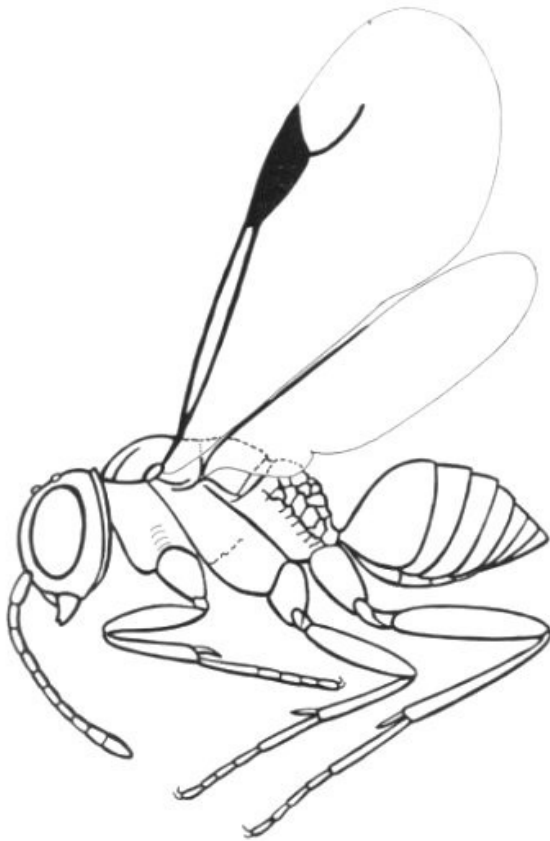


Figure 18. *Aphelopus* illustration from (17).

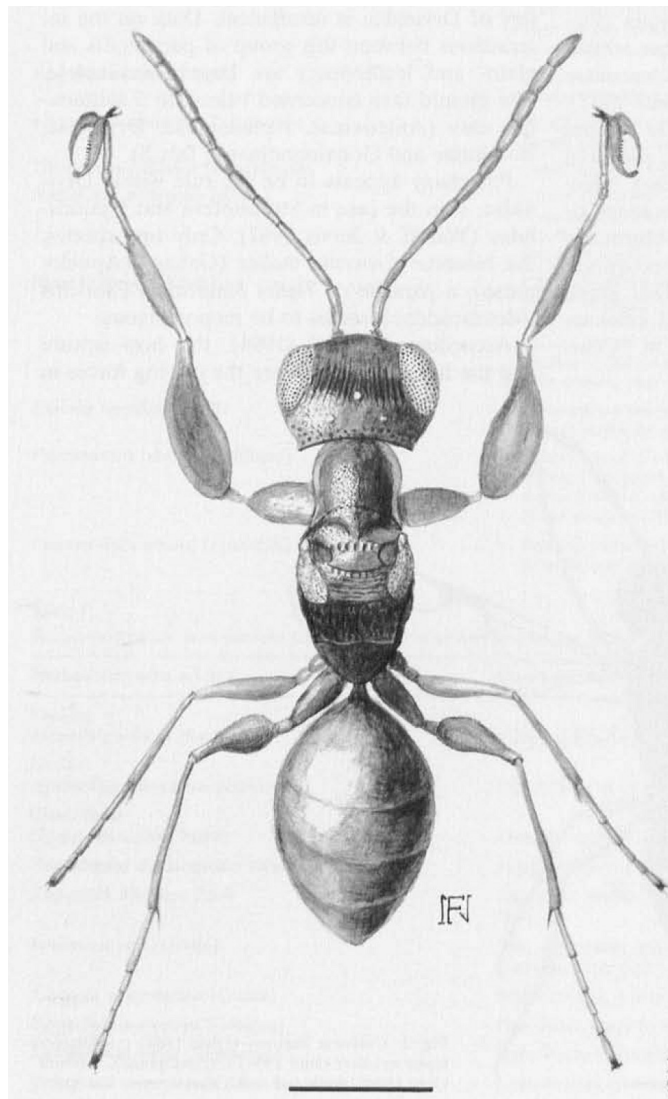


Figure 19. *Dryinidae* female with chelae. *Anteon buntini* Olmi (*Anteoninae*) from **Guglielmino (2000)**. Scale = 0.5 mm.



Figure 20. *Aphelopus atratus* illustration from (18).

species, of which four *Anagrus* species, were recorded in vineyard in Bourgogne (Sentenac, 2004), but *A. atomus* was by far the major species found. As *A. atomus* is cited in several articles as parasitoid of various species of Typhlocybinae leafhoppers and is currently commercialized as agent of biological control against leafhoppers (in UK). It may well be parasitizing the *E. decemonotata*. Nevertheless, Matteucig (2007) in Maugin and Sforza (2011), found for *E. zelleri*, a species very close to *E. decemonotata*, that *Anagrus atomus* had no impact on this species. They found that it was *Anagrus ustulatus* Haliday that parasitized *E. zelleri*.

### 3.3.2 *Aphelopus* Dalman (Hymenoptera: Dryinidae)

There are 80 relationships known to exist between *Dryinidae* and *Auchenorhynche* in France (Guglielmino, 2000). In Palearctic region, four families of *Dryinidae* are known to be parasitoid of *Cicadellidae*: *Aphelopinae* (*Aphelopus*), *Anteoninae* (*Anteion*, *Lonchodryinus*), *Bocchinae* (*Mirodryinus*, *Mystrophorus*) and *Gonatopodinae* (*Gonatopus*, etc.) Summing up to 166 the *Cicadellidae* taxa known to be hosts for *Dryinidae* in Palearctic region. Only one genus was identified as a parasitoid of *Typhlocybinae* leafhoppers – the genus *Aphelopus* (**fig. 18.**, **fig. 20.**) in France (Guglielmino, 2000). In the catalogue of hosts-parasites, Guglielmino *et al.* (2013), list *Aphelopus melaleucus* (Dalman), *Aphelopus atratus* (Dalman), *Aphelopus camus* Richards, *Aphelopus serratus* Richards, *Aphelopus nigriceps* Kieffer, *Aphelopus querceus* Olmi as species parasitizing *Typhlocybinae* leafhoppers. A species of *Diapriidae* family (Hymenoptera), *Ismarus dorsinger* Curtis is a hyperparasitoid of *Aphelopus* species (Jervis, 1980a).

#### Feeding behaviour

In all of the subfamilies of *Dryinidae*, the females resemble ants and are easily distinguishable by the “pincers” on the front legs - ‘the chelae’ (**fig. 19.**), except for the subfamily *Aphelopinae*, not having this trait (Guglielmino, 2000). The reproduction of *Dryinidae* can be bisexual and/or parthenogenic. The males do not feed or feed on sugar solutions (honey dew of the hosts), while females can be mono-or poly-phagous. They may feed on sugar solutions (honey dew) and/or predate. The females can feed on their host destructively using the chelae, such as specialised subfamilies *Dryininae* and *Gonatopodinae*. Some can feed non-destructively, such as less specialised subfamily *Anteoninae* (Olmi, 1994). Thus they are both parasitoids and predators. It is known that in the four subfamilies *Gonatopodinae*, *Dryininae*, *Bocchinae* and *Anteoninae* the predation is as important as parasitism (Guglielmino, 2002). Host feeding is absent in the species without chelae (“pincers”); which is the case of *Aphelopinae* subfamily and therefore for the genus *Aphelopus* (Olmi, 1994), thus being exclusively parasitoid.



Figure 21. *Chalarus spurius*, photo by J. Kahanpää from (19)

## Life cycle

The ovipositing *Aphelopinae* female grasp the host by the front legs and possibly with the help of mandibles, while other *Dryinidae* use their chelae. They inject a paralysing substance, which does not act long and lay the eggs into haemocoel. *Aphelopus* has five larval stages, of which two take place inside the host, but during the later three stages, the larvae protrudes on the side of the host's body in form of a cyst called "thylacium". The development in the host's body lasts six weeks (Jervis, 1980b). The mature larvae eats out the content of its host, killing it, splits open the thylacium, crawls into soil and pupates in a silk cocoon prepupa (Olmi, 1994). In non-diapausing species the adult emerge after three weeks, and the adults live for a maximum of two weeks (Jervis, 1980b). The development of larvae within a typhlocybine leafhopper causes degeneration of the host, such as, delayed development, degeneration of genital ducts of both males and females, feminisation of males and loss of stridulatory organ (Olmi, 1994).

The *Dryinidae* males are "lethargic", not moving much, except for searching for females, while females are very active - searching for hosts (Olmi, 1994). The ant-like look and honey-dew feeding allow some of the *Dryinidae* to approach closely their hosts without suspicion. Ants often feed on the honey dew of the hemipterans and protect them against predators. Such facilitation was observed in apterous females of the genus *Gonatopus* (Guglielmino, 2002). *Dryinidae* are used in biological control programs around the world (Guglielmino, 2002)

## *Dryinidae* in leafhopper control

During the experimentation in 1999-2000 in France Nusillard (2001) found that parasitism by *Aphelopus atratus* reached between 20 and 40 %, observed on hundreds of individuals, though the observation was done without any protocol. Contrary to the literature data, he found the parasitic cyst on the adults and not the larvae. He claims that the adult stage of *Aphelopus* is a predator of leafhoppers, but as stated above, *Aphelopinae* is the only non-predatory subfamily of *Dryinidae*. Similarly, Bouillant *et al.* (2004) found cysts of *Dryinidae* (no further identification given) on leafhoppers' larvae. In *Empoasca vitis*, Sentenac, (2004) found only around 1% parasitism by *Dryinidae*.

### 3.3.3 *Chalarus* Walker (*Diptera:Pipunculidea*)

The *Chalarus* species (**fig. 21.**) are rather well distinguishable by their large, often globular heads, almost completely covered by eyes. The name of the genus *Pipunculus* and name of the family is maybe a derivation of latin *pepunculus*, meaning little pumpkin (Jervis, 1992). The name *Chalarus*

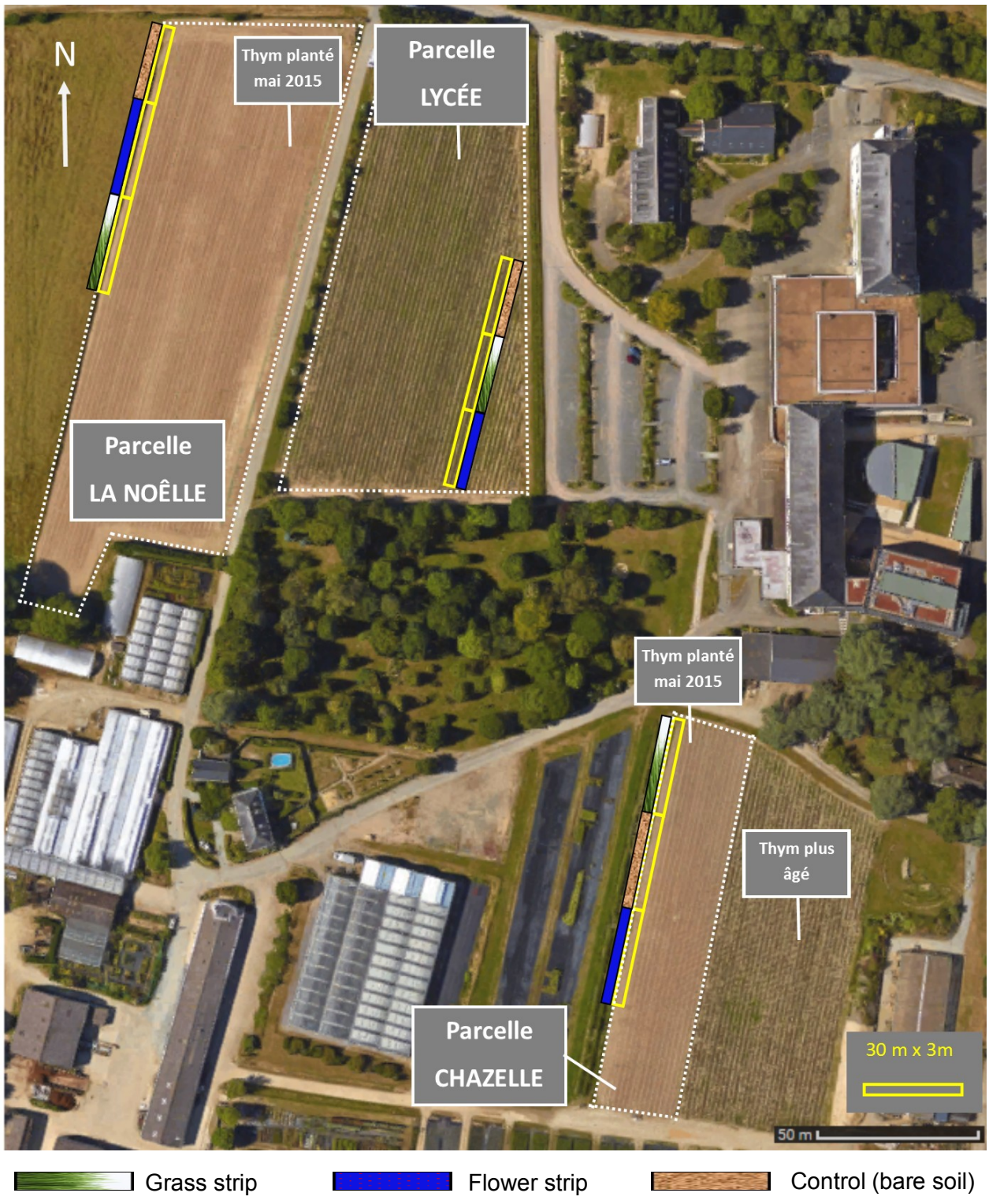


Figure 22. Overview of the thyme fields at the “Le Fresne” horticultural farm and layout of the experimental treatments.

is derived from Greek *khalaros*, meaning languid, probably describing their flying movements (Jervis, 1992).

The species of the genus *Chalarus* parasitize exclusively the typhlocybinae (Jervis, 1980a). In Europe 24 species are described (Kehlmaier and Assmann, 2008), of which 11 are recorded in France, including the three species that were reared from *Eupteryx* sp; *Chalarus spurius* (Fallen), from *E. aurata*, *E. cyclops* and *E. urticae* on *Urtica dioica* and from *E. melissae* on *Salvia* sp., *Chalarus pughi* (Coe) was reared from *E. aurata* and *E. urticae* on *Urtica dioica* and *Chalarus fimbriatus* Coe from *E. urticae* on *Urtica dioica* (Jervis, 1992). The parasitic range can be quite wide. For example, as many as five *Chalarus* species have been recorded to have *Empoasca vitis* for a host and *C. indistinctus* Jervis is already known to parasitize ten typhlocybinae species. (Kehlmaier and Assmann, 2008). To my knowledge, there are no records on *Chalarus* parasitizing *Eupteryx decemnotata*.

The females lay eggs in 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> larval stage hosts. In some *Chalarus-Typhlocybinae* relationship as much as 20% of mortality in progeny was observed (Jervis, 1980a). Only around 5% of parasitism was observed on *Empoasca vitis* by *Chalarus* sp. in vineyards in France by Sentenac, (2004). *Chalarus* species have two larval stages, with the second stage taking place in an adult host. The presence of the parasitoid within the host causes parasitic castration (abnormal genitalia), sometimes decolouration. After consuming the host's body, the larva drops into soil and pupates (Jervis, 1980a).

A few cases of multiparasitism were observed between *Chalarus* and *Aphelopus* in *Fagocyba cruenta* (Herrich-Schaeffer). The *Chalarus* larva reached the maturity and therefore indirectly killed the *Aphelopus* larva (Jervis, 1980b).

### 3.4 Natural enemies – Predators

There are indices that generalist predators, such as arachnids, coccinellids, carabids and true bugs may contribute towards *Eupteryx decemnotata* control. Sentenac (2004), observed predation on *Empoasca vitis* by larvae of lacewing *Chrysopidae* (*Chrysoperla*), but never in the field conditions. Grohs (2013) states similar results for several species of natural enemies. A few of them had some positive effect on leafhoppers under laboratory or greenhouse conditions. Though they were inefficient in controlling leafhopper population in field, among them, lacewing *Chrysoperla carnea* (Stephens), true bugs *Macrolophus pygmaeus* Rambur, *Orius majusculus* (Reuter) and *Orius laevigatus* Fieber. The author states that no effect was observed for the ladybirds or parasitic nematodes *Steinernema carpocapsae* (Weiser) and *S. bicornutum* Tallosi, Peters & Ehlers.

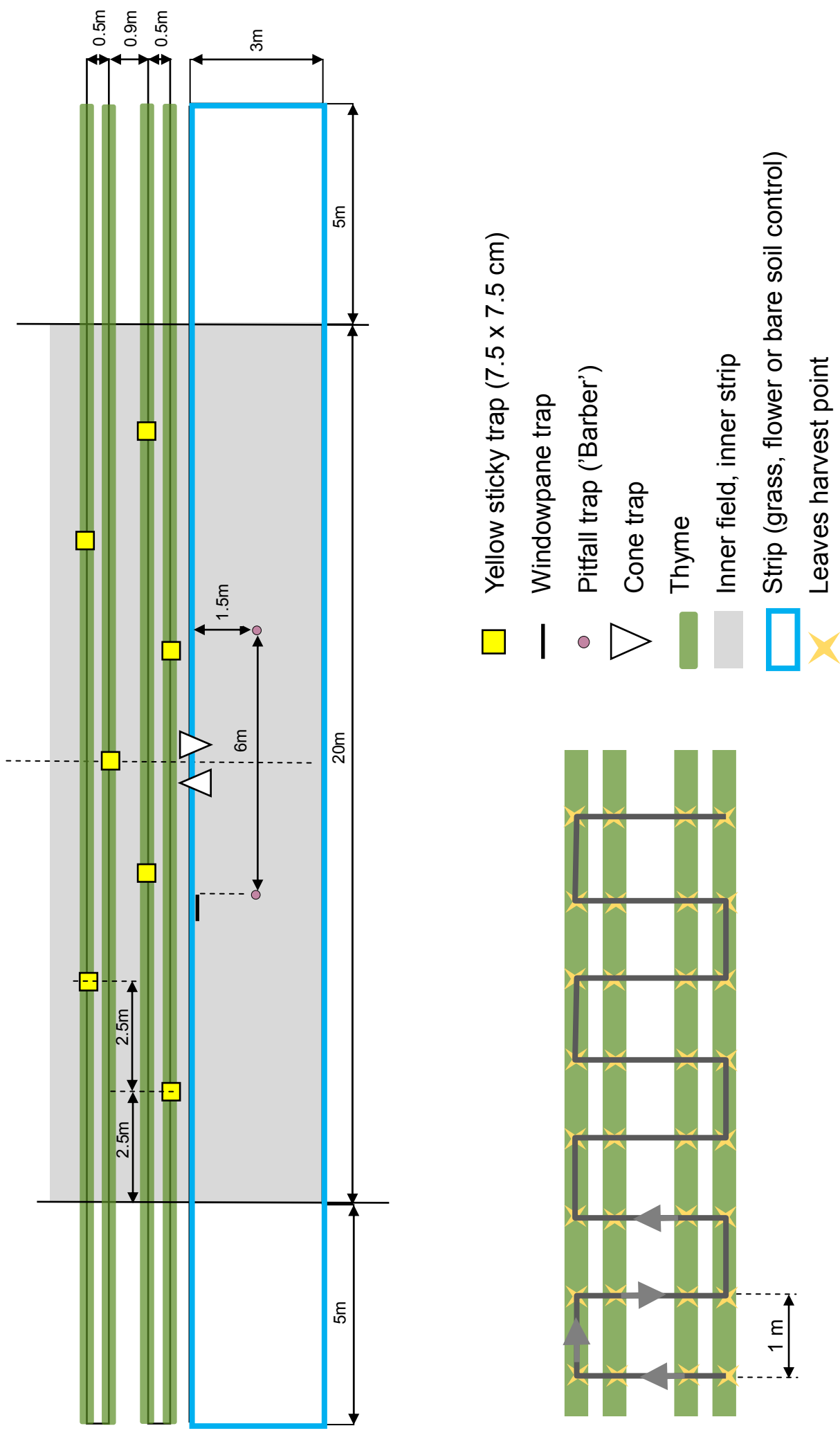


Figure 23. Layout of a the experimental plot (20m long) and a scheme of leaves harvest path.



## 4 The experiment

---

### 4.1 Materials and Methods

The experiment was conducted during the spring and summer 2017, on the thyme fields of the secondary agricultural school “Lycée Le Fresne”, located in Sainte-Gemmes-sur-Loire, near the city of Angers, France (47°26'32.5"N 0°35'16.5"W). The three separate thyme fields are named “Chazelle”, “Noëlle” and “Lycée”. The fields were planted in 2013 (Lycée, 0.98 ha) and 2015 (Chazelle 0.92 ha, Noëlle 1.19 ha). These fields are conducted the same way as farmers’ fields with an objective of essential oil production, although they may host several experiments. The management of the fields is not organic. A herbicide is used to deal with some of the persistent weeds, but strictly respecting the necessary delay between herbicide application and harvest cuts. The fields are planted with thyme of two chemotypes (mixed), thyme ‘thujanol’ and thyme ‘linalol’ due to error of the nursery providing the plantings. Once distilled, the essential oil is of a ‘thujanol’ chemotype (based on % thujanol content). The thyme is planted in double-rows, spaced 0.3 m within the row, 0.5 m between the rows, and 0.9 m between the double rows (pattern 0.5 m-0.9 m-0.5 m)(**fig. 23.**). The “Chazelle” field has rather open surroundings and a young hedge of trees and shrubs on its western side. The “Lycée” field is neighbouring with a arboretum park on its southern side. Both “Chazelle” and “Lycée” are situated above the “Noëlle” field (altitude). The last one is slightly slopping down from the road (east) towards a nearby retention zone of Maine river (west) (**fig. 22.**).

#### The experimental design

In each field a block (replicate) consisting of three plots was delimited along the field margin. The outer dimensions of plots were 30 m x 3 m. Due to technical obstacles; four of the nine experimental plots were reduced to 24 m. To secure the independence of the observations, at least 10m separated the observational plots (inner plots) in contiguous treatments. Hence, the inner dimensions of plots were 20 m x 3 m (or 14 m x 3 m for the smaller plots). The three treatments in each block were: F- Flower strip margin, G- Grass strip margin, C – Control (margin with bare soil) (**fig. 22.**).

The distribution of treatments within each block was randomised, except for two strips. The “Chazelle” flower strip matched the location of the flower strip of the previous year in order to avoid unwanted flower growth from the previous year’s seeds in the other treatments. In the “Lycée” field, the northern end of the experimental plot was very uneven (many thyme plants missing, leaving the ground bare - an opportunity for invading weeds). It was therefore decided to assign a control strip



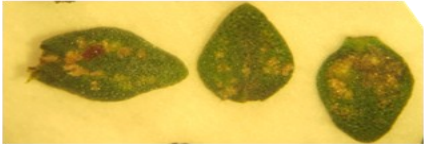
Table II. List of species sown in the flower strip with scientific, common French and common English names.

Species	Espèces	Species
<i>Calendula officinalis</i> L.	Souci officinal	Common marigold
<i>Centaurea cyanus</i> L.	Bleuet des champs	Cornflower
<i>Coriandrum sativum</i> L.	Coriandre cultivée	Coriander, Cilantro, Chinese parsley
<i>Fagopyrum esculentum</i> Moench	Sarrasin	Common buckwheat
<i>Foeniculum vulgare</i> Mill.	Fenouil commun	Fennel
<i>Malva sylvestris</i> L.	Mauve sylvestre	Common mallow
<i>Matricaria chamomilla</i> L.	Matricaire camomille	German chamomile
<i>Phacelia tanacetifolia</i> Benth.	Phacélie	Lacy phacelia, Phacelia
<i>Sinapis alba</i> L.	Moutarde blanche	White mustard
<i>Vicia sativa</i> L.	Vesce cultivée	Garden vetch, Common vetch

Table III. List of species sown in the flower strip with scientific name, botanical family, flowering period and colour, adapted from Villeneuve (2015).

Family	Species	Flowering period	Colour
Apiaceae	<i>Coriandrum sativum</i> L.	APR - SEP	white
Apiaceae	<i>Foeniculum vulgare</i> Mill.	MAY - OCT	yellow
Asteraceae	<i>Calendula officinalis</i> L.	APR - OCT	orange
Asteraceae	<i>Centaurea cyanus</i> L.	MAY - SEP	blue
Asteraceae	<i>Matricaria chamomilla</i> L.	MAY - OCT	yellow
Boraginaceae	<i>Phacelia tanacetifolia</i> Benth.	APR - SEP	blue
Brassicaceae	<i>Sinapis alba</i> L.	MAY - OCT	yellow
Fabaceae	<i>Vicia sativa</i> L.	APR - SEP	purple
Malvaceae	<i>Malva sylvestris</i> L.	MAY - OCT	purple/pink
Polygonaceae	<i>Fagopyrum esculentum</i> Moench	APR - OCT	white/pink
Number of species probably flowering		APR 3 MAY 5 JUN 9 JUL 10 AUG 10 SEP 9 OCT 5 NOV 1	

Table IV. Leaf damage categories.

Category	Criteria	Visual
No damage	no visible stippling observed	
Moderate stippling	less than 30% of the leaf surface damaged less than five spots	
Heavy stippling	more than 30% of the leaf surface damaged more than five spots	

to this part. Due to technical errors on the “Lycée” field, the strips were set on the east side of the experimental plots, and the plots were reduced to 24 m length.

#### 4.1.1 The experimental field management

The flower strips and grass strips were sown on 30<sup>th</sup> March 2017, using a mixture of seeds containing ten species (**tab. II.**), provided by ITEIPMAI, and ray grass/fescue mixture respectively. The flower mixture was conceived and tested in another project (Floregul, 2015-2017). The mixture was designed to contain non-expensive, adaptable and easy-to-obtain species of various flower colour, height and flowering period (**tab. III.**). It contains also two species providing extra-floral nectar (vetch and cornflower, Villeneuve, 2015). Both strips were hand sown by Mr. Eric Duclaud and rolled with hand-tracked roll. On 4<sup>th</sup> April, the missing flower species *Matricaria chamomilla* (L.) Rydb was sown separately. Due to windy and dry weather, the grassy strips had to be resown, once on “Chazelle” and twice on “Lycée” (last re-sowing on 10<sup>th</sup> May). The need for re-sowing thus delayed the development of the grass strips. The grass strip of the “Noëlle” was not sown as it is a part of semi-permanent grassland installed for several years on the western side of the field. It was cut on 30<sup>th</sup> May.

The strips were irrigated when needed. The “Chazelle” field is equipped with irrigation on the side of the strips, but the other two fields were irrigated manually (water container with showerhead). Later on in the season, a mobile sprinkler was used on the “Noëlle” field.

The first two double rows of the experiment, as well as the control strips were weeded regularly. However, it was impossible to keep it free of weeds at all times. The priority was given to keep the control clean (bare soil), usually by rototiller (FR: motoculteur) and mostly before the trapping periods (pitfall traps).

The harvest cut (removing the 15 cm of thyme height) was done during the week of 14<sup>th</sup> June. The two double rows used for leafhopper trapping were kept uncut. But the technicians accidentally cut a part of the second double-row (but outside the “inner border”) near the flower strip of the “Chazelle” field. Again, on the same spot, on the 3<sup>rd</sup> July, the second double row (from strip) was cut all along up to the middle of the next treatment (control strip).

#### 4.1.2 Botanical survey of the strips

To estimate the success of installation of the grass and flower strips a botanical survey was done once on 16<sup>th</sup> June 2017. The plant species were recorded in each grass and flower strip (20 m x 3 m or 14 m x 3 m). For each species, the cover area (%) and the percentage of flowers in bloom were estimated. The control strips had been rototilled just a few days prior to the survey,

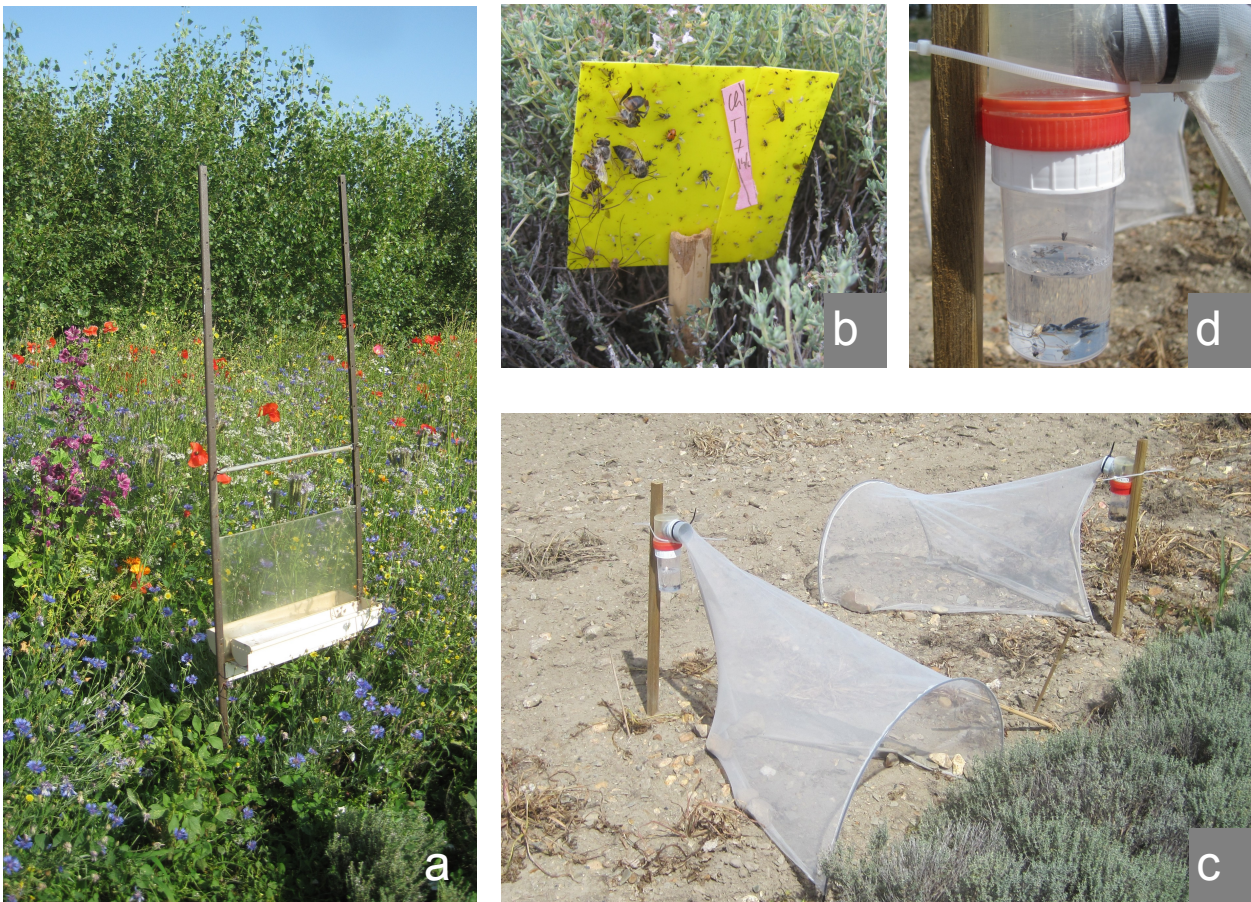


Figure 24. a - windowpane trap, b - yellow sticky trap, c—two cone traps, d—detail of the collecting pot.

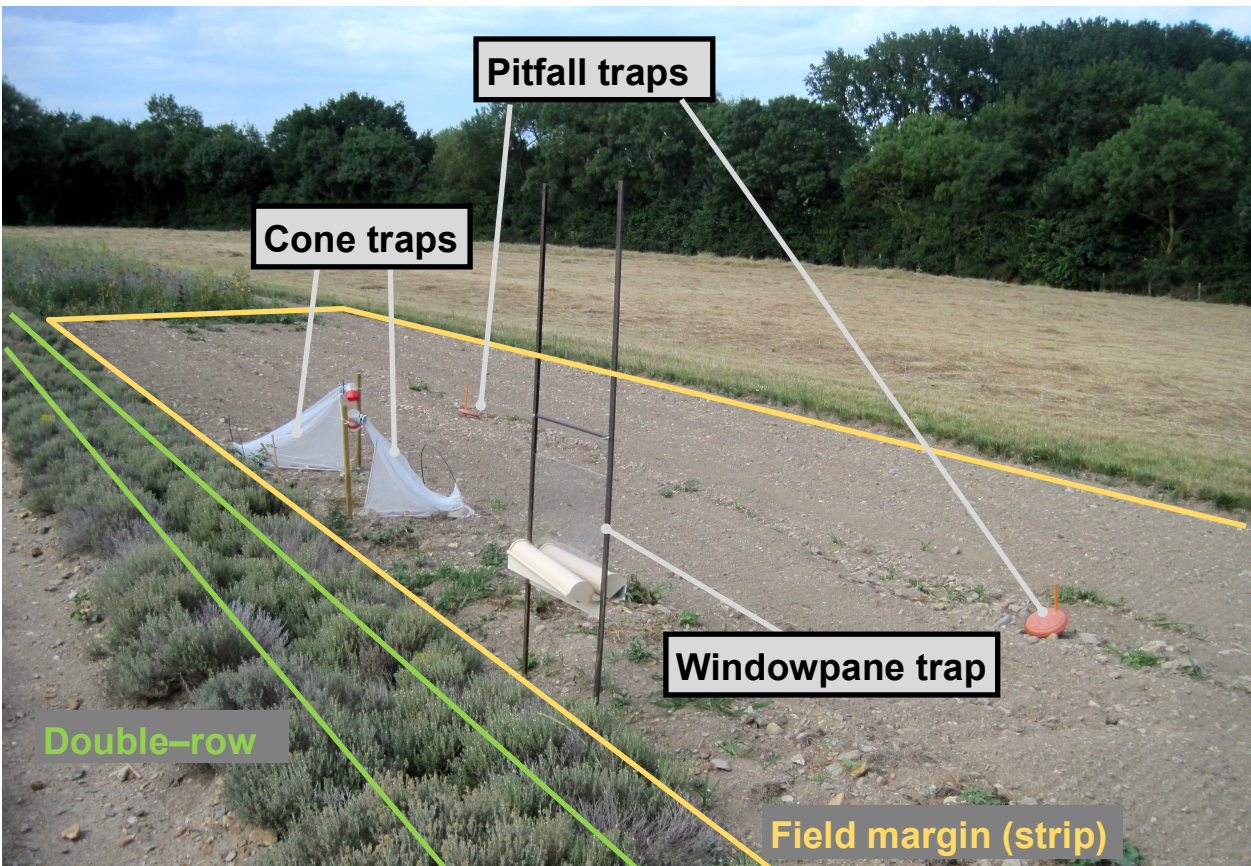


Figure 25. Position of traps within the field margin (strip) in the bare soil control of the “Noëlle” field.

therefore we did not record specie present. Anna Pollier, PhD, who is familiar with the botanical species present in the region and visual estimation of cover (%) and of flowers in bloom (%), was as kind as to lead the survey.

#### 4.1.3 Leaf damage estimation

It is quite difficult to distinguish the plants of the two chemotypes and therefore I did not. Thyme sprigs were harvested weekly from 12<sup>th</sup> April till 2<sup>nd</sup> August, with an exception of one week (21<sup>st</sup> June), due to extreme heat (impossibility to harvest). Fifty sprigs were collected in each plot, following a zigzag transect scheme (**fig. 23.**), placed into sealable plastic bags and kept in a fridge upon arrival to lab, until visual observation (within 48 hours). Having much more leaf samples to process (three times more) than previous year, we opted for observing the leaves directly on the sprigs (not detaching them), with a head-loupe. Only the top three leaf layers were considered, therefore accounting to 300 leaves observed per plot and total of 2700 leaves per experiment each week. The sprigs were submerged into tap water prior to inspection. The leaf wetting allowed washing e.g. dried dirt that could be mistaken for stippling. The thyme on 'Le Fresne' fields has a silvery aspect due to cuticle and trichomes, with rather silver-grey leaf colour, which reflects light. Submerging the leaves allowed having more contrast between the healthy and chlorotic parts of the leaves (dark green vs. yellowish). Leaf damages (leaf stippling) were measured on an ordinal scale with three levels (**tab. IV.**).

#### 4.1.4 Leafhopper population abundance and parasitoid identification

The estimation of leafhoppers densities in thyme followed the same method as in 2016. Yellow sticky traps, which serve generally to monitor and control e.g. white flies in greenhouses (supplier CRISOP), were installed in the first two double-rows only.

Last year, the ratio of trapping area over experimental plot area was approximately  $2.7 \cdot 10^{-3} \text{ m}^2/\text{m}^2$ , equivalent to ten traps per 30 m length of the plot (that is 60 m length of double-rows). In order to keep a similar ratio, seven and five traps were equally spaced 2.5 m apart lengthwise along the 20 m and 14 m long inner plots, respectively (**fig. 23., appendix II.**)

Ratio 2016:  $30/10 = 3 \text{ m/trap}$

Ratio 2017:  $20/7 = 2.86 \text{ m/trap}$  and  $14/5 = 2.8 \text{ m/trap}$

The sheets of yellow sticky traps were cut into squares of 7.5 cm x 7.5 cm. Upon installing in the field, each square trap was inserted into slit of a bamboo stick and the protective film was removed on both sides (**fig 24.b**). The bamboo sticks were installed beforehand, based on the experimental

Table V. Overview of the experiment calendar.

Experiment week	Calendar week	Calendar month								Yellow sticky trap sampling	Leaf damage estimation sampling	Arthropod diversity sampling
			Mo	Tu	We	Th	Fr	Sa	Su			
1	14	APRIL	3	4	5	6	7	8	9			
	15		10	11	12	13	14	15	16			
2	16		17	18	19	20	21	22	23	1	1	
	17		24	25	26	27	28	29	30	2	2	
3	18	MAY	1	2	3	4	5	6	7	3	3	
	19		8	9	10	11	12	13	14	4	4	
4	20		15	16	17	18	19	20	21	5	5	
	21		22	23	24	25	26	27	28	6	6	
7	22	JUNE	29	30	31	1	2	3	4	7	7	1
	23		5	6	7	8	9	10	11	8	8	
8	24		12	13	14	15	16	17	18	9	-	
	25		19	20	21	22	23	24	25	-	9	
9	26		26	27	28	29	30	1	2	-	10	2
	27	JULY	3	4	5	6	7	8	9	-	11	
28	10		11	12	13	14	15	16	10	12		
13	29		17	18	19	20	21	22	23	11	13	
	30		24	25	26	27	28	29	30	12	14	
16	31	AUGUST	31	1	2	3	4	5	6	13	15	3

Botanical survey

plan, and stayed on the field during the experimental season. The sticky traps were replaced each week. First traps were installed on April 12<sup>th</sup>. While many bees were found trapped (possibly attracted by the trap), the height of the bamboo sticks was lowered from around 30 cm the first two weeks to more-or-less the height of the surrounding plants.

The two sampling periods were from 12<sup>th</sup> April till 14<sup>th</sup> June (nine weeks) and from 5<sup>th</sup> July till 2<sup>nd</sup> August (four weeks). Because (harvest) cut is a big disturbance for the leafhopper population, a three-week pause (14<sup>th</sup> June – 5<sup>th</sup> July) followed the harvest. The cut may drastically change spatial distribution of leafhoppers over the crop as well as its age structure (differential stage mortality) hence generating potentially biased data (population peak).

The leafhoppers were counted on both sides of the sticky traps and an average was calculated per plot (total nb of individuals/ nb of traps) Adult parasitoids of the three genera *Aphelopus*, *Chalarus* and *Anagrus* were searched for on the yellow sticky traps, as well, under a binocular loupe, using a simplified identification method. I based my search on easy-to-spot morphological traits, such as size, colour, wing venation specific for the researched genus (**appendix I**). Only the traps of the three dates covering first five weeks of experimental period were inspected so far (week 1,3 and 5 corresponding to 19<sup>th</sup> April, 3<sup>rd</sup> May and 17<sup>th</sup> May).

#### 4.1.5 Arthropod diversity and natural enemies functional diversity

The effects of the different types of strips established along the thyme fields, on arthropod biodiversity and more specifically on leafhoppers natural enemies were tested according to the protocol elaborated during the Floregul project. Three types of traps were used within/on the border of the field margins in each plot (**fig. 25**): Two pitfall traps - for ground-crawling arthropods (e.g. spiders, carabids), two cone traps – for insects flying close to the soil or floating/gliding and crawling insects (**fig. 24.c**) and one two-sided windowpane trap – for the flying insect (**fig. 24.a**). The trapping period of seven days once in four/five weeks was applied, resulting in three trapping dates. (24<sup>th</sup>-31<sup>st</sup> May, 21<sup>st</sup>-28<sup>th</sup> June, 26<sup>th</sup> July-2<sup>nd</sup> August) (**tab. V**). The pitfall traps were established six meters apart from each other and 1.5 m within the strip width. The windowpane traps of adjustable height were placed three meters sideways from the centre of the plot, on the border between the last thyme row and F/G/C –strip. A plexiglas windowpane (45 x 25 cm) covered the trapping area of 50 to 80 cm height above the ground. The pitfall traps and the “gutters” on both sides of the windowpane, collecting trapped insect, were maintained filled with salty solution (sea salt 125 g/l + bio odourless detergent). Nevertheless, the gutters were sometimes found completely dry, with crystallized salt. Cone traps were constructed using insect-proof textile available at the local gardening store (brand Nortene, model Climabio). The cone traps are directional traps,





allowing the fauna to pass one-way only through the funnel-like passage towards a trapping “pot” - a modified plastic container filled with 40% ethanol (**fig 24.d**). Two cone traps were placed side by side on the border between the last thyme row and a strip, with one trap facing thyme and the other facing the strip. Upon sample collection, the content of the pitfall traps was united to form one sample. The two sides of windowpane trap, as well as the two cone traps were kept as separate samples. The pitfall trap and windowpane trap contents were filtered through a metallic mesh and plastic mesh filter, and transferred by rinsing with 40 % alcohol into a plastic container with screw-on lid. The cone traps containers were simply unscrewed from the traps and closed with a lid.

The samples will be processed using the RBA (Rapid Biodiversity Assessment) method tested for the first time by Cranston and Hillman (1992) and Oliver and Beattie (1993). This method was used in the previous year (2016) of the study. The arthropods are firstly sorted into taxonomic order. Within each order the identification minimum is the taxonomic family. Within taxonomic family, morphotypes are created based on visual criteria (colour, shape, etc). Size should be taken into account only for holometabolic arthropods. If possible more precise identification can be done based on time availability and skills of the observer. When trying the method for the first time, I realised certain families are not possible nor useful to identify fast and precisely enough (e.g. aphids, flies, small *Nematocera*). I decided to focus only on the groups of interest, i.e. possible natural enemies. I will try to follow the morphotypes described in 2016 where possible, with the help of the 2016 collection. After pseudo identification, the samples will be stored in 70 % alcohol.

## 4.2 Data analysis

We used the R Studio (version 1.0.136) statistical software and Excel 2007® of the MS Office® package to carry out the statistical and descriptive analyses.

### Botanical composition of AEI

The percentage cover data for each species were adjusted based on total vegetation cover (%) for each plot (taking bare ground into account). Similarly, percentage of flowering plants per species were adjusted based on a given species cover (%) and total vegetation cover in each plot (%). We calculated a flower resource index, by summing up the percentage of flowering plants (adjusted to the vegetation cover and species cover as described above) over all the species per plot. The data of botanical survey were analysed by a PCA (Principal Component Analysis) for species composition as presence/absence data, and for species composition as relative percentage cover, for grass and flower strips. Another PCA was done for the species composition (% cover) and percentage of flowering plants of the ten sown species in the flower strips.



#### 4.2.1 Leaf damage estimation

During experiment, we collected data corresponding to 15 out of 16 consecutive weeks (week 16 without harvest) (**tab. V**). The data on leaf damage were expressed as percentage of damaged leaves (two categories of damage combined) out of total leaves checked per each plot and date, resulting in 9 x 15 data entries. Curves per field and per treatment showing leaf damage evolution over time (with error bars) were made to be visually comparable. As evolution over time resembled a polynomial curve of second order (square) and differences between fields were observed, we tested the effect of treatment accounting for these factors. We created a statistical linear mixed model with treatment (factor), week squared (week<sup>2</sup>, continuous variable) as explicative variables, and field as block (random effect). We also tested the interaction between week and treatment as we expect variations over time (increase in leaf damage over time). Because observations were not independent over time, we also added corrections for time auto-correlations. Assumption of residual normal distribution was fulfilled (Shapiro-Wilcoxon test).

#### 4.2.2 Leafhopper population abundance and parasitoid identification

Only the number of *Eupteryx decemnotata* and *Anagrus sp.* could be analysed because other groups / families showed too little number of individuals to obtain reliable results (for the weeks 1, 3 and 5). The number of *E. decemnotata* and *Anagrus sp.* were averaged (nb individuals/nb traps) per plot, obtaining one variable per plot. This was done because the number of yellow traps per plot varied. Data were treated in the same way as for the leaf damage, although only three weeks are available at the time this report is written; creating curves per field and per treatment over time. Both *E. decemnotata* and *Anagrus sp.* showed non-linear increase over time differences between fields. Therefore, we created a statistical model with treatment, week and week squared as explicative variables and field as block.. No auto-correlation over time was observed. The criteria of normal distribution of residuals was not fulfilled, so we used a Poisson distribution as it is often the case for count data. The end model was therefore a generalized linear mixed model. Finally, we looked for the relationship between *E. decemnotata* and the proportion of leaf damage and the number of *Anagrus sp.* (correlation), to confirm functional relations.

#### 4.2.3 Arthropod diversity and natural enemies functional diversity

Once the data are obtained through RBA pseudo-identification (October-November 2017), the data will be analysed through PCA on abundance, to compare species composition across treatment. Species richness (or rather morphotype richness) would also be compared across treatment and would be plotted/correlated to the plant species richness and to leafhopper abundance to validate

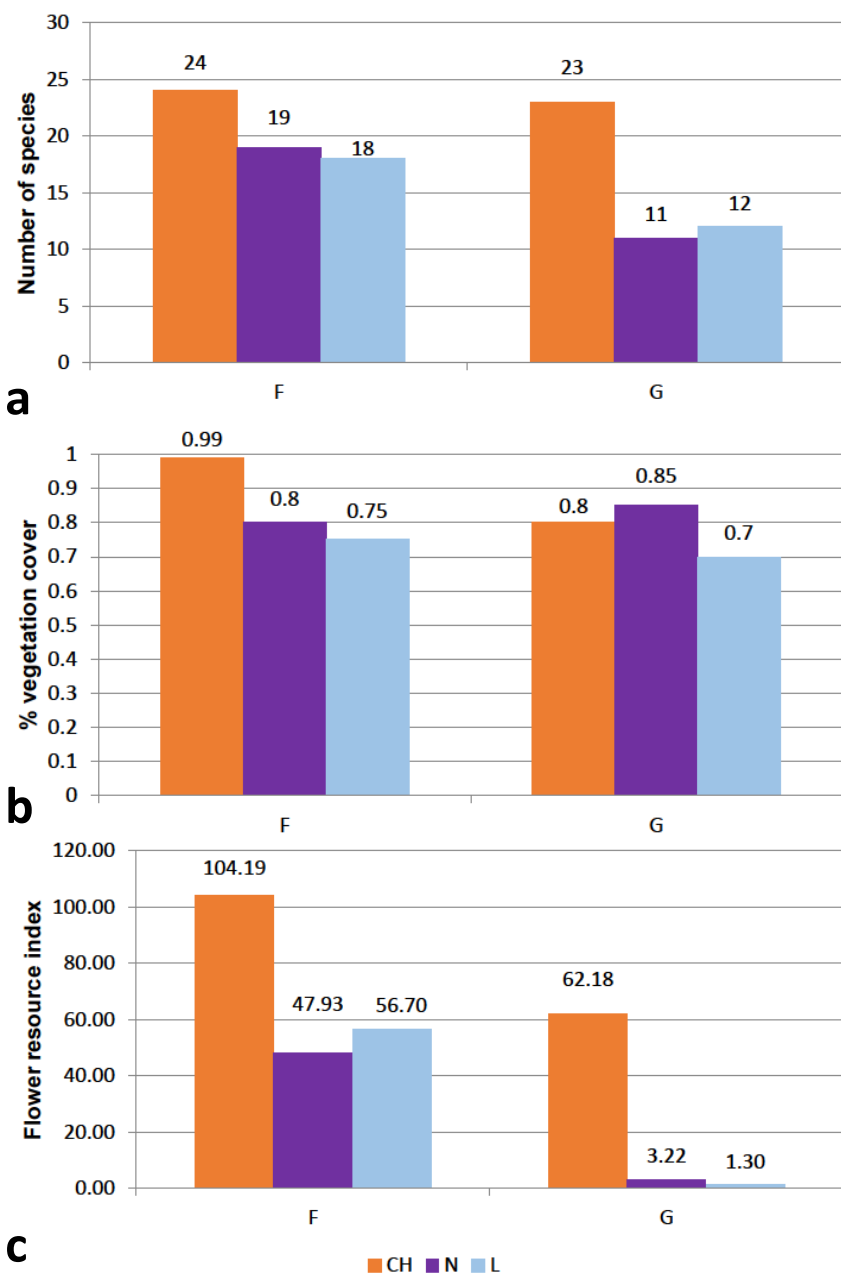


Figure 26. Number of species (a) , total vegetation cover (b) and flower index (c) of Flower strips (F) and grass strips (G), C-Control, CH–Chazelle, N–Noëlle, L–Lycée.

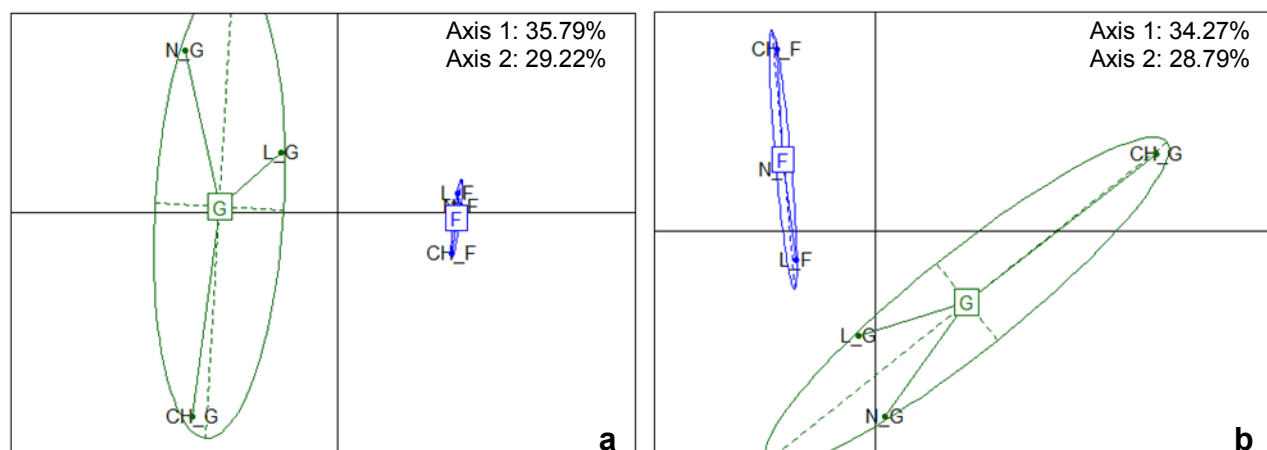


Figure 27. PCA of (a) plant species composition (presence/absence) and (b) plant species composition (cover %) in grass strips and flower strips (CH–Chazelle, N–Noëlle, L–Lycée, F–Flower, G–Grass).

or not the hypotheses stating that flower richness enhances arthropod richness and that higher arthropod richness/abundance translates into better biological control of the pest.

## 4.3 Results

### 4.3.1 AEIs development and their botanical composition

#### Observation

During the season, we observed that the development of the grassy strip of the “Chazelle ” field was not even and the botanical and cover sampling on 16<sup>th</sup> confirmed this. On the “Lycée” field, the grassy strip developed in delay, hardly taller than 15 cm and with low ground cover. The flower strips differed too; at the “Chazelle” field it was well developed, tall (above 1.7 m) and dense, while on the other two fields, its development was slightly delayed and it was much less tall (about 1 m) and more sparse. The flowering species were well established and all the species have been recorded flowering (except one – fennel).

#### Data analysis

In total 49 species were recorded. The “Chazelle” field had richer AEIs than the two other fields (23.5 species on average compared to 15 for the other two). Flower strips were botanically richer than grassy strips (20.3 vs. 15.3 species respectively, on average), as expected. The grass strip of the “Chazelle” field contained almost as many species as the flower strip of this field (24 vs. 23 species), thus being the second most diverse plot of all. The other two grass strips have around 50% less species than the flower strips and “Chazelle” grass strip (11 and 12) (**fig. 26.a**). The average vegetation cover was highest in the “Chazelle” field (0.9%) and lowest in the “Lycée” field (0.73%). It was slightly higher for flower strips (0.85) than in grass strips (0.78%). The percentage cover per strip and per plot is on (**fig. 26.b**). The average flower resource index was higher for flower strips (69.6) than in grass strips (22.23), and it was highest for the “Chazelle field (83.19) compared to “Noëlle” and “Lycée” (25.57 and 29.00) (**fig. 26.c**, averages not shown).

The PCA plot confirmed (**fig. 27.a**) that flower strips differ from the grass strips in their species composition (presence/absence) and the flower strips are more-alike than the grass strips. The “Chazelle” grass strip differed more from “Noëlle” and “Lycée” than the two between each other. The two axes represented 65.01% of the total variance (35.79% and 29.22% for axis 1 and 2 respectively). The first axis differentiated flower strips from grass strips while the second axis mainly contrasted fields. As for the species composition accounting for the species percentage cover the PCA showed very similar results (**fig. 27.b**) though differentiating more flower strips

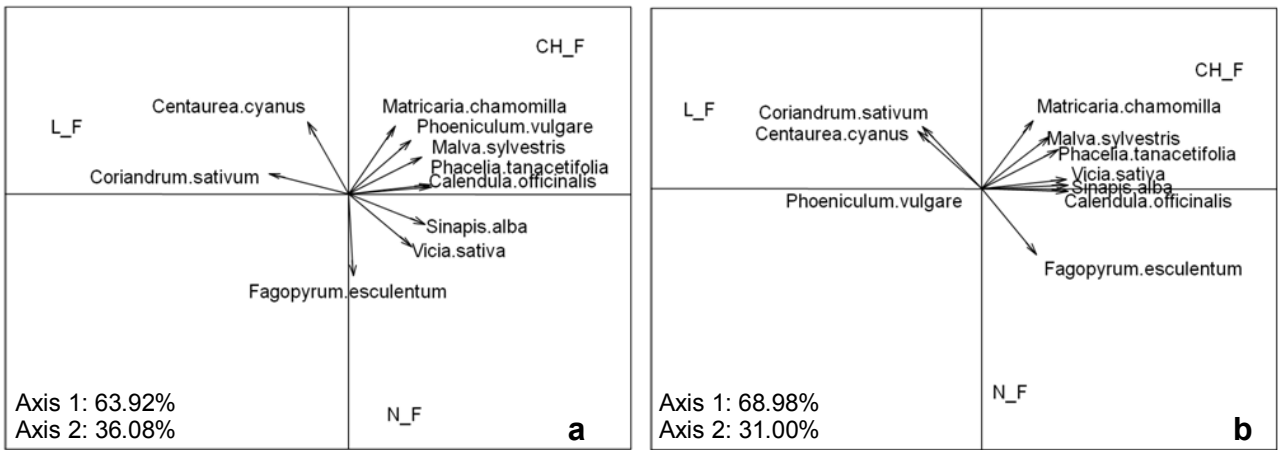


Figure 28. PCA of (a) plant species cover (%) and (b) flowering plants (%) of the sown species in flower strips (CH–Chazelle, N–Noëlle, L–Lycée).

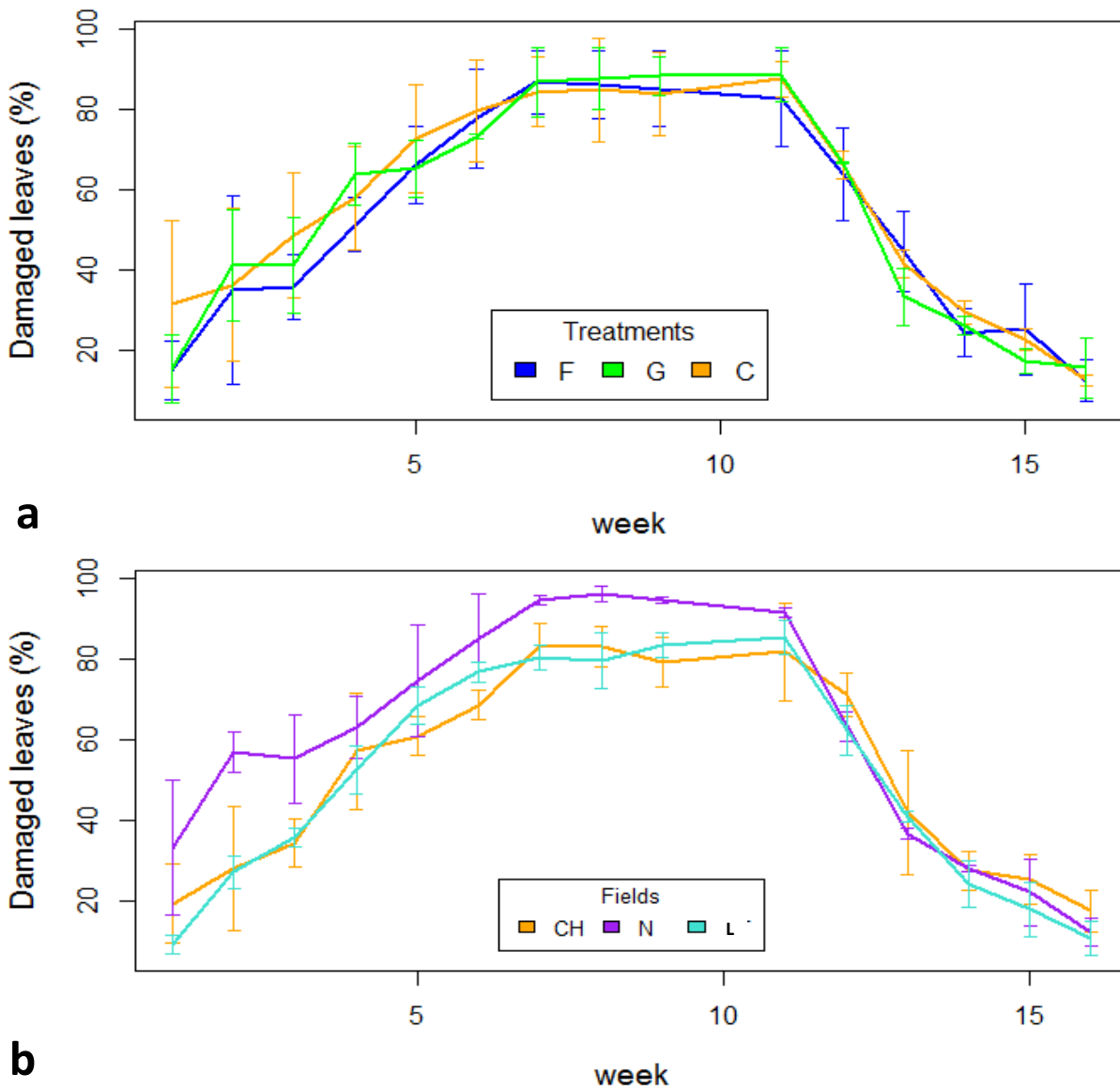


Figure 29. Evolution of leaf damage (%). **a** -by treatment over time, **b** - by field over time (CH–Chazelle, N–Noëlle, L–Lycée, F–Flower, G–Grass, C–Control).

among themselves than for the presence/absence PCA. The total variance accounted for is 34.27% and 28.79% for the axis 1 and 2 respectively. The PCA of the species composition (% cover) of the ten sown species in the flowers strips (**fig. 28.a**); differentiated the strips by higher presence (% cover) of coriander and cornflower in “Lycée” (axis 1 ~ 63.92% variance) and higher buckwheat, vetch and white mustard presence (% cover) in “Noëlle” (axis 2 ~ 36.08% variance). The results are very similar for the PCA of the % flowering plants (**fig. 28.b**). The only exception is fennel; which was not flowering yet at the time of survey (0% flowers). The axes account for 68.98% and 31.00% (axis 1 and 2), of that total variance. A list of all the species recorded is present in **appendix III**.

#### 4.3.2 Leaf damage estimation

We expressed the proportion of leaf damage (%) as the sum of the two categories of damage observed (more than 30% and less than 30% of leaf area damaged). The data on the different damage categories will be exploited in future.

Curves of leaf damage (%) per treatment over time (**fig. 29.a**) showed an increasing trend of leaf damage, from less than 30% in the first week, reaching the 90% plateau around week 7 (31<sup>st</sup> May) and decreasing from week 12 (5<sup>th</sup> July) onwards to less than 20%. The average per field showed a similar curve, with “Noëlle” field values slightly higher than the other fields during the first 12 weeks (**fig. 29.b**). The individual curves per plot show the same trend over all the treatments (**fig. 30.**). The curves per field seem more similar to each other (horizontally) than curves per treatment (vertically).

Statistical analysis confirmed these observations, showing a strong and significant effect of week and week squared (**tab. VI.**). However, there was no significant effect of treatment factor, even in interaction with time, showing leaf damage evolved similarly for the three treatments over time.

#### 4.3.3 Leafhopper and parasitoid population abundance

##### Observation

We could observe (naked-eye) the dynamics of leafhopper population, and very high abundance at certain periods, as well as certain differences in their abundance. This evolution is partly confirmed by the results available so far.

Several species of leafhoppers were recorded and distinguished; *Eupteryx decemnotata*, *E.atropunctata*, *E. aurata*, *Zyginidia scutellaris*, *Emelyanoviana mollicula*, *Empoasca sp.*, and *Hauptidia maroccana*. *E. decemnotata* was much more prevalent than the other species (estimated to be more than 90%). As for the parasitoids, several *Mymaridae* morphotypes were observed, being probably of genus *Polynema*, *Anaphes* and others, as well as what we consider to be

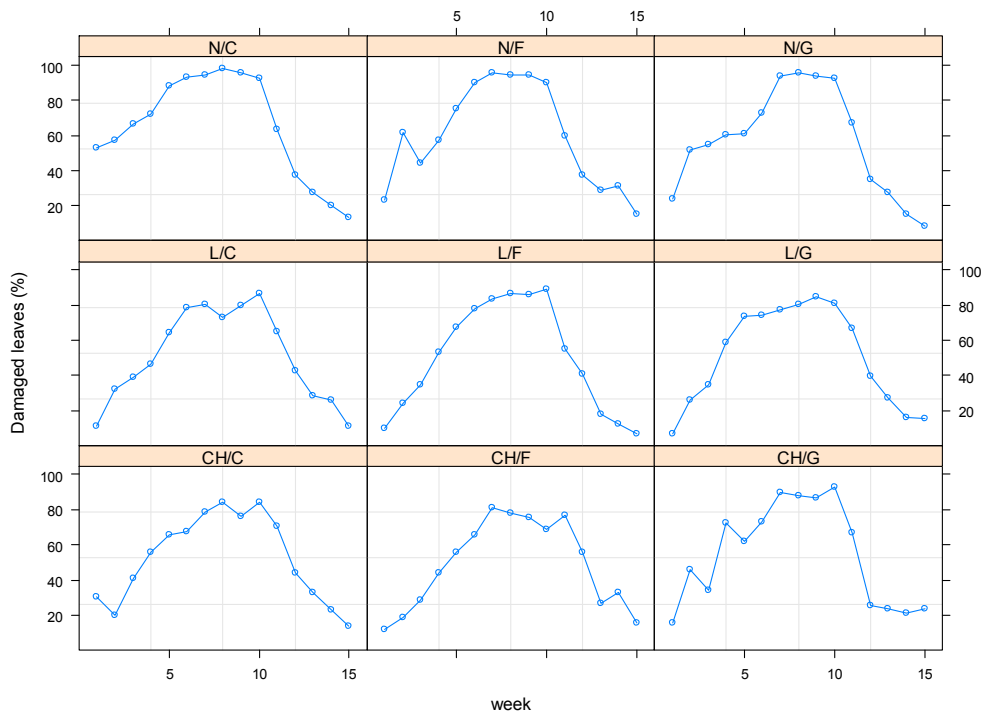


Figure 30. Evolution of leaf damage (%) in each plot over time. Rows= treatments, columns = fields, (CH–Chazelle, N–Noëlle, L–Lycee, F–Flower, G–Grass, C–Control).

Table VI. Results for the leaf damage model test.

	F-value	p-value
(Intercept)	283.6	<.0001
treatment	1.3	0.2882
week	8.1	0.0051
week <sup>2</sup>	250.2	<.0001
treatment:week	1.6	0.2033
treatment:week <sup>2</sup>	1.8	0.166



*Anagrus sp.* We therefore counted the number of *Anagrus sp.* and apart we grouped the number of other *Mymaridae*. No *Chalarus* specimen was observed and only three examples of *Aphelopus* were found. I did not observe presence of the cysts on larvae that we saw occasionally on the thyme plants during leaf-damage estimation, but I could observe a few adults of *Empoasca sp.* with cysts, on the yellow sticky traps. Only the results for *Anagrus sp.* and *E. decemnotata* are presented here.

#### Data analysis

Similarly to leaf damage, results showed an increase of *E. decemnotata* abundance and *Anagrus sp.* over the first five weeks (**fig. 31.a 31.b**). The average *E. decemnotata* abundance was higher (not significantly) in the crop adjacent the flower strips. This was the case also for the average per field, in “Noelle” (block effect) (**fig. 31.c 31.d**). The individual curves per plot can be seen at **fig 31.e and 31.f**. *Anagrus sp.* averaged abundances were not affected by treatment (**tab. VII**).

We found significant positive correlation (log) between *E. decemnotata* abundance (explanatory variable, axis x) and percentage of leaf damage over every experimental plot for the first weeks 1,3 and 5 ( $R^2 = 0.74$  p-value < 0.0001, **fig.32.a**), as well as a positive linear correlation between abundances of *E. decemnotata* (explanatory variable, axis x) and *Anagrus sp.* ( $R^2 = 0.84$ , p-value < 0.0001, **32.b**)

#### 4.3.4 Arthropod diversity and natural enemies functional diversity

We observed a strong presence of *Araneae* and *Opiliones* (*Phalagnum opilio* L.), ladybirds and true bugs (*Hemiptera: Heteroptera*) in the thyme crop. The flower strips attracted many pollinating species (butterflies, bees, bumblebees, and other *Hymenoptera*), as well as bugs, and beetles. A praying mantis (*Mantis religiosa* L.) was observed on one occasion in grass strip of the “Chazelle” field. The complete results of fauna studies will be available by December 2017.

## **4.4 Discussion**

The objective of this study was to evaluate the potential of AIE in leafhopper control in thyme. We present only preliminary and partial results, which will be completed once more data (arthropod abundance and richness and leafhopper and parasitoid abundances) are available (by the end 2017). Therefore, we are unable to confirm or reject the hypotheses tested on the effect of AEI. The results available so far compromise the possibility to enhance the leafhopper biological control by conservation (use of AEI). Nevertheless, our study may be regarded as the first attempt to describe the interactions among the arthropods in and near the PAMP crop with objective of

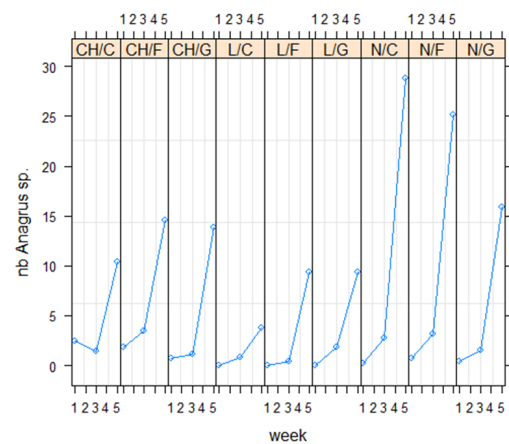
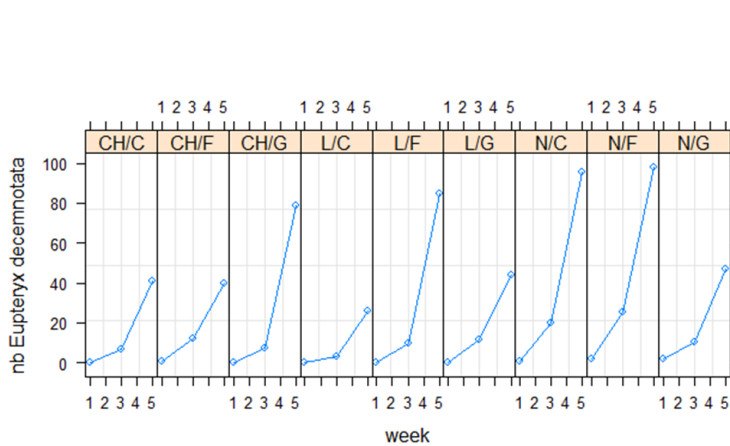
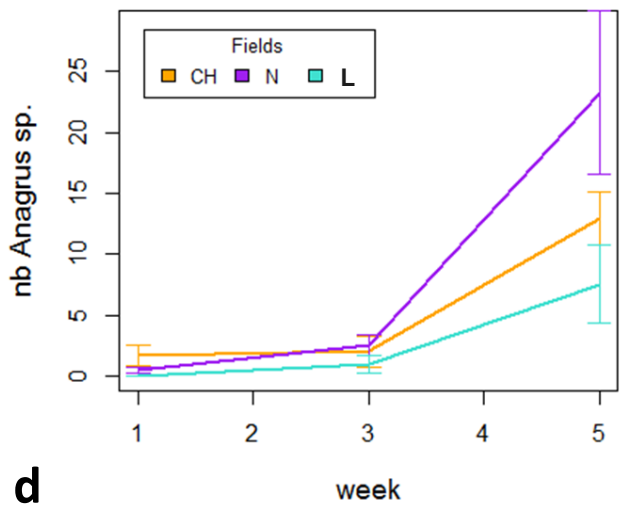
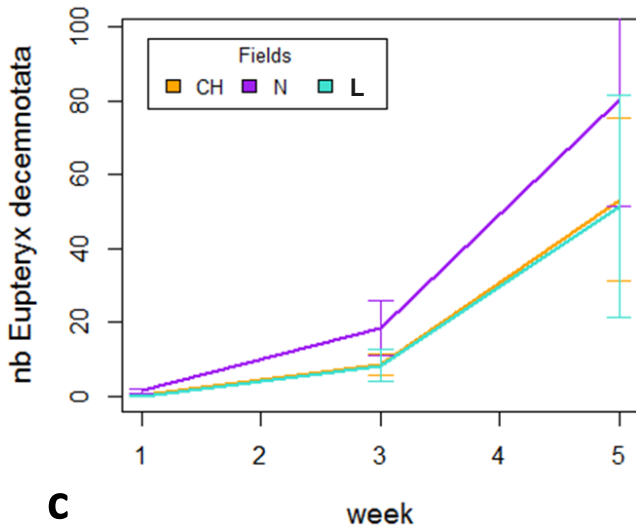
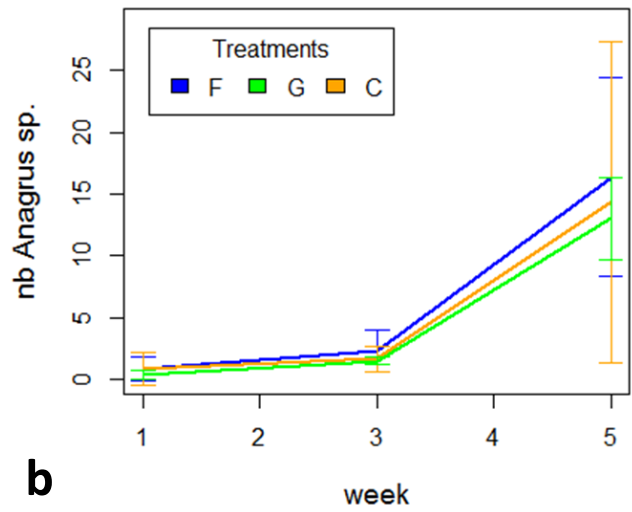
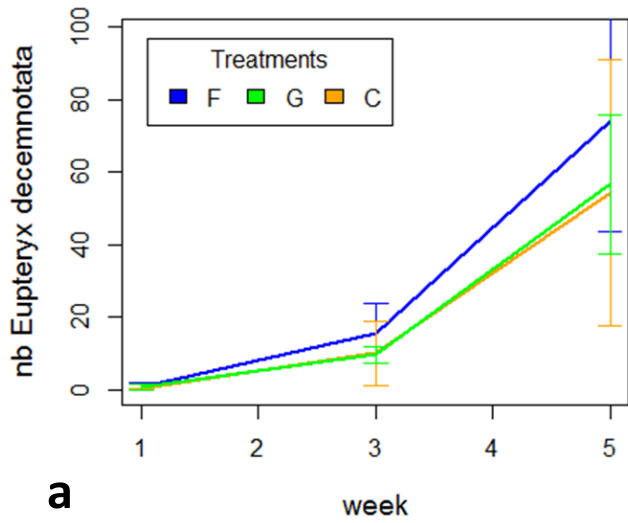


Figure 31. Evolution of *E. decemnotata* and *Anagrus sp.* abundance (Nb trapped) average and standard deviation, for the week 1, 3 and 5; **a** - *E. dec.* per treatment, **b** - *Anagrus*, per treatment, **c** - *E. dec.*, per field, **d** - *Anagrus* - per field, **e** - *E. dec.* - curve for each plot, **f** - *Anagrus* - curve for each plot. (CH - Chazelle, N - Noëlle, L - Lycee, F - Flower, G - Grass, C - Control).

biological control by conservation. So far, studies exist on major annual (what, oilseed rape) and perennial (orchards) crops no. We hope our study may contribute to filling a gap in the knowledge. Quite naturally it produces more questions than answers or applicable solutions.

The data on botanical composition of AEI confirms more species in flower strips and therefore more flower resources. We observed the evolution of leaf damage over time, common for all the treatments. More *E. decemnotata* were observed on average in the thyme crop adjacent the flower strips (non-significant). *Anagurs* sp morphotype was observed in the crop in the first five weeks, but this did not translate into control effect on the leafhopper abundance, nor on the leaf damage.

Over all the data available so far, we perceive the effect of block (field). It was the case already last year (2016). The grass strip treatment was situated at the “Noëlle” field, the flower strip and control were situated at “Chazelle” field (no replicates) (**fig. 33.**).

#### 4.4.1 Botanical composition of AEI

The development of grass strips and flower strips was not even over the three fields. Indeed, the three grass strips were quite different; in composition, height, density and age (“Noëlle” grass strip is a semi-permanent grassland). The flower strips were more homogenous, as they were all sown at the same time. Nevertheless, the different conditions of each field translated into difference in height density and % cover and % flowering plants. The block effect is accounted for in statistical analysis (in general). To obtain significant differences (for treatments), more replicates might be necessary. We recorded more botanical species and thus more flower resources were available to potential natural enemies in the flower strips. However, we cannot hypothesise about the effect of flower resources of flower strips on the presence of natural enemies during the first five weeks (data on parasitoid abundance available), because the flower strips were not developed enough to be flowering yet.

#### 4.4.2 Leafhopper and parasitoid populations

Several species of leafhoppers were simultaneously observed in the thyme crops, with *E. decemnotata*. being dominant. At the same time, several *Mymaridae* morphotypes were observed, belonging to different genera. Hence, we suspect that complex interactions between hosts, parasitoids and probably hyper parasitoids are taking place in thyme making the unravelling of underlying trophic networks challenging. This observation is shared with many other studies on arthropods and especially natural enemies in other crops. The complexity does not lie only in the species number only, but also in the diet (specialist-generalist spectrum) of every species, whatever its status (herbivore, omnivorous or predator) is. Moreover, the species seasonality

Table VII. Results of multiple comparison test (method Tukey) between the three treatments for the number of *E. decemnotata* and *Anagrus sp.* (average per plot). The original model accounted for time variation (significant) and field (block effect).

<i>E. decemnotata</i>	estimate	SE	z.ratio	p.value
control - flower	-0.44885633	0.2334495	-1.923	0.1323
control - grass	0.03401008	0.2601602	0.131	0.9906
flower - grass	0.48286641	0.2359143	2.047	0.1012

<i>Anagrus sp.</i>	estimate	SE	z.ratio	p.value
control - flower	-			
control - grass	0.1818455229	0.6030936	-0.302	0.9511
control - grass	0.0002563034	0.6299108	0.000	1.0000
flower - grass	0.1821018263	0.6031727	0.302	0.9510

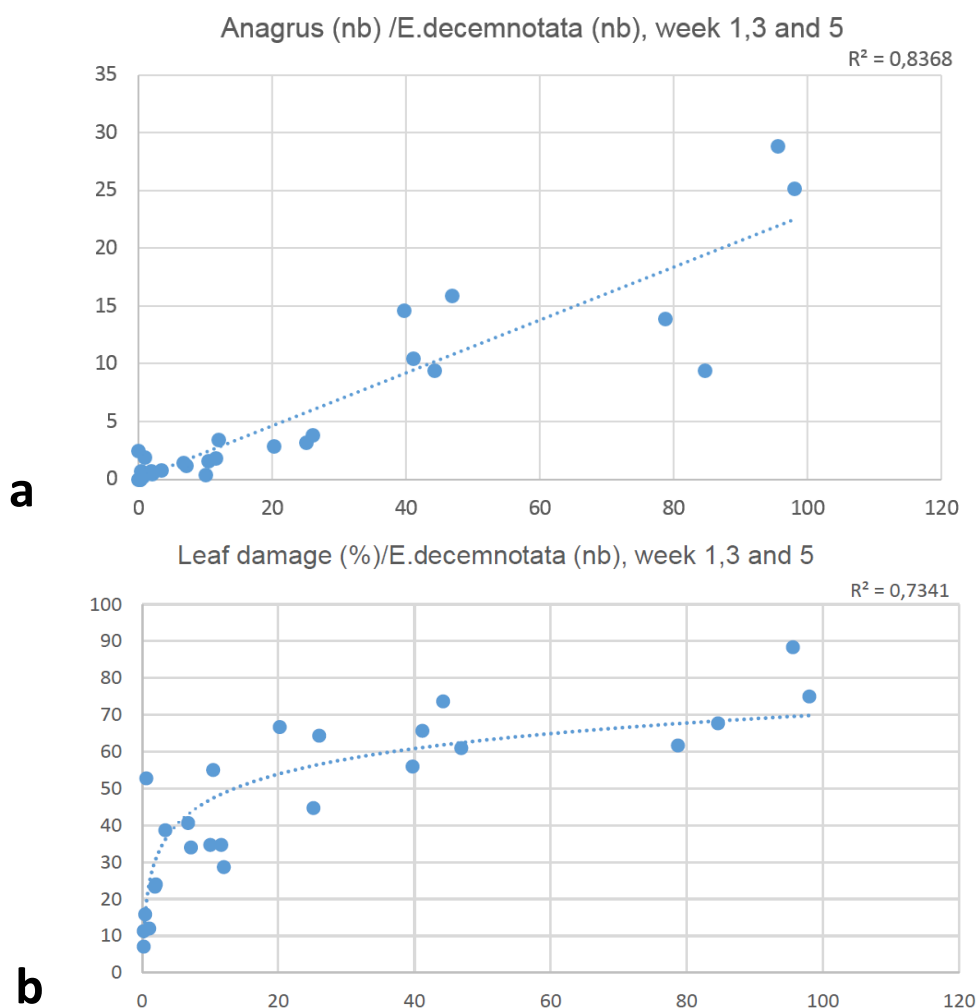


Figure 32. Correlations a - Linear correlation between *E. decemnotata* and *Anagrus sp.* abundance (nb trapped) and b- Log correlation between *E. decemnotata* and leaf damage for the week 1,3 and 5 (average values per plot were plotted)

(earlier vs. later species) or even behavioural ecology, may affect the observed interactions. For the observer, identifying species is already challenging, especially with tiny insects such as *Mymaridae*, and cryptic species may be mistakenly comprised in the same morphotype. Hence, we adopted a general approach of biodiversity assessment and correlation test to tackle this complexity and resume interactions that seem important for natural pest control.

It is suspected, that *Anagrus atomus* is parasitizing *E.decemnotata*. Despite profound efforts to find literature on *Anagrus* sp. parasitizing *E. decemnotata*, no such relation has been confirmed to my knowledge. The only source mentioning *A. atomus* parasitizing the eggs of *E decemnotata* is Arno (1988) in Bennison *et al.* (2001), on rosemary. Thought the authors of this project report doubt that a proper identification could had been done prior to 1989 when a major identification key was published for *Anagrus*. Based on our limited identification skills, *Anagrus* sp. is present in the thyme of 'Le Fresne'. The correlation between *E.decemnotata* and *Anagrus* sp.for the week 1,3 and 5 (19<sup>th</sup> April, 3<sup>rd</sup> May and 17<sup>th</sup> May) gives a hint on possible (host-parasitoid) interaction between these two species.

As observed in 2016, we suspect that *Anagrus* sp abundance would peak after the peak of *E.decemnotata*, suggesting that *Anagurs* is a late seasonal species. We may have observed the beginning of the *Anagrus* sp abundance peak (week 1,3,5) **(fig.34.)**

Results, where higher presence of *Anagrus* doe snot lead to efficient pest control were observed by (English-Loeb *et al.*, 2003). They studied the effect of nectar-producing cover crops in New York vineyards on parasitoid *Anagrus* sp.and therefore control of the pest leafhopper *Erythroneura* sp. Although the abundance or distribution of leafhoppers was not affected, buckwheat cover crop attracted more *Anagrus* over clover and sod (*Dactylis glomerata* L.) and the rate of parasitism was higher there, too. In Californian vinenyards, Daane *et al.* (1998) found lower leafhopper densities in vineyards with cover crops, but they suggest this may be explained by vine vigour (preference of leafhoppers for vigourous vines).

#### 4.4.3 Leaf damage

When comparing leaf damage and leafhopper population from the last year (2016), it is not possible to distinguish the block and treatment effect effect (only one replicate of each treatment, two treatments settled in the same field). Nevertheless, we clearly see that the two treatments on the "Chazelle" field (flower strip and control) are quite similar to each other in leafhopper abundance and % leaf damage **(fig.33.)**

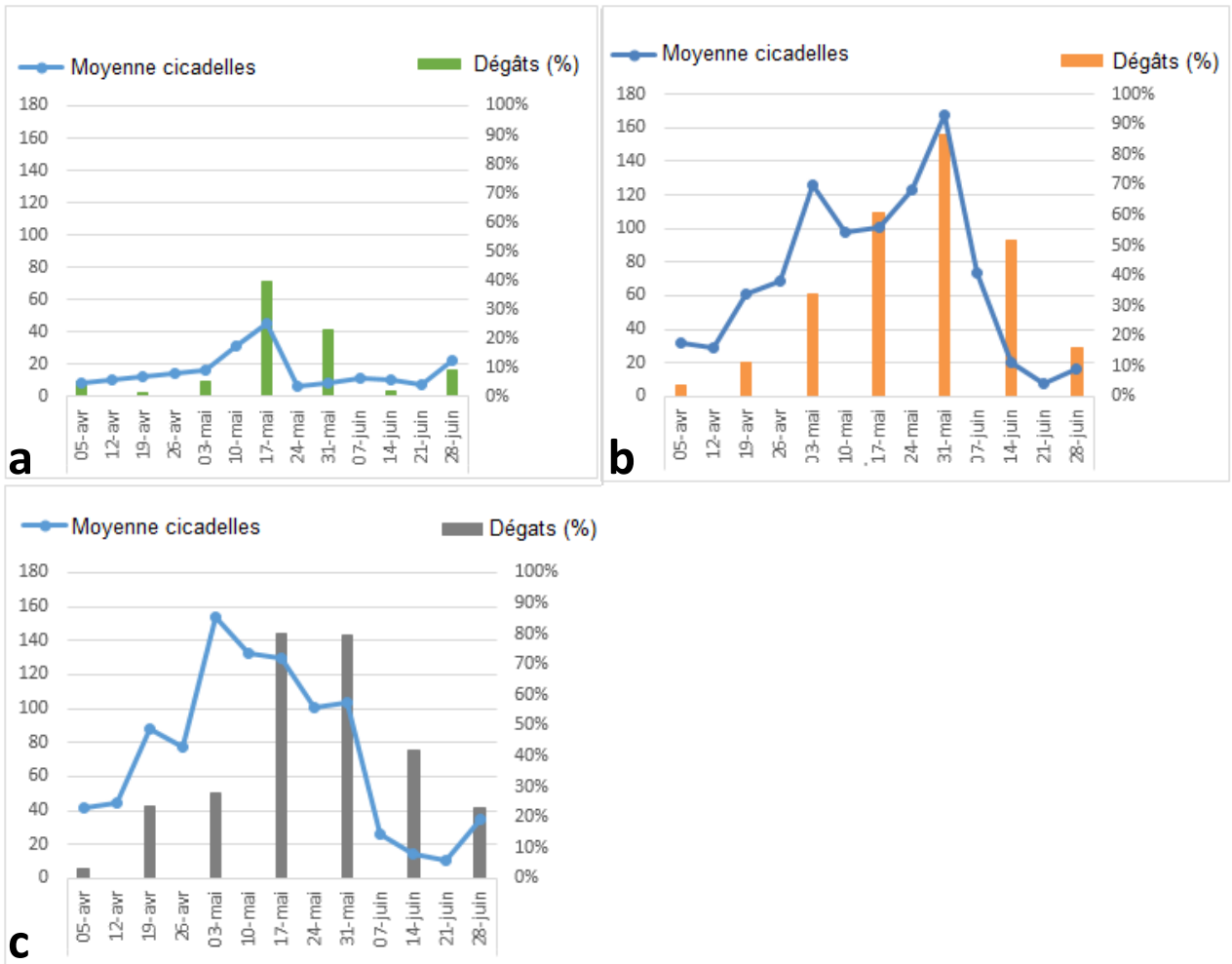


Figure 33. Evolution of leafhopper population (curve) and % leaf damage (bars) in 2016; a - Grass strip (Noëlle), b- Flower strip (Chazelle) c- Control (Chazelle). From Farcy (2016).

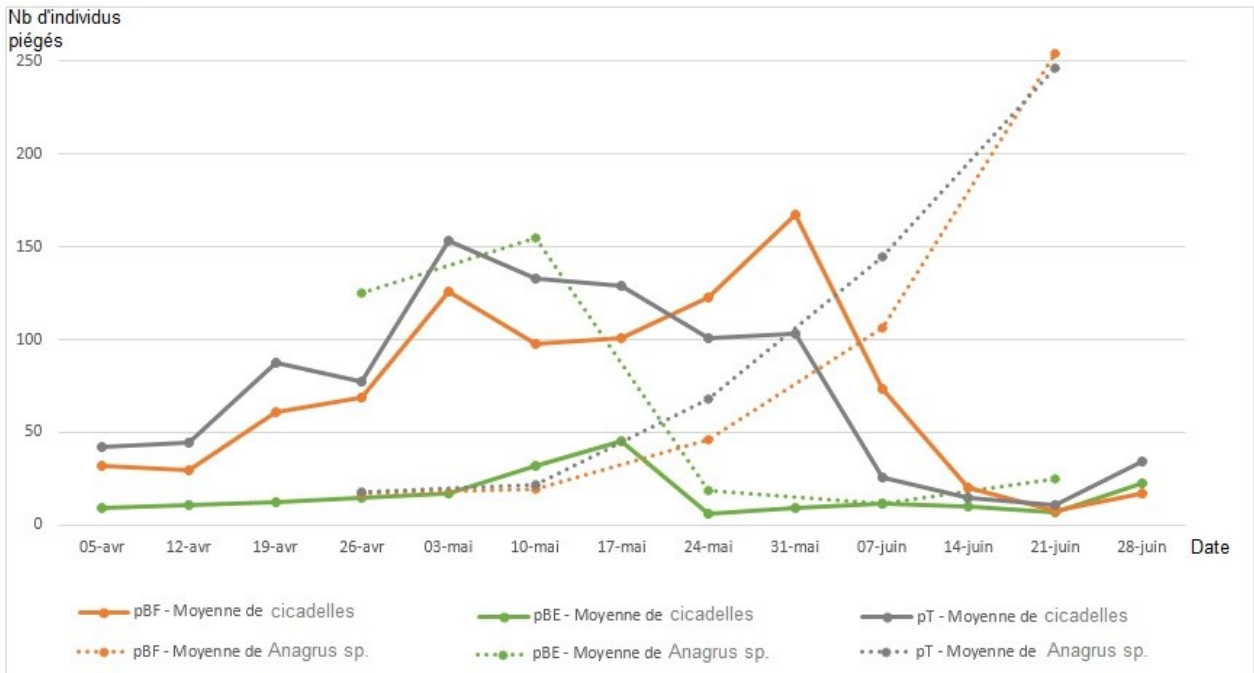


Figure 34. Evolution of leafhopper population (full line) and *Anagrus sp.* population (dotted line) in 2016; green - Grass strip (Noëlle), orange - Flower strip (Chazelle), grey - Control (Chazelle). From Farcy (2016).

In 2017 there were little differences between the treatments regarding the leaf damage. The period of highest leaf damage week 7 (31<sup>st</sup> May) till week 12 (5<sup>th</sup> July) coincides with the hottest period, as can be seen from the meteorological data for 2017 (**fig.35.**). These data were obtained from the meteorological station on the 'Le Fresne' farm. In 2016

Contrary to observations of the last year, this year (2017) (**fig. 33.**) "Noëlle" field seem to be more attacked than the other two fields, as more leaf damage and higher abundance of *E. decemnotata* was recorded in here. In 2016, an exceptionally wet season was observed while in 2017 it was all the contrary, dry and hot (**fig 35., fig.36.**). The precipitation were nearly double between April and July in 2016 compared to 2017 (**fig.36.**). We speculate that the "humid" "Noëlle" field (proximity to Maine River) was "advantaged" in 2016; by the excess humidity which may have lead to an under-optimal thyme growth and by the "apparition" of the entomopathogenic fungus, suspected of contribution towards leafhopper control. Indeed, Farcy (2016) observed the spread of this fungus from "Noëlle" through "Lycée" to "Chazelle". It seems that in 2017, hot and dry season "disadvantaged" this field. The thyme crop might have enjoyed better soil humidity conditions, but that made it more attractive to leafhoppers. Nusillard (2001) observed the same behaviour; leafhoppers preferred irrigated *Lamiaceae* crops during dry periods.

We detected leaf damage already from the first week of experiment (19<sup>th</sup> April), while the recorded leafhopper abundances were rather low. We suggest that this is due to the leaf damage caused in the previous season, or by leafhopper larvae that may have been already in the crop, but not trapped. The larvae were trapped only very seldom on the yellow sticky traps as they do not fly and they jump less.

We know from the literature that the harvest cuts interfere with leafhoppers' population (Bouillant *et al.*, 2004). The eggs and larvae present in the cut parts are exported from the field. Only a part of the crop is cut (top 15 cm), leaving major part of the plant and leaves. The experimental plots were not cut in 2016 and 2017 but the rest of the crop was. In 2016 the second leafhoppers' abundance peak of was observed around the 31<sup>st</sup> of May, just a week after the harvest cut (**fig.34.**). It may be possible that the adult leafhoppers escaping from the neighbouring rows (being harvested) end up trapped on the sticky traps. If this is the case, then the harvest may create a biased data with a false abundance peak. In ideal case, this could be tested by comparing the traps in the uncut field (whole) and partly cut field (as in our case).

In 2016 and 2017, only the first two double-rows adjacent to AElS were exploited in trapping experiments. It is highly possible that if the AElS attract the desired species, the spill-over effect would be observed further within the field. However, thyme is pollen- and nectar-producing crop

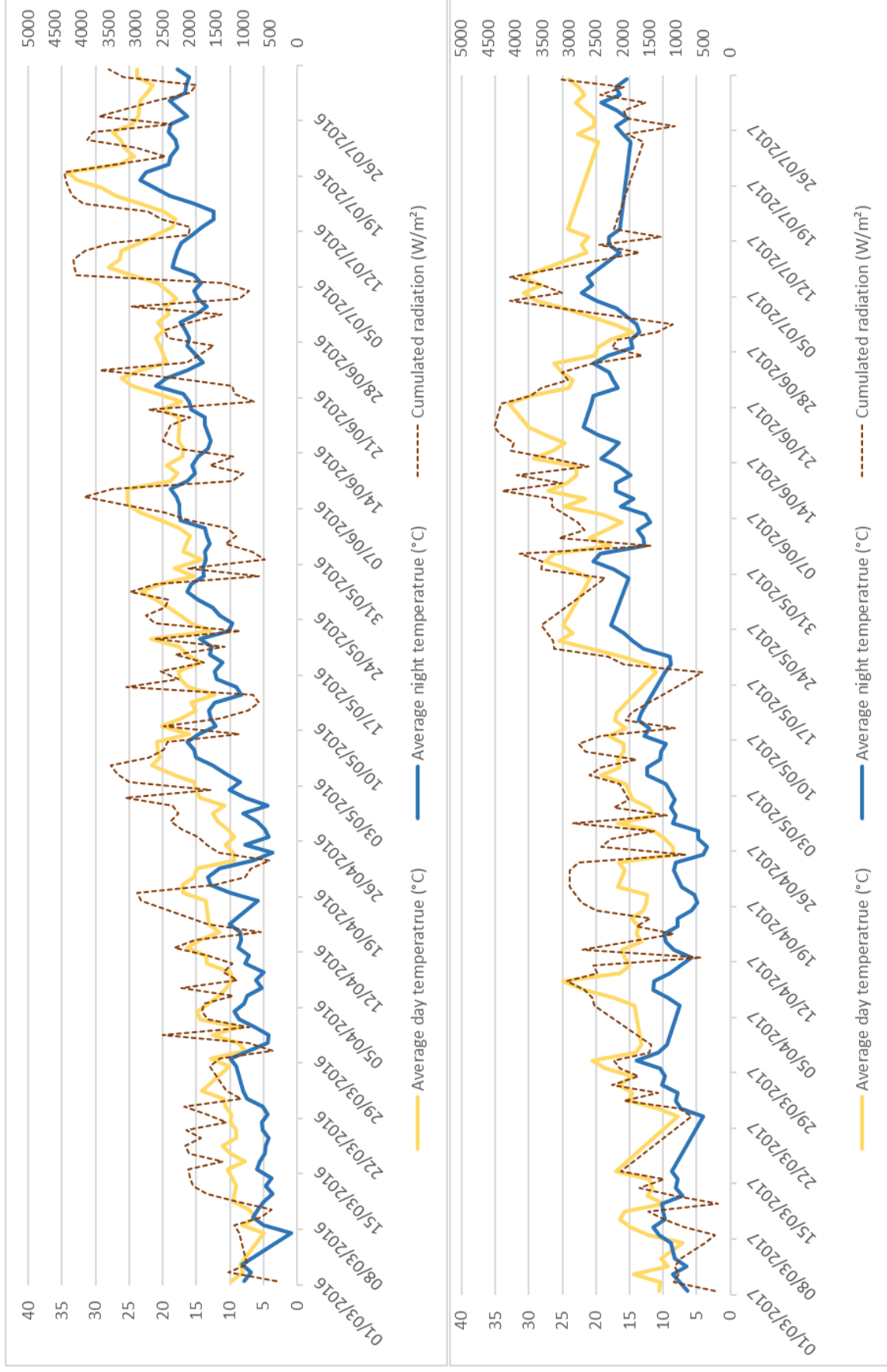


Figure 35. Evolution of the average day and night temperature (°C) and cumulated radiation (w/m<sup>2</sup>) during the experimental season 2016 (a) and 2017(b) at 'Le Fresne'.



and it may provide the necessary resources to natural enemies during its flowering season. The potential “sink” effect of AElS, especially flower strips, may not be detected in the flowering period of thyme. If, in fact, thyme provides sufficient flower resources for the natural enemies of the leafhoppers, the valuable potential of flower resources from flower strip could be early in the season, with early-flowering species. In the flower mixture we used, the Common marigold, Phacelia and Common vetch can flower as early as in April. In our study, the flower strip developed much later due to non-optimal weather conditions.

Based only on our naked-eye observations in fields, thyme is of a dense architecture (if healthy) and may provide habitat and refuge for potential predators. Moreover, the thyme crop remains in place for several years with limited perturbation except for cuttings. The grass and flower strips might provide more adapted refuge and habitat for the species requiring more shade, higher humidity or dense and tall vegetation cover. The IAE could maintain the fauna escaping the field at cutting, in its vicinity, thus facilitating its recolonization.

## 4.5 Conclusion

The pest leafhopper was present this year and last year (2016, 2017) in the thyme crop and so was the suspected parasitoid *Anagrus sp.* The data obtained so far do not show efficiency of AEI in leafhopper control and in reduction of leaf damage in thyme. However, our experiment show indices that *Anagrus sp.* may be a parasitoid of *E. decemnotata*.

## 4.6 Recommendations

To confirm the hypotheses, a proper identification of the captured *Mymaridae* is necessary. If the project continues in next year(s) a cooperation with entomologist specialised in *Mymaridae* and/or *Anagrus sp.* would be of great advance. Apart from identification, a proof that *Anagrus sp.* parasitizes *E. decemnotata* could be an interesting contribution to scientific literature. Direct observation in thyme fields or in the laboratory could provide an additional proof.

There are hints of predations on leafhoppers, but we found no literature proofs on *E. decemnotata*. Predatory studies under laboratory conditions are easier to set up than parasitism studies. I suggest collecting living potential predators (spiders, bugs etc.) and the pest leafhoppers on the crop and AElS (sweeping net) and observe their behaviour. Even though laboratory predation studies do not reflect the real situation, they give an indication. Information on who (potentially) eats whom would be a valuable knowledge.

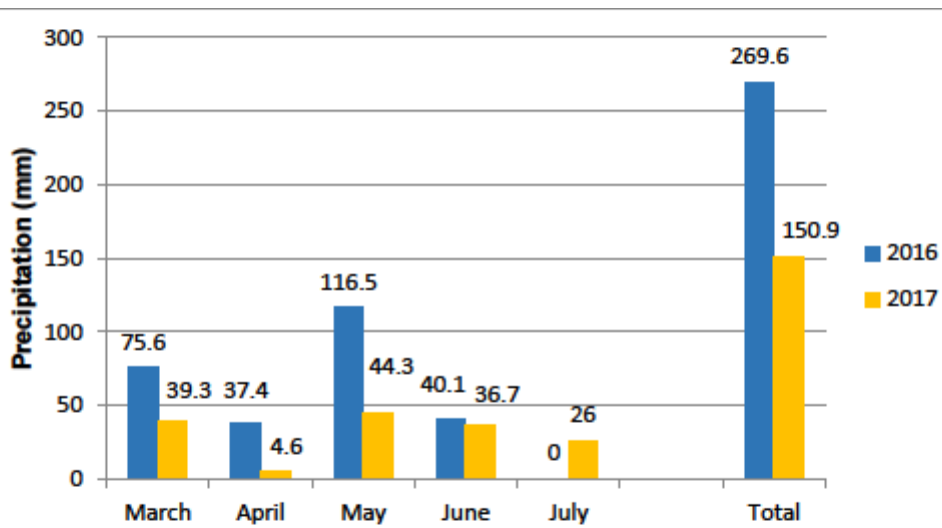


Figure 36. Precipitation (mm) during the experimental season 2016 and 2017 at “Le Fresne”.

The experimentation of the two years sampled the arthropod diversity only in the field margin. It would be useful to compare these data with the ones from within the crop. We may have data on parasitoids from both the AEI and the crop, but they were obtained by two different methods (cone / windowpane traps vs. sticky traps). Comparing data obtained by using the same trapping method could lead to information that is more precise. It could confirm that the desired species are present in the field margin and at the same time, they “travel” into the crop. On the other hand if compared with a control, another conclusion could be that they (the natural enemies) are already present and AEI does not play much role. Therefore, we propose some kind of sampling within the crop. For example, the arthropods captured on the yellow sticky traps could be identified. Some level of expertise is necessary for such identification, because the specimens are difficult to observe (restrained possibility to manipulate the stuck arthropods). For general sampling, other techniques (non-attractive), such as pitfall trap, cone trap or sweep net are probably better.

We propose a closer (regular) following of the development of the AEI and crop (e.g. % flowering) in next experimental seasons, to take into account the self-provisioning effect (nectar and pollen) of the thyme crop and developmental differences in AEIs.

Should the experimentation take place next year(s), I strongly advice more detailed communication among the technical personnel, head of the farm and the intern(s), to inform each other about operations that are to take place in the fields. Should an intern be in charge of insect identification (as was the case in the last two years) I propose some kind of short training (intensive) course at the beginning of the internship (focusing on using the identification keys and criteria and recognition of the major taxa). At Agro campus Ouest Angers I had the possibility to consult a qualified person in arthropod identification (Estelle Chenu). However, I believe I could have been more independent and faster, if I had followed some sort of short training (as described above). I prepared a simple identification sheet on the leafhoppers and their (suspected) parasitoids (**appendix I.**). Of course, I advise using proper identification keys for confirmation. I hope the work presented here will help the person who will work on this topic in the future.



## 5 List of references

---

(Baquero and Jordana, 1999; Chiappini, 2008; Le Quesne and Payne, 1981; Stewart, 1986)

- Agboka, K., Tounou, A.K., Al-moaalem, R., Poehling, H.-M., Raupach, K., Borgemeister, C., 2004. Life-table study of *Anagrus atomus*, an egg parasitoid of the green leafhopper *Empoasca decipiens*, at four different temperatures. *BioControl* 49, 261–275.
- Agboka, K., Tounou, A.K., Poehling, H.-M., Raupach, K., Borgemeister, C., 2003. Searching and oviposition behavior of *Anagrus atomus* L. (Hymenoptera: Mymaridae) on four host plants of its host, the green leafhopper *Empoasca decipiens* Paoli (Homoptera: Cicadellidae). *J. Insect Behav.* 16, 667–678.
- Agrimer (Ed.), 2016. Filières plantes à parfum, aromatiques et médicinales: Panorama 2015.
- Baquero, E., Jordana, R., 1999. Species of *Anagrus* Haliday, 1833 (Hymenoptera, Chalcidoidea, Mymaridae) in Navarra (Spain). *Miscel. L'ania Zoològica* 22, 39–50.
- Baroffio, C.A., Lenne, F., 2013. Stratégie “Push and Pull” contre les cicadelles. Agroscope.
- Bennison, J., Lole, M., Turner, N., 2009. Field-grown herbs: Evaluation of a mechanical method for the cultural control of leafhoppers (Project report). Agriculture and Horticulture Development Board.
- Bennison, J., Lole, M., Umpelby, R., Maher, H., Maulden, K., 2001. Protected herbs: Control of glasshouse whitefly and leafhoppers within IPM programmes (Project report). Horticultural Development Council.
- Bouillant, S., Mittaz, C., Cottagnoud, A., Branco, N., Carlen, C., 2004. Premier inventaire des populations de ravageurs et auxiliaires sur plantes aromatiques et médicinales de la famille des Lamiaceae. *Rev. Suisse Vitic. Arboric. Hortic.* 36, 113–119.
- CA Aude (Ed.), 2014. Fiche de référence: Thym (*Thymus vulgaris*).
- CA Rhône-Alpes, 2012. Thym bio: Produire du thym en AB.
- Chaieb, I., Bouhachem-Boukhris, S., Nusillard, B., 2012. *Eupteryx decemnotata* Rey.: a New Pest for Aromatic Plants in Tunisia, in: International Symposium on Medicinal and Aromatic Plants-SIPAM 2012 997. pp. 215–218.
- Chiappini, E., 2008. *Anagrus* Fairyflies (Hymenoptera: Mymaridae), in: Capinera, J.L. (Ed.), *Encyclopedia of Entomology*. Springer Netherlands, Dordrecht, pp. 152–154.
- Cranston, P., Hillman, T., 1992. Rapid assessment of biodiversity using biological diversity technicians. *Aust. Biol.* 5, 144–154.
- Daane, K., Costello, M., others, 1998. Can cover crops reduce leafhopper abundance in vineyards? *Calif. Agric.* 52, 27–33.
- Dimitriev, D., 2017. *Auchenorrhyncha* database (online) [WWW Document]. URL <http://dimitriev.speciesfile.org/search.asp?lng=En&keyN=&f=>
- English-Loeb, G., Rhainds, M., Martinson, T., Ugone, T., 2003. Influence of flowering cover crops on *Anagrus* parasitoids (Hymenoptera: Mymaridae) and *Erythroneura* leafhoppers (Homoptera: Cicadellidae) in New York vineyards. *Agric. For. Entomol.* 5, 173–181.
- Eriksson, A., Anfora, G., Lucchi, A., Lanzo, F., Virant-Doberlet, M., Mazzoni, V., 2012. Exploitation of Insect Vibrational Signals Reveals a New Method of Pest Management. *PLoS ONE* 7, e32954. doi:10.1371/journal.pone.0032954
- Farcy, C., 2016. Comment favoriser la régulation biologique des populations de cicadelles en culture de thym par la mise en place d'Infrastructure Agro-Ecologique? (Bachelor thesis). Université d'Angers, Agrocampus Ouest, Angers (France).
- Fernandez, X., Monge, R., Chailan, C., 2012. La production des huiles essentielles en France. *Vuibert*.
- Gillerfors, G., 2009. Fifth contribution to the presence of the *Auchenorrhyncha* in Sweden. Nine new species to the country and new provincial finds. *Entomol. Tidskr.* 130, 99–107.
- Goulet, H., Huber, J.T., 1993. *Hymenoptera of the world : an identification guide to families*. Agriculture Canada, Ottawa.



- Goust, J., 1999. Thym et sarriette. Actes Sud.
- Grohs, B., 2013. Blattzikaden im ökologischen Anbau von Lippenblütlern (Leafhoppers in the organic production of labiates). *Z. Arznei- Gewurzpflanzen J. Med. Spice Plants* 18, 47–52.
- Guglielmino, A., 2002. Dryinidae (Hymenoptera Chrysidoidea): an interesting group among the natural enemies of the Auchenorrhyncha (Hemiptera). na.
- Guglielmino, A., 2000. A contribution to the knowledge of Auchenorrhyncha-Dryinidae relationships in the Palaearctic Region. *Dtsch. Entomol. Z.* 47, 147–159. doi:10.1002/dez.200000018
- Guglielmino, A., Olmi, M., Bückle, C., 2013. An updated host-parasite catalogue of world Dryinidae (Hymenoptera: Chrysidoidea). *Zootaxa* 3740, 1. doi:10.11646/zootaxa.3740.1.1
- Hesami, S., Seyedoleslami, H., Ebadi, R., 2004. Biology of *Anagrus atomus* (Hymenoptera: Mymaridae), an egg parasitoid of the grape leafhopper *Arboridia kermanshah* (Homoptera: Cicadellidae). *Entomol. Sci.* 7, 271–276.
- Hill, P.S.M., 2014. The Tymbal: Evlution of a complex vibration-producing organ in the *Tymbalia* (Hemiptera excl. Sternorrhyncha), in: Cocroft, R.B., Gogala, M., Hill, P.S.M., Wessel, A. (Eds.), *Studying Vibrational Communication*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 13–30. doi:10.1007/978-3-662-43607-3\_2
- Jervis, M.A., 1992. A taxonomic revision of the pipunculid fly genus *Chalarus* Walker, with particular reference to the European fauna. *Zool. J. Linn. Soc.* 105, 243–352. doi:10.1111/j.1096-3642.1992.tb01232.x
- Jervis, M.A., 1980a. Ecological studies on the parasite complex associated with typhlocybine leafhoppers (Homoptera, Cicadellidae). *Ecol. Entomol.* 5, 123–136.
- Jervis, M.A., 1980b. Studies on oviposition behaviour and larval development in species of *Chalarus* (Diptera, Pipunculidae), parasites of typhlocybine leafhoppers (Homoptera, Cicadellidae). *J. Nat. Hist.* 14, 759–768. doi:10.1080/00222938000770651
- Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T., Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. *J. Nat. Hist.* 27, 67–105. doi:10.1080/00222939300770051
- Kehlmaier, C., Assmann, T., 2008. Kehlmaier, C. & Assmann, T. (2008): The European species of *Chalarus* Walker, 1834 revisited (Diptera: Pipunculidae).- *Zootaxa* 1936: 1-39; Brisbane. *Zootaxa* 1–39.
- Krugner, R., Johnson, M.W., Morgan, D.J.W., Morse, J.G., 2009. Production of *Anagrus epos* Girault (Hymenoptera: Mymaridae) on *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) eggs. *Biol. Control* 51, 122–129. doi:10.1016/j.biocontrol.2009.06.013
- Le Quesne, W.J., Payne, K.R., 1981. Cicadellidae (Typhlocybinae): With a Check list of the British Auchenorrhyncha (Hemiptera, Homoptera), Handbooks for the identification of British insects. Royal Entomological Society of London, London.
- Lubiarz, M., Musik, K., 2015. First record in Poland of the Ligurian leafhopper, *Eupteryx decemnotata* Rey 1891 (Cicadomorpha, Cicadellidae)—an important pest of herbs. *J. Plant Prot. Res.* 55, 324–326.
- Maczey, N., Wilson, M.R., 2004. *Eupteryx decemnotata* Rey (Hemiptera, Cicadellidae) new to Britain. *Br. J. Entomol. Nat. Hist.* 17, 111–114.
- Malenovský, I., Lauterer, P., 2010. Additions to the fauna of planthoppers and leafhoppers (Hemiptera: Auchenorrhyncha) of the Czech Republic. *Acta Musei Morav. Sci. Biol. Brno* 95, 41–122.
- Maugin, E., Sforza, R., 2011. Les cicadelles sèment la zizanie en culture ornementale. Presented at the Neuvième Conférence Internationale sur les Ravageurs en Agriculture, Montpellier.
- Mazzoni, V., Conti, B., 2006. *Eupteryx decemnotata* Rey (Hemiptera Cicadomorpha Typhlocybinae), Important Pest of *Salvia officinalis* (Lamiaceae), in: I International Symposium on the Labiatae: Advances in Production, Biotechnology and Utilisation 723. pp. 453–458.
- Nabavi, S.M., Marchese, A., Izadi, M., Curti, V., Daglia, M., Nabavi, S.F., 2015. Plants belonging to the genus *Thymus* as antibacterial agents: From farm to pharmacy. *Food Chem.* 173, 339–347. doi:10.1016/j.foodchem.2014.10.042





- Nickel, H., Holzinger, W.E., 2006. Rapid range expansion of Ligurian leafhopper, *Eupteryx decemnotata* Rey, 1891 (Hemiptera: Cicadellidae), a potential pest of garden and greenhouse herbs, in Europe.
- Nusillard, B., 2001. Les cicadelles Typhlocybines des Labiées aromatiques: des ravageurs méconnus. *Phytoma- Déf. Végétaux* 37–40.
- Oliver, I., Beattie, A.J., 1993. A possible method for the rapid assessment of biodiversity. *Conserv. Biol.* 7, 562–568.
- Olmi, M., 1994. The Dryinidae and Embolemidae (Hymenoptera: Chrysidoidea) of Fennoscandia and Denmark. Brill.
- Pollard, D.G., 1968. Stylet penetration and feeding damage of *Eupteryx melissae* Curtis (Hemiptera, Cicadellidae) on sage. *Bull. Entomol. Res.* 58, 55–71.  
doi:10.1017/S0007485300055863
- Pricop, E., 2013. Identification key to European genera of the Mymaridae (Hymenoptera: Chalcidoidea), with additional notes. *Extreme Life Biospeology Astrobiol.* 5, 69–81.
- Rung, A., Halbert, S.E., Ziesk, D.C., Gill, R.J., 2009. 0088. A Leafhopper Pest of Plants in the Mint Family, *Eupteryx decemnotata* Rey (Hemiptera: Auchenorrhyncha: Cicadellidae), Ligurian Leafhopper, New to North America. *Insecta Mundi* 2009, 1–4.
- Sarthou, J.-P., 2016. Infrastructure agroécologique – Dictionnaire d'agro-écologie.
- Sentenac, G., 2004. Les antagonistes naturels d' *Empoasca vitis* Göth en Bourgogne: Etude de faisabilité d'une lutte biologique par augmentation. Presented at the Mondiaiviti - Bordeaux, Institut Français de la Vigne et du Vin, pp. 25–37.
- Söderman, G., Gillerfors, G., Endrestöl, A., 2009. An annotated catalogue of the Auchenorrhyncha of Northern Europe (Insecta, Hemiptera: Fulgoromorpha et Cicadomorpha). *Cicadina* 10, 33–69.
- Stahl-Biskup, E., Sáez, F. (Eds.), 2003. *Thyme: the genus Thymus*. CRC Press.
- Stewart, A.J.A., 1986. Descriptions and key to the nymphs of *Eupteryx* (Curtis) leafhoppers (Homoptera: Cicadellidae) occurring in Britain. *Syst. Entomol.* 11, 365–376.
- Teall, E.K., 2014. Medicine and doctoring in ancient mesopotamia. *Gd. Val. J. Hist.* 3, 2.
- Thompson, J.D., Manicacci, D., Tarayre, M., 1998. Thirty-five years of thyme: A tale of two polymorphisms. *BioScience* 48, 805–815.
- Tschumi, M., Albrecht, M., Entling, M.H., Jacot, K., 2015. High effectiveness of tailored flower strips in reducing pests and crop plant damage. *Proc. R. Soc. B Biol. Sci.* 282, 20151369.  
doi:10.1098/rspb.2015.1369
- Tu-Saint Girons, F., Saint Girons, B., 2014. Le choix des huiles essentielles : santé, beauté et bien-être par l'aromathérapie, Les maxi pratiques. Jouvence DL, Thônex (Suisse).
- Vernet, P., Gouyon, R.H., Valdeyron, G., 1986. Genetic control of the oil content in *Thymus vulgaris* L: a case of polymorphism in a biosynthetic chain. *Genetica* 69, 227–231.
- Villeneuve, A., 2015. Mise au point d'un protocole de caractérisation de la flore et de l'entomofaune des bandes fleuries (Master thesis). Agrocampus Ouest.



## 6 Online sources

---

1. What are Ecosystem Services? – nerc-bess [Internet]. [cited 2017 Aug 31]. Available from: <http://www.nerc-bess.net/what-is-bess/what-are-ecosystem-services/>
2. [www.flaticon.com](http://www.flaticon.com) [Internet]. Available from: [https://www.flaticon.com/free-icon/spider-arthropod-animal-silhouette\\_47282](https://www.flaticon.com/free-icon/spider-arthropod-animal-silhouette_47282)
3. Vecteezy [Internet]. Available from: <https://www.vecteezy.com/vector-art/129481-free-wildflower-vectors>
4. Royalty Free vector clipart [Internet]. Available from: <https://rfclipart.com/wildflowers-poppies-and-cornflower-22878-vector-clipart.html>
5. <http://fr.vector.me/> [Internet]. Available from: [http://fr.vector.me/browse/212831/free\\_vector\\_grass\\_flowers\\_isolated\\_white\\_garden.cathrynhatfield.com](http://fr.vector.me/browse/212831/free_vector_grass_flowers_isolated_white_garden.cathrynhatfield.com) [Internet]. Available from: [http://garden.cathrynhatfield.com/wp-content/uploads/2011/10/14743\\_243545542624\\_242654157624\\_4130259\\_7257456\\_n.jpg](http://garden.cathrynhatfield.com/wp-content/uploads/2011/10/14743_243545542624_242654157624_4130259_7257456_n.jpg)
6. Spider arthropod animal silhouette free vector icons designed by Freepik [Internet]. Flaticon. 2017 [cited 2017 Aug 31]. Available from: [https://www.flaticon.com/free-icon/spider-arthropod-animal-silhouette\\_47282](https://www.flaticon.com/free-icon/spider-arthropod-animal-silhouette_47282)
7. grass clip art - Seivo Web | Clipart Panda - Free Clipart Images [Internet]. 2017 [cited 2017 Aug 31]. Available from: [http://www.clipartpanda.com/clipart\\_images/grass-clip-art-seivo-web-3030726](http://www.clipartpanda.com/clipart_images/grass-clip-art-seivo-web-3030726)
8. Creepy Beetle Sticker - Car Stickers [Internet]. 2017 [cited 2017 Aug 31]. Available from: <https://www.carstickers.com/proddetail.php?prod=8699>
9. Colorful Spiders [Internet]. Giggletimetoy.com. 2017 [cited 2017 Aug 31]. Available from: <http://www.giggletimetoy.com/product/1584.php>
10. British Bugs Gallery [Internet]. [cited 2017 Sep 1]. Available from: <https://www.britishbugs.org.uk/gallery.html>
11. Genot K. Kunzweb Gallery [Internet]. 2009 [cited 2017 Sep 1]. Available from: [http://gallery.kunzweb.net/main.php?g2\\_itemId=31743](http://gallery.kunzweb.net/main.php?g2_itemId=31743)
12. CRIEPPAM. La production française de lavande et lavandin [Internet]. Jardins de France. [cited 2017 Sep 3]. Available from: <https://www.jardinsdefrance.org/la-production-francaise-de-lavande-et-lavandin/>
13. Planthoppers: FLOW Website [Internet]. [cited 2017 Sep 3]. Available from: <https://www.hemiptera-databases.org/flow/?page=explorer&db=flow&lang=en&card=image&id=231&search=taxon>
14. Flavescence dorée 3 - Flavescence dorée - Wikipedia [Internet]. [cited 2017 Sep 3]. Available from: [https://en.wikipedia.org/wiki/Flavescence\\_dor%C3%A9e#/media/File:Flavescence\\_dor%C3%A9e\\_3.jpg](https://en.wikipedia.org/wiki/Flavescence_dor%C3%A9e#/media/File:Flavescence_dor%C3%A9e_3.jpg)
15. Scaphoideus titanus F - Scaphoideus titanus - Wikipedia [Internet]. [cited 2017 Sep 3]. Available from: [https://en.wikipedia.org/wiki/Scaphoideus\\_titanus#/media/File:Scaphoideus\\_titanus\\_F.jpg](https://en.wikipedia.org/wiki/Scaphoideus_titanus#/media/File:Scaphoideus_titanus_F.jpg)
16. INRA-OPIE. CHRYSIDOIDEA - Aphelopus [Internet]. Available from: <http://www7.inra.fr/opie-insectes/ch-chrysi1.htm>
17. Nederlandse Fauna 6. De wespen en mieren van Nederland - Dryinidae [Internet]. [cited 2017 Sep 2]. Available from: [http://www.naturalmedia.nl/NL/pags\\_jdr/onderzoek/publicaties/wespenatlas\\_2004\\_teksten/Dryinidae\\_2004.html](http://www.naturalmedia.nl/NL/pags_jdr/onderzoek/publicaties/wespenatlas_2004_teksten/Dryinidae_2004.html)
18. Kuvapankki [Internet]. [cited 2017 Sep 4]. Available from: <http://www.insects.fi/insectimages/browser?order=DIP&family=Pipunculidae&genus=Chalarus&species=spurius>



## Appendix I.

How to distinguish the (typhlocybinae) leafhopper species and parasitoids present in the thyme at “Le Fresne “ fields, Angers, France

### *Eupteryx decemnotata*, *Eupteryx zelleri* and *Eupteryx melissae*

- all have colourful wings

#### *Eupteryx decemnotata*

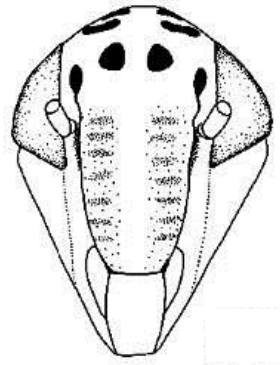
- **8-10 dark spots** on the head, often with the linear spot on the border between the vertex and the front



Lubiarz et Music (2015)



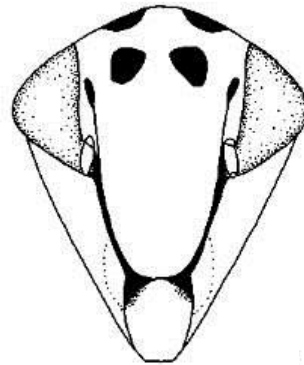
Photo Tristan Bantock; (1)



Blum *et al.* (2011).

#### *Eupteryx zelleri*

- only **up to 6 dark spots** on the head



Blum *et al.* (2011).

#### *E. melissae*

**central spot** on the vertex, near the pronotum

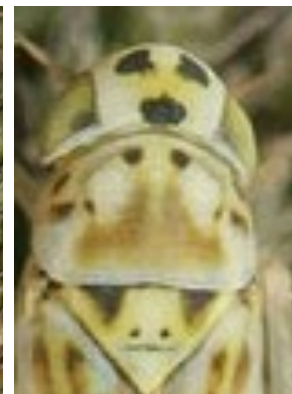


Photo Tristan Bantock (1)



### *E. atropunctata* and *E. aurata*

-both have two black spots on the pronotum and on vertex.

#### *E. atropunctata*

- has a **central spot** on the pronotum
- spots on the pronotum are **more bilobed**
- is tinted **greenish** and smaller (up to 3.5 mm)



Photo Tristan Bantock; www.britishbugs.org.uk

#### *E. aurata*

- two lighter-coloured (greyish) spots on the pronotum
- tinted **orange** and is slightly bigger (3.5-4.5 mm)



Photo Tristan Bantock; (1)

#### *Zyginidia scutellaris* (2-2.5 mm)

- altogether pale, **no markings** on the head, general yellowish colour,
- when dry: **3 triangular spots** on scutellum
- when in alcohol: **3 diamond-shaped spots** on the scutellum, a part of the, (close to head) hidden partially by the pronotum, and one triangular spot on the bottom of scutellum reaching "between the wings"



Photo Tristan Bantock; (1)

#### *Hauptidia maroccana* (3-3.5 mm)

- whitish, with **2 dark spots** on the vertex and two oval black stripes on the scutellum,
- wings pale with **2 darker stripes** (brown-grey)



Photo Tristan Bantock;(1)





**Zygina sp.**

white coloured leafhopper, with orange zig-zag pattern on the wings



Photo Tristan Bantock; (1)

**Empoasca**

**Emelyanoviana mollicula (3.2-3.6 mm )**

- no markings, pale green-yellow tinge
- dorsal surface of the abdomen is dark



Photo Tristan Bantock; (1)

“



## Distinguishing the parasitoids (on the yellow sticky traps)

### Mymaridae

- the smallest insect on the traps (together with thrips, mites and larvae of aphids).
- around 1.5 mm or less
- filiform antennae
- the scape of the antenna not very long,
- 9 (female) or 13 (male) antennae articles  
= (6 funnicle articles for females+ scape, pedicel and clava)

### **Hymenoptera of the World key (Goulet et Hubert, 1993)**

The toruli are closer to the eye than to another toruli

Hind wing has no other connection to the body than the vein (no membrane).

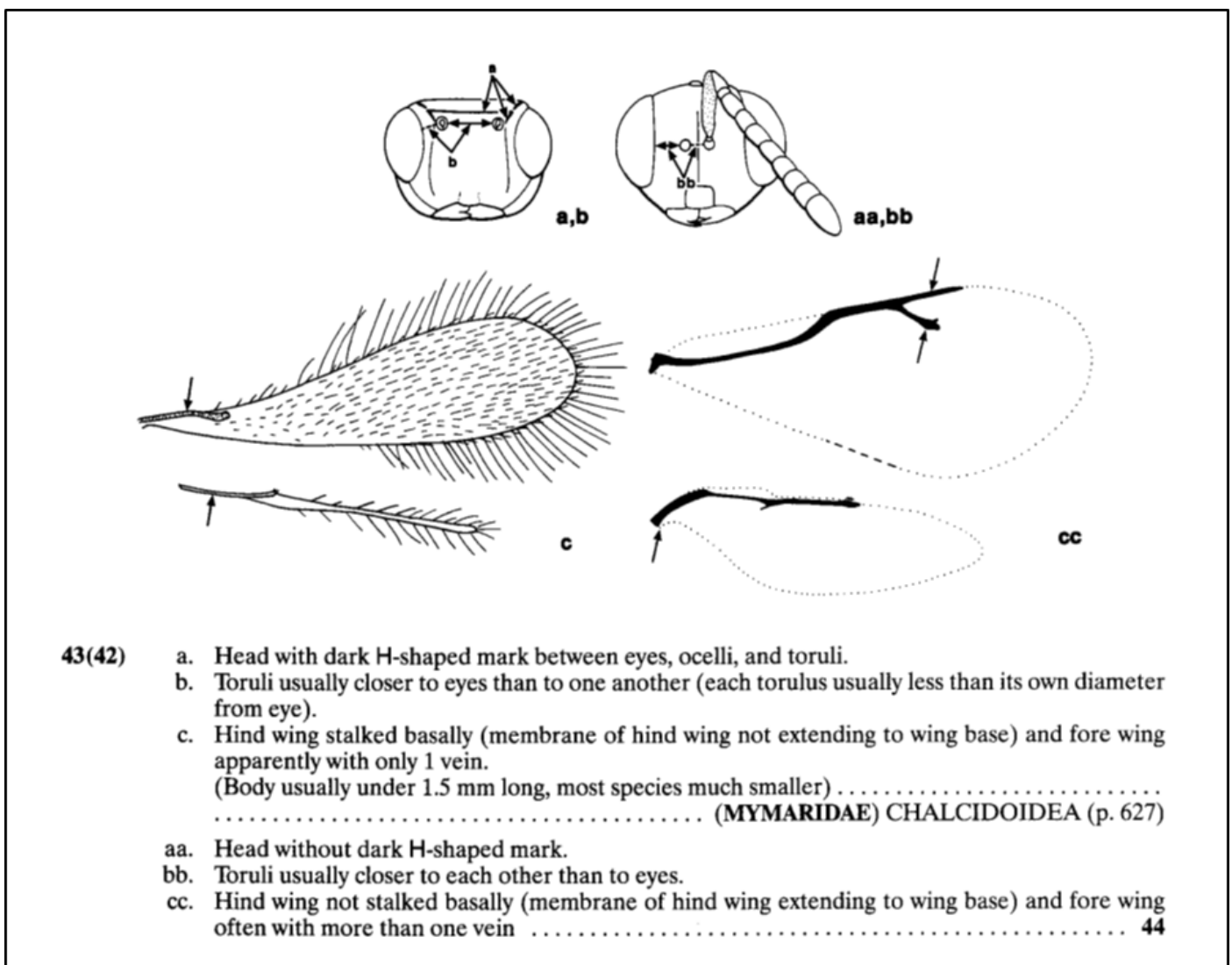


Figure X. Distinguishing *Mymaridae* from "Hymenoptera of the World " (Goulet et Hubert, 1993).



**Anagrus sp.**

- 4-segmented tarsi
- brown, slightly transparent on the yellow trap

**Other Mymaridae (**

5-segmented tarsi

Genus *Camptotera* –wings much narrower with very long cilia

4-segmented tarsi

Genus *Gonatocerus*

Genus *Anaphes* : slightly bigger (+/- 1 mm) and wings less narrow, bit more oval

Genus *Polynema* : (long petiole and abdomen of specific form), wings much wider

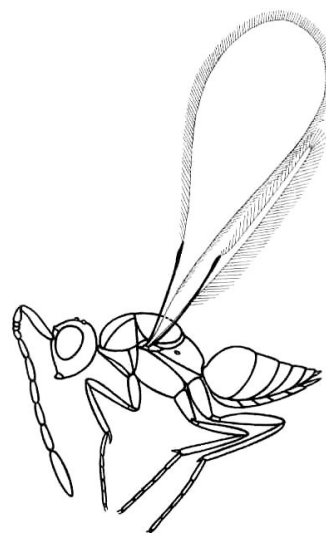


Figure X. *Gonatocerus* sp. From (2)

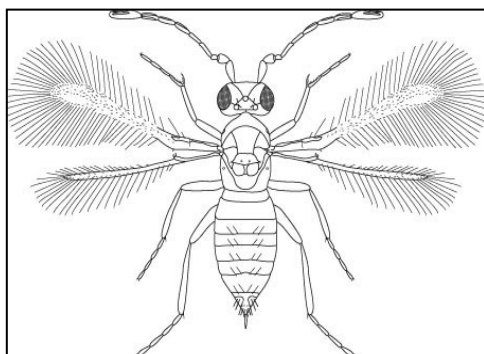
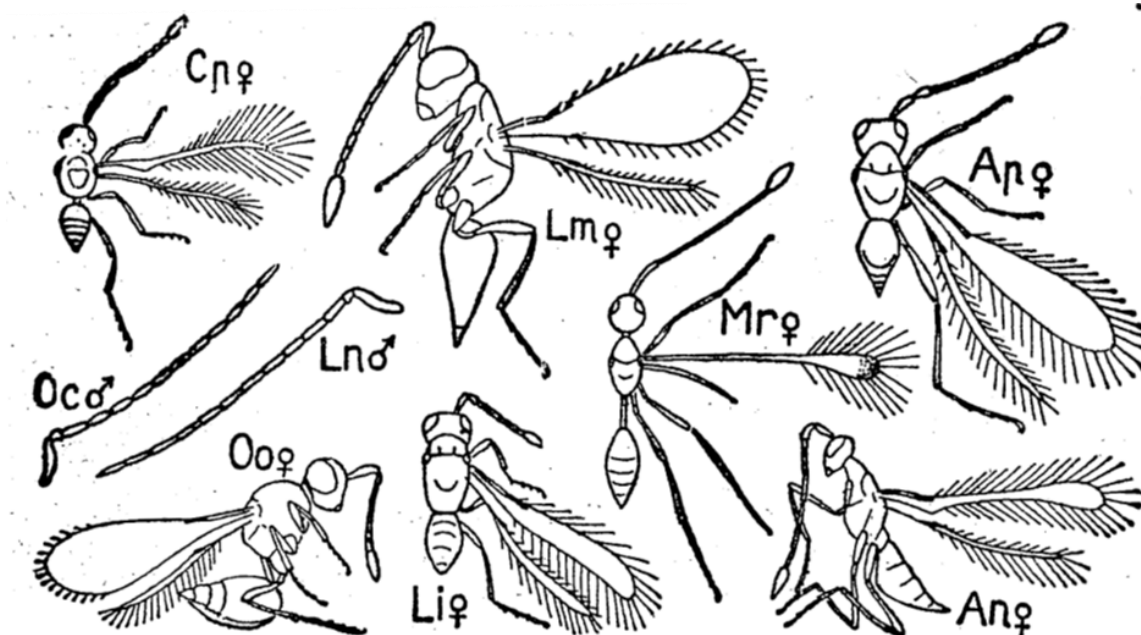


Figure. *Anagrus* sp Fig X from (Chiappini, 2008)



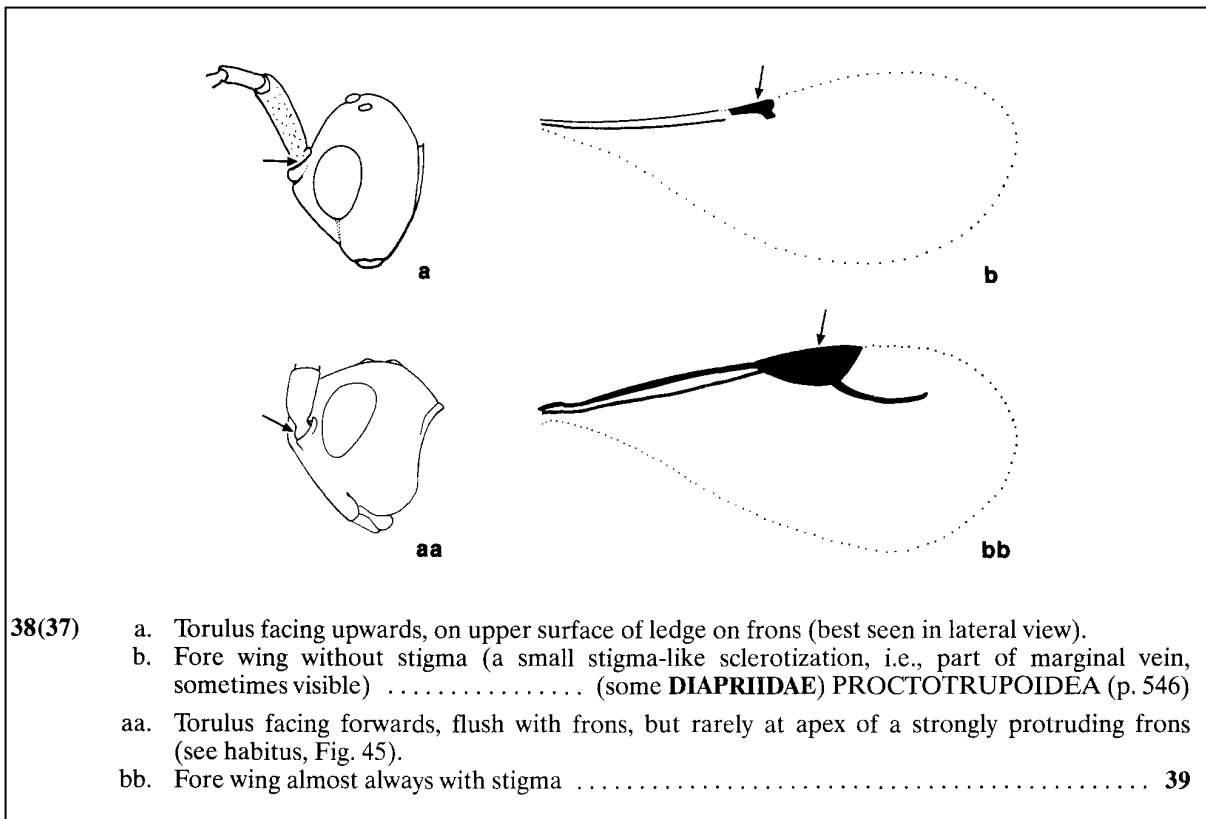
**Cp, *Camptoptera* sp. ♀ (d'après KIEFFER). — Oo, *Ooctonus* sp. ♀. — Oc, *Ooctonus* sp. ♂ (antenne). — Lm, *Limaenon* sp. ♀. — Ln, *Limaenon* sp. ♂ (antenne). — Li, *Litus* sp. ♀. — Mr, *Mymar regalis* Enoch ♀ (d'après SCHMIEDEKNECHT). — Ap, *Anaphes* sp. ♀. — An, *Anagrus* sp. ♀.**

Figure. Illustration of some genera of Mymaridae and Mymaromatidae, from (Berland, 1971)



**Aphelopus**

- size about 2-3 mm
- antennae seemingly without “elbow” and not bending forward
- antennae with 8 flagelomeres
- specific reduced front wing venation, wing with a stigma



Distinguishing *hymenoptera* superfamilies. From “Hymenoptera of the World” (Goulet et Hubert, 1993).

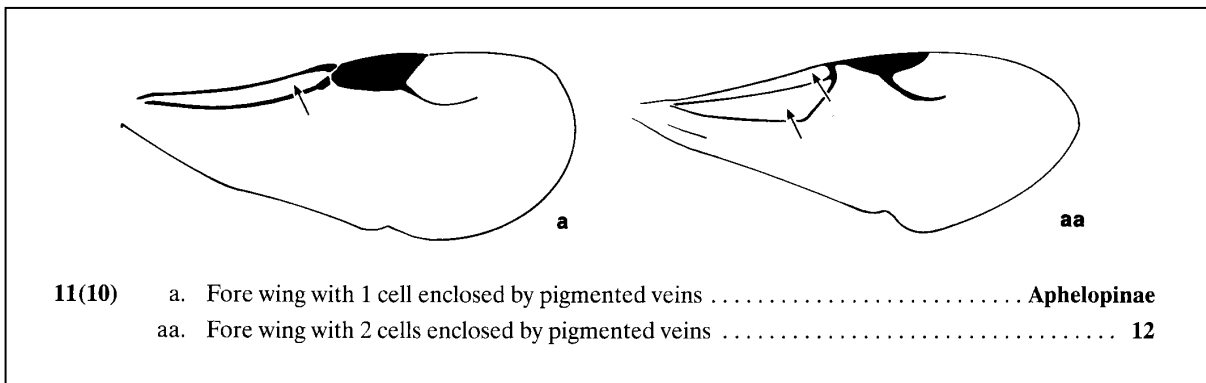


Figure X . Distinguishing *Aphelopinae* subfamily From Hymenoptera of the World (Goulet et Hubert, 1993)







Figure *Aphelopus* sp. (2)



*Aphelopus atratus* (3)



*Aphelopus atratus* ©Bees Wasps & Ants Recording Society (4)



**Chalarus sp.**

Diptera—searching for something resembling a fly

*Pipunculidea*

globular head, made almost only of eyes

Wings longer than abdomen

**Chalarus**

3-5 mm long

Specific wing venation: incomplete cell M open, cross vein dm-cu absent, vein m reduced).

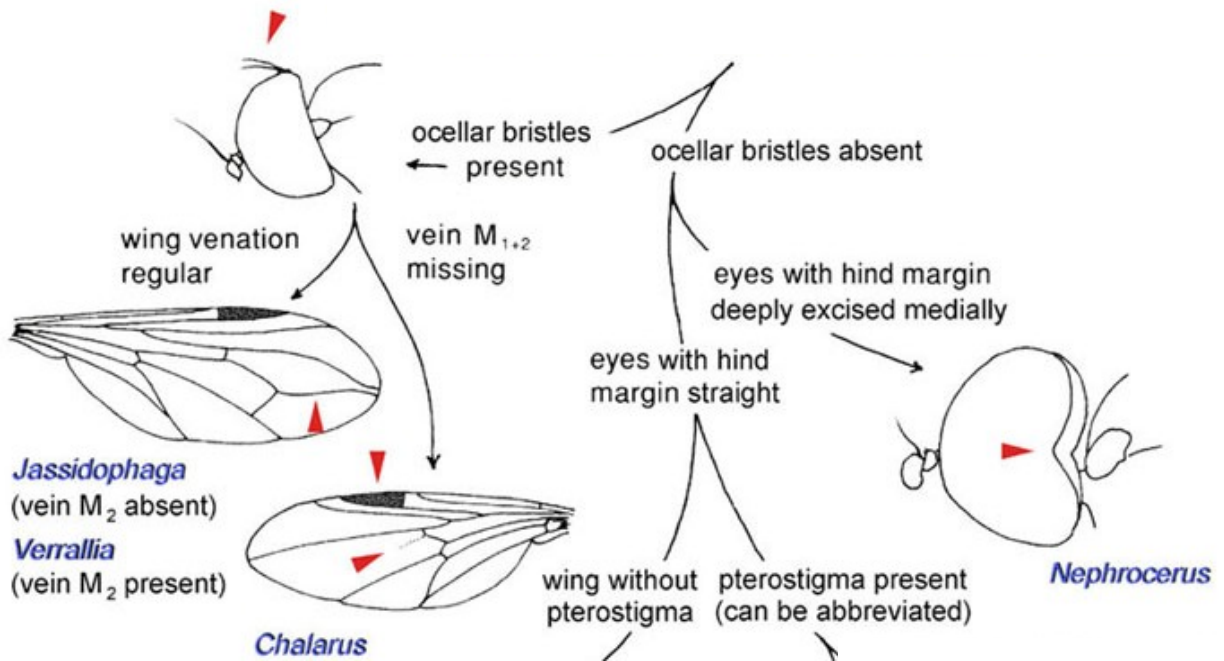
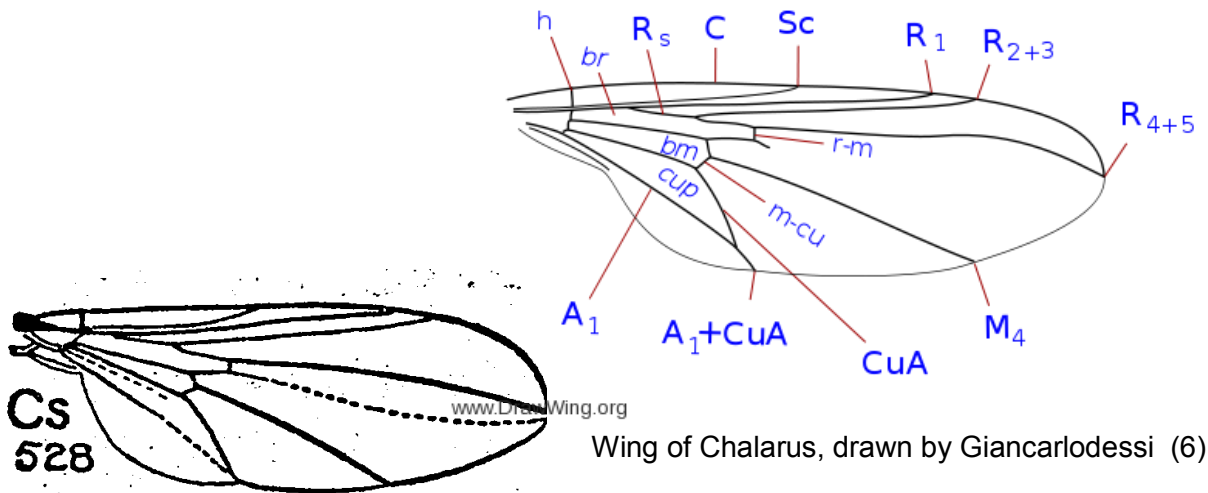


Figure X recognizing *Chalarus* in the Pipunculidae (5)



Wing of *Chalarus*, drawn by Giancarlo Dessi (6)

Figure X A wing of *Chalarus* Séguy(1937)





*Chalarus spurius*

Photo by J. Kahanpää (7)



Fig X *Chalarus sp* photo by John Rosenfeld (8)



## List of References (Appendix I.):

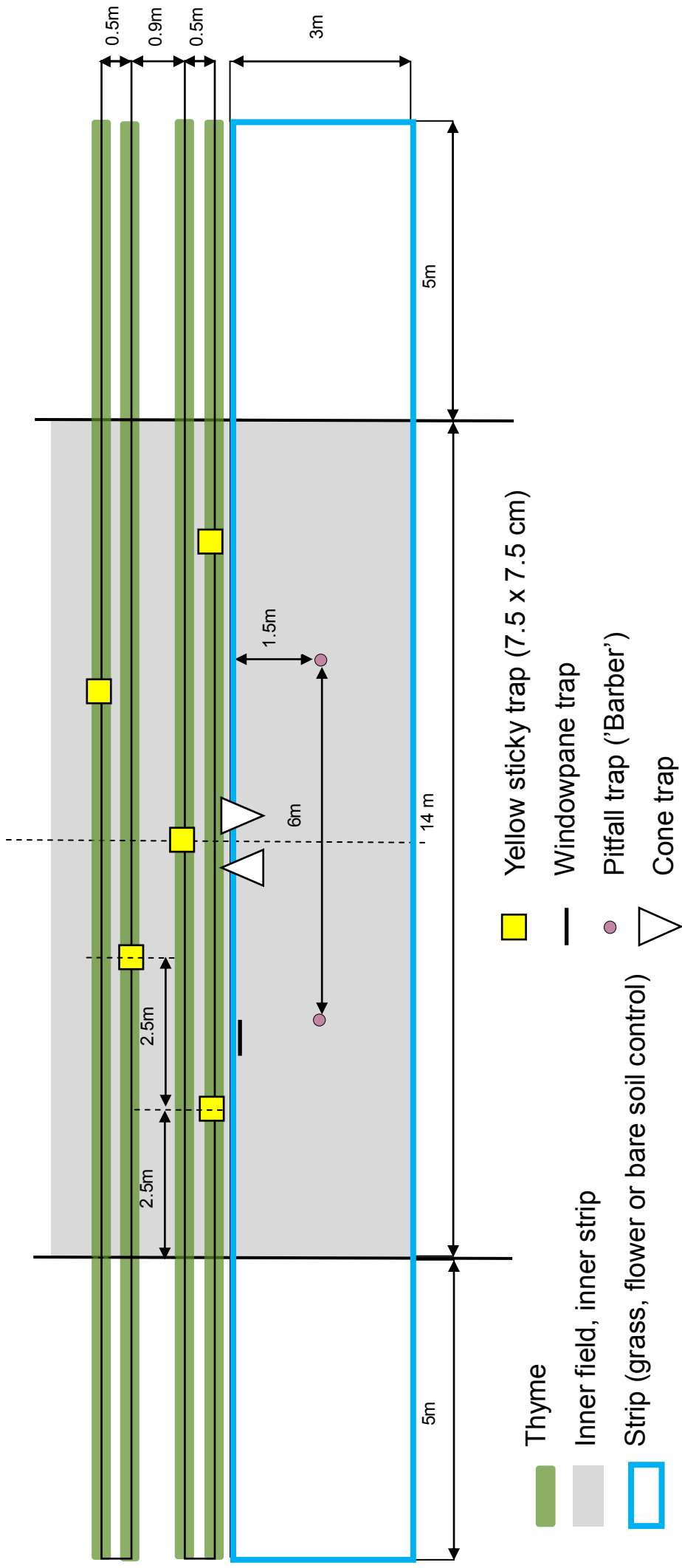
- Berland, L., 1971. La faune de la France Illustrée VII: Hymenoptères, Rémy Perrier. Delagrave.
- Blum, H., Jung, K., Nickel, H., Planer, J., 2011. Entwicklung praxistauglicher Strategien zur Regulierung von Zikaden im ökologischen Arznei- und Gewürzpflanzenanbau im Freiland und unter Glas - Practicable strategies to control leafhopper pests of organically grown medicinal herbs and spices in the field and under glass. Klein-Altendorf/Ahrweiler.
- Chiappini, E., 2008. Anagrus Fairyflies (Hymenoptera: Mymaridae), in: Capinera, J.L. (Ed.), Encyclopedia of Entomology. Springer Netherlands, Dordrecht, pp. 152–154.
- Goulet, H., Huber, J.T., 1993. Hymenoptera of the world : an identification guide to families. Agriculture Canada, Ottawa.
- Lubiarz, M., Musik, K., 2015. First record in Poland of the Ligurian leafhopper, *Eupteryx decemnotata* Rey 1891 (Cicadomorpha, Cicadellidae)—an important pest of herbs. Journal of Plant Protection Research 55, 324–326.
- Séguy, E., 1937. La faune de la France Illustrée VIII: Diptères, Aphaniptères, Rémy Perrier. Delagrave, Paris.

## Online sources :

1. British Bugs Gallery [Internet]. [cited 2017 Sep 1]. Available from: <https://www.britishbugs.org.uk/gallery.html>
2. INRA-OPIE. CHRYSIDOIDEA - Aphelopus [Internet]. Available from: <http://www7.inra.fr/opie-insectes/ch-chrysi1.htm>
3. Nederlandse Fauna 6. De wespen en mieren van Nederland - Dryinidae [Internet]. [cited 2017 Sep 2]. Available from: [http://www.naturalmedia.nl/NL/pags\\_jdr/onderzoek/publicaties/wespenatlas\\_2004\\_teksten/Dryinidae\\_2004.html](http://www.naturalmedia.nl/NL/pags_jdr/onderzoek/publicaties/wespenatlas_2004_teksten/Dryinidae_2004.html)
4. Aphelopus atratus (Dalman, 1823) | BWARS [Internet]. [cited 2017 Sep 4]. Available from: <http://www.bwars.com/wasp/dryinidae/aphelopus-atratus>
5. Kehlmaier. Pipunculidae [Internet]. 2011. Available from: <http://www.kehlmaier.de/pipunculidae1.html>
6. Chalarus, wing | DrawWing [Internet]. 2017 [cited 2017 Aug 24]. Available from: <http://drawwing.org/insect/chalarus-wing>
7. Kuvapankki [Internet]. [cited 2017 Sep 4]. Available from: <http://www.insects.fi/insectimages/browser?order=DIP&family=Pipunculidae&genus=Chalarus&species=spurius>
8. Big-headed Fly - Chalarus - BugGuide.Net [Internet]. [cited 2017 Sep 4]. Available from: <http://bugguide.net/node/view/963888/bgpape>







Appendix II. Layout of a the experimental plot (14m long) .



### Appendix III. List of plant species recorded in flower strips and grass strips 2016.

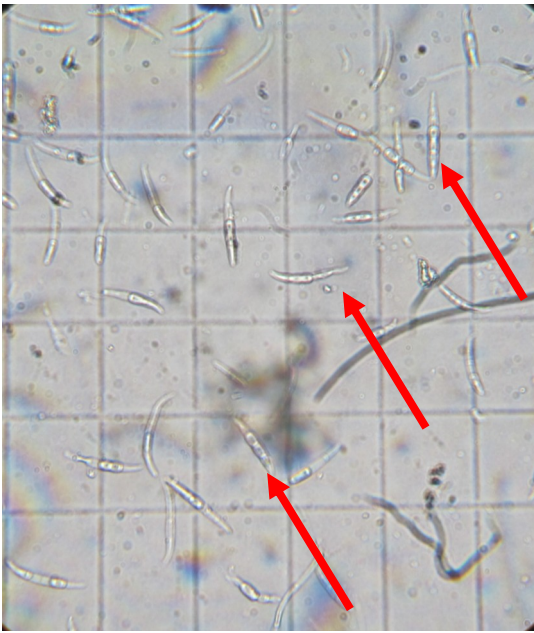
<b>Species</b>	<b>F</b>	<b>G</b>	<b>Sown in F</b>	<b>Sown in G</b>
1 <i>Avena fatua</i>	3	3		
2 <i>Anthemis arvensis</i>	2	3		
3 <i>Lolium multiflorum</i>	1	3		X
4 <i>Convolvulus arvensis</i>	3	2		
5 <i>Polygonum aviculare</i>	3	2		
6 <i>Solanum dulcamara</i>	2	2		
7 <i>Chenopodium album</i>	1	2		
8 <i>Matricaria chamomilla</i>	3	1	X	
9 <i>Amaranthus sp. (retroflexus)</i>	2	1		
10 <i>Echinochloa crus-galli</i>	2	1		
11 <i>Papaver rhoeas</i>	2	1		
12 <i>Capsella bursa-pastoris</i>	1	1		
13 <i>Erigeron sp.</i>	1	1		
14 <i>Mercurialis annua</i>	1	1		
15 <i>Sonchus oleraceus</i>	1	1		
16 <i>Calendula officinalis</i>	3		X	
17 <i>Coriandrum sativum</i>	3		X	
18 <i>Cyanus segetum</i>	3		X	
19 <i>Fagopyrum esculentum</i>	3		X	
20 <i>Malva sylvestris</i>	3		X	
21 <i>Phacelia tanacetifolia</i>	3		X	
22 <i>Phoeniculum vulgare</i>	3		X	
23 <i>Sinapis alba</i>	3		X	
24 <i>Vicia sativa</i>	2		X	
25 <i>Calystegia sepium</i>	1			
26 <i>Chrysanthemum segetum</i>	1			
27 <i>Convolvulus sepium</i>	1			
28 <i>Fumaria sp.</i>	1			
29 <i>Medicago sativa</i>	1			
30 <i>Polygonum sp.</i>	1			
31 <i>Rumex obtusifolius</i>	1			
32 <i>Elytrigia repens</i>		2		
33 <i>Festuca arundinacea</i>		2		X
34 <i>Poa trivialis</i>		2		
35 <i>Agrostis capillaris</i>		1		
36 <i>Anagalis arvensis</i>		1		
37 <i>Anthriscus sylvestris</i>		1		
38 <i>Arrhenaterum sp</i>		1		
39 <i>Brassica nanus</i>		1		
40 <i>Euphorbia helioscopia</i>		1		
41 <i>Holcus lanatus</i>		1		
42 <i>Hypocrepis radiata</i>		1		
43 <i>Lactuca seriolata</i>		1		
44 <i>Polygonum persicaria</i>		1		
45 <i>Ranunculus repens</i>		1		
46 <i>Senecio capensis</i>		1		
47 <i>Sonchus asper</i>		1		
48 <i>Veronica arvensis</i>		1		
49 <i>Vulpia muroides</i>		1		



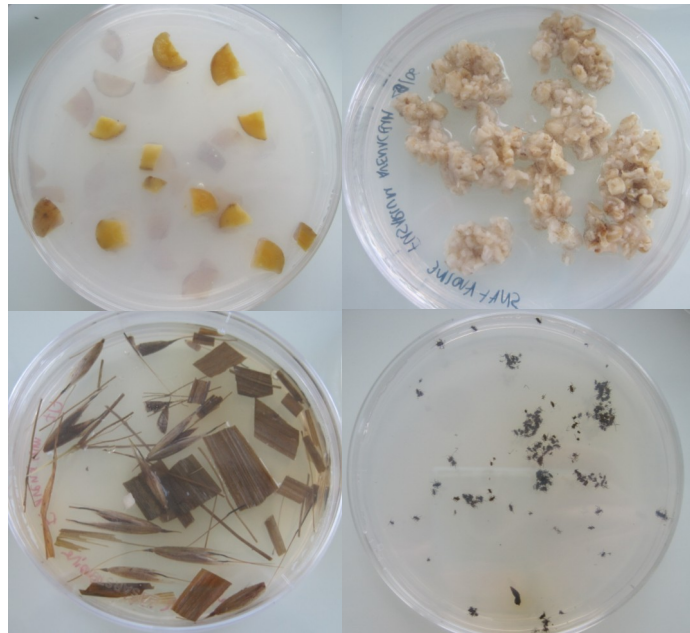
## Appendix IV. Flower strips and grass strips



Figure X. Grass strips and flower strips on the three fields on 14th June 2017 (Top to down; Chazelle, Noëlle, Lycee, Left to right Flower strip -Grass strip).



Conidia of *Fusarium avenaceum* in the Malassez counting cell.



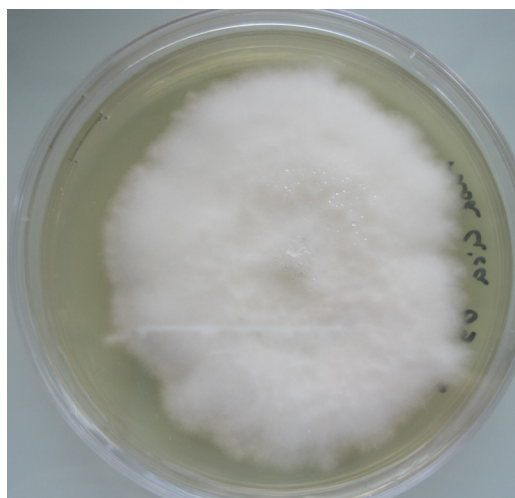
Different media with inclusions tested. Clockwise: oat flakes, aphids, rye, carrot.



DIY observation cage made of transparent plastic sheet, for checking on leafhoppers during the inoculation test.



Leafhopper covered in mycelium, inoculation test with *Fusarium avenaceum* 2017.



Mycelium of *Fusarium avenaceum* on PDA.

## Appendix V. Other activities carried out during the internship

### 1.1 Construction of the traps

The more extensive experimental protocol of this year (three replicates) demanded construction of more cone traps, as they were not available in any of the cooperating institutions. The order and delivery of necessary materials, as well as the construction demanded quite a lot of time. Thanks to a newly purchased sewing machine I was able to make 18 new cone traps, which may serve for future experiments. The construction took altogether about 1.5 weeks. Sometimes even the skills we expect to use the least come in handy, such as sewing.

### 1.2 The *Fusarium* fungus protocol

During the experimentation of the previous year (2016), an entomopathogenic fungus was detected on the thyme fields. The adult leafhoppers were found covered and attached by the mycelia to the leaves of thyme. The development of fungi was very probably enhanced by a particularly humid season of 2016 with high precipitation and low temperatures. The intern of previous year (2016), Cyril Farcy was able to isolate the fungus through several subcultures from the mummified leafhoppers. In a small group project, the students of the 'Le Fresne' performed an essay on aphids at the end of year 2016, with rather encouraging results. The identification of fungus species was done by Thomas Guillemette (IRHS) and Sylvie Leclerc (SNES/GEVES). The fungus was identified as *Fusarium avenaceum* (Fr.) Sacc., which is a non-sexual form of *Gibberella avenacea* R.J. Cook. To continue these efforts, I cooperated closely with Muriel Marchi, research engineer (IRHS).

Firstly we tried to obtain viable spores for an inoculation essay. This was more difficult than expected. We tested PDA medium with inclusions of rye, wheat, aphids and organic oat flakes (always twice autoclaved), as well as a specific sporulation medium for *Fusarium* found in the literature. Nevertheless, it was a medium with the inclusion of carrots given by a colleague of Muriel Marchi that provided us with partial success. We were able to harvest spores from one and only Petri dish for three inoculation tests. Having done the tests at the end of the season, I met with the difficulty to harvest enough larvae to perform all three essays. Therefore, the second and third test was done on adult leafhoppers of a *Zyginidia scutellaris* species, still present in the thyme crop at the time. The elaboration of the protocol was a lot of trial-and-error. At the present moment, no clear conclusions can be made. We found, however that a part of inoculated leafhoppers developed mycelia or were found mummified. It seems that leafhoppers in the control treatment lived a bit longer than those in *Fusarium* treatment. A small report including a literature review and detailed results will be available by the end of the year 2017.








### **1.3 Pedagogical part**

I had the opportunity to present the experiment to the students of the 'Le Fresne' school, during a 30-min, highly illustrative presentations, including samples of traps and leafhoppers.

The co-supervisor of my internship, Mme Melissa Leloup proposed to all interns of 'Le Fresne' to make short videos about their experiments. Summing up my internship into a 5-minute video was a welcome experience. I was responsible for the preparation of text and talk, as well as translation for the English subtitles. Mme Leloup did all the takes as well as the film and sound editing. The videos should be available shortly on the website of the school.





  	Diplôme / Mention : Biologie et Technologie du végétal Spécialité : Production et Technologie du Végétal (ProTeV) Parcours : Productions Végétales Spécialisées (PVS) Option : Filières de l'horticulture et végétal urbain
Auteur(s) : Ivana BILKOVA Date de naissance : 14/12/1984	Organisme d'accueil : EPLEFPA Angers le Fresne - Segré Adresse : 38, chemin du Fresne, 49130 Sainte Gemmes sur Loire BP 43627 49036 ANGERS Cedex 01 Maître de stage : Yann TRICAULT Co-encadrants : Mélissa LELOUP, Éric DUCLAUD
Nb pages : 32. Annexe(s) : V.	
Année de soutenance : 2017	
Titre français : <b>Evaluation d'aménagements agro-écologiques (bandes fleuries, bandes enherbées) en culture de thym, dédiés à la régulation des cicadelles</b> Titre anglais : <b>Evaluation of the of the agro-ecological infrastructures (grass strips, flower strips) for the leafhopper control in thyme crop</b>	
Résumé : Les infrastructures agro-écologiques sont utilisées dans le contrôle biologique par conservation pour certaines cultures, mais ce concept est moins étudié pour les cultures mineures, tel que les plantes à parfum, aromatiques et médicinales. Un ravageur majeur du thym et d'autres cultures de la famille des <i>Lamiaceae</i> sont les cicadelles du groupe <i>Typhlocybae</i> . Une revue bibliographique sur les cicadelles <i>Typhlocybae</i> et leurs potentiels ennemis naturels est présentée. Nous avons comparé par l'expérimentation les bandes fleuries, bandes enherbées et un témoin sol nu pour leur attractivité vis-à-vis des arthropodes auxiliaires (parasitoïdes et prédateurs), et leurs effets sur la population des cicadelles et des dégâts de feuilles qu'elles causent. Des résultats partiels sont présentés. Un genre de parasitoïde <i>Anagrus</i> sp ( <i>Mymaridae</i> ) a été recensé. Les résultats préliminaires ne montrent aucun effet de la présence de bandes fleuries ou enherbée sur la population de cicadelles ou sur les dégâts causés aux feuilles, par comparaison au témoin. Cependant, une forte corrélation entre le nombre de <i>Eupteryx decemnotata</i> (la principale espèce de cicadelle trouvée) et <i>Anagrus</i> sp. a été démontré, suggérant une relation hôte-parasitoïde.	
Abstract: Agro-ecological infrastructures are used in biological pest control by conservation in some crops, but the topic is less studied for minor crops, such as aromatic and medicinal herbs. The major pest in thyme ( <i>Thymus vulgaris</i> L.) and other <i>Lamiaceae</i> crops in west France are <i>Typhlocybae</i> leafhoppers. A literature review of <i>Typhlocybae</i> leafhoppers and their potential natural enemies is presented. We compared in an experiment flower strip, grass strip and bare soil control for their attractiveness to natural enemies (parasitoids and predators), and their effect on leafhopper population and leaf damage they cause in thyme crop. Partial results are presented. <i>Anagrus</i> sp ( <i>Mymaridae</i> ), a parasitoid was detected. Preliminary results showed no effects of strips compared to control in terms of leafhopper population or leaf damage. However, a strong correlation between the number of <i>Eupteryx decemnotata</i> (the main leafhopper found) and <i>Anagrus</i> sp. was detected, suggesting a host-parasitoid relationship.	
Mots-clés : cicadelle, <i>Typhlocybae</i> , thym, <i>Lamiaceae</i> , parasitoïde, <i>Mymaridae</i> , <i>Anagrus</i> , infrastructure agro-écologique, bande enherbée, bande fleurie Key Words: leafhopper, <i>Typhlocybae</i> , thyme, <i>Lamiaceae</i> , natural enemy, parasitoid, <i>Mymaridae</i> , <i>Anagrus</i> , agro-ecological infrastructure, flower strip, grass strip	