UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Dept. of Agroforest Ecosystems

Thysanoptera species assemblages associated with sown cover crops in citrus agroecosystems

Master's Thesis

European Master Degree in Plant Health in Sustainable Cropping Systems

AUTHOR: Tamang , Suraj

Tutor: Rodrigo Santamalia, María Eugenia

External cotutor: MONZO FERRER, CESAR

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AUTHOR: SURAJ TAMANG

EXPERIMENTAL TUTOR: Dr. CÉSAR MONZÓ FERRER

ACADEMIC TUTOR: Dr. MARIA EUGENIA RODRIGO SANTAMALIA

Valencia, Spain

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RESUMEN

El Control Biológico por Conservación es uno de los tres tipos de control biológicos que se utilizan en la Gestión Integrada de Plagas (GIP). Las cubiertas vegetales como fuente de alimento son un componente principal del control biológico por conservación. Las especies de tisanópteros, *Pezothrips kellyanus* Bagnall, *Chaetanaphothrips orchidii* Moulton y *Scirtothrips auranti* Faure, son plagas clave de los cítricos en España. En este trabajo estudiamos las comunidades de Thysanoptera en 11 especies vegetales utilizadas para cubiertas vegetales de cítricos, en dos localidades durante un período de 5 meses en Valencia. Las especies vegetales de la cubierta presentes en invierno (*Calendula arvensis*, *Calendula officinalis*, *Diplotaxis erucoides*, *Lobularia maritima*, *Rumex acetosa*, *Sonchus asper* y *Sonchus tenerrimus*) mostraron más de 5 especies diferentes de Thysanoptera. Mientras que de la cubierta de verano (*Achillea millefolium*, *Ammi majus*, *Coriandrum sativum* y *Convolvulus arvensis*) mostró menor diversidad. Los campos de IVIA y Pego mostraron una diversidad similar de especies de Thysanoptera. Sin embargo, en IVIA, *Calendula officinalis* parece tener mayor abundancia de especies de Thysanoptera, mientras que en Pego, *Calendula officinalis* y *Diplotaxis erucoides* tienen una abundancia similar de especies de Thysanoptera. *Melanthrips fuscus* y *Thrips angusticeps* fueron especialmente abundantes a finales del invierno y principios de la primavera. *Frankliniella occidentalis* y *Thrips tabaci* comenzaron a aumentar sus poblaciones desde finales de la primavera a principios del verano. Nuestros resultados confirman que las cubiertas vegetales sembradas no albergan ninguna de las 3 plagas de Thysanoptera asociadas a los cítricos en España y que la diversidad de especies de Thysanoptera que se encuentran en cubiertas vegetales de invierno pueden servir como alimento alternativo para los enemigos naturales durante esa época del año.

Palabras clave: Gestión Integrada de Plagas (GIP), Control biológico por conservación, cubiertas vegetales, Thysanoptera, biodiversidad

Autor: Mr. Suraj Tamang

Lugar y fecha: València, September 2022

Tutor experimental: Dr. César Monzó Ferrer

Tutor académico: Dr. Maria Eugenia Rodrigo Santamalia

Abstract

Conservation Biological Control is one the three biological control strategies used in Integrated Pest Management (IPM). Ground cover crops as source of food serves is a main component of Conservation Biological Control. Thysanoptera species i.e., *Pezothrips kellyanus* Bagnall, *Chaetanaphothrips orchidii* Moulton and *Scirtothrips auranti* Faure are known to be major citrus pest in Spanish citrus orchards. We studied the Thysanoptera assemblages on 11 ground cover crop species in two locations over a period of 5 months in Valencia. Winter ground cover crops (*Calendula arvensis, Calendula officinalis, Diplotaxis erucoides, Lobularia maritima, Rumex acetosa*, *Sonchus asper* and *Sonchus tenerrimus*) showed a more than 5 different Thysanoptera species. While the summer ground cover crops (*Achillea millefolium, Ammi majus, Coriandrum sativum* and *Convolvulus arvensis*) showed less diversity. The fields of IVIA and Pego showed a similar diversity of the Thysanoptera species. However, in IVIA, *Calendula officinalis* seems to have highest abundance of Thysanoptera species while in Pego, *Calendula officinalis* and *Diplotaxis erucoides* had similar abundance of Thysanoptera species. *Melanthrips fuscus* and *Thrips angusticeps* were abundant in the late winter and early spring. *Frankliniella occidentalis* and *Thrips tabaci* started to increase from late spring and early summer. Our results confirm that the ground cover crops do not host the 3 main citrus pests in Spain and the diversity of Thysanoptera species found in the winter ground crops may serve as an alternative food resource for the natural enemies during the winter season.

Keywords: Integrated Pest Management, Conservation Biological Control, Ground cover crops, Thysanoptera, Biodiversity

Author: Mr. Suraj Tamang

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Experimental tutor: Dr. César Monzó Ferrer

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Abbreviation

AEMET = The Spanish Meteorological Agency

BC = Biological Control

CABI = Centre for Agriculture and Bioscience International

EPPO = European Plant Protection Organization

FAO= Food and Agriculture Organisation of the United Nations

GLM= Generalized Linear Model

IPM= Integrated Pest Management

IUPAC= International Union of Pure and Applied Chemistry

IVIA= Instituto Valenciano de Investigaciones Agraries

KCT = Kelly's Citrus Thrips

RBD= Randomised Block Design

RCBD= Randomised Complete Block Design

SACT = South African Citrus Thrips

Sp. = Species

Introduction

Citrus is an important fruit in the world originated from Asian region. The production of citrus globally have increased over years. Spain is the highest producer of citrus in Europe, comprises of 25% global fresh citrus export from approximately 121,500 hectares of land in Spain (FAO, bulletin 2020). In Spain, major citrus production is from Andalusia, Catalonia, Murcia and Valencia from which the later region accounts for 90% of the country's citrus production (Futch and Singerman, 2018).

The importance of intergrated pest management (IPM) in citrus has increased as there are new disease and pest emerging due to change in climate and environment and the increasing movement of invasive species. The extensive use of pesticides for control of pest and diseases have lead to a decline in number of beneficial insects, resistance of the pest to the pesticides, increase in the pesticide residue in the citrus produce and to the environment. IPM aims at sustainable production for citrus for future generations.

Biological control (BC) is an increasing field of interest for the control of the citrus pest. BC is an environmentaly safe method. It involves using plant extracts, parasitoids, and predators, pathogens, antagonists or competitor populations. Classical Biological Control, Augmentative Biological Control and Conservation Biological Control are the three types of biological control implemented in the field. Classical Biological Control mainly highlights on introduction or importations of biological control organisms from where the pest is native, Augmentative Biological Control deals with the increase in the populations of the natural enemies by means of massive releases of biological control agents while Conservation Biological Control involves the manipulation of the agricultural ecosystem by preserving and enhancing the populations of natural enemies (Pekas, 2011). One example of biological control is the control of California red scale (CRS) *Aonidiella aurantia* through IPM by using biocontrol parasitoid *Aphytis melinus* in California (Rosen and De Bach, 1990).

From the various citrus pest in citrus orchards, the damage from Thysanoptera species were considered rare or absent until 2005. In Spanish citrus orchards, the major Thysanoptera species which are citrus pest are *Pezothrips kellyanus* Bagnall (Kelly's Citrus Thrips (KCT)), *Chaetanaphothrips orchidii* Moulton (Orchid Thrips) and *Scirtotrips aurantii* Faure (South African Citrus Thrips (SACT)). *Pezothrips kellyanus* Bagnall were first identified in 1996 in Spain (zur Strassen, 1996), but it was recorded to cause damage in citrus fruits in Spain only from 2005 and 2007 (Navarro et al., 2008). This Thysanoptera species feeds on young and mature fruit causing scurfing and rind blemish (Baker, 2006). Despite the worldwide distribution and economic importance of *P. kellyanus*, its biological control is still under development (Baker et al. 2011; Navarro-Campos et al. 2012a). Therefore, chemical control is currently the only practical alternative for growers.

Chaetanaphothrips orchidii Moulton was first detected to cause damage in Spanish citrus orchards between January and March 2016 from the two orange orchards Lanelate cv. located in the south of Tarragona province (Spain). The losses were very severe, affecting a high percentage of the overall production. *C. orchidii* in citrus fruits causes the development of irregular or circular rind of mature fruits (Goane et al, 2013). Ripe fruit re-infestations may occasionally occur due to migrations from other host plants, such as ground coverplants (Childers and Nakahara., 2006).

Scirtothrips aurantii Faure is also a major citrus pest native to Africa. In 2022 it was recorded to cause damage in Spanish citrus orchards of Andalucia, Spain. *S. aurantii* is highly polyphagous and has been reported from about 70 host plant species, some of them are said to be ground cover crops (Gilbert, 1990). It is listed in A1 Quarantine list of EPPO (EPPO RS 2021/008). *S. aurantii* has been recorded to cause reduction in citrus yields through serious damage to young leaves and reducing the size of the fruits for export quality (CABI, 2022).

Majority of Thysanoptera species are known to be phytophagous but there are some species like *Scolothrips sexmaculatus* which preys on spidermites, *Aeolothrips spp.* which are known to prey on genus *Thrips* and *Frankliniella* (Bournier et al., 1979; Loomans and van Lenteren., 1995) and *Franklinothrips* species which have been known to predate on Thysanoptera species globally. *Franklinothrips megalops,* a generalist predator native to Africa have been identified as potential predator of *C. orchidii* which is a major pest in the citrus orchards of Valencian community, Spain and was found to predate on all instars of *C. orchidii* (Thesis Qazi,2020 ; Thesis Montealegre-Morales, 2021)

Conservation Biological Control exploits resident, either native or naturalized, natural enemies. This strategy is especially useful in permanent ever-green crops (Barbosa and Benrey, 1998; Landis et al., 2000), such as citrus, where both pests and their natural enemies are active and abundant throughout the year (Garrido and Ventura, 1993). The plants used as ground cover crops in this experiment were native plants of the Mediterranean region as they would be better useful in habitat management for pollinators and natural enemies. These species are adapted for growing under local conditions and are less likely to be invasive (Fiedler et al., 2007; Isaacs et al., 2009). The use of native ground cover crops can help to ensure year round provision of resources to support beneficial arthropods, such as overwintering sites (Fiedler et al., 2008; Frank et al., 2008). These plants are locally adapted, they will have lower water, nutrient, and pest control requirements compared with non-native species (Isaacs et al., 2009).

Gómez-Martínez et al., (2016) found that when planting ground cover of *Festuca arundinacea* Schreber (Poaceae) in the Clementine orchards *A. obscurus* can act as a key alternative host for the generalists predator *N. barkeri* and *E. stipulatus* which allows better regulation of the citrus key pest *T. urticae*. Probably future management strategies that use ground cover crops should take into account the interaction between Thysanoptera species and flowering species.

We actually do not know whether the use of flower ground cover crops may have a positive or negative impact on the incidence of Thysanoptera species pests in Citrus orchards.The aim of this study is to investigate whether the use of ground cover crops of flowering plant species has an effect on the incidence of Thysanoptera species pests in citrus agroecosystems i.e 1. To study the Thysanoptera species assemblage associated to the cover crop, 2. To study specific relationships between plant species of the ground cover crop and Thysanoptera species and 3. To investigate seasonal changes in abundance and species composition of the Thysanoptera species assemblages in citrus ground cover crops.

Note: A. The term Thysanoptera species is used to generalize all the genus under Thysanoptera order, to reduce the confusion with the Genus *Thrips*.

B. The collective floral part of all the ground cover crops were considered as flowers during the sampling.

C. In each box plot, means with the same letters are not significantly different at the 0.05 level while with different letters indicate significantly different.

Material and Methods

A composite mixture of seeds of ground cover crops were sown on the two fields located at Instituto Valenciano de Investigaciones Agraries (IVIA) and Pego. Flowering stage of ground cover crops were more prefered during the sampling. Based on the flowering period the ground cover crops were sampled from February till June (Fig.1)

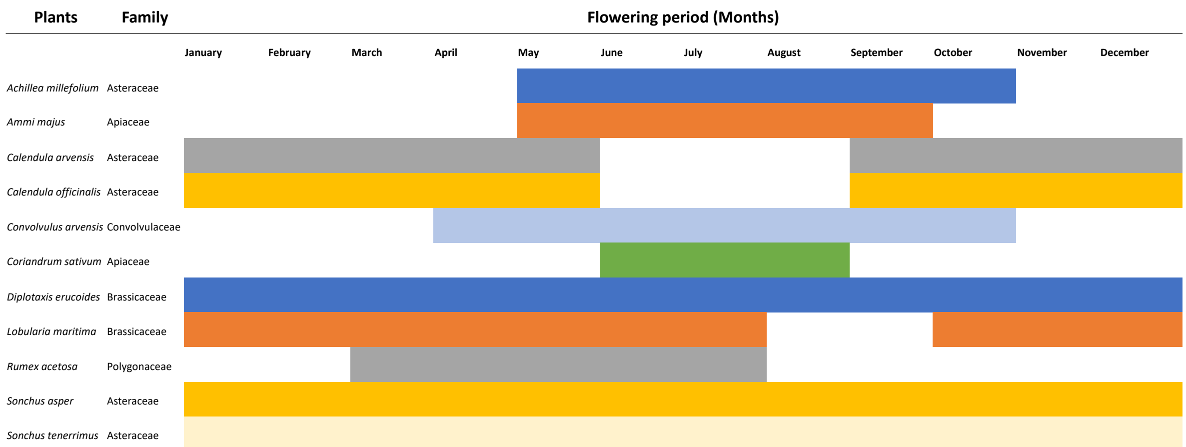


Fig 1. Groundcover crops sampled and their flowering period. (Source: *http://herbarivirtual.uib.es/*)

5.1 Experimental design:

a. *Instituto Valenciano de Investigaciones Agraries (IVIA)*

One of the groves (experimental) is located at Instituto Valenciano de Investigaciones Agraries (IVIA), Moncada. IVIA is in the province of Valencia, with an elevation of 37 m located at 39.5887° N, 0.3953° W. Latitude: 39° 32' 43" N - Longitude: 0° 23' 40" W. The area has no important incidence of citrus Thysanoptera species pest. The design of the field is Randomised Block Design (RBD) where each row serves as a block (Fig.2). Composite mixture of ground cover crops consisting of flowering plant species (Dicotyledonae) and Poaceae grass were planted in 8 rows in June 2021 (Table. 1). The field consist of buffers in all the sides. Citrus was planted in between the rows with the spacing of 1m. The diversity of the ground cover crops changed with the changing season (Fig.3 A, B, Fig.4 A, B, C, Fig.5 A, B and Fig. 6)

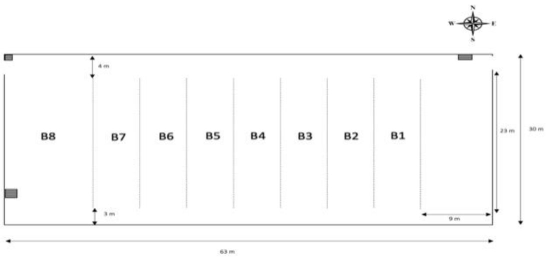


Fig.2 The RBD plot in IVIA.

Cover crops	Proportion
Lobularia maritima	3%
Diplotaxis eurcoides	2%
Medicago truncata	43%
Ammi majus	3%
Achillea millefolium	1.50%
Calendula officinalis	1.50%
Onobrychis viciifolia	43%
Rumex acetosa	3%

Table.1 The composite mixture of ground cover crops sown in IVIA.

Fig. 3 (A and B). The field with the ground cover crops during the month of March.

Fig. 4 (A, B and C) The growth of the ground cover crops in the month of April.

Fig. 5 (A and B). The field during the month of May.

Fig. 6 The field during the month of June.

b.Pego

The other grove i.e., a commercial grove is located at Pego, the area in which *Chaetanaphothrips orchidii* (Moulton), is known to cause important damages in citrus orchards. Pego is in the province of Alicante, with an elevation of 82 m above sea level. The area is 21.604 sq. m and is located at Latitude of 38° 50' 31" N and Longitude of 0° 7' 4" W. The design of the field in Pego is of Randomized Complete Block Design (RCBD) with 3 treatments (*Festuca*, Flowers, alternate rows). Each treatment have 4 replicates (Fig.7). Ground cover crops were planted in October 2021. *Festuca* treatment consist of a monospecific cover crop sown with *Festuca arundinacea* Schreb (Poaceae) while flower treatment consist of composite mixture of flowering plants (Dicotyledonae) (Table.2). Only the plots consisting of flowers were sampled during this experiment. The diversity and abundance of ground cover crops changed with changing season (Fig. 8 A, B, Fig.9 A, B, and Fig.10 A, B). The two sites were located in two different locations in Valencian Community (Fig.11).

Fig. 7 The RCBD plot in Pego where Brown signifies alternative rows, Green signifies *Festuca* treatment and Blue signifies Flower treatment.

Covercrops	Proportion %
Onobrychis vicifolia	65
Medicago trunculata	15
Lobularia maritima	6
Calendula officinalis	5
Achillea millefolium	3
Coriandrum sativum	3
Ammi majus	3

Table.2 The composite mixture of ground cover crops sown in Pego.

Fig.8 (A and B). The Field during the month of February.

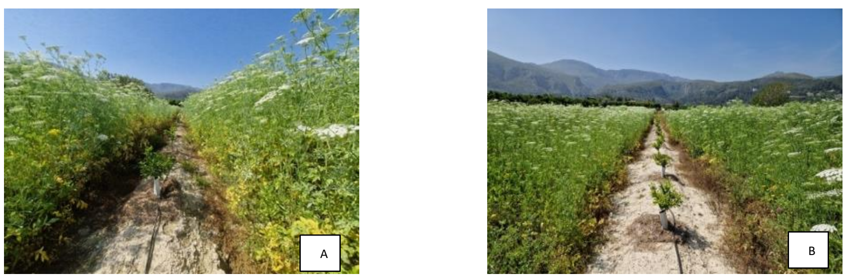


Fig.9 (A and B) The field during the month of May.

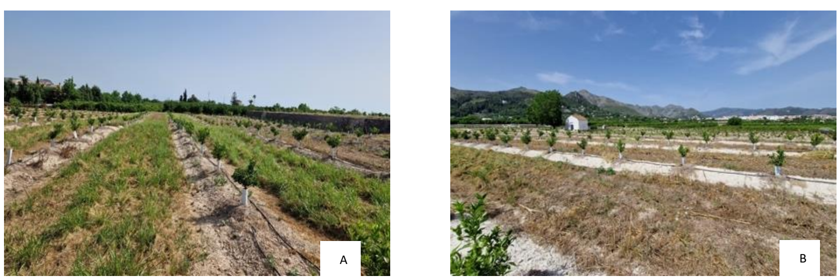


Fig. 10 (A and B) The field during the month of June after the mowing.

Fig. 11 The location of the two groves sampled. (A). IVIA and (B). Pego.

5.2 Sampling methodology:

All sampling was done at around 10 /11 am till 2:30 pm on dry and warm days for both the locations (20–30 °C).

a. *IVIA*

Plant species sampled were *Ammi majus, Achillea millefolium,Calendula arvensis,Calendula officinalis, Convolvulus arvensis*, *Diplotaxis erucoides, Lobularia maritima, Rumex acetosa, Sonchus asper* and *Sonchus tenerrimus*. A total of 8 flowers were sampled for each plant species in each row. Sample plants were identified among other plants by walking and Thysanoptera species were collected from random flowers based on the plant species available in the row. Sampling was done using a funnel (7.5cm in diameter) attached with the tube filled with 80% alcohol (Fig. 12). The flowers were hand shaken and the Thysanoptera species which dropped, were collected directly inside the tube (Fig. 13A). The sampling was done with the gap of 2 weeks in between. The sampling was done on 16th of February 2022, 1st of March 2022, 31st of March 2022, 19th of April 2022, 06th of May 2022, 24th of May 2022 and 06th of June 2022.

Fig. 12 The Funnel attached with the tube filled with 80% alcohol.

b. *Pego*

Plant species sampled were *Achillea millefolium, Ammi majus, Calendula arvensis, Calendula officinalis, Convolvulus arvensis, Coriandrum sativum, Diplotaxis erucoides, Lobularia maritima, Sonchus asper* and *Sonchus tenerrimus*. Only 4 plots (Plot 5, Plot 7, Plot 8 and Plot 11) with composite mixture of flower plants were sampled (Fig.7). A total of 5 flowers were taken from each plant species in the plot. Flowers were collected randomly from the each plant species. The samples were collected from the central rows of each plot as it would reduce the effects from the surrounding plots. Samples were collected in a plastic bags. They were stored in thermocol box with ice blocks, shifted to IVIA and stored in the refrigerator (-20^{O}C). Sampling was done once per month i.e. on 10th of March, 8th of April, 18th of May and 15th of June (After Mowing).

5.3 Preservation and Digestion of Thysanoptera species:

Using the stereoscope, Thysanoptera species were separated using fine brush and stored in eppendorf tubes with 80% alcohol which acts as dehydrating and preserving agent at room temperature (Fig. 13 B, C and D). The ground cover crop species, the row number (in case of samples from IVIA) or the plot number (in case of samples from Pego) and the date of collection was recorded on the top of each eppendorf (Fig.13 E).

Under the stereoscope Thysanoptera species from eppendorfs were transferred one by one onto the microscopic slides (26mm x 76mm) or excavated glass block with the help of fine brush (Fig. 13 F and G). In case of microscopic slides, 2 to 3 drops of lactic acid (90%) were applied based on the number of Thysanoptera species and a clean coverslip (18mm x 18mm) was placed onto it. The slides were labelled with the name of the ground cover crop species, row or plot number and the date of mounting. Lactic acid (IUPAC ID : 2-Hydroxypropanoic acid) 90% was used for clearing and as digester as it proved to be more effective and time saving in comparison with Nesbitt solution and Hoyer medium. The taxonomic characters of interest of the Thysanoptera species could be visualized properly with lactic acid. The microscopic slides were placed on the heating pan of 100 degrees for 3 hours as heat accelerated the rate of the clearing and digestion for identification and mounting (Fig. 13 H). After 3 hrs microscopic slides were left to cool for 5 to 10 minutes and then slides were placed into the microscope for identification.

For permanent slides (to be sent to the taxonomic experts and for record) the Thysanoptera species were placed on Hoyer liquid for mounting (one drop of Hoyer medium) with the help of fine brush using the stereoscope. The legs and wings were spread and the antennae were straighten by pressing on the basal segments with the brush or needle. Then coverslides were placed and left to dry and solidify for 10 to 15 minutes. Then nail polish was applied in the edges of the coverslides to seal the slides permanently. The permanent slides were stored in glass slide box. 240 temporary slides and 31 permanent slides were prepared in total during this experiment.

Fig. 13. (A) The collected samples in the tubes. The separation of the Thysanoptera species using stereoscope (B, C). The Thysanoptera species are screened out into the eppendorf (D). Labelling of the samples (E). Transfer of Thysanoptera species on the microscopic slides and excavated glass block (F, G). The heating of the samples on the heating pan for 3 hours (H).

5.4 Identification and counting

Only the adult males and females were mounted for identification as each Thysanoptera species adult could be distinguished easily in comparison with the nymphs and the eggs.

10x magnification in microscope was initially used as Thysanoptera species are small in size. But in order to identify further, 20x and 40x magnification helped in differentiation. The identification of the Thysanoptera species was based on the antennae (colour, number of segments, cones present, length of the antennae), the fore wings (setaes, pattern of colour, orientation of setaes), ocellar setaes (position and the length of the setaes), pronotum (setaes in lateral and marginal setaes), metanotum(the pattern, the presence or absence of campaniform sensillae) and the 8^{th} tergite segment (combs presense or not, combs pattern, length of the lateral setaes). The number of Thysanoptera species was recorded based on the species found, location, sampling date and ground cover crop species. The sources for identification of Thysanoptera species to the genus and species level were:

a. Thrips of the British Isles & Thrips of California, 2022 https://keys.lucidcentral.org/keys (Accessed on 01st July 2022).

b. Ozthrips (2022).Ozthrips, Thysanoptera in Australia. http://www.ozthrips.org/id-thrips/ (Accessed on 01st July 2022).

c. Palmer et al., 1992. IIE Guides to insects of importance to man. 2 Thysanoptera, CR, Betts. International Institute of Entomology (CAB).

d. Navarro-Campos et al., 2012. Trips en el cultivo de citricos: clave para distinguir la nueva plaga, *Pezothrips kellyanus*, de otras especies de trips.

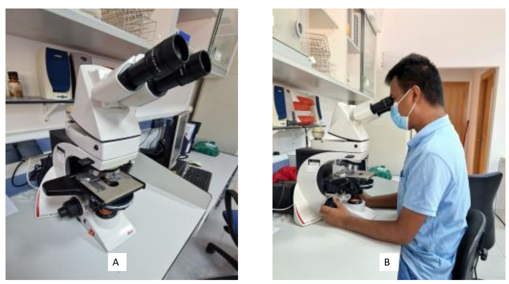


Fig.14 (A and B) The microscopic identification of the Thysanoptera species.

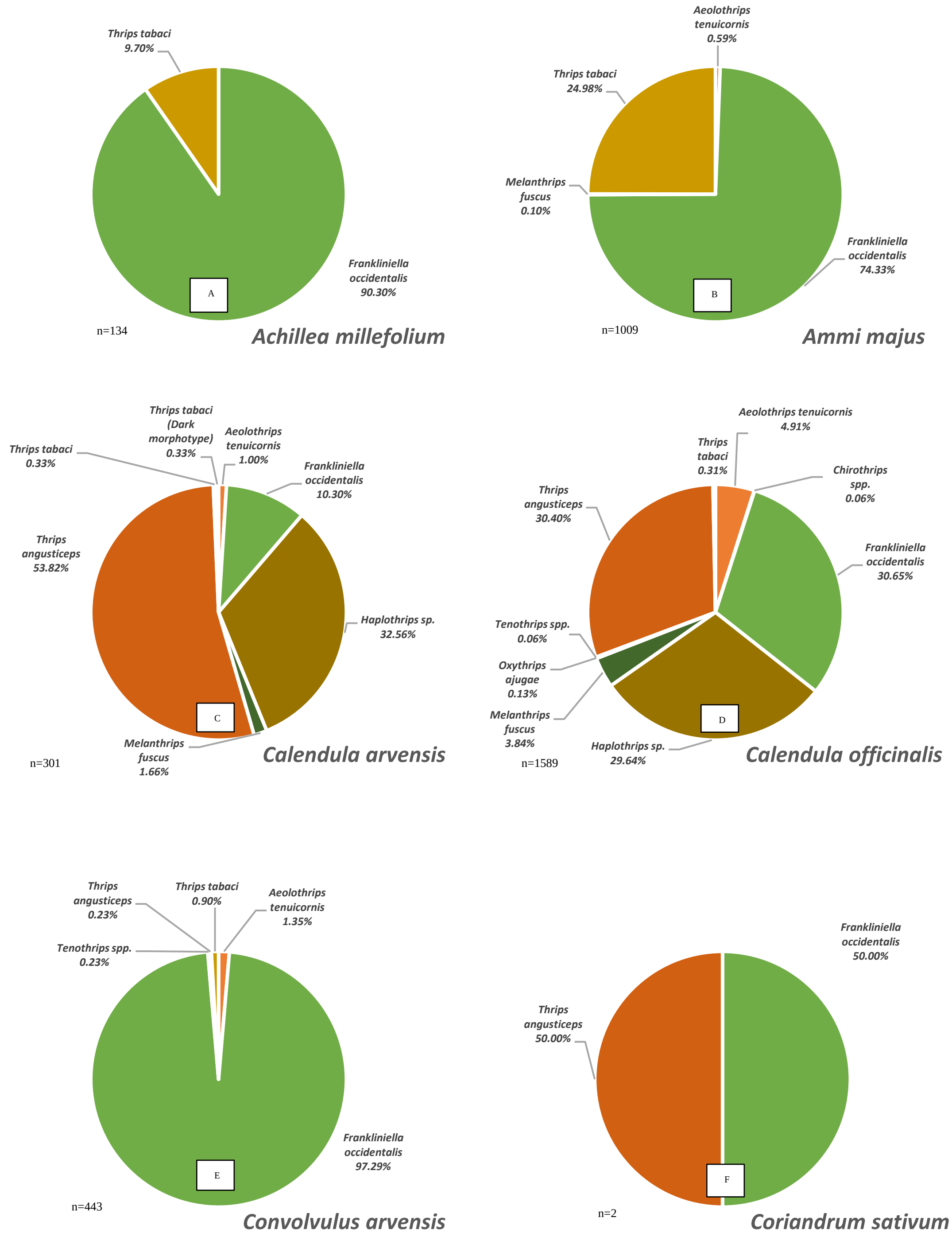
5.5 Data analyses:

Differences on the total number of Thysanoptera species collected from the different ground cover crops plant species in the two sampling sites as well as the seasonal changes in the Thysanoptera species throughout the various sampling dates in the two sampling sites were tested by Generalized Linear Model analysis (GLM) and post-hoc with Tukey test. Models assuming different error distributions (Gaussian, Poisson and Negative Binomial) were used. Error distribution in model selection was done based in AIC criterium. All the analyses were done using the program RStudio 2022.02.3+492 "Prairie Trillium" Release and its packages “agricolae”, “lsmeans”, “dplyr”, “ggplot2”, “mass”, “tidyverse”, “ggrepel”, “emmeans”, “multcomp” and “multcompView”.

Results

1.Species diversity and the percentage of Thysanoptera species present among the different ground cover crops sampled

Achillea millefolium flowers hosted mainly *Frankliniella occidentalis* (90.30 % of all the Thysanoptera specimens found), and *Thrips tabaci* (*9.70*%) (Fig. 15 A). *Ammi majus* hosted four different Thysanoptera species with highest being *Frankliniella occidentalis* (74.33% of the specimens) and the least *Melanthrips fuscus* (0.10%) (Fig.15 B). *Calendula arvensis* hosted seven different Thysanoptera species with highest being *Thrips angusticeps* (53.82%) and least of *Thrips tabaci* and *Thrips tabaci (Dark morphotype)* which represented only 0.33% of the captures (Fig.15 C). *Calendula officinalis* hosted the most diverse assemblage with nine different Thysanoptera species found, being the most abundant *Frankliniella occidentalis* (30.65%) and least *Tenothrips spp.,* and *Chirothrips spp.* representing just 0.06% of the specimens (Fig.15 D). *Convolvulus arvensis* flowers hosted five different Thysanoptera species with most abundant being *Frankliniella occidentalis* (97.29%) and least *Thrips angusticeps* and *Tenothrips spp.* (0.23%of the specimens) (Fig.15 E). *Coriandrum sativum* only hosted two species in equal proportion, i.e., 50% *Frankliniella occidentalis* and 50% *Thrips angusticeps* (Fig.15 F). *Diplotaxis erucoides* hosted seven species with *Melanthrips fuscus* (71.42% of the specimens) being the dominant species and *Frankliniella schultzei* the least (0.07%) (Fig.15 G). *Lobularia maritima* hosted seven different species with *Melanthrips fuscus* (62.29%) being the most common Thysanoptera species and *Haplothrips sp*. (0.85%) and *Thrips tabaci (Dark morphotype)* (0.85%) the least ones (Fig.15 H). *Rumex acetosa* hosted nine different Thysanoptera species with 60.32% *Frankliniella occidentalis* (60.32%) as the dominant species and *Aeolothrips tenuicornis, Chirothrips spp., Oxythrips ajugae* and *Thrips tabaci (Dark morphotype)* (all 1.59%) as the most uncommon species (Fig.15 I). *Sonchus asper* hosted nine different Thysanoptera species with *Thrips angusticeps* as the highest with 81.24% of the specimens found and *Frankliniella schultzei* as the least common species with just 0.19% of all the specimens found (Fig. 15 J). *Sonchus tenerrimus* hosted eight different Thysanoptera species being the most common *Thrips angusticeps* (68.33%) and the least *Thrips tabaci* (0.37%) (Fig. 15 K). In general, in the whole ground cover crops 11 Thysanoptera species were found, being the most abundant one *Frankliniella occidentalis* (32.6% of all the specimens).


Thrips tabaci 9.70%
Frankliniella occidentalis 90.30%
A
n=134
Achillea millefolium
Aeolothrips tenuicornis 0.59%
Thrips tabaci 24.98%
Melanthrips fuscus 0.10%
Frankliniella occidentalis 74.33%
B
n=1009
Ammi majus
Thrips tabaci 0.33%
Thrips tabaci (Dark morphotype) 0.33%
Aeolothrips tenuicornis 1.00%
Frankliniella occidentalis 10.30%
Thrips angusticeps 53.82%
Haplothrips sp. 32.56%
Melanthrips fuscus 1.66%
C
n=301
Calendula arvensis
Aeolothrips tenuicornis 4.91%
Thrips tabaci 0.31%
Chirothrips spp. 0.06%
Thrips angusticeps 30.40%
Frankliniella occidentalis 30.65%
Tenothrips spp. 0.06%
Oxythrips ajugae 0.13%
Melanthrips fuscus 3.84%
Haplothrips sp. 29.64%
D
n=1589
Calendula officinalis
Thrips angusticeps 0.23%
Thrips tabaci 0.90%
Aeolothrips tenuicornis 1.35%
Tenothrips spp. 0.23%
Frankliniella occidentalis 97.29%
E
n=443
Convolvulus arvensis
Frankliniella occidentalis 50.00%
Thrips angusticeps 50.00%
F
n=2
Coriandrum sativum

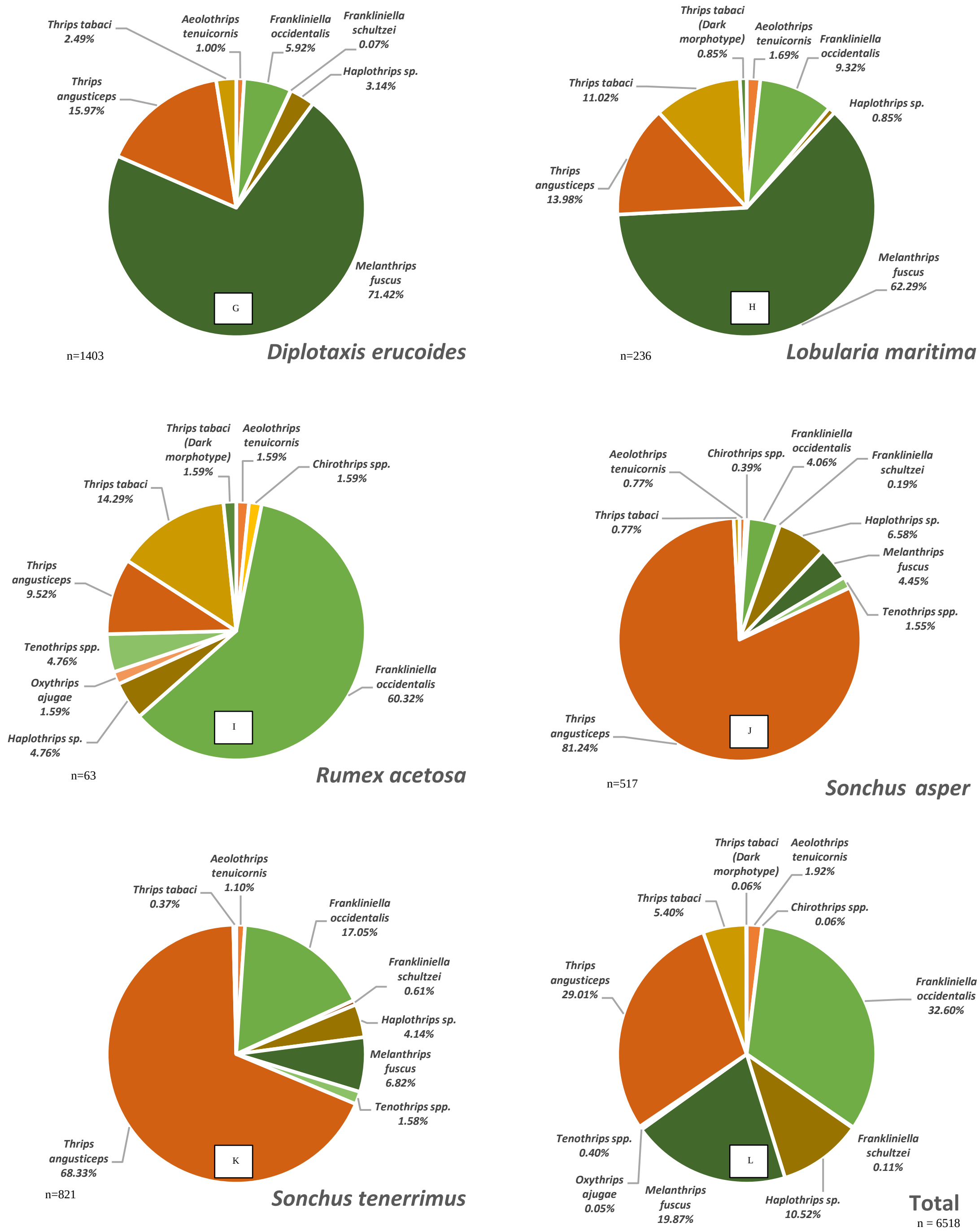


Fig.15 (A,B,C,D,E,F,G,H,I,J,K) Different ground covercrops with percentage of Thysanoptera species found.

Fig.15 (L) The total Thysanoptera species percentage from all the ground covercrops together.

2. Species diversity and percentage of Thysanoptera species present in the two sampling citrus groves.

The species diversity in IVIA consisted of all the 11 Thysanoptera species (including two different morphotypes of *Thrips tabaci*), while for Pego, the species diversity consisted of nine Thysanoptera species (Table. 3), with no *Frankliniella schultzei* and *Thrips tabaci (Dark morphotype)*. *Frankliniella occidentalis* was the most abundant Thysanoptera (43.77% of all the specimens found) while *Chirothrips spp.* (0.02%) was the least abundant species present in IVIA (Fig. 16 A). In Pego, *Melanthrips fuscus* represented 44.53% of all the specimens found while *Tenothrips spp.* was least common one with just 0.06% of all the specimens (Fig.16 B). In both the areas *Thrips angusticeps* represented more than 25% of all the specimens recorded.

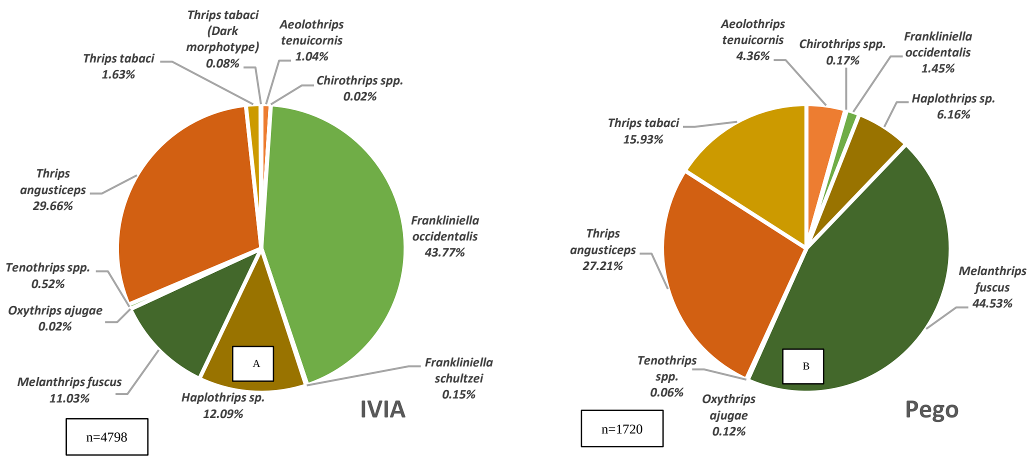


Fig. 16 (A and B) The total Thysanoptera species collected from IVIA and Pego respectively in percentage.

Thysanoptera species	IVIA	Pego	Total
Frankliniella occidentalis	2100	25	2125
Thrips angusticeps	1423	468	1891
Melanthrips fuscus	529	766	1295
Haplothrips sp.	580	106	686
Thrips tabaci	78	274	352
Aeolothrips tenuicornis	50	75	125
Tenothrips spp.	25	1	26
Frankliniella schultzei	7	0	7
Chirothrips spp.	1	3	4
Thrips tabaci (Dark morphotype)	4	0	4
Oxythrips ajugae	1	2	3
Total	**4798**	**1720**	**6518**

Table.3 Total number of Thysanoptera species collected from IVIA and Pego.

3. *Abundance of Thysanoptera species in each ground cover crops grown in the two sampling citrus groves*

a. IVIA

The mean number of Thysanoptera specimens collected from IVIA showed that *Calendula officinalis* hosted the significantly highest number of the specimens while the least was found on *Rumex acetosa* (Fig.17) (p<0.001).

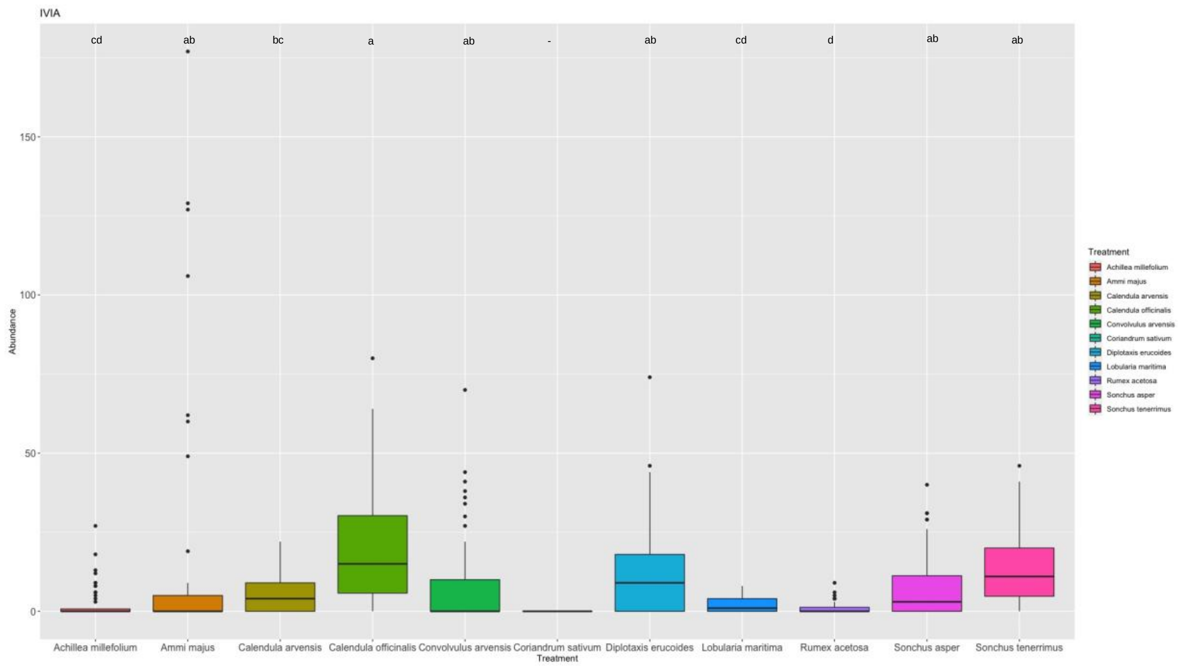


Fig. 17 Total Thysanoptera species collected from each cover crop in IVIA (p<0.001).

b. Pego

The mean number of Thysanoptera species collected per plant species from Pego showed significant differences between plant species (p<0.001) being *Calendula officinalis* and *Diplotaxis erucoides* the plant species hosting the most abundant assemblages while *Convolvulus arvensis* and *Coriandrum sativum* barely hosted a few individuals (Fig.18).

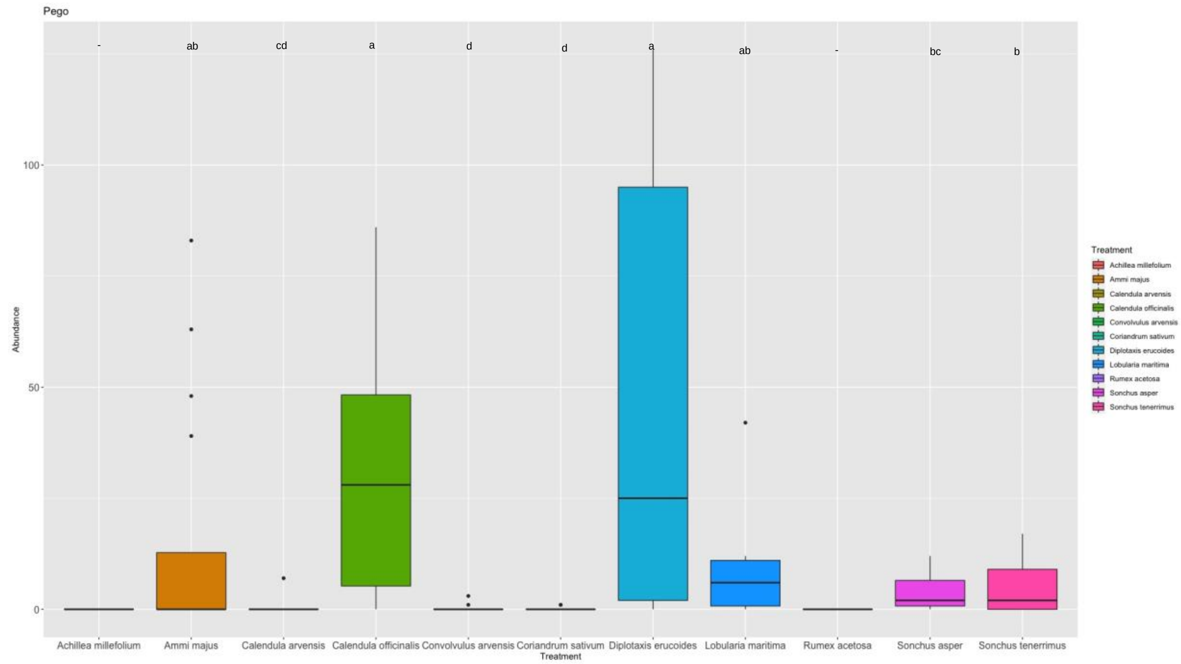


Fig.18 Total Thysanoptera species collected from each cover crop in Pego (p<0.001).

4. Seasonal changes in Thysanoptera species abundance.

a. *IVIA*

While some Thysanoptera species were uniformly present throughout all the sampling dates, some like *Frankliniella occidentalis* showed increases in numbers after May. *Thrips tabaci* was also increasing after May although in less numbers. *Melanthrips fuscus* and *Thrips angusticeps* abundance showed a declining trend after May. *Haplothrips sp*. decreased in its numbers earlier i.e., from last week of March (Fig.19 A).

On 16th February *Haplothrips sp*. number were significantly highest (p<0.001) followed by *Thrips angusticeps*. *Melanthrips fuscus* and *Frankliniella occidentalis* seemed to be in similar numbers. The abundance of the remaining Thysanoptera species were all significantly same (Fig.19 B).

On the 1st of March the number of *Thrips angusticeps* was significantly highest (p<0.001). *Haplothrips sp.* was second highest while *Melanthrips fuscus* and *Frankliniella occidentalis* were found in similar numbers. Others were all low and significantly same (Fig. 19 C).

On 31st March *Thrips angusticeps* and *Melanthrips fuscus* is significantly the highest number (p<0.001), followed by *Frankliniela occidentalis* while all other Thysanoptera species were low in number and significantly same (Fig.19 D).

On 14th April, *Thrips angusticeps* was the most abundant species, (p<0.001), followed by *Melanthrips fuscus*. *Frankliniella occidentalis* and *Haplothrips sp.* showed similar abundances. All other Thysanoptera species showed significantly lower abundances being similar between them (Fig.19 E).

On 06th of May *Thrips angusticeps* was the most abundant species (p<0.001). *Melanthrips fuscus* lied in between *Thrips angusticeps* and *Frankliniella occidentalis*. The rest of Thysanoptera species showed significantly lower in numbers (Fig. 19 F).

From 24th May only *Frankliniella occidentalis* showed significantly higher numbers than other Thysanoptera species. *Thrips angusticeps* and *Thrips tabaci* showed similar numbers. *Haplothrips sp.* abundance lied between, while other Thysanoptera species showed all significantly lower numbers (Fig. 19 G) (p<0.001) but with no differences between them.

On 06th June *Frankliniella occidentalis* was the most abundant species followed by *Thrips tabaci* (Fig.19 H) (p<0.001).

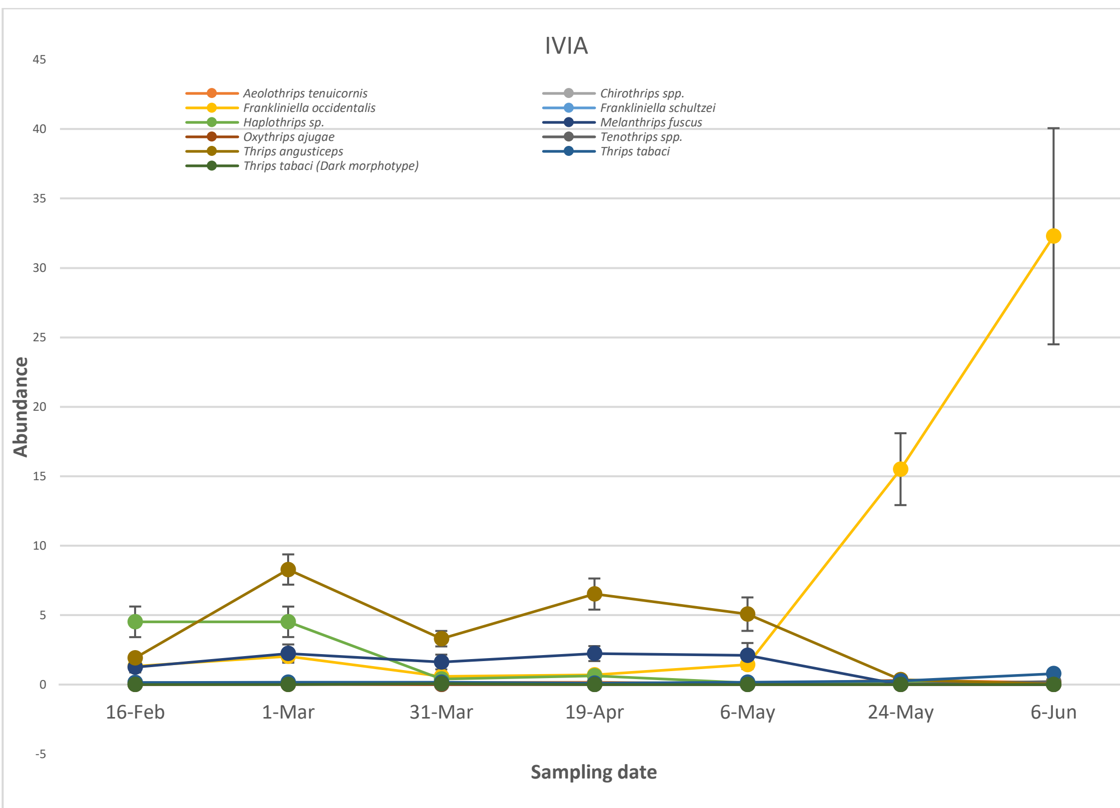


Fig. 19 (A) The seasonal changes in the Thysanoptera species abundance and diversity with mean ± SE.

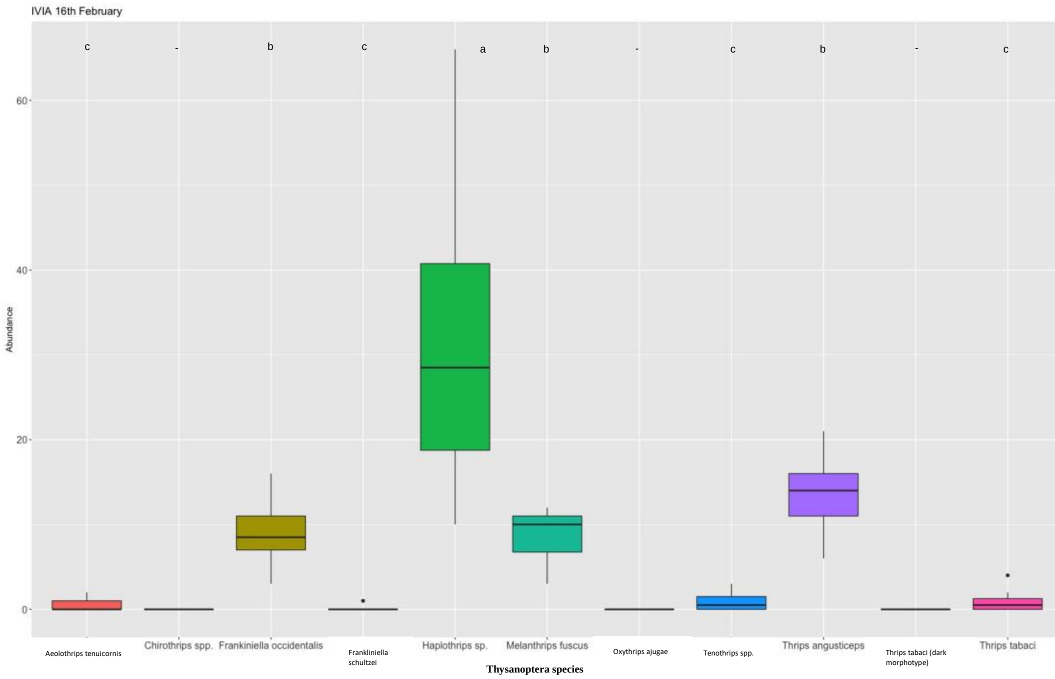


Fig.19 (B) Thysanoptera species abundance from ground cover crops on 16th February 2022 ($p<0.001$).

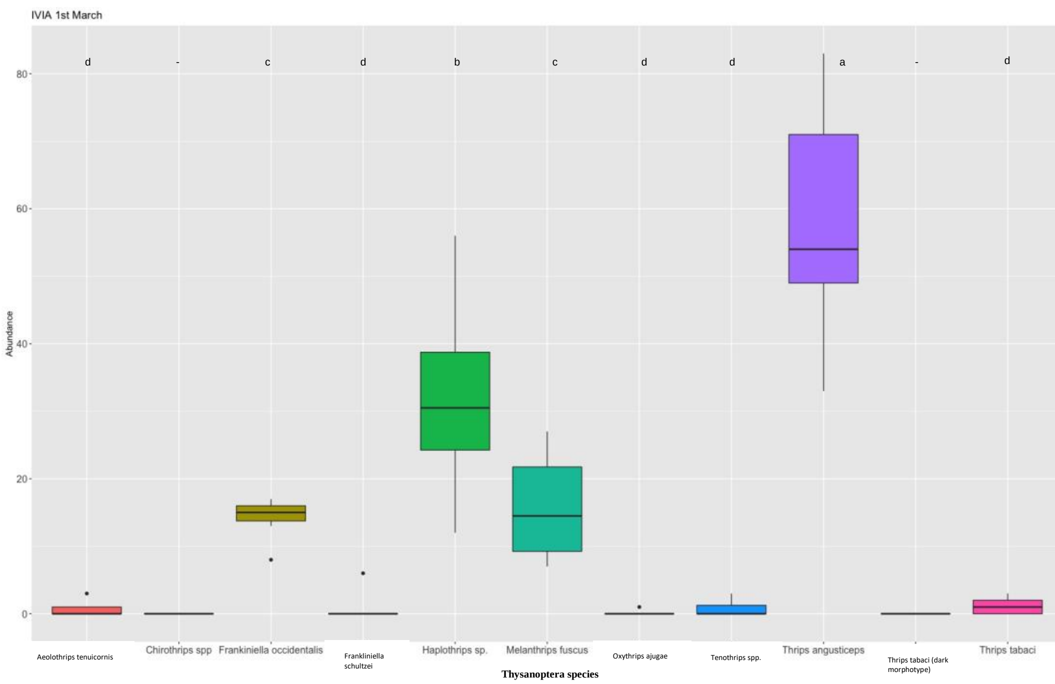


Fig. 19 (C) Thysanoptera species abundance from ground cover crops on 1stth March 2022($p<0.001$).

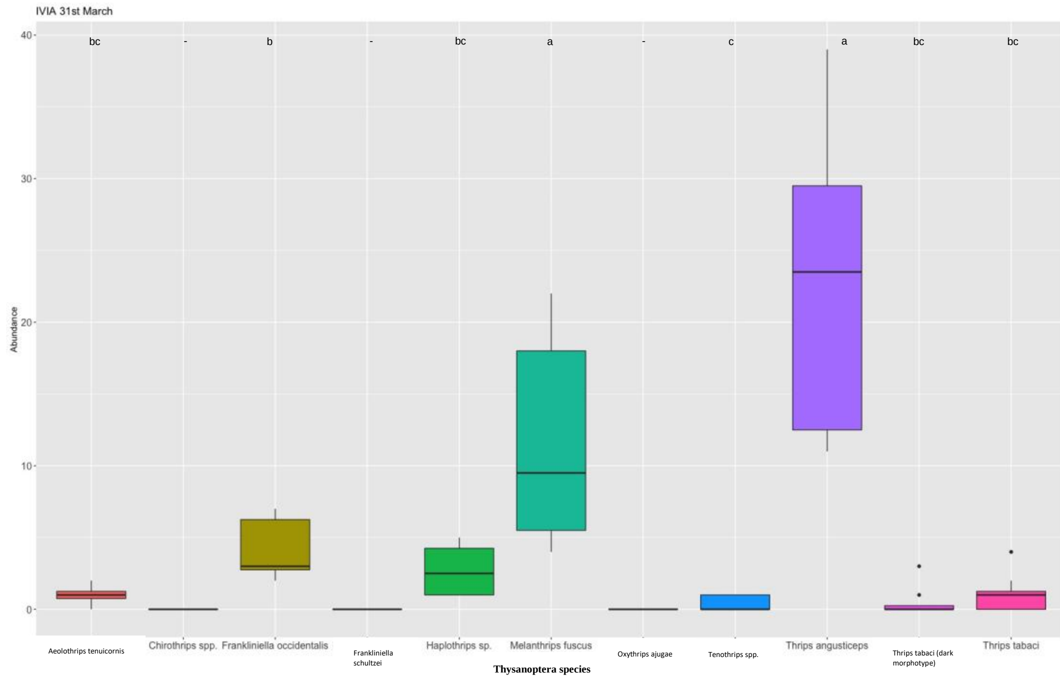


Fig. 19 (D) Thysanoptera species abundance from ground cover crops on 31st[th] March 2022 ($p<0.001$).

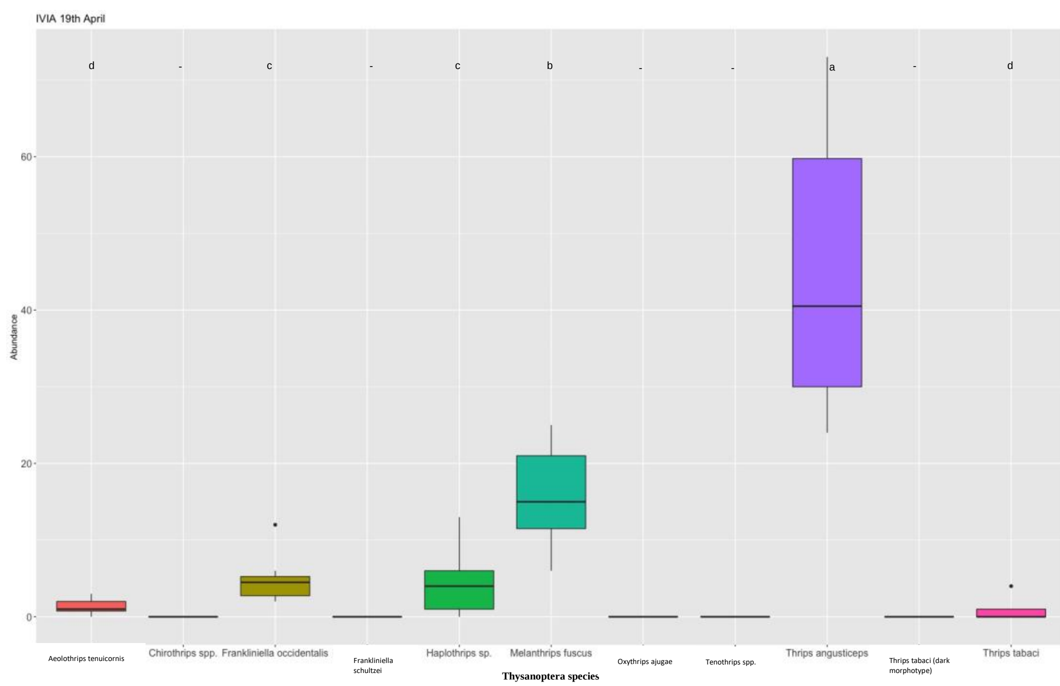


Fig. 19 (E) Thysanoptera species abundance from ground cover crops on 19[th] April 2022 ($p<0.001$).

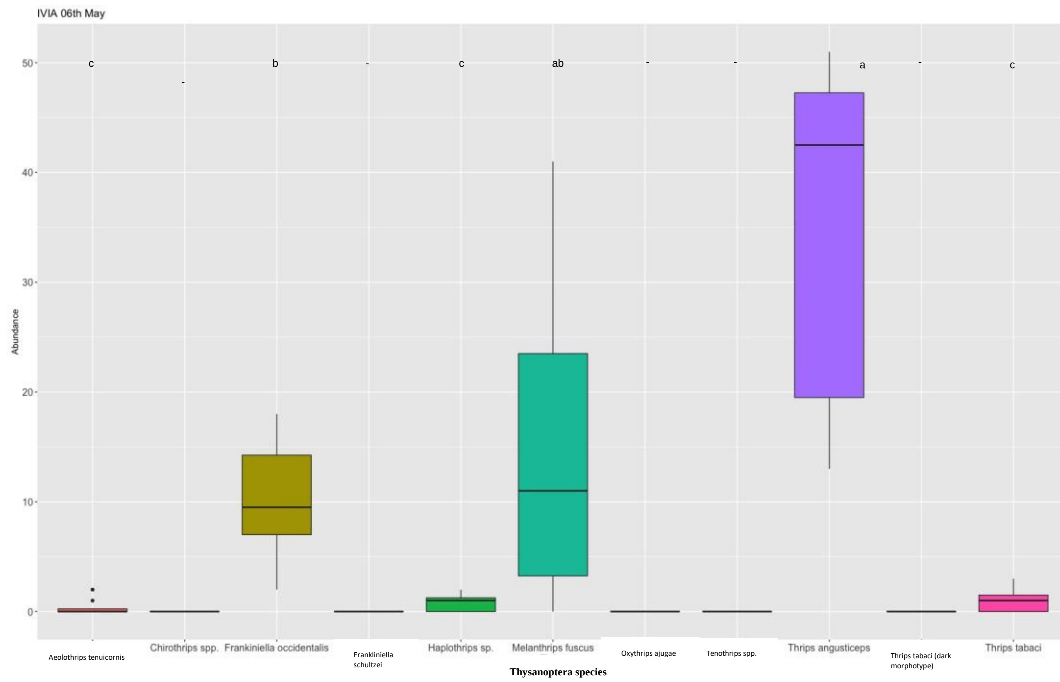


Fig. 19 (F) Thysanoptera species abundance from ground cover crops on 06th May 2022 ($p<0.001$).

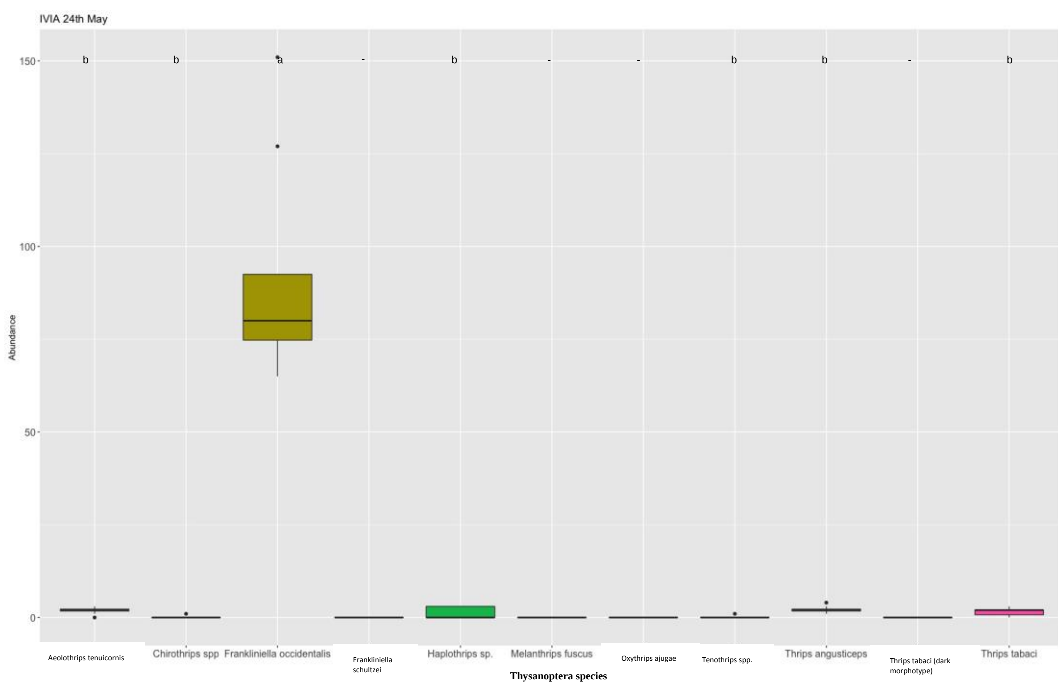


Fig. 19 (G) Thysanoptera species abundance from ground cover crops on 24th May 2022 ($p<0.001$).

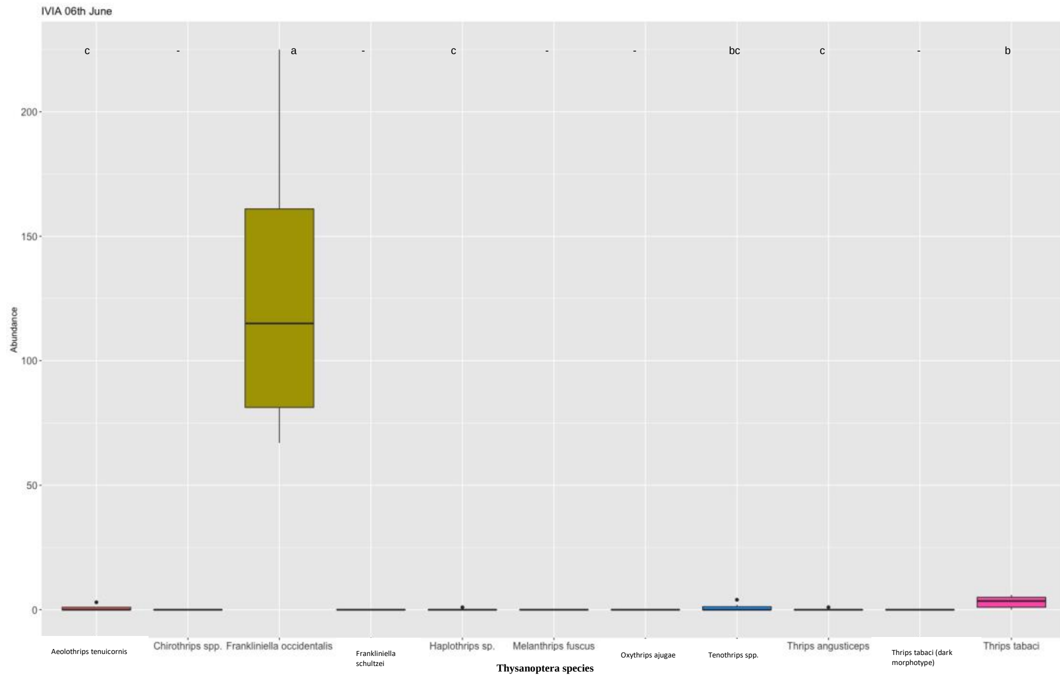


Fig.19 (H) Thysanoptera species abundance from ground cover crops on 06th June 2022 ($p<0.001$).

b.Pego

Melanthrips fuscus declined in numbers after May. *Thrips angusticeps, Haplothrips sp.,* and *Aeolothrips tenuicornis* seems to be decreasing from the April sampling. *Thrips tabaci* and *Frankliniella occidentalis* increased its number from May sampling. Other Thysanoptera species had lower numbers during the whole season (Fig.20 A).

Samples from 10th March (Fig.20 B) and 8th April (Fig. 20 C) showed *Melanthrips fuscus* as the most abundant species with significantly higher numbers, followed by *Thrips angusticeps* and *Frankliniella occidentalis*. On the contrary, 18th May data revealed that the most abundant species *Thrips angusticeps*, followed by *Haplothrips sp*. while *Chirothrips spp.,* had significantly lower abundance (Fig.20 D). From 15th June *Thrips tabaci* was the most abundant species with significantly higher numbers than *Frankliniella occidentalis*. The remaining Thysanoptera species were all significantly less abundant (Fig. 20 E) ($p<0.001$) but without differences between them.

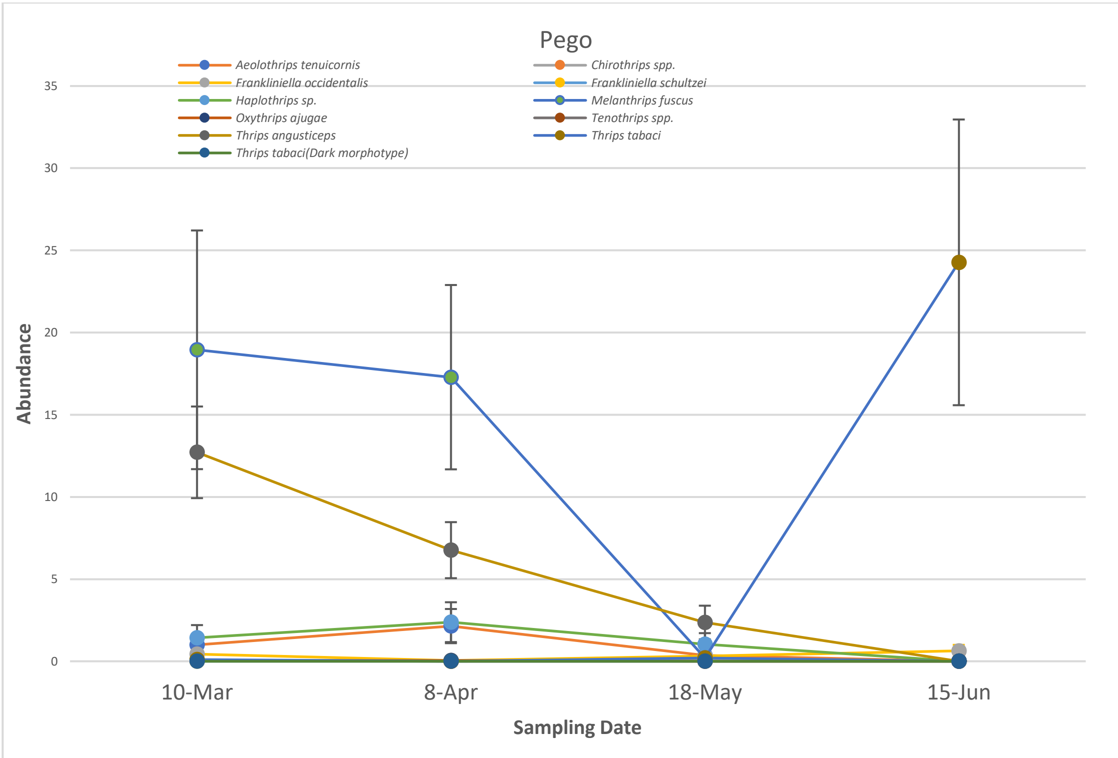


Fig. 20 (A) Seasonal changes in the Thysanoptera species abundance and diversity in Pego (Mean ± SE).

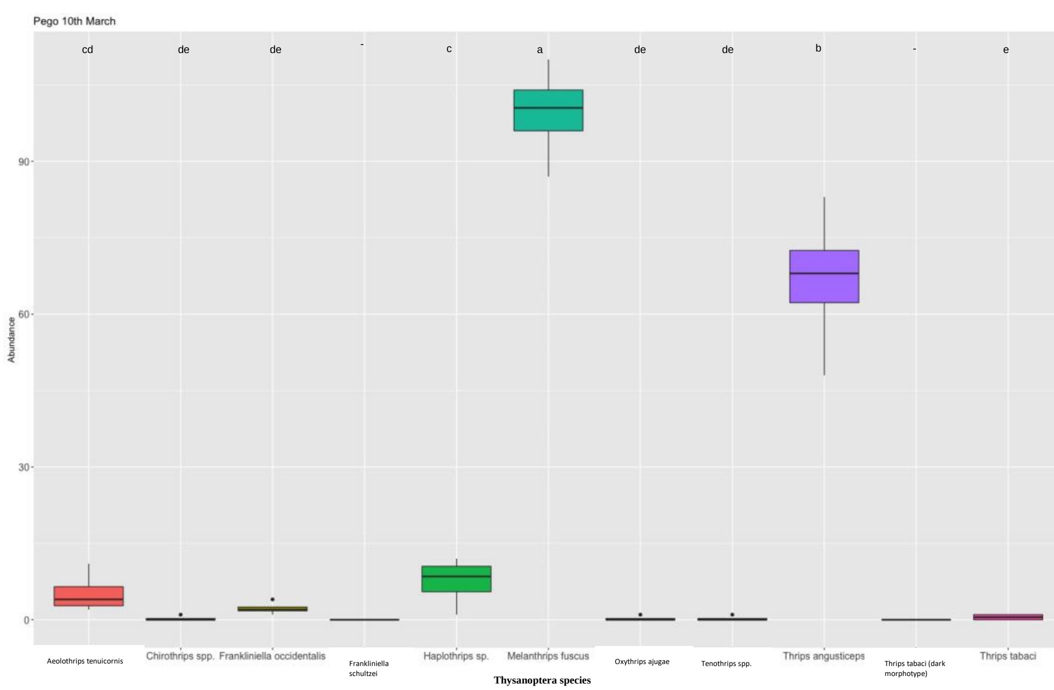


Fig. 20 (B) Thysanoptera species abundance from ground cover crops on 10th March 2022 ($p<0.001$).

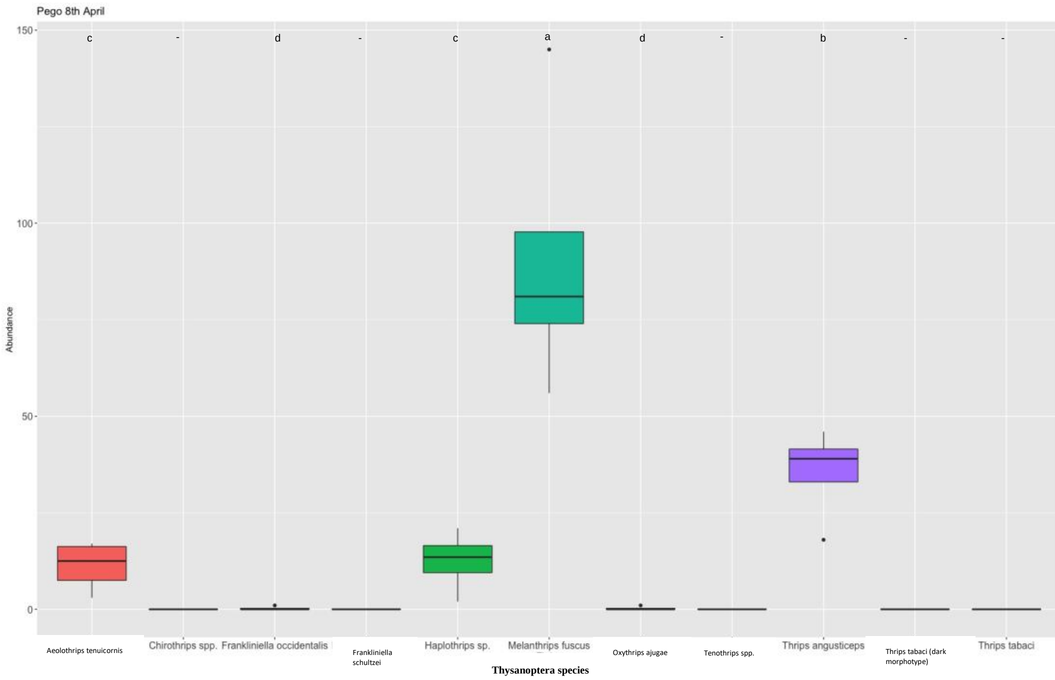


Fig. 20 (C) Thysanoptera species abundance from ground cover crops on 8th April 2022 ($p<0.001$).

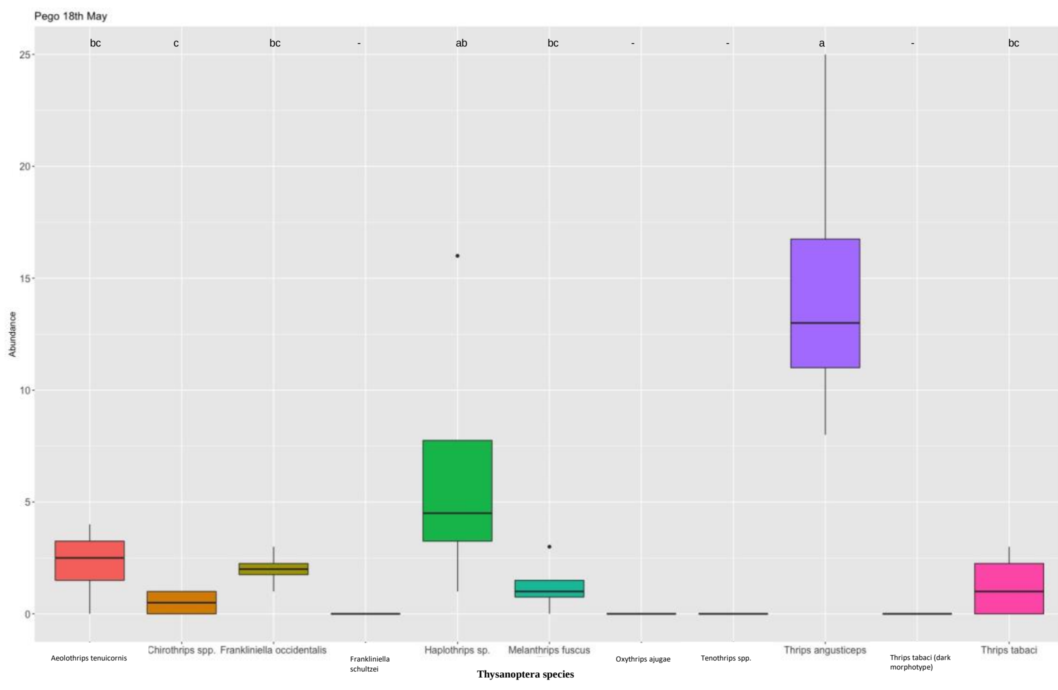


Fig. 20 (D) Thysanoptera species abundance from ground cover crops on 18th May 2022 ($p<0.001$).

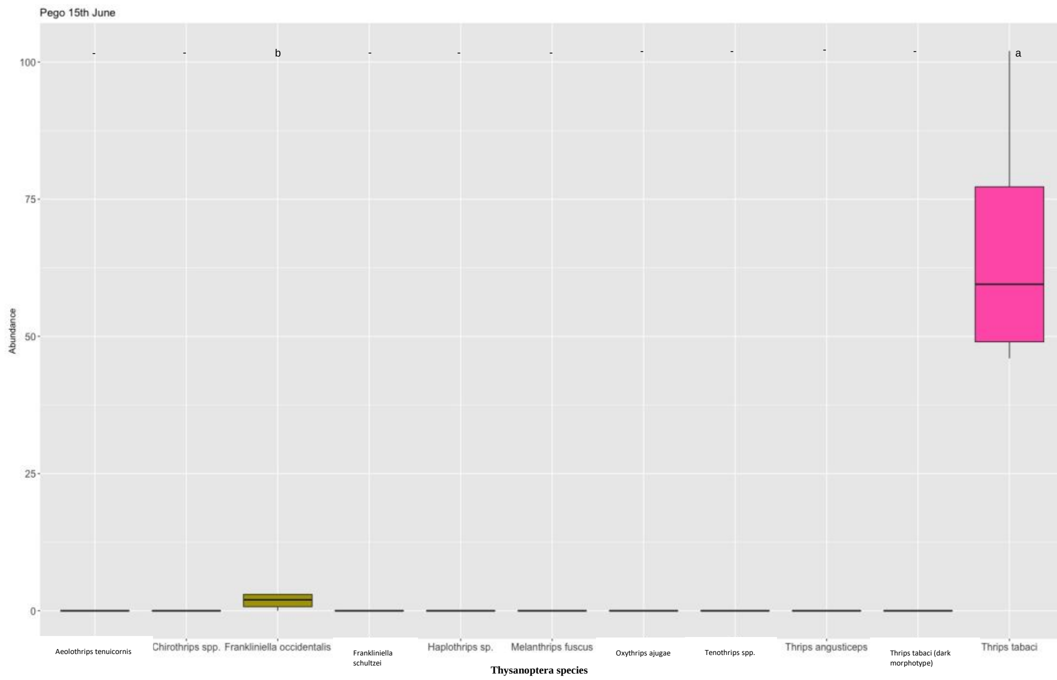


Fig. 20 (E) Thysanoptera species abundance from ground cover crops on 15th June 2022 ($p<0.001$).

Discussions

The ground cover crops species sampled in this study (*Achillea millefolium, Ammi majus, Calendula arvensis, Calendula officinalis, Convolvulus arvensis, Coriandrum sativum, Diplotaxis erucoides, Lobularia maritima, Rumex acetosa, Sonchus asper* and *Sonchus tenerrimus*) were all native to Meditaerrean region. As Conversation Biological Control is based on use of native ground cover crops species to increase the abundance of predators or parasitoids by providing nectars, pollen, shelter and water, these plants were preferred. Plant height of less than 1m not competing with citrus for light, self seeding for self reproduction (production of new plants without sowing again), provision to shelter beneficial insects in winter season low water competition, high erosion control potential and adaptivity to the local climate (Fiedler et al. 2008; Frank et al. 2008). Only the flower parts were sampled as flowers are the source of nectar and pollen.

The winter ground cover crop species (*Calendula officinalis, Calendula arvensis, Diplotaxis erucoides, Sonchus asper* and *Sonchus tenerrimus*) seems to host a wide variety of Thysanoptera species which could prove to be an effective alternative food resource for predators or parasitoids. Majority were *Frankliniella occidentalis*, *Haplothrips sp.*, *Melanthrips fuscus* and *Thrips angusticeps* which are not considered as major pests in citrus. The summer ground cover crops (*Ammi majus, Achillea millefolium*, *Convolvulus arvensis* and *Coriandrum sativum*) seems to host *Thrips tabaci* and *Frankliniella occidentalis*, which neither are deem as major citrus pests. Diversity of Thysanoptera species in ground cover crops may help in future if a new Thysanoptera species predator is found and also because some of the species could serve as an alternate food for natural enemies. In the ground cover crops from both the sites we found that *Frankliniella occidentalis* had the highest abundance followed by *Thrips angusticeps, Melanthrips fuscus, Haplothrips sp.*, *Thrips tabaci* and *Aeolothrips tenuicornis.* Navarro et al., (2008) recorded that from the Thysanoptera species identified, *Frankliniella occidentalis* was highest followed by *Thrips tabaci* and *Melanthrips fuscus* in the citrus orchards, from the Valencian Community, thereby showing the similarity in the Thysanoptera species abundance which are identified in this experiment.

Highest number of *Melanthrips fuscus* was identified from *Diplotaxis erucoides,* which Lacasa et al., (1996) also mentioned that *Melanthrips fuscus* was abundant in *Diplotaxis* *erucoides*. Thereby showing the preference of *Melanthrips fuscus* on the *Diplotaxis erucoides.*

Seasonal changes in the diversity of the Thysanoptera species from *Haplothrips sp., Melanthrips fuscus* and *Thrips angusticeps,* initially in high number during the sampling on February and March but as the spring started these species decreased and slowly by summer these species became very uncommon. On the contrary, new species like *Frankliniella occidentalis* in IVIA and *Thrips tabaci* in Pego showed increasing numbers at the end of the spring and the beginning of summer. These results demonstrate that the changing temperature and the ground cover crops species composition with changing season affects the Thysanoptera species assemblages associated to the ground cover crops.

The decrease in Thysanoptera species in Pego on the sampling of 15th June would be accounted for the cover crop mowing. The sampling was done after the mowing as the ground cover crops were more than 1m tall and to reduce the competition to the citrus plant, mowing was essential.

While sampling in two areas, the difference in the number of Thysanoptera species was visible based on the late flowering and the early flowering ground cover crops. Higher numbers of Thysanoptera species were noticed on the new flowering plant. This can lead to study how plant phenology effect of ground cover crops species affects on the abundance and diversity of the Thysanoptera species.

As pollen and nectars from the flower are the key nutrition for parasitoids in the Conversational Biological Control, some parasitoids and hyperparasitoids were found during the sampling. These parasitoids could be parasitoid of Thysanoptera species, as one parasitoid was found where there was abundance of *Thrips species*, shifting from one ground cover crop *Diplotaxis erucoides* to *Calendula officinalis* based on the seasons. Beltrà Ivars and Soto Sánchez., (2011) recorded the presence of *Thrips* parasitoid *Thripobius semiluteus* Bouček (Hymenoptera: Eulophidae*)* in Spain. This opens to the future possibility on identification and study on the different parasitoids and hyperparasitoids found on the flowers of these ground cover crops, which could be a parasitoid of Thysanoptera species. The hyperparasitoids found in the sample may also help to determine prey parasitoid hyperparasitoid relationships in relation to ground cover crops.

The effect of the rainy season on the Thysanoptera species abundance led to the decrease in the number of Thysanoptera species collected. The decrease in the number of Thysanoptera species collected on 31st March 2022 from IVIA can be mainly due to the continuous rain for 3 weeks after the last sampling i.e., on 1st of March 2022. This year in March it was recorded as one of the highest amounts of rainfall in Valencia which was about 195.3 l/m^2(Source; AEMET). The rainy and cloudy weather seems to affect the abundance of the Thysanoptera species in the ground cover crops maybe due to the decrease in temperature, and the non-opening of flowers to receive the sunlight.

The Thysanoptera species found in these ground cover crops were mainly pest of the flowers or vegetables and not of the citrus. Thereby it suggests avoiding planting these ground cover crops in the fields where vegetables and flowers are grown. For instance, *Frankliniella occidentalis* and *Thrips tabaci* which were increasing by the start of the summer are highly polyphagous pest of vegetables and flowers. Aguilar_Fenollosa and Jacas (2013) also found abundant *F.occidentalis* and *T.tabaci* during the period of citrus blooming and Petal falling (May and June). Planting these ground cover crops would enhance the damage in vegetables and flower crops.

As only two sites were used in this research. More sites in different locations where there are citrus orchards would help to test the variations in the Thysanoptera species in the ground cover crops. This would also help validate the results found in this experiment.

Conclusion

Thysanoptera species are known to cause damage in wide range of plants. In Spanish citrus orchards the pest from Thysanoptera species are known to be *Pezothrips kellyanus* Bagnall, *Chaetanaphothrips orchidii* Moulton and *Scirtotrips auranti* Faure. The damage is mainly caused in the citrus fruits which reduces the quality and the market price of the fruit. The biological control of this Thysanoptera species would help in reducing the losses. Conversation Biological Control is one of the three biological control methods which can be adapted to suppress these citrus pests.

In this experiment, the flowers of ground cover crops were sampled to find the diversity of the Thysanoptera species and the presence of any main citrus pest. The plant species showed no presence of any of the three citrus pest thrips. The sampling from February till June 2022 revealed that the ground cover crops sampled did not host *Chaetanaphothrips orchidii*. Non detection of *Pezothrips kellyanus* and *Scirtothrips aurantii* in Spanish citrus orchard is a further prove that these ground cover crops are non-host of this citrus pests. Thus, this ground cover crops are suitable to be grown in citrus orchards.

Different ground cover crop species showed different diversity of Thysanoptera species, the different was in line with the changing seasons. The winter ground cover crops showed more diversity of Thysanoptera species; therefore, these ground cover crops may serve as alternative food for the predators during the offseason time. The winter ground cover crops showed more of *Melanthrips fuscus* and *Thrips angusticeps* while the early summer showed an increase in *Frankliniella occidentalis* and *Thrips tabaci.* The diversity of Thysanoptera species between IVIA, Moncada and Pego, Alicante was found to be similar. There by suggesting these ground cover crops to be applicable in different locations.

This experiment opens on the future research on possibility of the Thysanoptera species related parasitoid and predators, identification and on their preference among the different ground cover crops. The effect of the time of flowering of the ground cover crops to attract Thysanoptera species is also another future research possibility.

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Annex 1 *Thysanoptera species.*

1. *Aeolothrips tenuicornis*

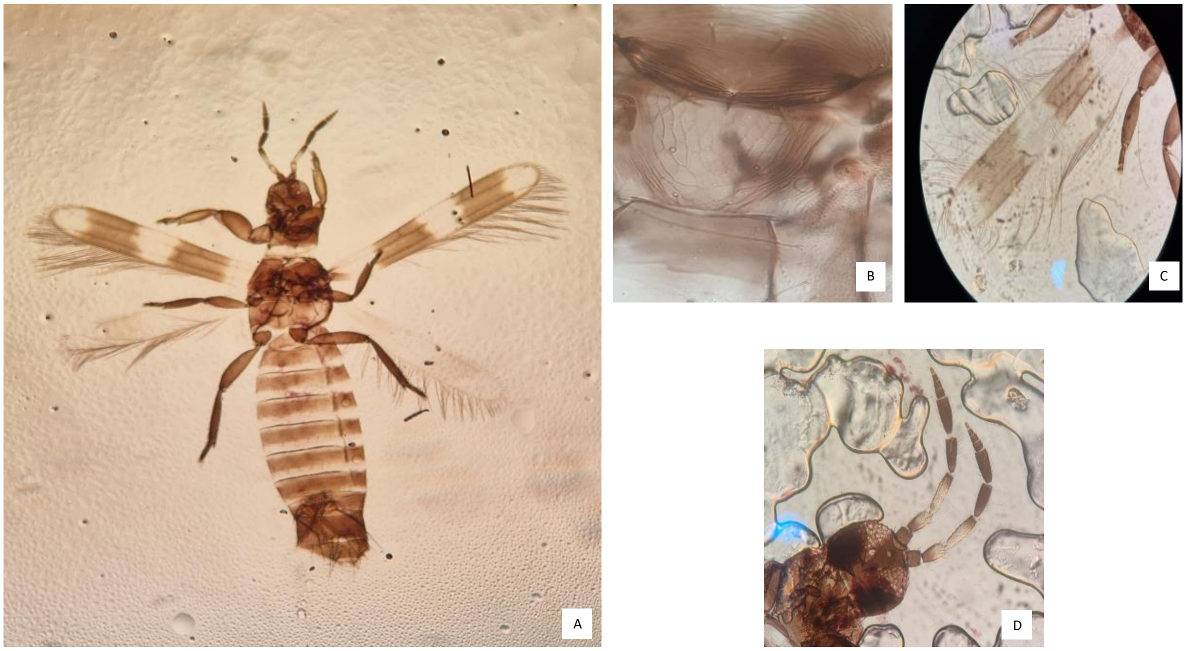

Fig. 1 (A) The adult *Aeolothrips tenuicornis.* (B)Metanotum. (C) Forewing. (D) Antennae

2. *Chirothrips spp.*

Fig. 2 (A) Adult *Chirothrips spp.* (B) Metanotum. (C) Forewing. (D) Antennae.

3. Frankliniella occidentalis

Fig. 3 (A) Adult *Frankliniella occidentalis.* (B)Metanotum. (C) Forewing. (D) Antennae.

4. Frankliniella schultzei

Fig. 4 (A) Adult *Frankliniella schultzei*. (B) Metanotum. (C) Antennae.

5. *Haplothrips sp.*

Fig. 5 (A) Adult *Haplothrips sp*. (B)Metanotum. (C) Wings. (D)Ovipositor. (E)Pronotum.

6. *Melanthrips fuscus*

Fig. 6 (A) Adult *Melanthrips fuscus.* (B) Metanotum. (C)Antennae.

7. *Oxythrips ajugae*

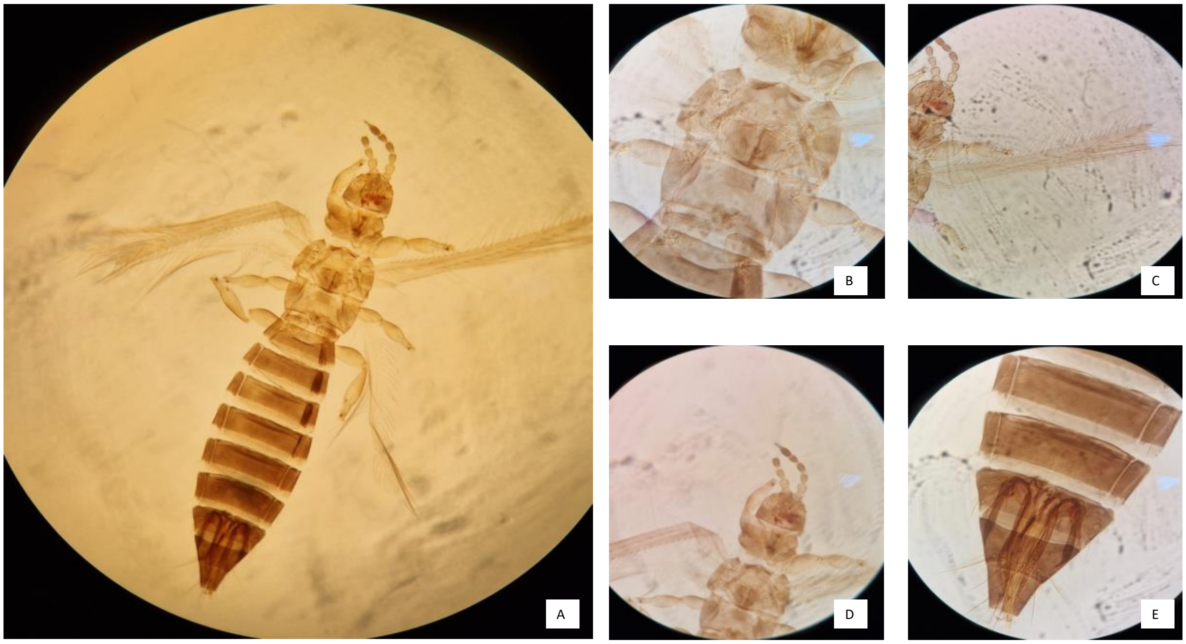

Fig. 7 (A) Adult *Oxythrips ajugae*. (B)Metanotum. (C)Forewing. (D)Antennae. (E)Tergite VIII.

8. *Tenothrips spp.*

Fig. 8 (A) Adult *Tenothrips spp.*(B)Metanotum. (C)Forewing. (D) Antennae. (E)Tergite VIII.

9. *Thrips angusticeps*

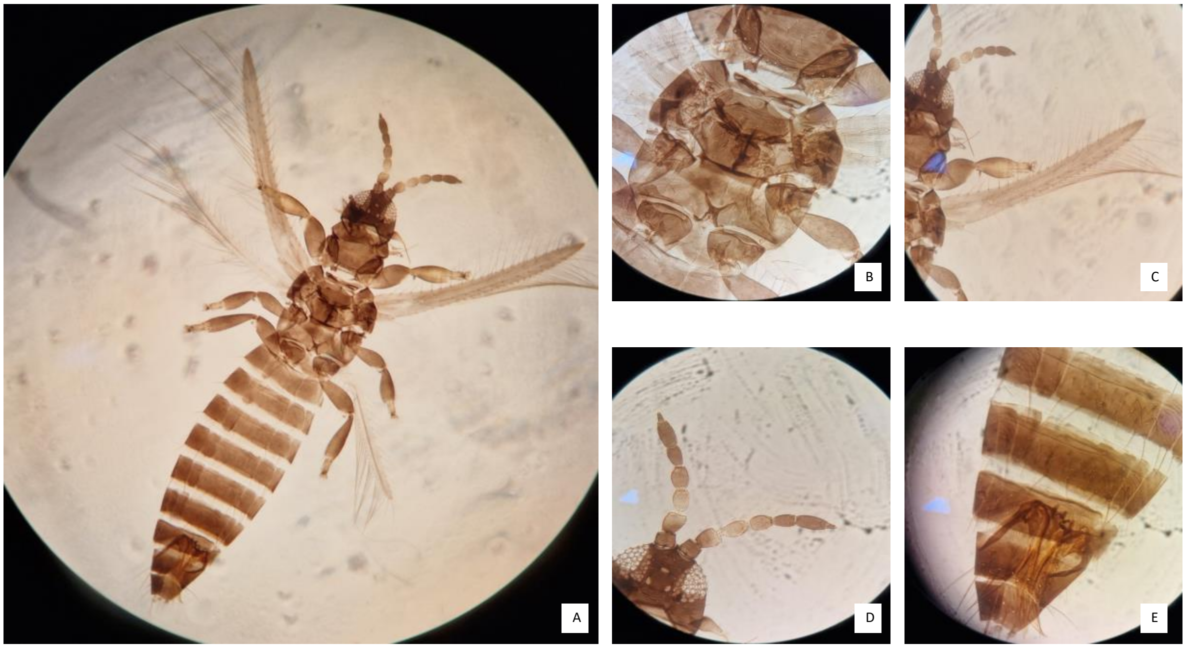

Fig. 9 (A) Adult *Thrips angusticeps.* (B) Metanotum. (C)Forewing. (D)Antennae. (E)Tergite VIII.

10. *Thrips tabaci* (Dark morphotype)

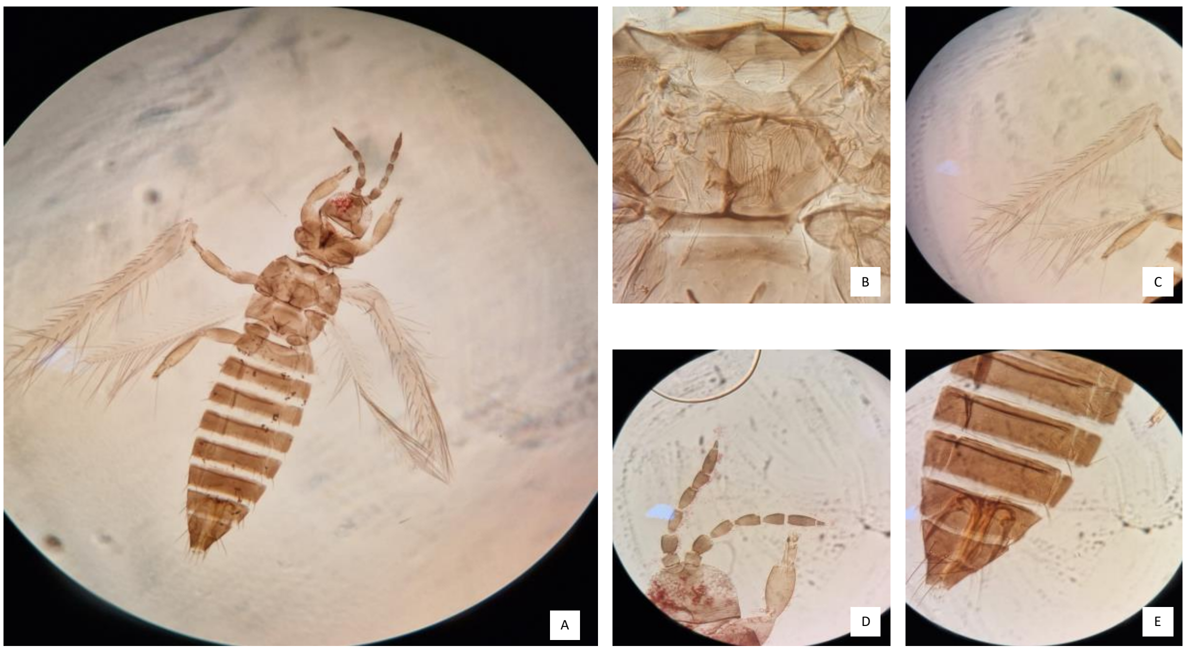

Fig.10 (A) Adult *Thrips tabaci* (Dark morphotype). (B) Metanotum. (C)Forewing. (D)Antennae. (E)Tergite VIII

11. Thrips tabaci

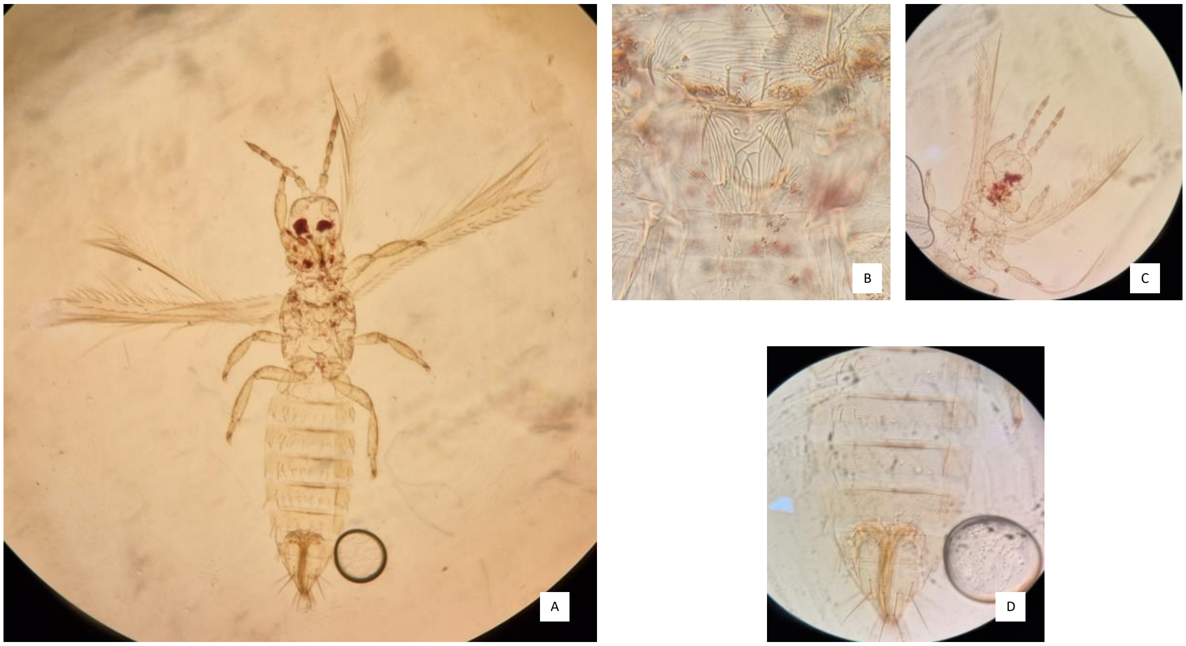

Fig.11 (A) Adult *Thrips tabaci.* (B)Metanotum. (C)Forewings and antennae. (D) Tergite VIII.

Annex 2 *Ground cover crops*

1. *Achillea millefolium*

Fig.1 (A) *Achillea millefolium* plant. (B) Seed package label. (C) Seeds.

2. *Ammi majus*

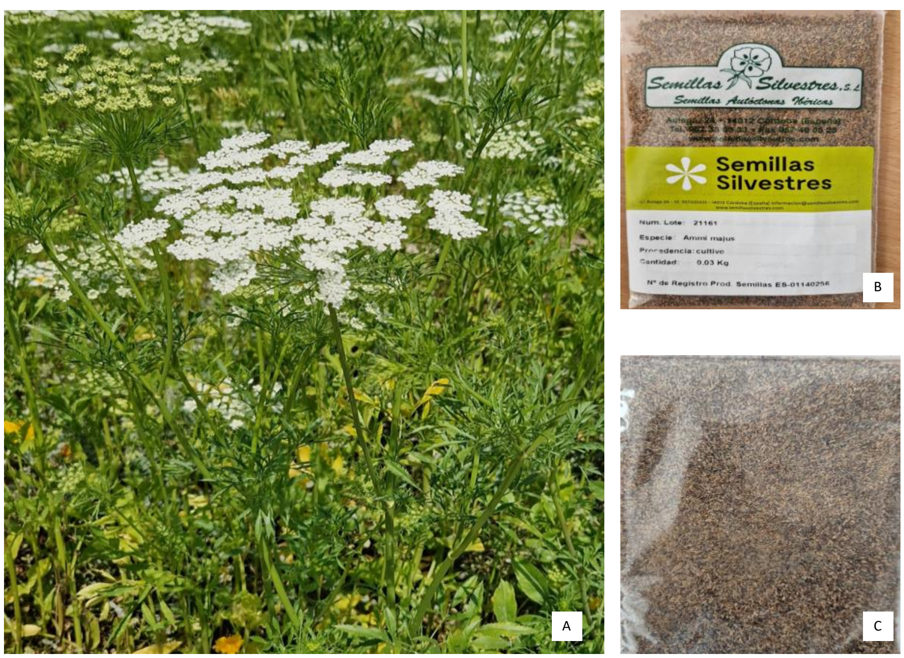

Fig. 2 (A) *Ammi majus* flower. (B) Seed package label. (C) Seeds.

3. *Calendula arvensis*

A (Image Source : Wikipedia)

Fig. 3 (A) *Calendula arvensis* flower. (B)Seed package label. (C) Seeds.

4. *Calendula officinalis*

Fig. 4 (A) *Calendula officinalis* flowers. (B) Seed package label. (C) Seeds.

5. Convolvulus arvensis.

Fig. 5 *Convolvulus arvensis* flowers.

6. Coriandrum sativum

Fig. 6 (A) *Coriandrum sativum* plant. (B) Seed package label. (C) Seeds.

7. *Diplotaxis erucoides*

Fig. 7 (A) *Diplotaxis erucoides* flowers. (B) Seed package label. (C) Seeds.

8. *Lobularia maritima*

Fig. 8 (A) *Lobularia maritima* flowers. (B)Seed package label. (C) Seeds.

9. Rumex acetosa

A(Image source : iNaturalist.org)

Fig. 9 *Rumex acetosa* flower.

10. Sonchus asper

Fig. 10 *Sonchus asper* flower.

11. Sonchus tenerrimus

Fig.11 *Sonchus tenerrimus* flowers.