Hellenic Plant Protection Journal

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Hellenic Plant Protection Journal

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REVIEW ARTICLE

Worldwide potential insect vectors of *Xylella fastidiosa* and assessment of their importance with a focus on Morocco

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Abstract   In Morocco, the climate conditions are favorable for the establishment and the spread of *Xylella fastidiosa (X.f)*. However, the successful establishment of the bacterium depends on many factors; mainly: bacterial subspecies and sequence type, host plants susceptibility, feeding behavior and transmission efficiency of the insect vectors. Knowledge of the relationship between the bacterium–affected crop agro-ecosystem and potential insect vectors is of crucial importance. In this work, we list the tritrophic interaction *X.f*-host plants-insect vectors that occur worldwide in order to apply it to the current situation in Morocco and for risk analysis on the bacterium in the country. Two most relevant *X.f* subspecies of the bacterium (in terms of impact on crops) were considered, namely, subsp. *fastidiosa* and subsp. *pauca*. Based on the international literature and public databases, the majority of the *X.f*-insect vectors are comprised in two families: Cicadellidae and Aphrophoridae. Among all cicadellid species recorded, a high number had the capacity to transmit *X.f* to hosts in America while this ability is null for other regions (except *Graphocephala versuta* Say (Hemiptera: Cicadellidae) recorded in Algeria). In Morocco, none of the cicadellid genera reported worldwide as vectors of *X.f,* have been so far reported, whereas many species of spittlebugs and leafhoppers are present. *Philaenus tesselatus* Melichar (Hemiptera: Aphrophoridae) is highly abundant in Morocco and could play a role as potential vector in case the bacterium is introduced in the country. With regard to the *X.f* hosts, citrus, olive, almond and grapevine, forest agroecosystems and oleander are considered the main susceptible species present in Morocco.

Additional keywords: *Graphocephala versuta, Philaneus tesselatus*, spittlebugs, subspecies *fastidiosa*, subspecies *pauca*, *Xylella fastidiosa*, *X.f* potential vectors

Introduction

Agriculture plays a key role in socio-economics and a significant role in improving the food security situation in Morocco. In the country, approximately the half area (43%) of arable land is devoted to cereals, which are located in rained zones and grown in rotation with other annual crops (e.g. beans, chickpeas, and lentils). However, olive, citrus, almond, and grapevine are among Morocco's leading agricultural products in terms of size of area and/or value. Other major agricultural crops in the country are mainly, pome and stone fruits, open field vegetables (e.g. tomatoes, potatoes, etc.) and other industrial crops, mainly in commercial greenhouse (e.g. cucumbers, peppers, blueberries, flowers, etc.). These crops are mainly located in the irrigated perimeters. All crops are located, mostly in the Center, western and northern parts of the country. However, many parameters that affect the annual production are needed to be

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taken into account in the near future, mainly technology development, adoption level and climatic conditions often characterized by their variation. In addition to water scarcity and climate change concerns, the disease threat from *Xylella fastidiosa* (*X.f*) (Xanthomonadaceae, Gamma proteobacteria) is maybe one of the most relevant concerns for crops that growers may face in the country.

Xylella fastidiosa is a xylem-restricted pathogenic, gram-negative bacterium, initially restricted to the American continent (Hopkins and Purcell, 2002). The main reported diseases for the bacterium include Olive quick decline syndrome (OQDS), Citrus variegated chlorosis (CVC), Pierce's disease (PD) in grapevine, Almond leaf scorch disease (ALSD) and Oleander leaf scorch disease (OLSD). *Xylella fastidiosa* is divided into three main groups of subspecies with allopatric distribution: *X.f* subsp. *fastidiosa* (*X.f.f*), *X.f* subsp. *multiplex* (*X.f.m*) and *X.f* subsp. *pauca* (*X.f.p*) (Nunney *et al.,* 2012, 2019; Coletta-Filho *et al.,* 2020). This bacterium was firstly identified as *X.f.* associated with PD with high impact and devastating consequences on the southern California grape industry (Chang *et al.,* 1993; Galvez *et al.,* 2010; Sanscartier *et al.,* 2012). *Xylella fastidiosa* subsp. *pauca* is also associated with different crops, causing several diseases: CVC is the most devastating disease, showing a high incidence on the citrus industry in Sao Paulo, and affecting almost 100 million of sweet orange trees (Coletta-Filho *et al.,* 2020). In *Medicago sativa* plants, *X.f* infection induces hypersensitive-response-like symptoms (Abou Kubaa *et al.,* 2019). In 2013, this bacterium was first reported in Europe and well characterized as *X.f.p* associated with leaf scorching with severe dieback and desiccation in olive causing OQDS in Apulia region (south of Italy) (Saponari *et al.,* 2013, 2017). A recent study demonstrated the physiological response to *X.f* infections by measurements of stomatal conductance and stem water potential in the infected olive trees (Surano *et al.,* 2022).

Xylella fastidiosa has a wide range of host plants infecting about 655 plant species (EFSA, 2022). Estimations in EU indicate that *X.f* has the potential of causing a 5.5 billion Euros of annual production loss once it is full spread across the entire EU. This would threaten more than 300.000 jobs across EU currently involved in that production. The economic threat of *X.f* is not limited to producers but should be contextualized as a societal problem (Schneider *et al.,* 2021).

In an open field crop or natural agro-ecosystems, the natural spread of *X.f* occurs in two main ways: (i) long-range transport of the infected plant (or part of the infected plant) and (ii) local and regional spread which can occur by an insect vector. For local spread, *X.f* is transmitted to host plants by specialized insect vectors belonging to order Hemiptera, suborder Auchenorrhyncha, more specifically spittlebugs (Aphrophoridae), sharpshooter leafhoppers (Cicadellidae, subfamily Cicadellinae), treehoppers (Membracidae) and Cicadas (Cicadidae and Tibicinidae), which feed on xylem sap (Janse and Obradovic 2010). Natural transmission of *X.f* by insects is considered the main factor in local dissemination and appears to be the most critical threat for *X.f* establishment and spread in Europe. When this pathogen overwinters in the xylem of the host plant, the long-distance spread is likely related to the movement of infected plant material (Chatterjee *et al.,* 2008; EFSA, 2018). When the bacterium is introduced into a new environment with well-suited abiotic conditions, it requires the presence of efficient vectors to establish and spread (Fereres, 2015).

The favorable factors that may cause a high risk of *X.f*-epidemic disease include: the presence of an efficient vector, favorable climatic conditions and the presence of a suitable host plant. This highlights the risks associated with the unintended introduction of organisms, e.g. subspec*ies* of *X.f*, into new regions. In the case of the Mediterranean basin countries, particularly Morocco, they are at high risk for the arrival and establishment of this bacterium (Frem *et al.,* 2020). Knowledge of potential insect vectors in association with the disease produced in the

host crops is of crucial importance for discovering emergent pathogens. The potential insect vectors of *X.f* around the world have been reviewed, except for North Africa (Cornara *et al.,* 2019; Di Serio *et al.,* 2019). In North America and South America, sharpshooter species are considered the main vectors of *X.f* that cause several serious diseases like PD on grapevine and CVC on citrus. In citrus orchards affected by CVC disease in Argentina, eleven leafhopper and treehopper species were tested positive for *X.f* by molecular assays (Dellap *et al.,* 2016). In Italy, three spittlebugs, *Philaenus spumarius* (Linnaeus), *Philaenus italosignus* Drosopoulos & Remane, and *Neophilaenus campestris* Fallén (Hemiptera: Aphrophoridae) demonstrated the ability to transmit *X.f* in the field (Cavalieri *et al.,* 2019; Panzavolta *et al.,* 2019). However, the two widespread species in Italy and other southern European countries are *P. spumarius* and *N. campestris* (Lopes *et al.,* 2014; Morente *et al.,* 2018; Thanou *et al.,* 2018; Tsagkarakis *et al.,* 2018; Bodino *et al.,* 2019; Theodorou *et al.,* 2021), in which *P. spumarius* alone is the main vector associated with OQDS in olive orchards in the south of Italy (Saponari *et al.,* 2017). Moreover, a recent survey conducted in Basilicata Region, Italy demonstrated that a *P. spumarius*, *N. campestris*, *Lepyronia coleoptrata* Linnaeus (Hemiptera: Aphrophoridae) and *Cicadella viridis* (Linnaeus) (Hemiptera: Cicadellidae) were the potential vectors of *X.f* captured in that region (Trotta *et al.,* 2021). A recent review describes control attempts and current management of *X.f* in olive, including control of the vector *P. spumarius* (Morelli *et al.,* 2021). In addition, subspecies of *X.f* have been well described considering plant diseases and sequence type, but limited data is available on subspecies of *X.f*, hosts and insect vector interrelationships.

In Morocco, *X.f* is considered a quarantine pest and since 2016, there has been a crop-agroecosystems insurance program to help producers in coping with the risk related to the national *X.f* (ONSSA, 2016; Afechtal *et al.,* 2018). Up to 2022, the bacterium has not been detected in Morocco, thus the country remained *X.f*-free (El Handi *et al.,* 2022). However, limited data is available on the relationship between worldwide potential insect vectors and plant heritage in North Africa, particularly in Morocco. The ability to carry out rational control strategies against *X.f* depends mainly on the knowledge and analysis of the risk assessment of worldwide potential insect vectors in the context of Moroccan crop agro-ecosystems. This study aims to give recent data and analyze the threat of the occurrence and interaction between the potential insect vectors with *X.f* around the world, including America, Europe, Asia and North Africa, in comparison with Morocco. This may provide promising prospects on how we could manage the bacterium–affected crop agro-ecosystem threat in Morocco.

Materials and Methods

1. Literature search

Available information on the main potential insect vectors of *X.f* associated with different plant species, including crops worldwide were retrieved upon the consultation of public databases ZENEDO (i.e. https://zenodo.org/record/3775537#.YitG-GXrMKUl) and through a literature search of articles published from 90's to 2022. For this, we used databases of EFSA (https://www.efsa.europa.eu/en/efsajournal/pub/5736), EPPO (www.eppo.org), Scopus (www.scopus.com), Springer (https://link.springer.com) and Google Scholar (https://scholar.google.com).

2. Data analysis

Global species distribution was divided into five main areas: America (North America and South America), Europe, Asia, and North Africa and for comparison Morocco (including the data previously collected in Morocco). The collected data were summarized as a data matrix including the following generated variables:
- Insect species belonging to the Cicadomorpha infraorder were categorized as:

a) main vector: the insect can transmit the bacterium to the plant in the natural conditions and induce disease that results with symptoms on the plant or the tree after infection, in situ, b) competent vector: the insect can transmit the bacterium to the plant and may induce symptoms on the plant or the tree after infection only in semi-field and laboratory conditions, c) potential insect vector: insect may be able to acquire and/or to transmit the bacterium.

- *Phylogeny diagram* of all recorded species groups.
- *Host plants*: the main host plants of vector or potential vector insects; monocotyledonous and dicotyledonous in ground cover and shrub tree crops.
- *Acquisition (Acq)*: number of the potential insect vector individuals that are able to acquire the bacterium from *X.f*-symptomatic plant divided by the total number of all specimens tested. Under laboratory conditions with three levels: *Acq-lab*: the insect acquires the bacterium; *Acq-no lab*: the insect does not acquire the bacterium and *Acq-un lab*: unknown information or no available data on insect acquisition. Under field conditions: *Acq-field*: the insect acquires the bacterium; *Acq-no field*: the insect does not acquire the bacterium; and *Acq-un field*: unknown information or no available data.
- *Transmission (Tra)*: number of the potential insect vector individuals that are able to transmit *X.f* in the plant after acquiring the bacterium divided by the total number of individuals tested. Under laboratory conditions: *Tra-lab*: the insect can transmit the bacterium; *Tra-no lab*: the insect cannot transmit the bacterium; and *Tra-un lab*: unknown information or no available data. Under field conditions:*Tra-field*: the insect can transmit the bacterium; *Tra-no field*: the insect cannot transmit the bacterium and *Tra-un field*: unknown information or no available data.
- *Nature of X.f subspecies transmission*: the subspecies *X.f* transmitted by the potential insect vector.

For some variables (e.g., species categorized, *Acq, Tr*), the number was determined and a percentage was calculated as the number of species reported for the parameter over the total number reported in the region. The data collected from these variables' determination, were used to generate graphs.

Results

In this study, a total of 218 species of Cicadomorpha were recorded as potential insect vectors of *X.f,* worldwide (Table S1). Infraorder, subfamilies, families and genera of these species, ordered phylogenetically are given in Figure 1. The largest numbers of genera were found to belong to four families Cicadellidae (40 genera, 79 species), Aphrophoridae (9 genera, 38 species), Membracidae (16 genera, 27 species), and Tibicinidae (8 genera, 47 species). The number of genera does not exceed two for other families. In Morocco, only 6 *X.f* potential insect vectors were recorded. The highest number (70) of *X.f* potential insect vectors belonging to Cicadellidae was identified in the America compared to Europe (7) and other areas (<5). However, the number of Aphrophoridae species recorded was higher in Europe (29) than in America (9), Asia (6), North Africa (9), and Morocco (6). The numbers of known potential insect vectors of *X.f* with the capacity to acquire and transmit the bacterium are provided in Figures 2 and 3. Among all identified *X.f*-potential insect vectors, only four families contain high numbers of species that have the capacity to acquire and to transmit the bacterium from the infected plant: Cicadellidae, Aphrophoridae, Clastopteridae and Membracidae. In terms of species able to acquire and transmit *X.f*, numbers were higher in America compared to the other areas: for cicadellids (51 and 43, respectively) and for membracids (13 and 2, respectively). These numbers were variables for Aphrophoridae (1 and 5) and for Clastopteridae (4 and 1).

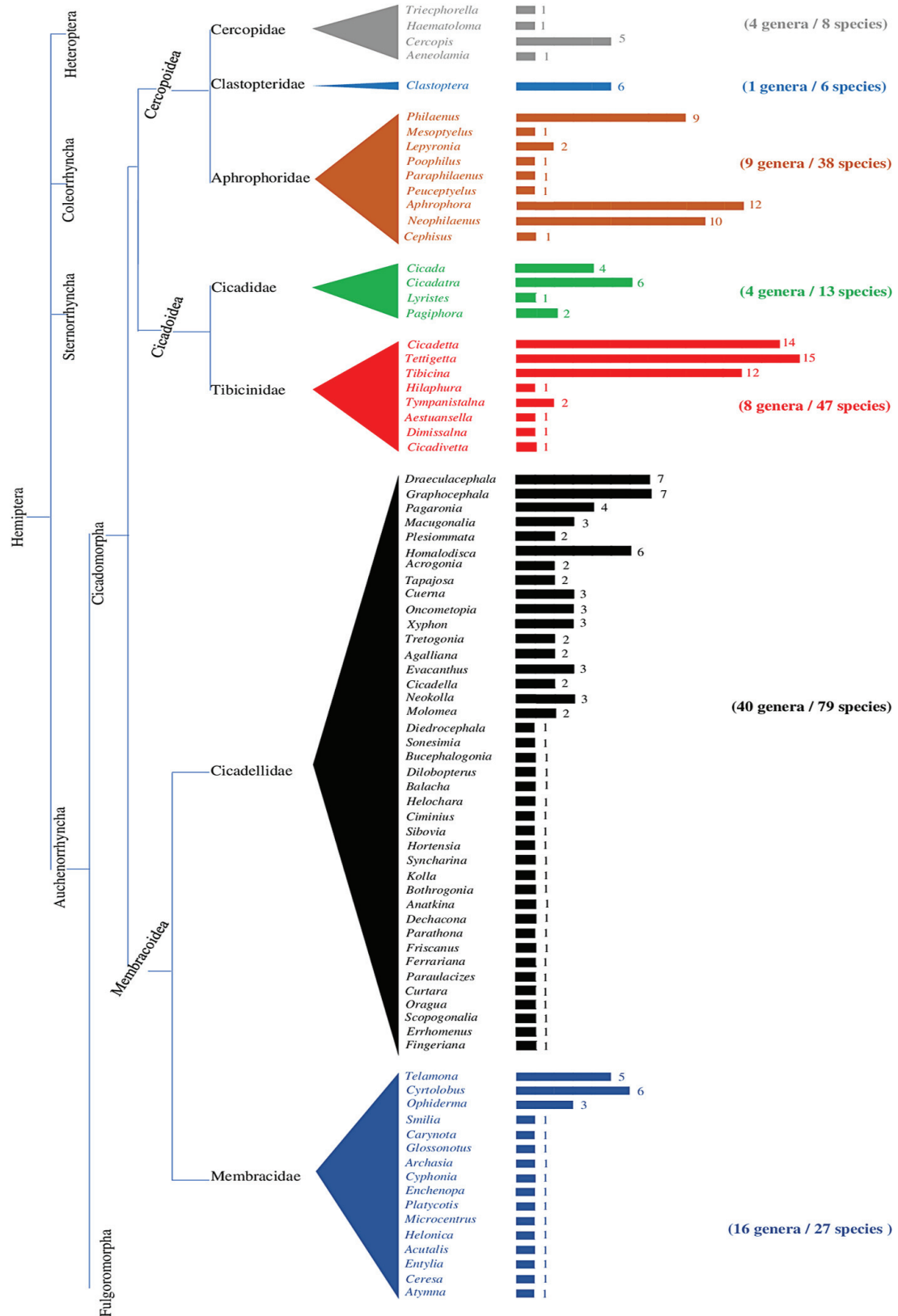Regarding the capacity to acquire *X.f,*

Figure 1. List of worldwide potential insect vectors of *Xylella fastidiosa*: infraorder, subfamilies, families and genera ordered phylogenetically, in America, Europe, Asia, North Africa and for comparison with Morocco. Histogram with number of potential insect vectors of *X.f* for each family.
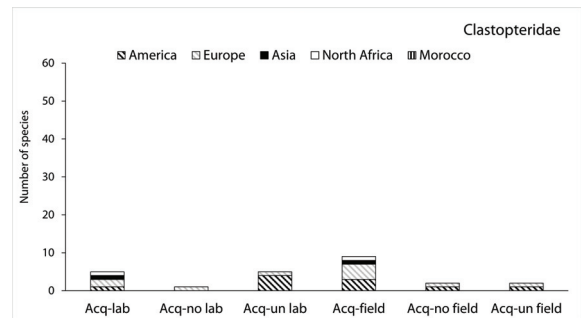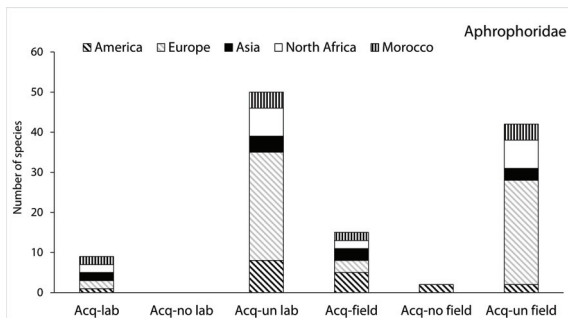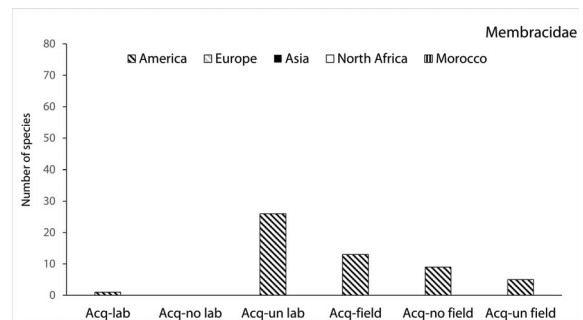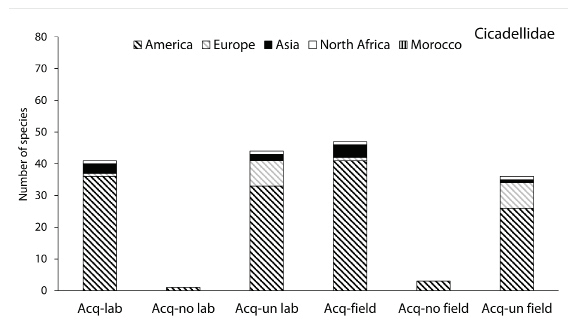
Figure 2a. Worldwide potential insect vectors of *Xylella fastidiosa*: Number of known species that able to acquire bacterium in the field and in the laboratory in America, Europe, Asia and North Africa and for comparison with Morocco. (Acq) Acquisition, (Tra) Transmission; (lab) laboratory, (un) unknown, (no) cannot.

Figure 2b. Worldwide potential insect vectors of *Xylella fastidiosa*: Number of known species that able to acquire bacterium in the field and in the laboratory in America, Europe, Asia and North Africa and for comparison with Morocco. (Acq) Acquisition, (Tra) Transmission; (lab) laboratory, (un) unknown, (no) cannot.

Figure 3a. Worldwide insect vectors of *Xylella fastidiosa*: Number of known species that able to transmit the bacterium in the field and in the laboratory in America, Europe, Asia and North Africa and for comparison with Morocco. (Acq) Acquisition, (Tra) Transmission; (lab) laboratory, (un)unknown, (no) cannot.
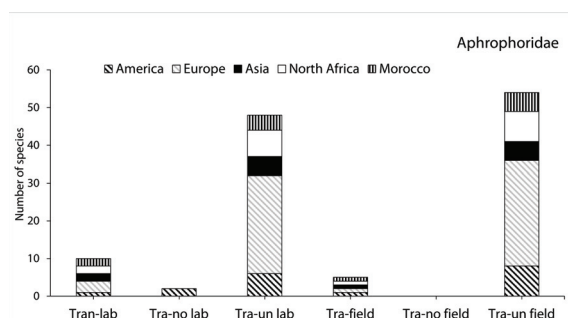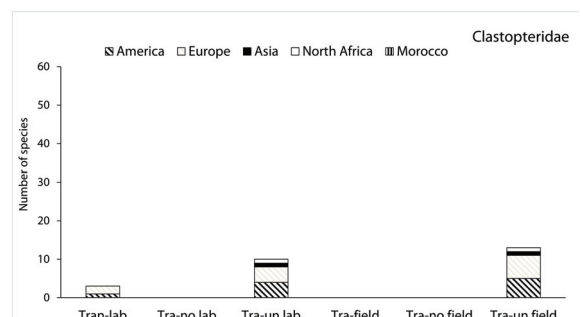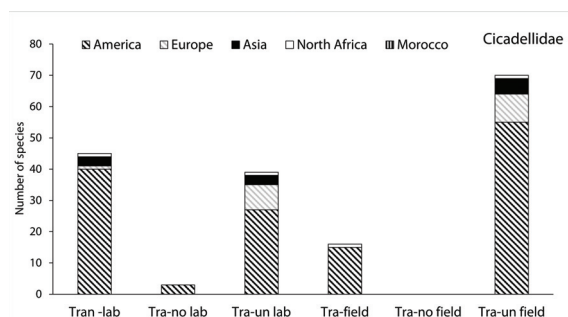
Figure 3b. Worldwide insect vectors of *Xylella fastidiosa*: Number of known species that able to transmit the bacterium in the field and in the laboratory in America, Europe, Asia and North Africa and for comparison with Morocco. (Acq) Acquisition, (Tra) Transmission; (lab) laboratory, (un)unknown, (no) cannot.

the cicadellids species number was higher in America compared to other areas, in the laboratory (36) and in the field (41). This number does not exceed two in laboratory conditions in Asia and one for North Africa. Among all cicadellid species, we identified a high number of species that able to transmit *X.f* to the host in America with 40

and 15 species under laboratory conditions and in the field, respectively. This number was 2 for Asia under laboratory conditions and one for North Africa. For aphrophorid species, the number of species that able to acquire *X.f* was very low in America (5) and Europe (3) either in the laboratory or in the field. This number does not exceed two in other areas. Even in America, among all aphrophorids, the number of species that able to transmit *X.f* ranged from two to five under laboratory conditions. However, only one species has the capacity to transmit this bacterium in the field in each region About 50% of all cicadellid species have no information related to their capacity to acquire and transmit *X.f*. In America, two Membracidae species can acquire *X.f* while only one species transmits the bacterium to crops. Many species with unknown information related to their capacity to acquire and transmit *X.f* were recorded. Few data has been reported on the tritrophic interaction *X.f*-host plant-insect vector, provided in Table S2.

Discussion

Xylella fastidiosa remains a major disease because it threatens natural areas and the horticultural, agricultural, and forestry sectors as a recognized agent of many serious and economically important diseases of crops and landscape plants (Almeida *et al.,* 2014). Morocco is among the Mediterranean countries of high risk for arrival, establishment, and spread of this bacterium (Afechtal *et al.,* 2018; Frem *et al.,* 2020). The most economically known insect-borne diseases (e.g. OQDS) in Europe from Cicadomorpha, particularly *P. spumarius*, are linked to the bacterium *X.f* (Saponari *et al.,* 2017). High numbers of *X.f*-potential insect vectors were identified for only two families; Cicadellidae and Aphrophoridae. For comparison, only six aphrophorids were recorded for Morocco.

Cicadellid species were the main vector of *X.f* in America. Twelve leafhopper species are known to be vectors of CVC in Bra-

zil (Lopes, 1996; Gravena *et al.,*1998; Parra *et al.,* 2003) and nine are the most important sharpshooter vectors of *X.f* to economically important crops in North America (Cornara *et al.,* 2019). Most Cicadellinae species have shown the ability to acquire and transmit *X.f* because of their xylem-fluid feeding habit (Redak *et al.,* 2004). The most important one, *C. viridis*, was reported by current studies in Europe (Bodino *et al.,* 2022; Rodrigues *et al.,* 2023). The last survey carried out in Morocco showed that none of the Cicadellid genera (e.g. *Cicadella, Evacanthus* and *Graphocephala* genera) has been reported as potential vectors of *X.f* were found in Morocco (Haddad *et al.,* 2021). However, it is most important to notice the presence of *G. versuta* during the survey carried out in Algeria between September 2013 and April 2014 (Boulaouad *et al.,* 2022). This ciccadellid is a vector of *X.f* in USA (Janse and obradovic, 2010). Some specialized phloem feeders such as leafhoppers, can come into contact with xylem vessels and become infected (El Beaino *et al.,* 2014), however, we cannot consider them as a potential insect vectors, since no current studies have clarified there transmission of the bacterium. This is the case of the American grapevine leafhopper, *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae), which is a specialist of *Vitis spp.* and a true mixed xylem/phloem-feeder (Chuche *et al.,* 2017).

Few aphrophorid species are able to acquire *X.f*, however, under field conditions; only one aphrophorid species has the capacity to transmit this bacterium. In America, 12,880 insect specimens were collected in pin, red oaks in New Jersey, 1.7% were Aphrophoridae and Clastopteridae, and only four spittlebug species DNA tested positive for *X.f* (Zhang *et al.,* 2011). However, in Europe, *P. spumarius* is the only important insect vector involved in the ongoing *X. fastidiosa* outbreak in olive groves in Italy (Saponari *et al.,* 2017; Cornara *et al.,* 2019). A recent survey of potential insect vectors in Morocco was carried out by Haddad *et al.* (2021) where only two main *X.f* potential insect vectors were recorded, *P. tesselatus* and *N. campestris* (Hemiptera: Aphrophoridae).

According to these authors, *P. spumarius* was not found. They suggest that *P. tesselatus* should be considered as the main potential insect vector of *X.f* in the country. According to Greco *et al.* (2021), in areas where the bacterium is not yet present, a qualitative-quantitative study on the entomofauna is important for the timely identification of potential vectors and for developing effective control strategies.

In America, two Membracidae species can acquire *X.f* but only one species transmits the bacterium to plant. In CVC-affected citrus agroecosystems in Argentina; two Membracidae species *Cyphonia clavigera* (Fabricius) and *Entylia carinata* (Forster) were tested positive for *X.f* (Dellape *et al.*, 2016). However, in transmission trials, only *C. clavigera* successfully transmitted *X.f* to plants. In addition, under field around the pin and red oaks leaf scorch-affected agroecosystem in New Jersey (USA), eleven treehopper species DNA tested, were positive for *X.f* (Zhang *et al.*, 2011).

The Cicadidae are xylem-sap feeders, and so at least in theory they are candidate vectors of *X.f.* However, few studies have been conducted on the ability to transmit the bacterium (Paiao *et al.*, 2002; Krell *et al.*, 2007). Recently, Cornara *et al.* (2020) showed that no successful transmission of *X.f* occurred using *Platypedia minor* Uhler and *Cicada orni* Linnaeus (Hemiptera: Cicadidae), infesting grapevine and olive plants. A study by Mesmin *et al.* (2023) showed similar results in Corsica (France). In Tunisia, four potential *X.f* vectors were identified recently: namely *N. campestris*, *N. lineatus*, *P. tesselatus* and *P. maghresignus* Drosopoulos & Remane (Boukhris-Bouhachem *et al.*, 2022). A new study showed similar results for spittlebugs community in European olive and grapevine (Antonatos *et al.*, 2021; Bodino *et al.*, 2020; 2021a, b; Rodrigues *et al.*, 2023). In Morocco, many species with unknown information related to their capacity to acquire and transmit *X.f* were identified. The lack of information is due to the limited trials worldwide on *X.f*-potential insect vectors. Thus, there is a serious necessity to make supplementary surveys to get new data in the country, as well as in North Africa.

Several pathogenic variants of the bacterium have been described and categorized by subspecies. Six subspecies are reported for *X.f*; worldwide (Schaad *et al.*, 2004; Schuenzel *et al.*, 2005; Randall *et al.*,2009; Nunney *et al.*,2014): *X.f* subsp. *fastidiosa*, *X.f* subsp. *multiplex*, *X.f* subsp. *sandy*, *X.f* subsp. *pauca*. *X.f* subsp. *tashke* which was isolated from the ornamental tree *Chitalpa tashkentensis* (Randall *et al.*, 2009) and finally, *X.f* subsp. *morus* that infects *Morus* spp. (Nunney *et al.*, 2014, 2019). Together, these subspecies of *X.f* have been reported to cause diseases after infection on more than 655 plant species belonging to 293 genera and 88 botanic families (EFSA, 2022). However, only two of subspecies are of worldwide important and most economically devastating; *X.f* subsp. *fastidiosa* which cause PD in grapevine in North America and *X.f* subsp. *pauca* which causes CVC and OQDS in South America and in Europe, respectively (Parra *et al.*, 2003; Dellape *et al.*, 2016; Saponari *et al.*, 2017). The other subspecies are associated with low economic damage.

The spread of the bacterium to forest areas could lead to an impact on oaks, elms, maples, and other tree species known to be affected in North America (EFSA, 2020; Desprez-Loustau *et al.*, 2021). Most researches have shown no specificity of insect vector transmission for different bacteria genotypes and strains (Almeida and Nunney, 2015; Esteves *et al.*, 2019; Coletta-Filho *et al.*, 2020). Except for the two tritrophic interactions (*X.f* subsp. *pauca*-olive-*P. spumarius* and *X.f* subsp. *fastidiosa*-grapevine-sharphopper), little specific data are available on transmitted *X.f* subspecies by a confirmed insect vector. This is the case of ALSD in California, which is caused by the interactions of two types of sequence of *X.f* subspecies; subsp *fastidiosa* (ST1) and subsp. *multiplex* (ST81) (Olmo *et al.*, 2021). The same authors reported that, to date, ALSD affects over 81% of almond trees and PD was widespread in vineyards across Mallorca. However, no available data was recorded on the role of the potential vec

tor of this bacterium. In addition, all knowledge on Apulian OQDS outbreak should not be generalized and applied to other pathosystems, since *Xylella-pathosystems* should be treated on a case-by-case basis (Desprez-Loustau *et al.,* 2021).

In Morocco, citrus, olive, almond, grapevine, the forest agroecosystem and oleander which is the main ornamental plant in the country, are identified as at high risk to be host plants of the bacterium. While *P. tesselatus* has not been tested for acquisition and transmission efficiency on any crop, its occurrence at high abundance suggest that it could be the main potential vector of *X.f* in Morocco once the latter introduced. In addition, taking into account all data which implies the role of *P. tesselatus* in the potential epidemiology of *X.f* in Mediterranean region and particularly in Morocco, it will be essential to conduct trials to assess the role of this spittlebug, as an insect vector, in the spread of different subspecies of *X.f* in to the main crop agroecosystems, e.g. citrus, olive, almond grapevine, forest system and oleander.

Conclusions

Knowledge of relationships between *X.f* subspecies and the worldwide potential insect vectors is of crucial importance to understand the risk of introduction and spread of the bacterium in new regions and countries such as in Morocco. The emergence of serious diseases associated with *X.f* in different EU countries and its easy spread to other countries in the Mediterranean region, represents a serious threat to all potential host species in each country. Furthermore, the favorite climatic conditions for *X.f* in Morocco, suggest that the bacterium will find a suitable environment for successful establishment. The present work highlights the main reported diseases caused by *X.f*, the associated subspecies, affected crops and the typical symptoms. According to the available data in the literature*, P. tesselatus* should be considered as the main potential insect vec-

tor of *X.f* in Morocco. While no data is available on the acquisition and transmission efficiency of *P. tesselatus* to any crop, this insect which is already associated with several susceptible crops in Morocco could play a major role in fast spread of *X.f* once introduced in Morocco. Indeed, it is of crucial importance to notice the presence of *G. versuta* in Algeria. There is a great need to carry out supplementary surveys on potential insect vectors of this bacterium. In addition, there is an urgent need to have the scientific tools to detect quickly the occurrence of this bacterium in the first bacteria inoculums that could be introduced in the near future.

Supplementary Material
Table S1. List of worldwide, families, genera, occurrence, acquisition and transmission of X.f, disease transmitted and host plants, vector, competent and potential vector of X.f, including America (AM), Europe (EU), Asia (AS), North Africa (NA) and Morocco (MO). Lab: Laboratory.
Table S2. Worldwide tritrophic interaction subspecies of X.f-host plant-insect vector.

Conflicts of Interest
The authors declare no conflict of interest.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ἔντομα δυνητικοί φορείς του βακτηρίου *Xylella fastidiosa* παγκοσμίως και αξιολόγηση της σημασίας τους με έμφαση στο Μαρόκο

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Περίληψη   Στο Μαρόκο οι κλιματικές συνθήκες είναι ευνοϊκές για την εγκατάσταση και εξάπλωση του φυτοπαθογόνου βακτηρίου *Xylella fastidiosa* (*X.f*). Ωστόσο, η επιτυχής εγκατάσταση του βακτηρίου εξαρτάται από πολλούς παράγοντες και κυρίως: τα υποείδη του βακτηρίου και τον τύπο αλληλουχίας, την ευαισθησία των φυτών-ξενιστών, τη διατροφική συμπεριφορά και την αποτελεσματικότητα μετάδοσης από τα έντομα-φορείς. Η γνώση της σχέσης μεταξύ του αγρο-οικοσυστήματος των καλλιεργειών που προσβάλλονται από το βακτήριο και των πιθανών εντόμων-φορέων είναι ιδιαίτερης σημασίας. Σε αυτήν την εργασία, παραθέτουμε τις τριτροφικές αλληλεπιδράσεις *X.f* – φυτά ξενιστές - έντομα φορείς, οι οποίες εμφανίζονται παγκοσμίως, με στόχο την αξιοποίησή τους στην τρέχουσα κατάσταση στο Μαρόκο και για την ανάλυση κινδύνου για το βακτήριο στη χώρα. Εξετάστηκαν δύο πιο σχετικά υποείδη *X.f* του βακτηρίου (όσον αφορά τις επιπτώσεις στις καλλιέργειες), συγκεκριμένα, το υποείδος *fastidiosa* και το υποείδος *pauca*. Με βάση τη διεθνή βιβλιογραφία και τις δημόσιες βάσεις δεδομένων, η πλειονότητα των εντόμων-φορέων του *X.f* ανήκει σε δύο οικογένειες: Cicadellidae και Aphrophoridae. Μεταξύ όλων των ειδών Cicadellidae που καταγράφηκαν, ένας μεγάλος αριθμός είχε την ικανότητα να μεταδώσει το *X.f* σε φυτά-ξενιστές στην Αμερική, ενώ αυτή η ικανότητα είναι μηδενική για άλλες περιοχές (εκτός από το *Graphocephala versuta* Say (Hemiptera: Cicadellidae) που καταγράφηκε στην Αλγερία). Στο Μαρόκο, κανένα από τα γένη της οικογένειας Cicadellidae που έχουν αναφερθεί παγκοσμίως ως φορείς του *X.f*, δεν έχει αναφερθεί μέχρι στιγμής ενώ υπάρχουν πολλά είδη Cicadellidae και Aphrophoridae. Το είδος *Philaenus tesselatus* Melichar (Hemiptera: Aphrophoridae) είναι σε μεγάλη αφθονία στο Μαρόκο και θα μπορούσε να παίξει ρόλο ως δυνητικός φορέας σε περίπτωση που το βακτήριο εισαχθεί στη χώρα. Όσον αφορά τους δυνητικούς ξενιστές του *X.f* στο Μαρόκο θεωρούνται τα εσπεριδοειδή, η ελιά, η αμυγδαλιά, το αμπέλι, τα δασικά αγροοικοσυστήματα και η πικροδάφνη.

Supplementary material

Table S1. List of worldwide, families, genera, occurrence, acquisition and transmission of *X.f*, disease transmitted and host plants, of confirmed vector, competent and potential vector of *X.f*, including America (AM), Europe (EU), Asia (AS), North Africa (NA) and Morocco (MO). Lab: Laboratory.

uk: Unknown; AD; Alfalfa dwarf; PD: Pierce's disease; PP: Phony peach; ALS: Almond Leaf Scorch; BLS: Bacterial leaf scorch; PPD: Phony peach disease; OLS, Oleander leaf scorch; CVC: Citrus variegated chlorosis; CLS: Coffee leaf scorch; PLS: Plum leaf scald; OQDS: Olive quick decline syndrome.

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Cicadellidae	*Draeculacephala*												
	D. anguilifera Walker, 1851	+	-	-	-	-	Yes	Yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	D. navicula Hamilton, 1985	+	-	-	-	-	no	yes	uk	uk	uk	unknown	Mitchell *et al.* 2009; Overall & Rebek 2015
	D. portola Ball, 1927	+	-	-	-	-	yes	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	D. californica Davidson Frazier, 1949	+	-	-	-	-	uk	yes	Yes	uk	AD/PD	unknown	Redak *et al.* 2004
	D. crassicornis VanDuzee, 1915	+	-	-	-	-	uk	yes	yes	uk	AD/PD	unknown	Redak *et al.* 2004
	D. noveboransis Fitch, 1851	+	-	-	-	-	uk	yes	no	uk	AD/PD	unknown	Redak *et al.* 2004
	D. minerva Ball, 1927	+	-	-	-	-	yes	yes	yes	yes	PD/ALS/AD	unknown	Redak *et al.* 2004; Overall & Rebek 2017; Cornara *et al.* 2019
	G. atropunctata Signoret, 1854	+	-	-	-	-	yes	yes	yes	yes	PD/ALS/AD	unknown	Redak *et al.* 2004; Daugherty *et al.* 2011; Overall & Rebek. 2017; Cornara *et al.* 2019; Redak *et al.* 2004; Daugherty *et al.* 2019; Redak *et al.* 2004; Overall & Rebek 2017; Cornara *et al.* 2019
	Graphocephala												
	G. cythura Baker, 1898	+	-	-	-	-	uk	uk	uk	uk	PD/AD	unknown	Redak *et al.* 2004
	G. coccinea Forster, 1771	+	-	-	-	-	yes	yes	uk	uk	uk	Blackberry, forsythia, cheese wood, cup plant, whole leaf rosinweed, rose, thistle, milkweed, goldenrod, and fragrant sumac	Zhang *et al.* 2011; Overall & Rebek 2015; Overall *et al.* 2017
	G. versuta Say, 1830	+	-	-	+	-	yes	yes	yes	yes	PD/PPD	Ragweed, sunflower, okra, plum, and blackberry	Redak *et al.* 2004; Mitchell *et al.* 2009; Zhang *et al.* 2011; Cornara *et al.* 2019; Boulaouad *et al.* 2022
	G. hieroglyphica Say, 1830	+	-	-	-	-	uk	yes	uk	yes	PPD/PD/AD	Willow, poplar, broad-leafed milkweed, and great ragweed	Redak *et al.* 2004; Overall & Rebek 2015; Overall *et al.* 2017
	G. confluens Uhler, 1861	+	-	-	-	-	yes	yes	yes	uk	PD/AD/ALS	unknown	Redak *et al.* 2004
	G. fennahi Young, 1977	+	+	-	-	-	uk	uk	uk	uk	uk	woody plants; *Acer platanoides, Azalea spp., Forsythia spp., Hedera helix, Ilex aquifolium, Platanus sp., Pyris japonica, Robina pseudoacacia, Rumex acetosa, Tilia spp.*	Olthoff, 1986; Sergel 1987; Tóth *et al.* 2017

Family/genus/species	species	AM	EU	AS	NA	MO	Capacity of acquisition Lab	field	Capacity of transmission Lab	field	Disease(s) transmitted	Host plants	References
Pagaronia	*P. confusa* Oman, 1938	+	-	-	-	-	yes	uk	yes	uk	PD	unknown	Redak et al. 2004; EFSA, 2019
	P. furcata Oman, 1938	+	-	-	-	-	uk	uk	uk	uk	PD	unknown	EFSA, 2019
	P. tredecimpunctata Ball, 1902	+	-	-	-	-	uk	uk	uk	uk	PD/AD	unknown	Redak et al. 2004; EFSA, 2019
	P. triunata Bal, 1902	+	-	-	-	-	uk	uk	uk	uk	PD/AD	unknown	Redak et al. 2004; EFSA, 2019
Macugonalia	*M. cavifrons* St al, 1862	+	-	-	-	-	yes	uk	yes	uk	PLS/CVC	*Citrus sinensis* (L.) Obs., *Citrus unshiu Marc.*	Dellape et al. 2013; 2016; Esteves et al. 2019; Müller et al. 2021
	M. sobrina Stål, 1862	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Dellape et al. 2016
	M. leucomelas Walker, 1851	+	-	-	-	-	yes	yes	yes	uk	CVC/PLS	unknown	Redak et al. 2004; Cornara et al. 2019; Esteves et al. 2019; Müller et al. 2021
Plesiommata	*P. corniculata* Young, 1977	+	-	-	-	-	yes	yes	yes	uk	CVC	unknown	Dellape et al. 2016; Cornara et al. 2019; Redak et al. 2004
	P. mollicella Fowler, 1900						uk	yes	yes		CVC	*Zea mays* L. (Poaceae), *Triticum aestivum* L. (Poaceae), *Avena sativa* L. (Poaceae) malezas asociadas, *Sorghum vulgare* (Poaceae), *Oriza sativa* L. (Poaceae), *Brassica* sp. (Brassicaceae), *Lycopersicon* sp. (Solanaceae), *Solanum* sp. (Solanaceae), *Citrus sinensis* Osb. (Rutaceae) malezas asociadas, *C. reticulata* (Rutaceae), *Andropogon gayanus* Kunth (Poaceae), *Carica papaya* L. (Caricaceae). *Vaccinium corymbosum* L. (Ericaceae)	Dellape et al. 2016
Homalodisca	*H. spottii* Takiya, Cavichioli & McKamey, 2006	+	-	-	-	-	uk	yes	uk	uk	CVC	unknown	Ringenberg et al. 2014; Takiya et al. 2006
	H. vitripennis Germar, 1821	+	-	-	-	-	yes	yes	yes	yes	PD/PPD/ALS/ OLS/BLS/CVC	Johnsongrass, sunflower, hollyhock, okra, lambsquarters, cotton, corn, cowpeas, oak, ash, silktree, crapemyrtle, elderberry and peach	Redak et al. 2004; Overall & Rebek 2015; Overall et al.2017; Daugherty et al. 2011; Krugner et al. 2012; Mitchell et al. 2011; Almeida et al. 2005; Sanderlin & Melanson 2010; Cornara et al. 2019
	H. liturata Ball, 1901	+	-	-	-	-	uk	yes	no	uk	PD/AD	unknown	Redak et al. 2004
	H. lacerta Fowler, 1899	+	-	-	-	-	yes	uk	yes	uk	uk	unknown	Daugherty et al. 2011
	H. ignorata Melichar, 1924	+	-	-	-	-	yes	yes	yes	uk	CVC/CLS	unknown	Redak et al. 2004; Marucci et al. 2008; Yamamoto et al. 2002
	H. insolita Walker, 1858	+	-	-	-	-	yes	yes	yes	yes	PD/PP/BLS	Texas millet, Johnsongrass and crabgrass; southern sandbur	Redak et al. 2004; Cornara et al. 2019; Overall & Rebek 2017; Sanderlin & Melanson 2010
Acrogonia	*A. virescens* Metcalf, 1949	+	-	-	-	-	yes	uk	yes	uk	CVC	unknown	Dellape et al. 2016 Redak et al. 2004
	A. citrina Marucci & Cavichioli, 2002	+	-	-	-	-	yes	uk	yes	uk	CVC	unknown	Redak et al. 2004; Cornara et al. 2019

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Tapajosa	*T. rubromarginata* Signoret, 1855	+	-	-	-	-	yes	yes	yes	uk	CVC	*Citrus sinensis* (L.) Obs. and *C. UnshiuMarc.*	Dellape *et al.* 2013, 2016
	T. fulvopunctata Signoret, 1854	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Ringenberg *et al.* 2014
Cuerna	*C. costalis* Fabricius, 1803	+	-	-	-	-	yes	yes	yes	yes	PD/PP/BLS	Bermudagrass, crabgrass, Johnsongrass, Texas millet, cotton, cowpea, sunflower, ragweed, and young peach tree	Overall & Rebek 2015; Overall *et al.* 2017; Cornara *et al.* 2019; Redak *et al.* 2004; Sanderlin & Melanson 2010
	C. occidentalis Osman & Beamer, 1944	+	-	-	-	-	uk	yes	yes	uk	PD/AD	unknown	Redak *et al.* 2004.
	C. yucca Osman & Beamer, 1944	+	-	-	-	-	uk	yes	yes	uk	PD/AD	unknown	Redak *et al.* 2004
Oncometopia	*O. orbona* Fabricius, 1798	+	-	-	-	-	yes	yes	yes	yes	PD/PPD	Ash, honeysuckle, Chickasaw plum , redbud, silktree, blackberry, septic weed, Dahlia, Johnsongrasss, greenbrier, hibiscus, pokeweed, sunflower, hollyhock, okra, lambsquarters, ragweed, and peach	Overall & Rebek 2015; Zhang *et al.* 2011; Cornara *et al.* 2019; Redak *et al.* 2004
	O. nigricans Walker, 1851	+	-	-	-	-	yes	yes	yes	yes	PPD/CVC/ PD	Crapemyrtle; Texas millet and Johnson grass	Overall & Rebek 2017; Cornara *et al.* 2019; Redak *et al.* 2004. Cornara *et al.* 2019; Redak *et al.* 2004; Marucci *et al.*2003
	O. facialis Signoret, 1854	+	-	-	-	-	yes	uk	yes	uk	CVC /CLS	unknown	Overall & Rebek 2015; Overall *et al.* 2017 Redak *et al.* 2004
Xyphon	*X. flaviceps* Riley, 1880	+	-	-	-	-	uk	yes	yes	yes	PD/AD	Bermudagrass; cotton, cucumber, alfalfa, beebalm, and prickly Russina thistle	Redak *et al.* 2004; Cornara *et al.* 2019; Overall & Rebek 2017
	X. fulgida Nottingham, 1932	+	-	-	-	-	yes	yes	yes	yes	PD/AD/ALS	unknown	
	X. triguttata Nottingham, 1932	+	-	-	-	-	uk	yes	yes	uk	PD/AD	*Cynodon* sp., *Trichostema lanceolatum*, *Chrysothamnus* sp.	Redak *et al.* 2004 Dmitriev, 2019
Tretogonia	*T. cribrata* Melichar, 1926	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Ringenberg *et al.* 2014
	T. notatifrons Melichar, 1926	+	-	-	-	-	uk	uk	uk	uk	uk	*Citrus sinensis* (L.) Obs	Dellape *et al.* 2013, 2016
Agalliana	*A. ensigera* Oman,1934	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Dellape *et al.* 2016
	A. sticticollis Stål, 1859	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Dellape *et al.* 2016
Evacanthus	*E. acuminatus* Fabricius, 1794	-	+	-	-	-	uk	uk	uk	uk	uk	Lamiaceae; *Urtica* sp;*Rubus* sp.	EFSA, 2013
	E. interruptus Linnaeus, 1758	-	+	+	-	-	uk	uk	uk	uk	uk	Asteraceae, *Urtica* sp., *Epilobium*sp.; *Angelica archangelica*; *Mentha aquatica*	EFSA, 2013; Mozaffarian, 2018
	E. rostagnoi Picco, 1921	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2013
Cicadella	*C. viridis* Linnaeus, 1758	-	+	-	-	-	yes	uk	yes	uk	uk	*Alnus* sp.; Asteraceae; *Carex* sp; *Corylus avellana*; *Crataegus* sp; Ericaceae; Fabaceae; *Juncus* sp; *Pinus* sp; Poaceae; *Primula* sp.; *Prunus laurocerasus*; *Quercus* sp.; Renonculaceae; *Rubus* sp.; *Silene* sp.; *Urtica* sp.	EFSA, 2013, 2015; Nickel & Remane 2002; Mozaffarian & Wilson 2016; Bodino *et al.* 2022
	C. lasiocarpae Ossiannilsson, 1981	-	+	-	-	-	uk	uk	uk	uk	uk	*Carex* sp, *Carex lasiocarpa*, *Carex nigra*, *Carex vesicaria* and others	EFSA, 2013, 2015; Nickel& Remane 2002; Malenovsky *et al.* 2013

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Bucephalogonia	*B. xanthophis* Berg, 1879	+	-	-	-	-	yes	yes	yes	uk	CVC/CLS	*Citrus senensis* Osb (Rutaceae), *Vitis vinifera* L. *Prunus domestica* L. (Rocaceae), *Coffea sp.* (Rubiaceae, *Vernonia condensata* (Asterceae), *Duranta repens* L.(Verbenaceae), *Brassicanapus* L, *var oleifera* (Brasscaceae),	Marucci *et al.* 2003; Paris *et al.* 2012; Dellape *et al.* 2013; Redak *et al.* 2004; Esteves *et al.* 2019
Ferrariana	*F. trivittata* Signoret, 1851	+	-	-	-	-	uk	yes	uk	uk	CVC	*Citrus sinensis* (L.) Osb. (Rutaceae), *Prunus domestica* L.	Redak *et al.* 2004; Cornara *et al.* 2019
Diedrocephala	*D. bimaculata* Gmelin, 1789	+	-	-	-	-	uk	no	yes	uk	uk	*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc	Dellape *et al.* 2013, 2016
Sonesimia	*S. grossa* Signoret, 1854	+	-	-	-	-	yes	yes	uk	yes	CVC	*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc	Dellape *et al.* 2016; Cornara *et al.* 2019; Redak *et al.* 2004
Dilobopterus	*D. costalimai* Young,1977	+	-	-	-	-	yes	uk	yes	uk	CVC/CLS	*Citrus sinensis* (Rutaceae), *Coffea arabica* L. (Rubiaceae), *Vernonia condensata* (Asteraceae), *Aloysiavirgata* (Verbenaceae), *Hibiscus rosa–sinensis* (Malvaceae)	Cornara *et al.* 2019 ; Redak *et al.* 2004; Marucci *et al.* 2008
Balacha	*B. melanocephala* Signoret, 1854	+	-	-	-	-	uk	uk	uk	uk	uk	*Eryngium* L (Apiaceae)	Dellape *et al.* 2016
Helochara	*H. delta* Oman, 1943	+	-	-	-	-	yes	yes	yes	yes	PD/AD	unknown	Redak *et al.* 2004; Overall & Rebek. 2017
Ciminius	*C. albolineatus* Taschenberg, 1884	+	-	-	-	-	uk	uk	uk	uk	uk	*Avena sativa* L. (Poaceae)	Dellape *et al.* 2016
Sibovia	*S. sagata* Signoret, 1854	+	-	-	-	-	yes	uk	yes	uk	PLS/CVC	*Zea mays* L. (Poaceae), *Citrus sinensis* Osb. (Rutaceae) *Pelargonium hortum* L. (Geraniaceae)"malvòn", *Phaseolus vulgaris* L. (Fabaceae), *Malva parviflora* L. (Malvaceae), *Vitis vinifera* L. (Vitaceae). *Mentha* sp. L. (Lamiaceae).*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc	Dellape *et al.* 2013,2016; Esteves *et al* 2019; Müller *et al.* 2021
Hortensia	*H. similis* Walker, 1851	+	-	-	-	-	yes	yes	yes	uk	CVC	*Sorghum halepense* L. (Poaceae), *Zea mays* L. (Poaceae), *Medicago sativa*L. (Poaceae), *Hordeum vulgare* L. (Poaceae), *Vigna unguiculata* L. (Fabaceae), *Panicum barbino* Trin. (Poaceae), *Dolichos* sp. (Fabaceae), *Bidens* sp. (Asteraceae), *Fragaria* sp. (Rosaceae), *Crotalaria* sp. (Fabaceae), *Phaseolus vulgaris* L. (Fabaceae), *Cynodon* sp. (Poaceae), *Paspalum* sp. (Poaceae), *Pennisetum* sp. (Arecaceae), *Lactuca* sp. (Asteraceae), *Brassica* sp. (Brassicaceae), *Carica* sp. (Caricaceae), *Ipomeas* sp. (Convolvulaceae), *Sida* sp. (Malvaceae), *Hibiscus* sp. (Malvaceae), *Digitaria* sp. (Poaceae), *Panicum* sp. (Poaceae), *Cynodon* sp. (Poaceae), *Eriochloa* sp. (Poaceae), *Cenchrus* sp. (Poaceae), *Coffea* sp. (Rubiaceae), *Lycopersicon* sp. (Solanaceae), *Capsicum* sp.	Dellape *et al.* 2013, 2016; Ringenberg *et al.* 2014; Hickel *et al.* 2001; Paris *et al.* 2012

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Syncharina	*S. argentina* Berg, 1879	+	-	-	-	-	uk	uk	Uk	uk	uk	*Oryza sativa* L. (Poaceae), *Sorghum halepense* L. (Poaceae), *Zea mays* L. (Poaceae), *Citrus sinensis* Osb. (Rutaceae), *Hordeum vulgare* L. (Poaceae), *Medicago sativa* L. (Poaceae), *Gossypium hirsutum* L. (Malvaceae), *Cynodon sp.* (Poaceae), *Triticum aestivum* L. (Poaceae) *Fragaria sp.* (Rosaceae).	Dellape et al. 2016
Kolla	*K. paulula* Walker, 1858	-	-	+	-	-	yes	yes	yes	uk	CVC/PD	unknown	Tuan et al. 2016
Bothrogonia	*B. ferruginea* Fabricius, 1787	-	-	+	-	-	yes	yes	yes	uk	CVC/PD	unknown	Tuan et al. 2016
Anatkina	*A. horishana* Matsumura, 1912	-	-	+	-	-	uk	yes	uk	uk	uk	unknown	Tuan et al. 2016
Dechacona	*D. missionum* Berg, 1879	+	-	-	-	-	yes	yes	yes	yes	CVC/CLS	*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc	Marucci et al. 2008; Redak et al. 2004; Dellape et al. 2013,2016; Cornara et al. 2019; Esteves et al. 2019
Parathona	*P. gratiosa* Blanchard, 1840	+	-	-	-	-	uk	-	uk	uk	CVC	unknown	Redak et al. 2004
Friscanus	*F. friscanus* Ball, 1909	+	-	-	-	-	uk	yes	yes	yes	PD/AD	unknown	Redak et al. 2004
Molomea	*M. lineiceps* Young, 1968	+	-	-	-	-	yes	yes	yes	uk	CVC	*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc.	Dellape et al. 2013
	M. consolida Schroder, 1959	+	-	-	-	-	uk	uk	uk	uk	CVC	*Citrus sinensis; Prunus salicina; Zea mays*	Logarzo et al. 2012; Schneider et al. 2017; Dmitriev, 2019; EFSA, 2019
Paraulacizes	*P. irrorata* Fabricius, 1794	+	-	-	-	-	yes	yes	uk	uk	uk	Virginia wildrye, tall thistle, horseweed, thistle, prickly lettu, wholeleaf rosinweed, and cultivated sorghum	Zhang et al. 2011; Overall & Rebek. 2015; Overall et al. 2017
Curtara	*C. samera* DeLong & Freytag, 1972	+	-	-	-	-	yes	yes	yes	uk	CVC	*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc	DeCool et al. 2000; Dellape et al. 2013, 2016; Ringenberg et al. 2014
Neokolla	*N. circullata* Baker	+	-	-	-	-	uk	uk	uk	yes	uk	unknown	Overall & Rebek 2017.
	N. severini DeLong, 1948	+	-	-	-	-	uk	yes	no	uk	PD	unknown	Redak et al. 2004; EFSA, 2018
	N. hyeroglyphica Say, 1830	+	-	-	-	-	yes	uk	yes	uk	PD	Willow, poplar and alfalfa	EFSA, 2018
Scopogonalia	*S. subolivacea* Stal, 1862	+	-	-	-	-	uk	-	uk	uk	uk	*Citrus sinensis* (L.) Osb. (Rutaceae)	De Coll et al. 2000; Redak et al. 2004
Errhomenus	*E. brachypterus* Fieber, 1866	-	+	-	-	-	uk	-	uk	uk	uk	unknown	Nickel & Remane 2002
Fingeriana	*F. dubia* Cavichioli, 2003	+	-	-	-	-	yes	uk	yes	uk	CVC	Caipira sweet orange	EFSA, 2018; Yamamoto et al. 2007
Oragua	*O. discoidula* Osborn, 1926	+	-	-	-	-	yes	yes	yes	uk	CVC	unknown	EFSA, 2018; Lopes & Krugner 2016
Clastopteridae — Clasteroptera	*C. brunnea* Ball, 1919	-	+	-	-	-	yes	yes	yes	uk	PD	unknown	Redak et al. 2004; Severin, 1950; EFSA, 2013
	C. lawsoni Doering, 1929	+	+	-	-	-	uk	yes	uk	uk	uk	*Corynephorus canescens*	Nickel & Remane 2002; Mitchell et al. 2009; EFSA, 2013

Family/genus/species	Occurrence					Capacity of acquisition		Capacity of transmission		Disease(s) transmitted	Host plants	References
	AM	EU	AS	NA	MO	Lab	field	Lab	field			
C. xanthocephala Germar, 1839	+	+	-	-	-	uk	yes	uk	uk	uk	unknown	Mitchell *et al.* 2009; EFSA, 2013
C. achatina Germar, 1839	+	+	-	-	-	yes	uk	yes	uk	BLS	Stipa sp.	Lodos & Kalkandelen 1983; Biedermann 2003; Sanderlin & Melanson 2010
C. obtusa Say, 1825	+	+	+	+	-	uk	yes	uk	uk	uk	mostly on meadow and Graminae weeds; Nardus stricta, Phragmites, Thuja sp; Trifolium spp., T. armenicum	Nickel & Remane 2002; Zhang et al. 2011; Mozaffarian & Wilson 2015
C. lineatocolis Stål, 1854	+	+	-	-	-	uk	no	uk	uk	uk	unknown	Mitchell *et al.* 2009; EFSA, 2013
Aphrophoridae **Aphrophora**												
A. permutata Uhler, 1876	+	-	-	-	-	uk	yes	no	uk	PD/AD	unknown	Redak et al. 2004;
A. angulata Ball, 1899	+	-	-	-	-	uk	yes	no	uk	PD	unknown	Beal et al. 2021 Redak et al. 2004
A. quadrinotata Say, 1831	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang et al. 2011
A. cribrata Walker, 1851	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang et al. 2011
A. alni Fallen, 1805	-	+	+	+	-	uk	uk	uk	uk	uk	Alnus spp., Amygdalus communis, Betula pendula, Castanea sativa, Cornus mas, Corylus avellana, Cynodon vulgaris, Elaeagnus spp., Fagus ylvatica, Juglans sp., Mespilus germanica, Myrica gale, Oryza sativa, Populus sp., Prunus domestica, P. avium, P. cerasus, Quercus spp., Rubus fructicosus, Rosa sp., Salix sp., Ulmus sp., Urticia sp, Verbascum sp., Vitis uinijera	Nickel & Remane 2002; EFSA, 2015; Mozaffarian & Wilson 2015; Antonatos et al. 2019
A. salicina Goeze, 1778	-	+	+	+	-	uk	uk	uk	uk	uk	Betula pendula, Robinia pseudoacacia, Rubus fruticosus, Salix spp, sugar beet	Antonatos et al. 2019; Mozaffarian & Wilson 2015; EFSA, 2015; Nickel & Remane 2002
A. corticea Germar, 1821	-	+	-	-	-	uk	uk	uk	uk	uk	Conifers ; Arctostaphylos uva-ursi, Cupressus sp., Pinus sylvestris, Pyrus communis, Quercus sp., Verbascum sp.	Antonatos et al. 2019; EFSA, 2015; Nickel & Remane 2002
A. major Uhler, 1896	-	+	-	-	-	uk	uk	uk	uk	uk	Salix spp, Betula pendula	Antonatos et al. 2019; EFSA, 2013; Nickel & Remane 2002
A. pectoralis Matsumura, 1903	-	+	-	-	-	uk	uk	uk	uk	uk	Salix caprea, Salix purpurea and mas Univoltine	Antonatos et al. 2019; EFSA, 2013; Nickel & Remane 2002
A. costalis Matsumura, 1903	-	+	-	-	-	uk	uk	uk	uk	uk	Salix spp.	EFSA, 2015
A. similis Lethierry, 1888	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
A. willemsi Lallemand, 1946	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Philaenus	*P. marginellus* Linnaeus, 1758	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
	P. tesselatus Melichar, 1899	-	+	-	+	+	uk	uk	uk	uk	uk	unknown	Remane & Drosopoulos 2001; Drosopoulos *et al.* 2010
	P. tarifa Remane & Drosopoulos, 2001	-	+	-	-	-	uk	uk	uk	uk	uk	wildshrubs and trees; particularly on *Quercus* spp.	Drosopoulos & Remane 2000
	P. maghresignus Remane & Drosopoulos, 2000	-	+	-	+	+	uk	uk	uk	uk	uk	unknown	Drosopoulos & Remane 2000; Drosopoulos *et al.* 2010
	P. signatus Melichar, 1896	-	+	-	+	+	uk	uk	uk	uk	uk	lily *Asphodelus microcarpus* and later on shrubs and trees like *Quercus* and *Castanea sativa*	Drosopoulos & Remane 2000; EFSA, 2015
	P. italosignus Remane & Drosopoulos, 2000	-	+	-	-	-	uk	yes	yes	uk	uk	*Asphodelus* spp.	Cavalieri *et al.* 2019; EFSA, 2013 ; Bodino *et al.* 2020
	P. pallidus Linnaeus, 1903	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	P. spumarius Linnaeus, 1758	+	+	+	+	+	yes	yes	yes	yes	OQDS/ALS/AD	Mainly dicotyledonous herbs, broadleafs hrubs	Schedl, 1988; Redak *et al.* 2004; Zhang *et al.* 2011; Cavalieri *et al.* 2019; Rodrigues *et al.* 2014; Ben Moussa *et al.* 2016 ; Bodino *et al.* 2020
	P. lukasi Drosopoulos & Asche, 1991	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
Neophilaenus	*N. campestris* Fallén, 1805	-	+	+	+	+	yes	yes	yes	uk	uk	Conifers, Graminae, *Abies* sp.,*Corylus aoeluma, Juncus* sp., *Medicago sativa, Pinus* sp.,*Pyrus communis, Quercus* sp., *Rhododendron* sp.,*Ulmus* sp., *Triticum* sp., *Zeamays*	Lodos & Kalkandelen 1983; Elbeaino *et al.* 2014; Nickel & Remane 2002; Mozaffarian & Wilson 2015; Ben Mooussa *et al.* 2016; Cavalieri *et al.* 2019 : Bodino *et al.* 2020
	N. lineatus Linnaeus, 1758	-	+	+	+	+	uk	uk	uk	uk	uk	mostly on meadow and Graminaeweeds; *Nardusstricta, Phragmites, Thuja* sp.; *Trifolium* spp., *T. armenicum*	Mozaffarian & Wilson 2015; Nickel & Remane 2002
	N. exclamationis Thunberg, 1884	-	+	-	-	-	uk	uk	uk	uk	uk	*Festuca ovina, Deschampsia flexuosa*	Nickel & Remane 2002; EFSA, 2013
	N. infumatus Haupt, 1917	-	+	-	-	-	uk	uk	uk	uk	uk	*Festuca ovinas* sp.	Nickel & Remane 2002; EFSA, 2013
	N. limpidus Wagner, 1935	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2013
	N. albipennis Fabricius,1798	-	+	-	-	-	uk	uk	uk	uk	uk	*Brachypodium pinnatum*	Nickel & Remane 2002; EFSA, 2013
	N. longiceps Putton, 1895	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2013

Family/genus/species			Occurrence					Capacity of acquisition		Capacity of transmission		Disease(s) transmitted	Host plants	References
		AM	EU	AS	NA	MO		Lab	field	Lab	field			
	N. minor Kirschbaum, 1868	-	+	-	-	-		uk	uk	uk	uk	uk	*Festuca ovina, Corynephorus canescens*	EFSA, 2013; Nickel & Remane, 2002
	N. pallidus Haupt, 1917	-	+	-	-	-		uk	yes	uk	uk	uk	unknown	EFSA, 2013
	N. modestus Haupt, 1922	-	+	-	-	-		uk	uk	uk	uk	uk	unknown	EFSA, 2015
Lepyronia	*L. quadrangularis* Say, 1825	+	-	-	-	-		uk	uk	uk	uk	BLS	unknown	Redak *et al.* 2004; Sanderlin & Melanson 2010; Overall & Rebek. 2017
	L. coleoptrata Linnaeus, 1758	-	+	+	+	-		uk	uk	uk	uk	uk	*Poa annua, Trifolium repens;* mainly Poaceae, dicotyledonous herbs	Nickel & Remane 2002; Mozaffarian & Wilson 2015; Antonatos *et al.* 2019; Theodorou *et al.* 2021
Cephisus	*C. siccifolius* Walker, 1926	+	-	-	-	-		uk	uk	uk	uk	uk	*Citrus sinensis* (L.) Obs. *Citrus unshiu* Marc	Dellap *et al.* 2013
Mesoptyelus	*M. petrovi* Grigoriev, 1910	-	+	-	-	-		uk	uk	uk	uk	uk	unknown	EFSA, 2015
Poophilus	*P. costalis* Walker, 1851	-	-	+	-	-		uk	yes	uk	uk	PD	unknown	Tuan *et al.* 2016; Mozaffarian, 2018
Peuceptyelus	*P. coriaceus* Fallén, 1826	-	+	-	-	-		uk	uk	uk	uk	uk	unknown	Holzinger *et al.* 2003
Paraphilaenus	*P. notatus* Mulsant & Rey, 1855	-	+	-	-	-		uk	uk	uk	uk	uk	*Stipa sp.*	Lodos & Kalkandelen 1983; Biedermann, 2003
Membracidae *Cyphonia*	*C. clavigera* Fabricius, 1803	+	-	-	-	-		yes	yes	yes	uk	CVC	unknown	Dellape *et al.* 2016
Entylia	*E. carinata* Forster, 1771	+	-	-	-	-		uk	yes	uk	uk	CVC	unknown	De Coll *et al.* 2000; Zhang *et al.* 2011; Dellape *et al.* 2016
Ceresa	*C. ustulata* Fairmair, 1846	+	-	-	-	-		uk	uk	uk	uk	uk	unknown	Dellape *et al* 2013
Cyrtolobus	*C. maculifrontis* Emmons,	+	-	-	-	-		uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
	C. discoidalis Emmons, 1854	+	-	-	-	-		uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	C. fenestratus Fitch, 1851	+	-	-	-	-		uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	C. fuscipennis Van Duzee, 1908	+	-	-	-	-		uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
	C. gratiosus Woodruff	+	-	-	-	-		uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
	C. puritanus Woodruff, 1924	+	-	-	-	-		uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Ophiderma	*O. definita* Woodruff, 1919	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	O. evelyna Woodruff, 1919	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Zhang *et al.* 2011
	O. flava Goding, 1893	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
Telamona	*T. concava* Fitch, 1851	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
	T. extrema Ball, 1903	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	T. monticola Fabricius, 1803	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	T. tiliae Ball, 1925	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
	T. unicolor Fitch, 1851	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
Glossonotus	*G. acuminatus* Fabricius, 1775	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
Helonica	*H. excelsa* Fairmaire, 1846	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
Platycotis	*P. vittata* Fabricius, 1803	+	-	-	-	-	uk	no	uk	uk	uk	unknown	Zhang *et al.* 2011
Smilia	*S. fasciata* Amyot & Serville,	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
Enchenopa	*E. binota* Say, 1824	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
Acutalis	*A. tartarea* Say, 1830	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Zhang *et al.* 2011
Archasia	*A. belfragei* Stål, 1869	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
Atymna	*A. querci* Fitch, 1851	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Zhang *et al.* 2011
Carynota	*C. mera* Say, 1830	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Zhang *et al.* 2011
Microcentrus	*M. perditus* Amyot & Serville, 1843	+	-	-	-	-	uk	yes	uk	uk	uk	unknown	Zhang *et al.* 2011
Cicadidae													
Cicada	*C. barbara* ssp. *lusitanica* Boulard, 1982	-	+	-	-	-	uk	uk	uk	uk	uk	*Eucalyptus globulus, Ceratonia siliqua, Olea europea,* Quercus sp, *Pinus pinaster, Pistacia lentiscus*	Sueur *et al.* 2004

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease(s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
	C. barbara Stål, 1866	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	C. orni Linné, 1758	-	+	-	-	-	uk	yes	uk	no	uk	Cypressus spp., *Eucalyptus globulus*, *Olea europeae*, *Pinus pinaster*, *P. pinea*, *P. alpensis*, *Quercus* spp., *Vitis vinifera*	Nickel & Remane 2002; Antonatos *et al.* 2019; Cornara *et al.* 2019, 2020
	C. mordoganensis Boulard, 1979	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
Cicadatra	*C. alhageos* Kolenati, 1857	-	+	-	-	-	uk	uk	uk	uk	uk	*Vitis* spp.	Mozaffarian & Sanborn 2010; Mehdipour *et al.* 2016
	C. atra Olivier, 1790	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	C. persica Kirkaldy, 1909	-	+	-	-	-	uk	uk	uk	uk	uk	*Malus domestica*	Dardar & Belal 2013; Dardar *et al.* 2013; De Jong *et al.* 2014
	C. hyalina Fabricius, 1798	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	C. hyalinata Brullé, 1832	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	C. querula Pallas, 1773	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
Pagiphora	*P. annulata* Brullé, 1832	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	P. aschei Kartal, 1978	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
Lyristes	*L. plebejus* Scopoli, 1763	-	+	-	-	-	uk	uk	uk	uk	uk	*Olea europeae*, *Pinus pinaster*, *Zea mays*, *Vitis vinifera*	Sueur *et al.* 2004
Tibicinidae													
Aestuansella	*A. aestuans* Fabricius, 1794	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	De Jong *et al.* 2014
Cicadetta	*C. albipennis* Fieber, 1876	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	De Jong *et al.* 2014
	C. dubia Rambur, 1840	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	C. flaveola Brullé, 1832	-	+	-	-	-	uk	uk	uk	uk	uk	Grasses	Gogala & Drosopoulos 2006
	C. mediterranea Fieber, 1876	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
	C. montana Scopoli, 1772	-	+	-	-	-	uk	uk	uk	uk	uk	*Crataegus* sp, *Betula pendula*, *Pinus* sp.	Meineke, 2012; Tsagkarakis *et al.* 2018
	C. petryi Schumacher, 1924	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015

Family/genus/species	Occurrence AM	EU	AS	NA	MO	Capacity of acquisition Lab	field	Capacity of transmission Lab	field	Disease (s) transmitted	Host plants	References
C. podolica Eichwald, 1830	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
C. sibillae Hertach & Trilar, 2015	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	Puisant & Gurcel 2018
C. undulata Waltl, 1837	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
C. caucasica Kolenati, 1857	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
C. concinna Germar, 1976	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
C. fangoana Boulard, 1976	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
C. hageni Fieber, 1872	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
C. lobulata Fieber, 1876	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
Cicadivetta C. tibialis Panzer, 1798	-	+	-	-	-	uk	uk	uk	uk	uk	Pinus spp.	Mol, 2017
Dimissalna D. dimissa Hagen, 1856	-	+	-	-	-	uk	uk	uk	uk	uk	Quercus pubescens, Platanus orientalis	Puissant & Sueur 2011
Hilaphura H. varipes Waltl, 1837	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
Tettigetta T. argentata Olivier, 1790	-	+	-	-	-	uk	uk	uk	uk	uk	Cistus ladanifer, Eucalyptus globulus, Olea europea, Pinus sp, Quercus sp.	Sueur et al. 2004
T. baenai Boulard, 2000	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
T. brullei Fieber, 1876	-	+	-	-	-	uk	uk	uk	uk	uk	unknown	EFSA, 2015
T. dimissa Hagen, 1856	-	+	-	-	-	uk	uk	uk	uk	uk	Rubus ulmifolius, Pistacia terebinth	Schedl, 1988
T. leunami Boulard, 2000	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. manueli Boulard, 2000	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. pygmaea Olivier, 1790	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. aneabi Boulard, 2000	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015

Family/genus/species	Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
	AM	EU	AS	NA	MO	Lab	field	Lab	field			
Tibicina												
T. atra Gómez-Menor Ortega, 1957	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. carayoni Boulard, 1982	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. dimissa Hagen, 1856	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. estrellae Boulard, 1982	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. josei Boulard, 1982	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. mariae Quartau & Boulard, 1995	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. musiva Germar, 1830	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. cisticola larestifi Boulard, 1980	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. cisticola Hagen, 1855	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. corsica corsica Rambur, 1840	-	+	-	-	-	uk	uk	uk	uk	uk	*Bituminaria bituminosa, Foeniculum vulgare, Thymus vulgaris, Cistus spp.*	Holzinger et al. 2003; Sueur & Aubin 2004; Sueur et al. 2004
T. corsica faimairei Boulard, 1983	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA 2015
T. garricola Boulard, 1983	-	+	-	-	-	uk	uk	uk	uk	uk	*Cistusspp., Olea europeae, Pistacea lentiscus, Quercus coccifera*	Sueur et al. 2004
T. haematodes Scopoli, 1763	-	+	-	-	-	uk	uk	uk	uk	uk	*Laurus nobilis; Quercus ilex, Vitis vinifera*	Sueur & Aubin 2003, 2004; Schedl 1988
T. luctuosa Costa, 1883	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. nigronervosa Fieber, 1876	-	+	-	-	-	uk	uk	uk	uk	uk	*Arbutus unedo*	Sueur & Aubin 2004
T. picta Fabricius, 1794	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
T. quadrisignata Hagen, 1855	-	+	-	-	-	uk	uk	uk	uk	uk	*Arbutus unedo, Castanea sativa, Cistus ladanifer, Olea europea, Pinus pinaster, Quercus pyrenaica*	Sueur & Aubin 2004; Sueur et al. 2004
T. steveni Krynicki, 1837	-	+	-	-	-	uk	uk	uk	uk	uk	*Quercus pubescens, Prunus mahaleb, Acer opulus, Pynus sylvestris, Ononis rotundifolia, Astragalus monspessulanus, Vitis spp.,*	Sueur et al. 2003

Family/genus/species		Occurrence					Capacity of acquisition		Capacity of transmission		Disease (s) transmitted	Host plants	References
		AM	EU	AS	NA	MO	Lab	field	Lab	field			
Tympanistalna	T. tomentosa Olivier, 1790	-	+	-	-	-	uk	uk	uk	uk	uk	Cistus spp.,Juncus maritimus, Foeniculum vulgare	Sueur & Aubin 2004; Sueur et al. 2004
	T. distincta Rambur, 1840	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015
	T. gastrica Stal, 1854	-	+	-	-	-	uk	uk	uk	uk	uk	Pinus alepensis, Quercus ilex	Sueur et al. 2004
Cercopidae Cercopis	C. arcuata Fieber, 1844	-	+	-	-	-	uk	uk	uk	uk	uk	Pinus spp., Quercus spp.; grasslands	Holzinger et al. 2003
	C.intermedia Kirschbaum, 1868	-	+	-	+	-	uk	uk	uk	uk	uk	Acacia sp., Alnus sp., Astragalus sp.,Medicaoa sativa, Onopordon sp., Pistaciavera, Prunus domestica, Salix sp.,Verbascum sp.	Holzinger et al. 2003
	C. sabaudiana Lallemand, 1949	-	+	-	-	-	uk	uk	uk	uk	uk	Unknown	EFSA, 2015
	C. sanguinolenta Scopoli, 1763	-	+	-	-	-	uk	uk	uk	uk	uk	weeds; Castanea vesca, Malus domestica, Medicago sativa, Pyrus communis	Lodos & Kalkandelen 1983; Theodorou et al. 2021
	C. vulnerata Rossi, 1807	-	+	-	-	-	uk	uk	uk	uk	uk	Aegopodium sp.,Alnus sp., Arrhenatherum sp., Chrysanthemum sp.,Crataegus sp., Filipendula sp., Linum usitatissimum, Lupinus sp., Malus sp., Populus nigra, Prunus sp., Rubus sp., Solidago sp., Ulmus sp., Urtica sp., Lupinus sp., Vitis vinifera	Lodos & Kalkandelen 1983; Holzinger et al. 2003; Mazzoni, 2005; Holzinger, 2008
Haematoloma	H. dorsata Ahrens, 1812	-	+	-	-	-	uk	uk	uk	uk	uk	Abies sp., Cedrus sp., Crataegus sp., Cupressus sp., Juniperus sp., Linum usitatissimum, Picea sp., Pinus sylvestris, Populus sp., Prunus sp., Pseudotsuga sp., Quercus sp., Rosa sp.	Lodos & Kalkandelen 1983; Nickel & Remane 2002; Paris et al. 2012
Aeneolamia	A. colon Germar, 1821	+	-	-	-	-	uk	uk	uk	uk	uk	unknown	Ringenberg et al. 2014
Triecphorella	T. geniculata	-	+	-	-	-	uk	uk	uk	uk	uk		EFSA, 2015

Table S2. Worldwide tritrophic interaction subspecies of *X. f*-host plant-insect vector.

Subspecies of *X.f*	Diseases	Insect vectors	References
X.f multiplex	PLS	*M. cavifrons* *M. leucomelas* *S. sagata*	Mûller *et al.* 2021
X.f pauca	OQDS	*P. spumarius*	Saponari *et al.* 2014
X.f pauca		*P. italosignus,* *N. campestris*	Cavalieri *et al.* 2019
X.f pauca ST13	CVC	*B. xanthophis* *M. leucomelas*	Esteves *et al.* 2019
X.f pauca ST53	OQDS	*P. spumarius*	Cavalieri *et al.* 2019; Catalano *et al.* 2022
X.f fastidiosa	CVC	*O. nigricans* *H. vitripennis*	Hopkins and Purcell 2002
X.f fastidiosa	PD	*H. vitripennis*	Kyrkou *et al.* 2018; Burbank *et al.* 2020
X.f ST1	PD	*P. spumarius*	Moralejo *et al.* 2019
X.f fastidiosa	PD	*K. paulula* *B. ferruginea*	Lin and Chang 2012; Tuan *et al.* 2016

SHORT COMMUNICATION

First report of prunus necrotic ringspot virus infecting rose (*Rosa* spp.) in Lebanon

R. Abou Kubaa[1,2*], E. Choueiri[3], F. Jreijiri[3] and A. Minafra[1]

Summary Prunus necrotic ringspot virus (PNRSV) was detected in several rose plants showing symptoms of rose mosaic disease (RMD) in Beqaa valley, Lebanon. PNRSV was found in 29 plants by molecular and serological analyses, while other viruses associated with RMD were absent. Although PNRSV is known to have a wide host range, the present paper reports the first occurrence of PNRSV on rose plants in Lebanon.

Additional keywords: PNRSV, RMD, RT-PCR, Sequencing

Rose (*Rosa* spp.), a woody perennial flowering plant, belongs to *Rosa* genus, in the family Rosaceae and is considered the most common type of flowering shrub. More than 200 species belong to the *Rosa* genus, of which more than half are polyploids (Fougère-Danezan *et al*., 2015). Rose is the most popular ornamental plant with high economic and cultural importance as garden plant and raw material in the perfume industry in Lebanon as well as worldwide.

Rose mosaic disease (RMD) is generally caused by viruses belonging to two genera (i) *Ilarvirus* [prunus necrotic ringspot virus (PNRSV) and apple mosaic virus (ApMV)] and (ii) *Nepovirus* [arabis mosaic virus (ArMV) and strawberry latent ringspot virus (SLRSV)]. These viruses may occur in rose plants in single or mixed infections. In general, variable symptoms can be associated with RMD, depending primarily on rose cultivars, virus agents and environmental factors (Chen *et al*., 2022). Symptoms on leaves usually associated with RMD include ring spots and intense yellow spots, severe distortion and mottling, chlorotic line patterns, yellow vein banding and puckering (da Silva *et al*., 2018). Low flower production and flower deformation sometimes accompanied by reduced size are also observed. Infected plants drop leaves easily in autumn and become more sensitive to cold (Cochran, 1972; 1982; Secor *et al*., 1977; Thomas, 1984).

During spring 2021, RMD symptoms consisting of chlorotic zigzag pattern, squiggly discoloration with jagged edges on leaves and/or ringspots were observed on several red rose plants (*Rosa* spp.) in Douris, Zahle, Kab-Elias and Saghbine (Beqaa valley), Lebanon (Figure 1). Leaf samples from 29 symptomatic plants were collected and subjected to enzyme-linked immunosorbent assay (DAS-ELISA) tests, using commercial antibodies (LOEWE, Germany) against PNRSV, ApMV and ArMV, which are the three most common viruses associated with RMD (Yardimci and Çulal, 2009). Asymptomatic rose seedlings taken from a nursery located in Beqaa Valley were included as negative controls. All symptomatic plants reacted positively with the PNRSV antiserum, while ArMV and ApMV were not detected, thus excluding the occurrence of mixed infections with these viruses. The presence of PNRSV was confirmed by RT-PCR us-

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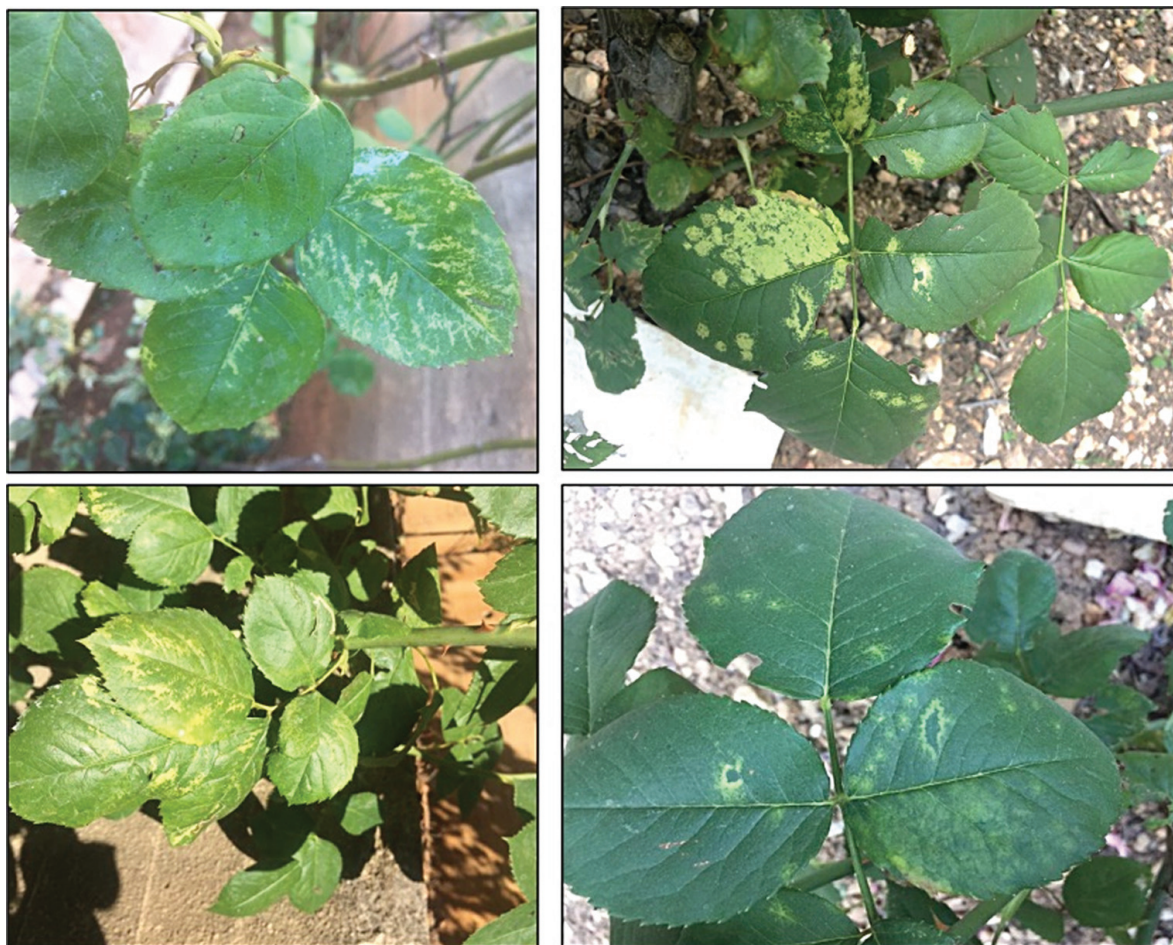
Figure 1. Symptoms of rose mosaic disease (RMD) caused by PNRSV on naturally infected rose plants in Lebanon.

ing primers: NRSCPF3/NRSCPR4 (Malinowski and Komorowska, 1998). Two amplicons (700 bp) were selected, purified by QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced (Macrogen, Korea) in both directions. Bioinformatics tools used for sequence analyses and for local alignments at nucleotide level included NCBI BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi), BioEdit (Hall, 1999) and MEGA11 (Tamura *et al.*, 2021) software. Sequences from the two different isolates showed high similarity (99.8%) at nucleotide level.

The Lebanese isolate PNRSV-M2 was deposited in GenBank under accession number OM650244. Blast analysis revealed 99.2% nucleotide identity with isolates RM-2 (AY948440) and RM-5 (AY94841), two PNRSV isolates causing oak leaf pattern and mosaic symptoms on rose in India. Moreover, a total of 13 different isolates of PNRSV were obtained from NCBI database in order to assess the phylogenetic relationships between the detected PNRSV isolate from Lebanon and other isolates distributed all over the world. Phylogenetic analysis clustered the Lebanese isolate in the same clade with the two isolates from India (Figure 2A). These results were further confirmed by a sequence dramatic tool (SDT) that can show pairwise identity scores using a color-coded pairwise identity matrix (Muhire *et al.*, 2014). Nucleotide identities between PNRSV-M2 and isolates from other countries are shown in Figure 2B. PNRSV has been isolated in many rose growing regions worldwide (Rakhshandehroo *et al.*, 2006). In Lebanon, PNRSV has already been reported on peach trees (Choueiri *et al.*, 2001) showing high level (61.2%) of infection.

To our knowledge, this is the first report of natural occurrence of PNRSV on rose
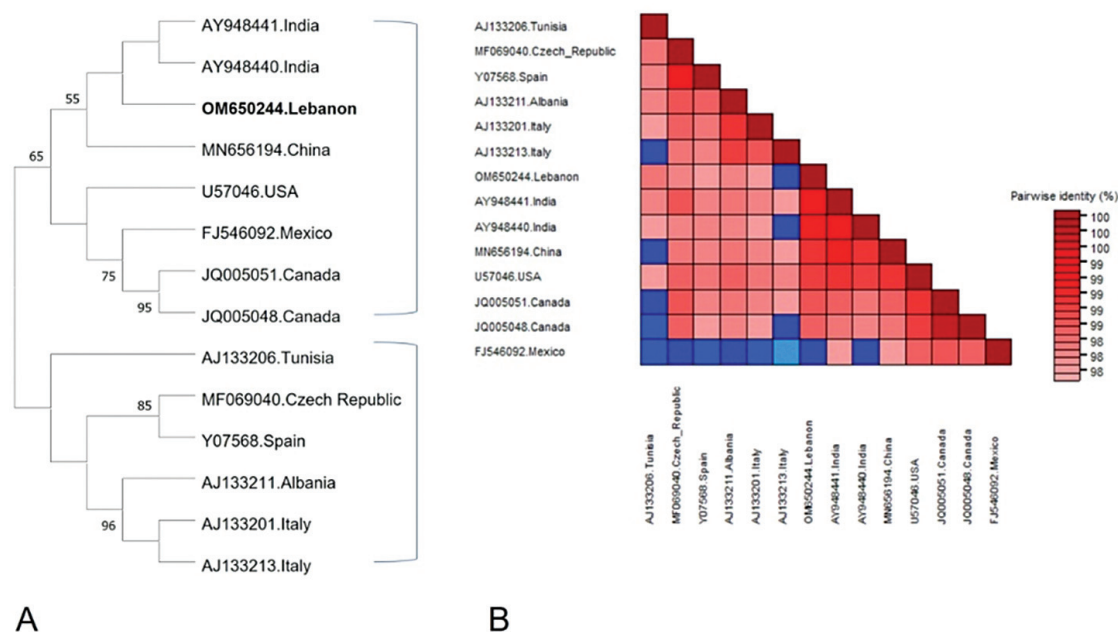
Figure 2. (A) Phylogenetic tree of prunus necrotic ringspot virus (PNRSV) isolates constructed by the Neighbor-Joining method using the 700 nucleotide sequences of the coat protein genes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Only node values above 50% are shown. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.,* 2021), (B) Color-coded pairwise identity matrix generated using the Sequence Demarcation Tool (SDT) software by alignment of 700 bp fragment from CP gene of PNRSV isolate from Lebanon and another 13 isolates distributed worldwide. Each colored square represents a percentage identity score between two sequences. The colored scale key indicates the correspondence between the colors appeared in the matrix and pairwise identities between isolates.

plants in Lebanon. This finding rises important issues concerning rose production. In fact, PNRSV is difficult to control, because it is transmitted in several modes, mainly by grafting (Seitadzhieva *et al*., 2022), by seeds and by some pollinator insects. This is additive to the absence of certified virus-free plants, which is one of the main problems in commercial rose production in Lebanon. Phytosanitary aspects need to be addressed in the next future to limit spread of PNRSV and further studies need to be undertaken to evaluate the impact of PNRSV on rose plants in Lebanon, as well as on other host plant species in the country.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη αναφορά του ιού της νεκρωτικής δακτυλιωτής κηλίδωσης των πυρηνοκάρπων σε φυτά τριανταφυλλιάς (*Rosa* spp.) στον Λίβανο

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Περίληψη   Ο ιός της νεκρωτικής δακτυλιωτής κηλίδωσης των πυρηνοκάρπων (prunus necrotic ringspot virus, PNRSV) ανιχνεύθηκε σε φυτά τριανταφυλλιάς που εμφάνιζαν συμπτώματα της ασθένειας του μωσαϊκού της τριανταφυλλιάς (RMD) στην κοιλάδα Beqaa, Λίβανος. Ο PNRSV βρέθηκε σε 29 φυτά με μοριακές και ορολογικές αναλύσεις, ενώ δεν ανιχνεύτηκαν άλλοι ιοί που σχετίζονται με το μωσαϊκό της τριανταφυλλιάς. Αν και είναι γνωστό ότι ο PNRSV έχει ευρύ φάσμα ξενιστών, η παρούσα εργασία αποτελεί την πρώτη αναφορά εμφάνισης του PNRSV σε φυτά τριανταφυλλιάς στον Λίβανο.

Effects of adjuvants on the effectiveness and rainfastness of rimsulfuron in potato

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Summary   Adjuvants are used with herbicides to increase their efficacy. In this study, it was demonstrated that the application of 0.1% of the non-ionic surfactant (NIS, Contact) and 0.5 L ha^{-1} of a crop oil concentrate (COC, Renol) did not improve the efficacy of rimsulfuron on *Amaranthus retroflexus*. In contrast, the same treatments enhanced performance and rainfastnes of rimsulfuron in *Chenopodium album*. Increasing non-ionic surfactant concentration to 1 L ha^{-1} increased rimsulfuron performance around 10-fold for *A. retroflexus* compared to around 3-fold for *C. album*. The same treatment reduced 12-fold the rainfastness of rimsulfuron on *A. retroflexus* while improving 2-fold the rainfastness of rimsulfuron on *C. album*. Measured ED_{50} and ED_{90} doses of rimsulfuron indicated that the addition of the 0.2 L ha^{-1} of NIS improved the recommended (60 g a.i. ha^{-1}) and the reduced (30 g a.i. ha^{-1}) dose effect of rimsulfurom in potato crop in the field. The highest potato yield was recorded (60 tons per ha) when 60 g ai. ha^{-1} of rimsulfuron was applied at three growth stages [leaf development (S1) + vegetatively propagated organs (S4) + development of tuber (S7)] of the crop without using a NIS; not significant differences were measured when the same dose of rimsulfuron was applied at the three (S1, S4, and S7) and two (S1, S4) growth stages with NIS.

Additional keywords: chemical control, dose-response, growth stages, herbicide efficacy, surfactant

Introduction

The most important weeds in potato crop in Iran are *Amaranthus* spp., *Chenopodium album* L., *Portulaca oleracea* L., *Polygonum* spp., *Setaria* spp., *Echinochloa crus gali*, *Hordeum leporinum* and *Lolium* spp. (Hasaninasab Farzaneh *et al*., 2018). Rimsulfuron is a sulfonylurea herbicide (applied at both pre- and post- emergence) which is selective and effective in potato crop for the control of a range of grass and broadleaved weeds except from *Solanum sarrachoides* (L.) (Robinson *et al*., 1996). Pre- and post- emergence application of rimsulforun effectively controlled *C. album* and *A. retroflexus* (Tonks and Eberlin, 2001; Alebrahim *et al*., 2012). On the other hand, the intensive use of metribuzin has caused an increasing number of resistant species (Alebrahim *et al*., 2012), including *S. sarachoides* and *Solanum triflorum* (Eberlein *et al*., 1992). Also some potato cultivars are not tolerant to metribuzin (Arsenault and Ivany, 1996).

It is generally accepted that the optimization of herbicide use allows dose reductions. To optimize herbicide use, aspects such as the best time of application (Samadi Kalkhoran and Alebrahim, 2016) and appropriate use of adjuvants are investigated and in many studies have shown efficient weed control with reduced herbicide doses (Blachshaw *et al*., 2006). An adjuvant is a biologically inert substance, but when it is added to herbicide spraying solutions could enhance retention, coverage, and efficacy of the applied herbicide (Green and Beestman, 2007). Improving the penetrability of the active ingredients will increase the effect of herbicide and will reduce side effects risk (Penner, 2000). Numerous types of adjuvants exist such as surfactants, oils, and fertilizers while surfactants are classified as non-ionic, anionic, cationic, or zwitterionic ones (Schramm, 2003). Herbicide applications carry the risk of reduced efficacy if

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rainfall occurs shortly after application. Adjuvants would affect the herbicide's rainfastness which is another reason to consider when selecting an adjuvant (Kudsk *et al.*, 1989).

Rimsulfuron needs an adjuvant to optimize its efficacy and rainfastness similarly to other ALS inhibitors (Green and Green 1993; Mathiassen and Kudsk, 2002; Pannacci, 2010). Since the type and the concentration of the applied adjuvant could influence rimsulfuron activity, Tonks and Eberlein (2001) applied rimsulfuron at 4 different dosages (9, 18, 26, 35 g ha^{-1}) with different types of adjuvants such as a nonionic surfactant (NIS), a crop oil concentrate (COC), a methylated seed oil (MSO), or an organosilicon surfactant (SIL). The mean control of the common lambsquarters (*C. album*) reached 75%, regardless of the type of adjuvant or rimsulfuron dose .

Amaranthus retroflexus and *C. album* are two problematic weeds in cultivation of potato (Alebrahim *et al.*, 2012), given the limited available herbicides for their control. Therefore, it is important to investigate solutions for their effective control. The objectives of the current study are to demonstrate the efficacy and rainfastness of optimized doses of rimsulfuron when applied together with NIS and COC adjuvants in controlling *A. retroflexus* and *C. album* in greenhouse and field experiments.

Materials and methods

First outdoor pot experiment

The experiments were carried out at the Department of Agroecology, the University of Aarhus, Denmark (55° 19′ N, 11° 23′ E) in 2011. Two experiments were conducted in pots outdoors. *Chenopodium album* and *A. retroflexus* seeds were obtained from the seedbank of the University of Aarhus, Denmark. For each plant species, twenty seeds were sown per pot in 2 L plastic pots filled using an automatic seed sowing machine. The potting mixture included: sandy loam soil, sand, and peat (2:1:1 w/w/w respectively, 4.2% organic matter and a pH of 6.9). The pots were sub-irrigated five times a day by deionized water after seedling emergence, only four plants were kept in each pot. At four to six-leaf growth stages, plants were treated by the herbicide. Commercial rimsulfuron (Titus DF, 250 g kg^{-1}) were used in a laboratory pot sprayer equipped with two flat–fan nozzles (4110-14; Hardi; Taastrup, Denmark) working at 260 kpa and delivering 155.3 l ha^{-1}. Rimsulfuron was used solo and in a mixture of surfactants. The treatments used are shown in Table 1. The plants were subjected to 5 mm rain at different times by a rain simulator which was working at an intensity of 9 mmh^{-1} (Pannacci *et al.*, 2010). A factorial experiment with three factors was applied: The first factor was the rimsulfuron doses (seven levels: 0, 1.872, 3.75, 7.5, 15, 30, 60 g a.i. ha^{-1} for *C. album* and 0, 0.2343, 0.468, 0.9375, 1.875, 3.75, 7.5 g ha^{-1} for *A. retroflexus*); the second factor was the type of surfactant (two levels: Contact (a non-ionic surfactant, 980 g/l linear alcohol ethoxylate, from Nufarm company (NIS), 0.1%), Renol (a crop oil concentrate (COC), 0.5 L ha^{-1})); the third factor was the time of 5 mm rain (four levels: 0, 1, 2, 4 hours after treatment (HAT) (Table 1). The pots were located outdoors adopting a factorial experiment of a completely randomized block design with three replications. Plants were harvested three weeks after the herbicide application and fresh weight (all plants/pot) of the above ground biomass was measured. Afterwards, the fresh biomass was oven-dried at 75°C for 48 h and then the dry weight was measured.

Second outdoor pot experiment

In the second outdoor pot experiment, plants of *C. album* and *A. retroflexus* were sprayed with the same rimsulfuron doses and exposed to the same rain treatments, as in the first outdoor experiment. The NIS (Contact) was used in 0, 0.04, 0.2, and 1 L ha^{-1} for *C. album* and *A. retroflexus* (Table 1). The plants were harvested three weeks after the herbicide application and fresh weight (all plants/pot) of the above ground biomass

Table 1. The list of treatments for all the experiments that refer to rimsulfuron and surfactants doses; the first and second outdoor pot experiments and the two field experiments. NIS: non-ionic surfactant, COC: crop oil concentrate, and HAT: hours after treatment, the application timings (see Table 2) were as follows: i) S1: Leaf development; ii) S1+S4: leaf development (S1) + vegetatively propagated organs (S4); and iii) S1+S4+S7: leaf development (S1) + vegetatively propagated organs (S4) + Development of tuber (S7).

First outdoor pot experiment				
Species	Rimsulfuron (g a.i. ha^{-1})	Contact (NIS)	Renol (COC)	Rain
Chenopodium album	0, 1.875, 3.75, 7.5, 15, 30, 60	0.1%	0.5 L ha^{-1}	0, 1, 2 ,4 HAT
Amarathus retroflexus	0, 0.2343, 0.468, 0.9375, 1.875, 3.75, 7.5	0.1%	0.5 L ha^{-1}	0, 1, 2 ,4 HAT
Second outdoor pot experiment				
Species	Rimsulfuron (g a.i. ha^{-1})	Contact (NIS)	Renol (COC)	Rain
Chenopodium album	0, 1.875, 3.75, 7.5, 15, 30, 60	0.04. 0.2, 1 L ha^{-1}	-	0, 1, 2 ,4 HAT
Amarathus retroflexus	0, 0.2343, 0.468, 0.9375, 1.875, 3.75, 7.5	0.04. 0.2, 1 L ha^{-1}	-	0, 1, 2 ,4 HAT
Field experiment (Reduced dose)				
Species	Rimsulfuron (g ai. ha^{-1})	Contact (NIS)	Renol (COC)	Application time
Chenopodium album	0, 5, 10, 15, 20, 25, 30	0.2 L ha^{-1}	-	S1, S1+S4, S1+S4+S7
Amarathus retroflexus	0, 5, 10, 15, 20, 25, 30	0.2 L ha^{-1}	-	S1, S1+S4, S1+S4+S7
Field experiment (Recommended dose)				
Species	Rimsulfuron (g ai. ha^{-1})	Contact (NIS)	Renol (COC)	Application time
Chenopodium album	0, 10, 20, 30, 40, 50, 60	0.2 L ha^{-1}	-	S1, S1+S4, S1+S4+S7
Amarathus retroflexus	0, 10, 20, 30, 40, 50, 60	0.2 L ha^{-1}	-	S1, S1+S4, S1+S4+S7

was measured. Afterwards, the fresh biomass was oven-dried at 75°C for 48 h and then the dry weight was measured.

Field experiments

Two field experiments were conducted at Sheikh Kalkhoran of Ardabil (48° 20′ N, 38° 15′ E) in Iran, on loam soil (42% sand, 32% silt, and 26% clay), in 2015. Soil organic matter and pH were 0.9% and 7.68, respectively. Fertilizers were applied through the irrigation system according to soil test recommendations during the growing season. Seed potatoes (cv. Agria) were planted at 10 cm depth and 25 cm distance in rows separated 75 cm apart from each other on April 21, 2015, after soil tillage on October 13, 2014. Plots were sprayed against in-

sects and diseases as needed and irrigated during the growing season with overhead sprinklers to obtain 65% minimum soil water content. Two factorial experiments of a complete randomized block design were conducted with three replications at a plot size of 2*4 m^2. There were three factors on *C. album* and *A. retroflexus* with reduced or the recommended doses. The first factor was rimsulfuron doses, either reduced (0, 5, 10, 15, 20, 25, 30 g ai. ha^{-1}) or the recommended (0, 10, 20, 30, 40, 50, and 60 g ai. ha^{-1}); the second factor was Contact surfactant doses (0 and 0.2 L ha^{-1}) and the third factor was the time of application (S1, S1+S4, S1+ S4+S7) on *C. album* and *A. retroflexus* (Table 1). In both experiments, rimsulfuron was applied at leaf development (S1, one stage), leaf develop-

ment (S1) + vegetatively propagated organs (S4) (two stages), and leaf development (S1) + vegetatively propagated organs (S4) + development of tuber (S7) (three stages) (Meier *et al.*, 2009) (Table 2). Commercial rimsulfuron (Titus DF, 250 g kg^{-1}) was applied by using a back-pack sprayer equipped with two flat–fan nozzles (8001; Inter) worked at 260 kpa and delivering 250 L ha^{-1}. Weed control was evaluated by harvesting weed plants within a quadrat of (0.75× 0.50 m^2) at three weeks after treatment. Three weeks after the herbicide application, all of the plants in each plot were harvested and dried in an oven at 75°C for 48 h and then weighed. For determination of total tuber yield per hectare, the tubers of center rows in each plot were harvested and weighted.

Statistical Analyses

The dose-response data were analyzed using the R (R Core Team, 2020) program (Version 4.0.1) with the drc package (Version 4.0.1). The log-logistic regression of dry weight of *C. album* and *A. retroflexus* on dose was fitted with a log-logistic regression using a three parameters log-logistic model (Streibig *et al.*, 1993):

$$y = \frac{d}{1 + \exp[b(\log(z) - \log(ED_{50})]} \quad (1)$$

where y is dry weight z, d is the upper limit where the dose is zero, ED_{50} denotes the dose required for reducing dry weight at 50%, and bis proportional to the slopes of the curves around ED_{50}. The ED_{50} parameter in Eq. (1) can be replaced by any ED level (Pannacci and Covarelli, 2009), (e.g., ED_{10}, ED_{90}).

The relative potency (RP) is the horizontal displacement between two curves at a chosen ED_x (Kudsk *et al.*, 1989). The biological exchange rate in a single application, i.e. the relative potency between the herbicides was calculated as follows:

$$RP = ED_{50xf}/EDC_{50xf+v} \quad (2)$$

Where, ED_{xf} = the ED_x of herbicide alone; and ED_{xf+v}= the ED_x of herbicide in mixture with a surfactant. If RP = 1, the addition of surfactant does not affect herbicide performance. If RP was significantly higher or lower than 1, the herbicide in the mixture with the surfactant was more or less potent than the herbicide alone, respectively. Similarly, the rainfastness can be assessed by calculating the ratio between the ED_x dose with rain and the corresponding ED_x dose without rain.

Statistical analyses of yield data were performed using SAS (9.1) software. An ANOVA was performed using PROC GLM (PC-SAS) and when the F test indicated significant effects, means were compared using LSD Fisher (*P < 0.05*) test.

Table 2. Application times of rimsulfuron in relation to the principal potato growth stages in the field experiments.

Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth/shoot development (main shoot)
4	Development of harvestable vegetative plant parts or vegetatively propagated organs/ booting (main shoot)
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

Results

First outdoor pot experiment

Regarding the efficacy, the results showed no significant improvement on rimsulfuron performance on *A. retroflexus* with the addition of the adjuvant 0.1% NIS or 0.5 COC (compare ED_{50} values of the 'no rain' treatments) (Table 3). In contrast, the inclusion of the 0.1% NIS adjuvant significantly improved the performance of rimsulfuron on *C. album* (Table 3).

Regarding the rainfastness, 0.1% NIS and 0.5 COC provided a high level of rainfastness comparable to that of COC on *A. retroflexus* (Table 3). In *C. album*, the ED_{50} doses were much higher in comparison with *A. retroflexus* and the non-ionic surfactant strongly improved the efficacy of rimsulfuron and had a strong effect on rainfastness while no significant impact of rain with Renol was shown (Table 3). Also, the results showed that rainfastness was low without adjuvant and with 0.5 L ha^{-1} COC, but the inclusion of 0.1% NIS significantly improved the rainfastness; full rainfastness of rimsulfuron was obtained only after 4 hours (Table 3).

Second outdoor pot experiment

In the second pot experiment, the re-

Table 3. The effect of NIS and COC adjuvants on the effectiveness and rainfastness of rimsulfuron [applied at various timings: 1, 2, 4 HAT (hours after treatment)] on *Amaranthus retroflexus* and *Chenopodium album*. Relative rainfastness is the necessary length of a rain-free period following a herbicide application. Different superscripts in the ED_{50} column indicate differences between ED_{50} values for each weed species.

Adjuvant	Rain	ED_{50} (g a.i. ha^{-1})	Confidence intervals	Relative rainfastness
Amaranthus retroflexus				
None	No rain	0.28[abc]	0.201-0.368	1.00
	1 HAT	4.68[ijk]	3.318-6.036	16.71
	2 HAT	1.59[fgh]	1.069-2.103	5.68
	4 HAT	1.34[f]	0.937-1.743	4.79
0.1 % NIS	No rain	0.26[ab]	0.182-0.345	1.00
	1 HAT	0.55[de]	0.372-0.732	2.12
	2 HAT	0.51[d]	0.363-0.657	1.96
	4 HAT	0.28[abc]	0.184-0.382	1.08
0.5 L ha^{-1} COC	No rain	0.25[a]	0.179-0.330	1.00
	1 HAT	3.76[ij]	2.294-5.235	15.04
	2 HAT	3.74[i]	2.407-5.064	14.96
	4 HAT	1.44[fg]	0.910-1.970	5.76
Chenopodium album				
None	No rain	26.47[e-i]	-14.78-67.72	1.00
	1 HAT	24.56[e-h]	6.13-42.98	0.93
	2 HAT	27.34[e-k]	-3.60-58.29	1.03
	4 HAT	31.16[e-l]	6.55-55.77	1.18
0.1 % NIS	No rain	1.89[a]	1.11-2.69	1.00
	1 HAT	4.18[d]	1.60-6.77	2.20
	2 HAT	2.06[ab]	0.85-3.28	1.08
	4 HAT	2.36[abc]	0.92-3.80	1.24
0.5 L ha^{-1} COC	No rain	13.28[e]	1.60-24.96	1.00
	1 HAT	21.44[efg]	5.09-37.79	1.61
	2 HAT	26.58[e-j]	3.39-49.77	2.00
	4 HAT	16.69[e-i]	-2.26-35.65	1.26

sults showed that increasing the concentration of the NIS up to 1 L ha^{-1} improved the performance of rimsulfuron (Table 4). At 1 L ha^{-1}, the surfactant increased the efficacy of rimsulfuron on *A. retroflexus* around 10-fold compared to rimsulfuron alone. On the contrary, increasing the concentration of the NIS sharply improved the performance of rimsulfuron on *C. album* (Table 4). Herbicidal performance was slightly more enhanced as concentration increased from 0.04 to 1 L ha^{-1}. At 1 L ha^{-1}, surfactant increased rimsulfuron performance on *C. album* around 3-fold compared to rimsulfuron alone which was less compared to *A. retroflexus* (Table 4).

The rainfastness of rimsulfuron on *A. retroflexus* reduced with an increase in surfactant concentration up to 1 L ha^{-1} up to 12-fold (Table 4). On the contrary, increasing the concentration of the NIS sharply improved the rainfastness of rimsulfuron on *C. album* to 2.5-fold (Table 4). Each species had some distinct differences to the single surfactant properties. Regarding herbicidal performance, the non-ionic surfactant was less efficient on *C. album* compared to *A. retroflexus,* but when tested for rainfasness showed a higher effectiveness especially at 1 L ha^{-1} for *C. album* (Table 4). The spray deposit on the leaf surface is strongly related to cuticular

Table 4. The effect of NIS adjuvant doses on the effectiveness and rainfastness of rimsulfuron [applied at various timings: 1, 2, 4 HAT (hours after treatment)] on *Amaranthus retroflexus* and *Chenopodium album*. Relative rainfastness is the necessary length of a rain-free period following a herbicide application. Different superscripts in the ED$_{50}$ column indicate differences between ED$_{50}$ values for each weed species.

Adjuvant	Rain	ED$_{50}$ (g a.i. ha^{-1})	Confidence intervals	Relative rainfastness
Amaranthus retroflexus				
0.04 L ha^{-1} NIS	No rain	0.33^{cd}	0.219-0.437	1.00
	1 HAT	0.96^{ij}	0.701-1.219	2.38
	2 HAT	0.59^{fg}	0.433-0.757	1.49
	4 HAT	0.43^{de}	0.302-0.553	1.16
0.2 L ha^{-1} NIS	No rain	0.12^{b}	0.051-0.196	1.00
	1 HAT	1.10^{jk}	0.789-1.406	4.22
	2 HAT	0.47^{def}	0.305-0.640	2.30
	4 HAT	0.43^{de}	0.306-0.559	1.73
1 L ha^{-1} NIS	No rain	0.03^{a}	-0.006-0.069	1.00
	1 HAT	0.73^{hi}	0.513-0.939	24.3
	2 HAT	0.70^{gh}	0.523-0.878	23.3
	4 HAT	0.29^{c}	0.191-0.396	9.67
Chenopodium album				
0.04 L ha^{-1} NIS	No rain	5.37^{b-f}	1.44-9.30	1.00
	1 HAT	23.38^{ij}	7.89-38.86	4.35
	2 HAT	23.91^{jk}	1.62-46.21	4.45
	4 HAT	13.47^{e-i}	6.15-20.79	2.51
0.2 L ha^{-1} NIS	No rain	3.66^{a-d}	2.26-5.05	1.00
	1 HAT	9.86^{d-h}	4.50-15.21	2.69
	2 HAT	6.61^{b-g}	3.39-9.83	1.81
	4 HAT	5.17^{a-f}	2.71-7.62	1.41
1 L ha^{-1} NIS	No rain	1.75^{a}	0.84-2.66	1.00
	1 HAT	4.20^{a-e}	2.24-6.16	2.40
	2 HAT	2.97^{abc}	1.46-4.47	1.70
	4 HAT	2.38^{ab}	1.21-3.56	1.36

structure and the adjuvant chemical tendency for retention on the leaf. Thus, the appropriate surfactant type and concentration increased rimsulfuron effectiveness by 10-fold. NIS efficacy was much higher compared to Renol. A surfactant concentration of 1 L ha^{-1} was required for maximum herbicidal performance. However, regarding to rainfastness, the results were different between the weed species.

Field experiments

The results showed that increasing the doses of rimsulforun up to 60 g ai. ha^{-1} increased control of both *C. album* and *A. ret-* *roflexus*. The assessment at three weeks after treatment demonstrate that rimsulforun at 40, 50, and 60 g ai. ha^{-1} in two (S1+S4) and three (S1+S4+S7) stages application provided 100% control of *C. album* and *A. retroflexus* (Figures 1, 2). Application of rimsulforun at 25 and 30 g ai. ha^{-1} (i.e. 0.25X and 0.5X of the recommended dose) applied at three crop stages (S1+S4+S7) provided excellent control of both weed species (Figures 1, 2). When rimsulforun was combined with 0.2 L ha^{-1} NIS, *C. album* and *A. retroflexus* control was only slightly increased.

Tables 5 and 6 show the ED$_{50}$ values of the surfactants applied alone and in combi-
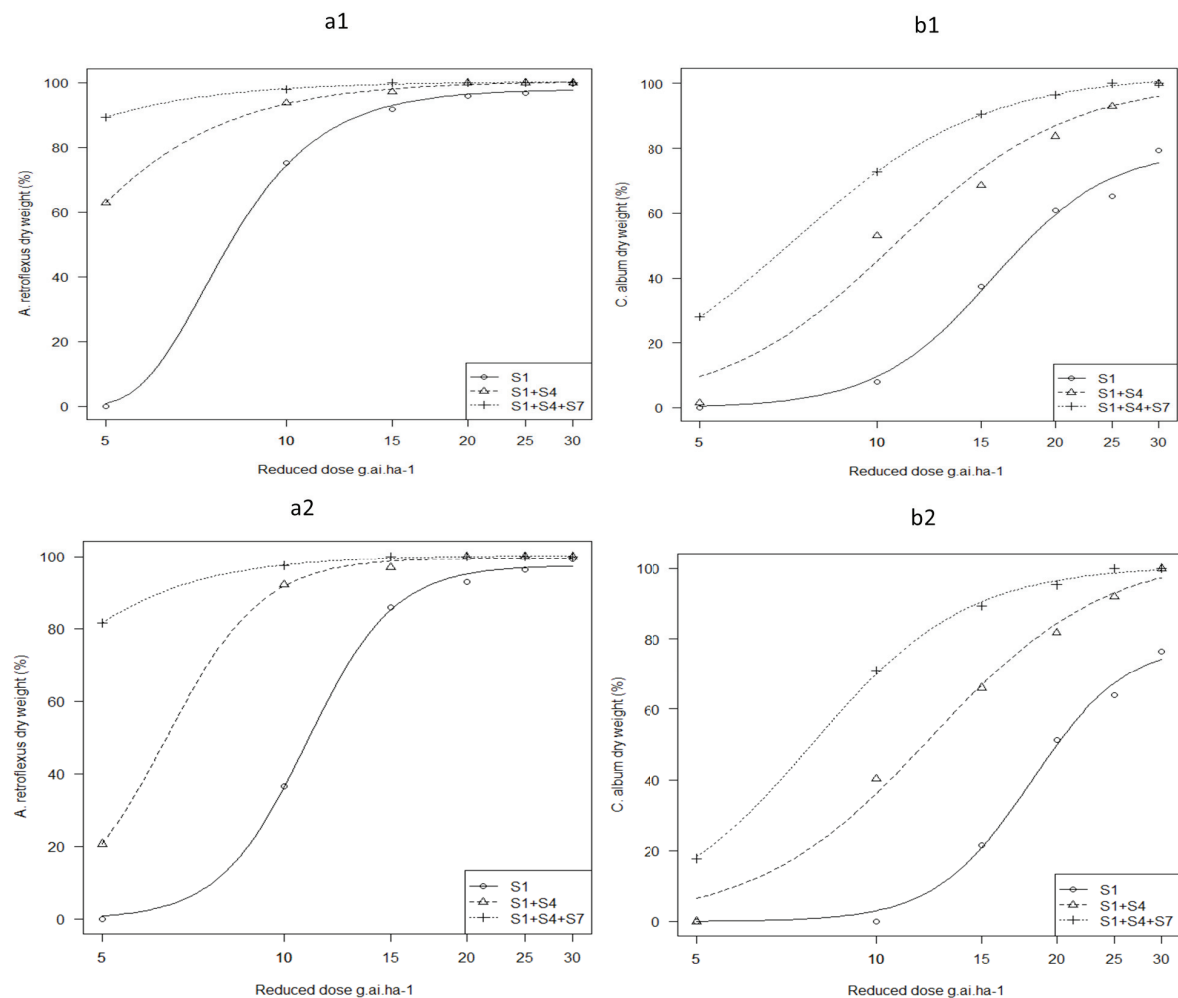
Figure 1. The effect of rimsulfuron application on dry weight of (a) *Amaranthus retroflexus* and (b) *Chenopodium album* when applied at a reduced dose (1X: 30 g a.i. ha-1) without (a1, b1) and with (a2, b2) NIS surfactant (0.2 L ha-1) at three cropping timings. The application timings (see Table 2) were as follows: i) S1: Leaf development; ii) S1+S4: leaf development (S1) + vegetatively propagated organs (S4); and iii) S1+S4+S7: leaf development (S1) + vegetatively propagated organs (S4) + Development of tuber (S7).
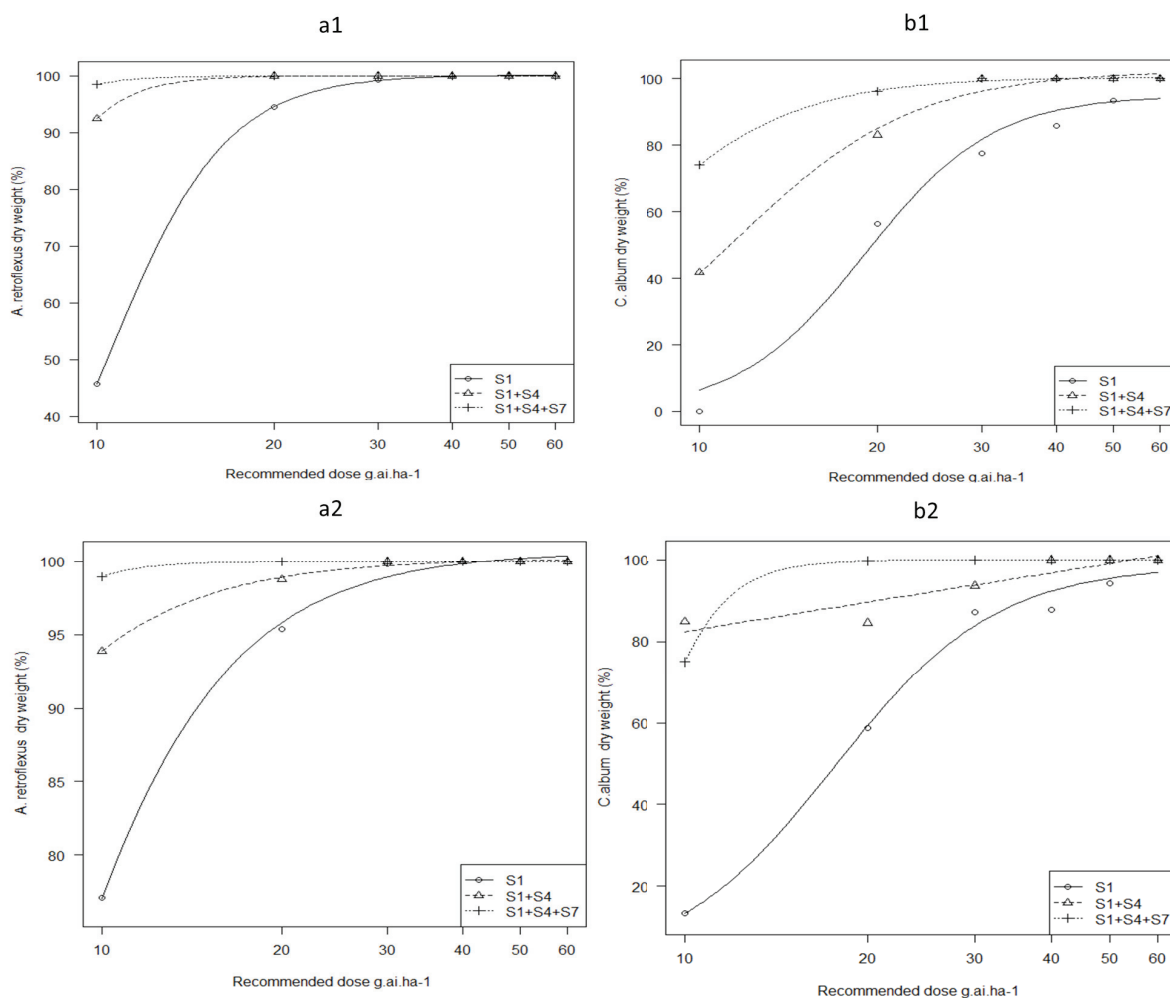
Figure 2. The effect of rimsulfuron application on dry weight of (a) *Amaranthus retroflexus* and (b) *Chenopodium album* when applied at the recommended dose (1X: 60 g a.i. ha-1) without (a1, b1) and with (a2, b2) NIS surfactant (0.2 L ha-1) at three cropping timings. The application timings (see Table 2) were as follows: i) S1: Leaf development; ii) S1+S4: leaf development (S1) + vegetatively propagated organs (S4); and iii) S1+S4+S7: leaf development (S1) + vegetatively propagated organs (S4) + Development of tuber (S7).

nation with rimsulfuron, at different growth stages of potato. The curves' upper limits were different for various doses and application time for each species, in some cases, the curves were crossing. The parameters ED_{10}, ED_{50}, ED_{90}, and b were estimated at each application time by fitting Eq. (1) for the *C. album*, and *A. retroflexus* biomass reduction. The ED_{50} values of rimsulforun were significantly reduced two to three times for *A. retroflexus* at reduced dose of rimsulfuron. For *A. retroflexus*, the ED_{50} values of the reduced doses rimsulforun experiment (applied at one, two, three stages without surfactant), were 10.88, 6.37 and 3.09 g ai ha^{-1} vs. 7.27,

3.77 and 2.03 g ai ha^{-1} (applied at one, two, three stages with 0.2 L ha^{-1} NIS), (Table 5).

The reduced dose-response curves described the response well. The ranking of ED_{50} at reduced dose for the three application times was S1>S1+S4>S1+S4+S7 with and without surfactant (Table 5). Based on the ED_{50} values given in Table 5, surfactants can improve the efficiency of rimsulfuron at reduced dose compared to rimsulforun alone. The ED_{50} values of rimsulfuron at reduced dose without surfactant were estimated at 18.11, 12.31, 7.81 g a.i. ha^{-1} on *C. album* after application at one, two or three potato growth stages, respectively; when

Table 5. The estimated regression parameters of the reduced rimsulfuron dose effect (1X: 30 g a.i. ha^{-1}) on *Amaranthus retroflexus* and *Chenopodium album* dry weight, when applied with and without NIS surfactant (1L ha^{-1}) at three application times as follows: i) S1: Leaf development; ii) S1+S4: leaf development (S1) + vegetatively propagated organs (S4); and iii) S1+S4+S7: leaf development (S1) + vegetatively propagated organs (S4) + Development of tuber (S7). The response variable is 0-100-percent weed control (dry weight reduction). The Relative potency (Rp) is explained in section 2.4. Different superscripts in the ED$_{50}$ column indicate differences between ED$_{50}$ values within each weed species.

Application time	slope	upper	ED$_{10}$	ED$_{50}$	ED$_{90}$	Rp
Reduced dose without surfactant on *Amaranthus retroflexus* dry weight						
S1	-6.04± 0.33	97.46 ± 0.78	7.56 ± 0.14	10.88 ± 0.09[f]	15.64 ± 0.38	1.49
S1+S4	-5.48 ± 0.27	99.58 ± 0.59	4.27 ± 0.07	6.37 ± 0.09[d]	9.52 ± 0.29	1.68
S1+S4+S7	-3.07 ±0.76	100.29 ± 0.73	1.51 ± 0.44	3.09 ± 0.35[b]	6.32 ± 0.43	1.52
Reduced dose with surfactant on *Amaranthus retroflexus* dry weight						
S1	-4.09 ± 0.13	97.94 ± 0.33	5.93 ± 0.09	7.27 ± 0.07[e]	12.60 ± 0.16	1.00
S1+S4	-2.69 ± 0.14	100.49 ± 0.39	2.77 ± 0.08	3.77 ± 0.06[c]	8.70 ± 0.28	1.00
S1+S4+S7	-2.38 ± 0.45	100.36 ± 0.42	1.43 ± 0.33	2.03 ± 0.33[a]	5.22 ± 0.15	1.00
Reduced dose without surfactant on *Chenopodium album* dry weight						
S1	-5.40 ± 0.47	79.05 ± 2.86	12.06 ± 0.32	18.11 ± 0.40[f]	27.20 ± 1.46	1.17
S1+S4	-3.00 ± 0.18	104.00 ± 2.66	5.92 ± 0.20	12.31 ± 0.34[d]	25.59 ± 1.72	1.13
S1+S4+S7	-3.37 ± 0.18	100.64 ± 1.21	4.07 ± 0.15	7.81 ± 0.16[b]	15.00 ± 0.68	1.08
Reduced dose with surfactant on *Chenopodium album* dry weight						
S1	-4.41 ± 0.58	79.74 ± 3.90	9.51 ± 0.47	15.46 ± 0.58[e]	25.73 ± 2.43	1.00
S1+S4	-2.97 ± 0.27	100.36 ± 3.32	5.09 ± 0.27	10.66 ± 0.43[c]	22.31 ± 2.22	1.00
S1+S4+S7	-2.71 ± 0.23	102.67 ± 2.15	3.19 ± 0.21	7.19 ± 0.25[a]	16.17 ± 1.44	1.00

combined with surfactant the ED$_{50}$ values were 15.46, 10.66, 7.19 g a.i. ha^{-1} , respectively (Table 6). The ED$_{50}$ values at reduced dose and various time of application for *C. album* were higher than *A. retroflexus*. Table 6 shows the same ED$_{50}$ ranking of the potency of the three application times at the recommended dose of the dose-response curves of *C. album* and *A. retroflexus*. The results of rimsulfuron on *C. album* dry weight showed that the NIS surfactant could not improve the efficacy of the recommended dose of rimsulfuron at S1+S4 and S1+S4+S7. The estimated ED$_{50}$ doses demonstrated that a reduced dose of rimsulfuron at S1+S4 and S1+S4+S7 provided full control of the two species at 30 g ai. ha^{-1}. The relative potency (RP) values were different when NIS were added to the herbicide. RP values showed that surfac-

tants can improve the efficacy of reduced and recommended doses of rimsulfuron for *A. retroflexus* at all application times, and reduced and recommended doses at all application times at S1 stage.
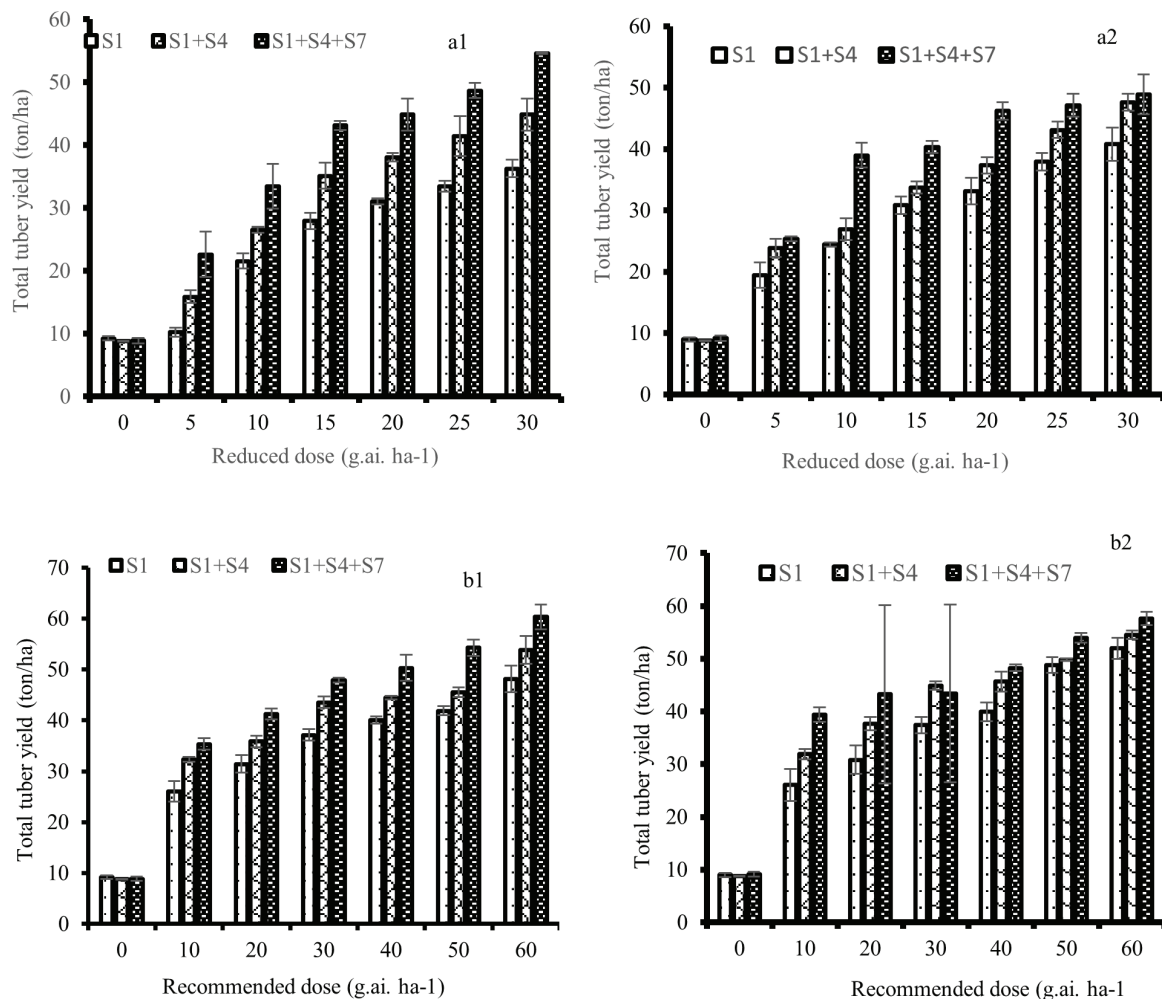
The effect of rimsulfuron application on total tuber yield, when applied at a reduced dose (1X: 30 g a.i. ha^{-1}) and the recommended dose (1X: 60 g a.i. ha-1) without and with NIS surfactant (0.2 L ha-1) at three crop stages (S1, S4, and S7), is presented in Figure 3. The highest potato yield was measured (60 tons per ha) when 60 g ai. ha^{-1} rimsulfuron was applied at all stages without using NIS. No significant differences were measured when the same dose of rimsulfuron was applied with NIS at three (S1, S4, and S7) and two (S1, S4) potato crop stages.

Table 6. The estimated regression parameters of the recommended rimsulfuron dose effect (1X: 60 g a.i. ha^{-1}) on *Amaranthus retroflexus* and *Chenopodium album* dry weight, when applied with and without NIS surfactant (1L ha^{-1}) at three application times as follows: i) S1: Leaf development; ii) S1+S4: leaf development (S1) + vegetatively propagated organs (S4); and iii) S1+S4+S7: leaf development (S1) + vegetatively propagated organs (S4) + Development of tuber (S7). The response variable is 0-100-percent weed control (dry weight reduction). The Relative potency (Rp) is explained in section 2.4. Different superscripts in the ED_{50} column indicate differences between ED_{50} values within each weed species.

Recommended dose without surfactant on *Amaranthus retroflexus* dry weight						
Application time	slope	upper	ED_{10}	ED_{50}	ED_{90}	Rp
S1	-4.32 ± 0.25	100.19 ± 0.44	6.28 ± 0.17	10.41 ± 0.07[a]	17.27 ± 0.55	1.63
S1+S2	-6.18 ± 8.04	100.02 ± 0.37	4.67 ± 4.61	6.67 ± 3.49[a]	9.51 ± 0.61	2.07
S1+S2+S3	-6.73 ± 49.78	100.00 ± 0.36	3.87 ± 27.11	5.37 ± 24.61[a]	7.44 ± 16.14	1.14
Recommended dose with surfactant on *Amaranthus retroflexus* dry weight						
S1	-2.60 ± 0.33	100.68 ± 0.64	2.73 ± 0.44	6.35 ± 0.36[a]	14.76 ± 0.85	1.00
S1+S2	-2.37 ± 1.25	100.21 ± 0.68	1.27 ± 1.36	3.21 ± 1.86[a]	8.11 ± 0.87	1.00
S1+S2+S3	-6.00 ± 43.54	100.00 ± 0.39	3.24 ± 26.42	4.67 ± 25.69[a]	6.74 ± 19.15	1.00
Recommended dose without surfactant on *Chenopodium album* dry weight						
S1	-4.04 ± 0.35	94.93 ± 1.68	11.06 ± 0.49	19.05 ± 0.42[a]	32.81 ± 1.96	1.08
S1+S2	-2.85 ± 0.23	102.37 ± 1.43	5.30 ± 0.34	11.44 ± 0.30[a]	24.68 ± 1.83	0.88
S1+S2+S3	-3.10 ± 0.87	100.43 ± 1.27	3.54 ± 1.02	7.18 ± 0.67[a]	14.57 ± 1.71	0.83
Recommended dose with surfactant on *Chenopodium album* dry weight						
S1	-3.27 ± 0.51	98.69 ± 3.53	9.02 ± 0.97	17.62± 0.92[a]	3.44 ± 4.31	1.00
S1+S2	-0.24 ± 0.25	170.19 ± 145.92	0.001 ± 0.005	12.86 ± 89.96[a]	9.79 ± 1.56	1.00
S1+S2+S3	-7.19 ± 26.69	100.00 ± 2.00	6.32 ± 10.72	8.58 ± 4.82[a]	1.16 ± 6.68	1.00

Discussion

The optimum concentration of the active ingredient in post-emergence herbicides should be able to preserve its effectiveness for the critical period otherwise it could lead to inadequate weed control (Hess and Richard, 1990). Our results showed that ED_{50} rimsulfuron was ranked as S1+S4+S7< S1+S4< S1 with and without surfactant. Renner and Powel (1998) concluded that post-emergence application of rimsulforun at 18, 27 and 35 g ha^{-1} provided full control of *A. retroflexus* and 77, 84, and 91% of *C. album*, respectively. Also, Robinson *et al.* (1996) reported that post-emergence application of rimsulfuron at 9, 18, 27, 35, 70 g ha-1 controlled 67, 100, 100, 100, 100 % *A. retroflexus*, and 60, 90, 97, 90, 98% *C. album*, respectively. Rimsulfuron

at 30 g a.i ha^{-1} controlled *A. retroflexus* and *C.album* 100% at S1+S4 and S1+S4+S7 stages. Lanfranconi *et al.* (1993) reported that rimsulforun applied post-emergence at 9, 18, 27, 35, and 70 g ha^{-1} controlled *A. retroflexus* 67, 100, 100, 100, 100% and *C.album* 60, 90, 97, 90, and 98%, respectively. Similar results were reported by Renner and Powell (1998) that concluded that post-emergence application of rimsulfuron at 18, 27, 35 g ha^{-1} controlled *C. album* 77, 84 and 91% and of *A. retroflexus* 100, 100, and 100%, respectively. Khatami *et al.* (2017) reported that post-application of rimsulfuron provided 87.37, 99.75, and 96.87% control of *C. album* at 30, 40, and 50 g a.i ha^{-1} and 86.73, 89.91 and 95.50% for *A. retroflexus,* respectively.

For the enhanced activity of a post-emergence herbicide, high absorption and

translocation are necessary. Using the appropriate type of adjuvants is necessary to increase the efficacy of most post-emergence herbicides by, mostly, surface tension reduction (Kudsk, 2008). Our results in greenhouse and field trials showed that NIS was more effective to improve the rimsulfuron performance and rainfastness on both weed species. Increase of NIS concentration increased rimsulfuron activity around 10-fold for *A. retroflexus* compared to around 3-fold for *C. album;* also it reduced the rainfastness of rimsulfuron on *A. retroflexus* while it improved the rainfastness of rimsul-

furon on *C. album*. Previous studies showed that NIS slightly improved the performance of rimsulfuron on *C. album* and *A. retroflexus*in in field potato due to lower values of surface tension where ED_{50} was calculated. Hutchinson *et al.* (2004) suggested that using NIS with rimsulforun controlled *C. album* and *A. retroflexus* by 89% and 97%, respectively. Tonks and Eberlin (2001) concluded that rimsulfuron applied at 4 different doses (9, 18, 26, or 35 g/ ha) together with different types of surfactants, i.e. NIS, COC, a methylated seed oil (MSO), or an organosilicon surfactant (SIL), had moderate (average

Figure 3. The effect of rimsulfuron application on total tuber yield, when applied at (a) a reduced dose (1X: 30 g a.i. ha-1) and (b) the recommended dose (1X: 60 g a.i. ha-1) without (a1, b1) and with (a2, b2) NIS surfactant (0.2 L ha-1) at three cropping timings, The application timings (see Table 2) were as follows: i) S1: Leaf development; ii) S1+S4: leaf development (S1) + vegetatively propagated organs (S4); and iii) S1+S4+S7: leaf development (S1) + vegetatively propagated organs (S4) + Development of tuber (S7) (LSD=4.33).

75%) control of common lambsquarters, regardless of adjuvant or dose. Mamnoii *et al.* (2016) concluded that the use of rimsulfuron (60 g ha^{-1}) + 2.5 % citowet (non-ionic surfactant) controlled *A. retroflexus*, at an average of 54% and 96% in Jiroft and Karaj of Iran, respectively, in a two-year field experiment.

Chenopodium album had higher ED$_{50}$ than *A. retroflexus*. One explanation is that *A. retroflexus* possess leaf areas that are easier to be wettable compared with those of the *C. album* (Ren *et al.*, 2007). The present results are in line with findings in previous similar experiments (Medd *et al.*, 2001). Harr *et al.* (1991) report there were 66% polar components in the leaf surface of *C. album,* in comparison with 55% for *A. retroflexus*. Other studies report that a) the hair covering on the abaxial side of the *C. album* leaves and crystalline structure of hair led to less herbicide retention and penetration into leaf tissues, b) *A. retroflexus* leaf surface possessed smooth cuticular layer; c) the angle of the spray droplet was 76º on the *C. album* leaves in comparison with of 54º *on A. retroflexus* (Harr *et al.*, 1991). Therefore, we can possibly assume that higher amounts of herbicide are required to be absorbed, transferred, and reach the target in the photosynthetic system in *C. album*. Hence, weak herbicide performance of *C. album* could be associated with lower wettability of the leaf surface.

On the other hand, due to the hairy cuticle of *C. album* it appears that utilization of a NIS adjuvant could increase the herbicide rainfastness. The improvement of the rimsulfuron efficacy by NIS could be associated with the solubilization, softening, or disordering of the nature of cuticular waxes by the utilization of the non-ionic surfactant. Hence, it is possible that the surfactant could lead to better cuticle penetration and stromal infiltration and subsequently, better absorption and translocation of rimsulfuron. Also, the non-ionic surfactant could possibly lower the surface tension of the spray droplet until the known as the Critical Micelle Concentration (CMC) (Sharma *et al.*, 1996; Penner, 2000).

Conclusions

Utilization of a NIS surfactant enhanced rimsulfuron efficacy and rainfastness on *C. album* and *A. retroflexus.* Increasing non-ionic surfactant concentration from 0.04 to 1 L ha^{-1} increased rimsulfuron activity around 10-fold for *A. retroflexus* compared to around 3-fold increase for *C. album.* Also, increasing rimsulfuron dosages up to 60 g ai. ha^{-1} enhanced weed control to 100% at potato field and increased total tuber yield up to 60 ton ha^{-1}. The application time was critical, however, post-application of rimsulfuron at S1+S4 and S1+S4+S7 stages of potato enhanced weed control and potato yield.

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Επίδραση επιφανειοδραστικών στην αποτελεσματικότητα και την αντοχή στην έκπλυση του ζιζανιοκτόνου rimsulfuron στην πατάτα

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Περίληψη Τα επιφανειοδραστικά χρησιμοποιούνται μαζί με ζιζανιοκτόνα για να αυξήσουν την αποτελεσματικότητά τους. Στην παρούσα μελέτη, αποδείχθηκε ότι η εφαρμογή 0,1% του μη ιονισμένου επιφανειοδραστικού (NIS, Contact) και 0,5 L ha^{-1} συμπυκνώματος φυτικού ελαίου (COC, Renol) δεν βελτίωσε την αποτελεσματικότητα του rimsulfuron εναντίον του ζιζανίου *Amaranthus retroflexus*. Αντίθετα, οι ίδιες επεμβάσεις ενίσχυσαν την αποτελεσματικότητα και την αντοχή στην έκπλυση του rimsulfuron εναντίον του ζιζανίου *Chenopodium album*. Η αύξηση της συγκέντρωσης του μη ιονισμένου επιφανειοδραστικού σε 1L ha^{-1} αύξησε την αποτελεσματικότητα του rimsulfuron περίπου κατά 10 φορές εναντίον του *A. retroflexus* ενώ κατά 3 περίπου φορές εναντίον του *C. album*. Η ίδια επέμβαση μείωσε κατά 12 φορές την αντοχή στην έκπλυση του rimsulfuron εναντίον του *A. retroflexus* ενώ τη βελτίωσε κατά 2 φορές εναντίον του *C. album*. Οι δόσεις ED$_{50}$ και ED$_{90}$ του rimsulfuron έδειξαν ότι η προσθήκη 0,2 L ha^{-1} NIS βελτίωσε την αποτελεσματικότητα της συνιστώμενης (60 g δ.ο. ha^{-1}) και της μειωμένης δόσης (30 g δ.ο. ha^{-1}) του rimsulfurom σε καλλιέργεια πατάτας στον αγρό. Η υψηλότερη απόδοση της πατάτας καταγράφηκε (60 τόνοι ανά εκτάριο) όταν 60 g δ.ο. ha^{-1} rimsulfuron εφαρμόστηκαν σε τρία στάδια ανάπτυξης [ανάπτυξη των φύλλων (S1), βλαστικά πολλαπλασιαστικά όργανα (S4) και ανάπτυξη κονδύλου (S7)] της καλλιέργειας χωρίς τη χρήση NIS. Δεν παρατηρήθηκαν σημαντικές διαφορές όταν εφαρμόστηκε η ίδια δόση rimsulfuron μαζί με NIS σε τρία (S1, S4 και S7) και δύο (S1, S4) στάδια ανάπτυξης της καλλιέργειας.

New data on the scale insect pests of *Ephedra foeminea* in Greece

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Summary   The dioecious gymnosperm self-sown ornamental plant *Ephedra foeminea* Forssk (Ephedraceae: Gnetales) has a special interest due to its nutraceutical and pharmaceutical properties. The present study contributes with data about the phenology of the plant in Attica, Greece, and the complex of its scale insect pests (Hemiptera: Coccomorpha), *Stotzia ephedrae* (Newstead) (Coccidae) (morphology, phenology, biology, natural enemies), *Dynaspidiotus ephedrarum* (Lindinger) (morphology, natural enemies) and *Leucaspis riccae* Targioni Tozzetti (Diaspididae) (natural enemies), based on observations made from April 2021 to June 2023. *Stotzia ephedrae* which was the scale species found in higher numbers on *E. foeminea* is a univoltine, oviparous and biparental species recorded as settled 1st instar nymph on the shoots of *E. foeminea* from May to January. The fecundity of the scale fluctuated between 370 and 598 eggs per female. The results on the phenology of *S. ephedrae* contribute to the knowledge of the critical period for the pest control, i.e. from May to January during which the scale remains in the sensitive first instar. In addition, the records of parasitoids and predators in the colonies of the scale insects infesting *E. foeminea* provide information on the available natural enemies for potential use in biological control schemes of these pests.

Additional keywords: ecology, parasitism, phenology, predators, scale insects

Introduction

Ephedra foeminea Forssk (Ephedraceae: Gnetales) is a gymnosperm dioecious endemic plant species to the Mediterranean region. It is a plant of special interest. Among the ten species of genus *Ephedra, E. foeminea* and *E. aphylla* Forssk. are the two insect pollinated species by Diptera and Lepidoptera, many of them being nocturnal able to pollinate the plants during the full moon of July (Bolinder *et al.,*2015a; 2015b; 2016). A correlation is referred between its pollination period and the lunar cycle (Bolinder *et al.,* 2015b; Rydin and Bolinder, 2015). Additionally, the bibliography supports evidence for its medicinal properties, composing a promising source of new selective antioxidant and anti-cancer drugs (Rydin and Bolinder, 2015;

Mpingirika *et al.,* 2020; Al-Saraireh *et al.,* 2021), as well as for its nutraceutical value especially of the fruits (Hegazy *et al.,* 2019; Khalil *et al.,* 2020).

Twenty-five (25) scale insect species (Hemiptera: Coccomorpha) have been reported in genus *Ephedra*: four of the family Coccidae, 15 of Diaspididae, five of Eriococcidae and one of Pseudococcidae. Records of scale insects in genus *Ephedra* in Greece include: the diaspidids (Hemiptera: Diaspididae) *Leucaspis (ephedrae) riccae* Targioni Tozzetti on *E. (vulgaris) distachya* in Athens and on *E. (campylopoda) foeminea* in Athens (on Lecabettus hill) and in Ano Lechonia (Magnessia) (Koroneos, 1934); *Dynaspidiotus (Aspidiotus) ephedrarum* (Lindinger) on *E. (campylopoda) foeminea* in Athens (on Lecabettus hill) (Koroneos, 1934); *Dynaspidiotus ephedrarum* on *Ephedra distachya* in East Attica (near Athens - Kato Sounio) (Szita, *et al.,* 2017); the coccid (Hemoptera: Coccidae) *Stotzia ephedrae* (Newstead) on *E. foeminea* in Athens (on Lecabettus hill) (Stathas *et al.,* 2023). Reports of other scale insects on *E. foeminea* in other countries include *Diaspidiotus cecconii* (Leonardi) in Palestine and

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Diaspidiotus distinctus (Leonardi) in Turkey (García Morales *et al.*, 2016)

In April 2021, plants of *E. foeminea* were found infested by a complex of three species of scale insects: *S. ephedrae* (Stathas *et al.,* 2023), *D. ephedrarum* and *L. riccae.*

Host plants of *S. ephedrae* belong to six plant genera of five families: *Bupleurum* (Apiaceae), *Asparagus* (Asparagaceae), *Ephedra* (Ephedraceae), *Coronila* and *Genista* (Fabaceae) and *Tamarix* (Tamaricaceae) (García Morales *et al.* 2016). *Dynaspidiotus ephedrarum* has been recorded worldwide to infest three plant genera of three families: Asparagaceae (*Asparagus* sp.), Ephedraceae (*Ephedra alata*, *E. altissima*, *E. distachya*, *E. foeminea*, *E. foliate*, *E. major*, *E. procera,* and *E. tilhoana*) and Pinaceae (*Pinus sylvestris*). *Leucaspis riccae* has been found on 6 plant genera of 6 families: Apocynaceae (*Nerium oleander*), Ephedraceae (*Ephedra alata*, *E. distachya*, *E. vulgaris*, *E. foeminea*, *E. campylopoda*, and *E. fragilis*), Ericaceae (*Erica arborea, E. manipuliflora*, Euphorbiaceae (*E. dendroides*), Moraceae (*F. carica*) and Oleaceae (*Olea europaea*) (García Morales *et al.,* 2016).

Morphological description of female adults of *S. ephedrae* is available by Ezzat and Hussein (1969) and by Hodgson (1994) but there is lack of information on biology and phenology of the scale in bibliography. Also concerning its natural enemies, the only records are the endoparasitoids *Metaphycus stephaniae* sp. nov. Guerrieri (Hymenoptera: Encyrtidae) in Israel (Guerrieri, 2006) and *Metaphycas hodzhevanishvilii* Yasnosh (Hymenoptera: Encyrtidae) in Iran (Fallahzadeh and Japoshvili, 2017).

In the present study, data on morphology of the developmental stages and on biology, phenology and natural enemies primarily of *S. ephedrae,* and to an extent of *D. ephedrarum,* are provided. *Dynaspidiotus ephedrarum* and *L. riccae* were found in low populations, therefore it was not possible to have clear information on their phenology, but their records were considered important for completing the complex of the existing scale insects entomofauna of *E. foeminea* in the area of the study. Further-

more, the records of active natural enemies against *D. ephedrarum* and *L. riccae,* contribute to the knowledge of their ecology and could possibly partly explain their weakness to develop high populations.

Materials and Methods

The study was conducted with scales recorded on *E. foeminea* in Athens (Lecabettus Hill: 37º58´ N, 23º44´ E, altitude: 270m) from 20 April 2021 to 5 June 2023. The phenological stages of the host plant *E. foeminea* were recorded weekly, including the dates of appearance of the new vegetation and the blossom of *E. foeminea* bushes.

To study the morphology of *S. ephedrae*, measurements (length, width) were made in the laboratory under a stereoscopic microscope on 20 individuals of each developmental stage, except for crawlers: eggs, settled 1st instar nymphs, 2nd instar nymphs, the scale cover of the male adult nymphs, preovipositing female adults ovipositing female adults (female body with ovisac included). For *D. sephedrarum*, the same dimensions were measured in 12 individuals from each of the following stages, 2nd instar nymphs (19/2/2022), female adults and scale covers of males (15/10/2022). The mean number of the scales' length and width and Standard Error (SE) were calculated. Permanent slides of the scale insect species were made in the Laboratory of Entomology and Agricultural Zoology of the Department of Agriculture of the University of the Peloponnese, where identified. The confirmation of the scale species was made by Professor Giuseppina Pellizzari (Dipartimento di Agronomia, Animali, Alimenti), University of Padua, Italy. Vouchers of permanent slides of the scale insect species are kept in the Laboratory of Agricultural Entomology, Department of Agriculture, University of the Peloponnese.

To study the phenology of *S. ephedrae*, samples of 20–30 infested shoots of approx. 15 cm length, to ensure adequate populations of more than 300 individuals of *S. ephedrae* per sampling, were collected weekly.

The samples were transferred to the Laboratory of Entomology (Department of Agriculture, University of the Peloponnese) and were examined under the stereoscopic microscope. The developmental stage and the numbers of all the alive *S. ephedrae* individuals were recorded and were expressed as percentage (%) of the total alive scales.

The biological parameters of *S. ephedrae* studied were the fecundity and sex ratio. The fecundity was measured by counting the total number of eggs in 30 ovipositing female adults on 23 April 2022. The sex ratio was estimated on 26 March 2022, on 375 individuals of the scale, by counting the female adults and the total number of males (alive and dead male nymphs + empty male scale covers).

The records of natural enemies included observations for the presence of predators in the field during the sampling of the aforementioned infested shoots and in the laboratory during the examination of the samples under the stereoscopic microscope. The parasitized scales that were found during the examination of the above samples were kept in cylindrical glass vials (diameter: 2.5 cm, height: 5cm covered by organtine) which were kept in incubators under controlled conditions (65-70% humidity and photoperiod 16:8 hours light per day) until the emergence of parasitoids. The parasitism was expressed as percentage (%) of parasitized scales out of the total number of the alive scales. The identification of the parasitoid of genus *Metaphycus* was made by Professor George Japoshvili (Entomology and Biocontrol Research Centre Agrarian), University of Tbilisi, Georgia.

Results

Morphology, phenology and biology traits of the scales

As regards the phenological stages of *E. foeminea*, sprouts of the new vegetation were grown on 10/4/2021, 17/4/2022, and 26/3/2023. From the end of August to the middle of October 2021 the bushes were significantly dehydrated but recovered their vitality until the next period. Blooming occurred during 10-17/7/2021, 1-23/7/2022 and 21/9/2022 – 17/10/2022. The secretion period of pollination drops on the female and male blossoms in the year 2022 were recorded during 9-16/7/2022 and 2-13/10/2022 (Fig. 1). The full moon of July and October 2022 occurred on 13/7/2022 and 10/10/2022, respectively.

Regarding the morphology of *S. ephedrae,* the settled 1st instar has an elliptic and thin flat shape with a light brownish color (Fig. 2a). The 2nd instar nymph of female has a darker brownish color, elliptic shape, with a longitudinal carina that arises on median site of the dorsum (Fig. 2b). The male 2nd instar nymph develops a white thin waxy cover (Fig. 2c). The pre-ovipositing female adult get a dark olive-green color secreting honeydew from dorsum (Fig. 3a). The color of

Figure 1. Pollination drops on male (a) and female (b) blossoms of *Ephedra foeminea* in Athens (Lecabettus Hill), Greece.

Figure 2. Nymphs of *Stotzia ephedrae* on *Ephedra foeminea*: (a) settled 1st instar nymphs, (b) 2nd instar nymphs of females, (c) 2nd instar nymphs of males, (d) larva of parasitoid in 2nd instar nymph.

the body of the ovipositing female is purple brownish (Fig. 4a). The ovipositing female adult creates a white convex waxy ovisac that covers the eggs and the whole body of the female (Fig. 4b). The male adult is similar with the female in color and has two long waxy filaments in abdomen (Fig. 3b). Fig. 3 shows the empty male scale cover (3c) and male adult under the male scale cover before its emergence (3d). The eggs are oval ellipsoid light purple-reddish, laid under the ovisac to the posterior part of the body of the female (Fig. 4a). The crawler is elliptic, rather flattened with a slightly lighter tint than the eggs (Fig. 4b). The dimensions of the developmental stages of the scale are presented in Table 1.

Figure 3. *Stotzia ephedrae* adults on *Ephedra foeminea:* (a) preovipositing female, (b) male adult, (c) empty male scale cover and (d) male adult under the scale cover before its emergence.
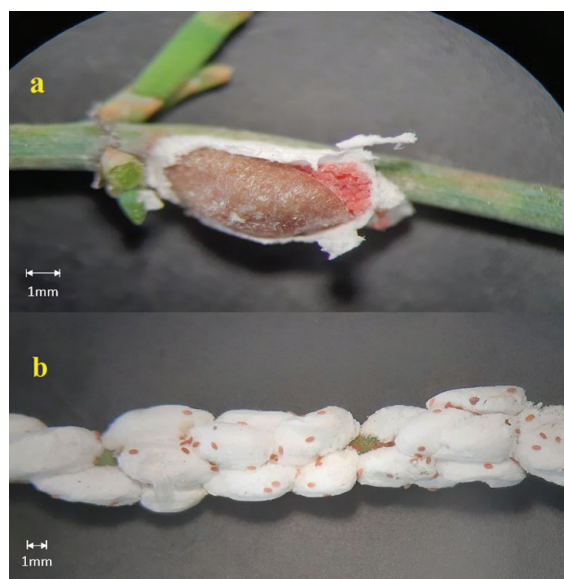
Figure 4. Ovipositing female adults of *Stotzia ephedrae* on *Ephedra foeminea*: (a) the body of ovipositing female, (b) female adults covered by their scale covers, and hatched crawlers.

Table 1. Dimensions (mm) of the developmental stages of *Stotzia ephedrae* (n=20).

Developmental stage	Length Mean ± SE (min – max)	Width Mean ± SE (min-max)
Egg	0.22 ± 0.001 (0.21-0.22)	0.14 ± 0.003 (0.12-0.15)
Settled 1st instar nymph	1.48 ± 0.03 (1.21-1.87)	0.63 ± 0.02 (0.43-0.76)
2nd instar nymph	2.08 ± 0.09 (1.62-3.10)	0.86 ± 0.03 (0.60-1.06)
Preovipositing female adult	3.46 ± 0.09 (3.10-4.58)	1.39 ± 0.02 (1.20-1.52)
Ovipositing female (including ovisac)	5.80 ± 0.09 (5.24-6.41)	3.15 ± 0.09 (2.43-3.75)
Male adult	1.27 ± 0.01 (1.10-1.35)	0.44 ± 0.02 (0.32-0.56)

Stotzia ephedrae is an oviparous biparental species. The recorded sex ratio (females/males) was 52.8/47.2. The eggs laid by females fluctuated between 370 to 598 per female. The mean (± SE) was 493.13 ± 15.90 eggs per female. The percentages of the developmental stages of *S. ephedrae* in the sampled shoots are presented in Fig. 5. As it is shown in the Figure, the hatching of crawlers was recorded in May (2021, 2022 and 2023). The settled 1st instar nymphs were present until the end of January (2022, 2023), thus this instar covers the longest period of the year, comparatively with the other stages of the scale. The 2nd instar nymphs were recorded from the end of December 2022 to the end of February 2023 and from the end of December 2022 to the beginning of March 2023. The immature stages of males (nymphs under their cover) were recorded between the end of February 2022 to the end of March 2022 and by the middle of February 2023 to middle of March 2023. Pre-ovipositing females were recorded from the beginning of March 2022 to the beginning of April 2022 and from the end of February 2023 to the beginning of April 2023. Ovipositing females were recorded from the start of the study (20/4/2021) to the end of April 2021, from the end of March 2022 to the end of April 2022 and from the middle of March to the middle of April 2023. Until the crawler hatching the ovisacs remained settled on the host plant, containing the dehydrated body of the dead females under their frontal portion and alive eggs under the posterior portion (Fig. 5). The mean number of *S. ephedrae* eggs per female was 493.13 ± 15.90.

Stotzia ephedrae is settled only on the shoots of *E. foeminea* and the development of the scales is not affected by the development of the shoots. The infestation by *S. ephedrae* damages the plant by sucking the sap, dehydrating the shoots (Fig. 2) and by the secretion of honeydew by the preovipositing female adults, which encourages the growth of sooty molds and obstructs photosynthesis.

Dynaspidiotus ephedrarum is an ovipa-rous biparental species. The dimensions of the 2nd instar nymphs, of the scale covers of male adults and of female adults, are presented in Table 2. The scale cover of female adult is convex white to grey - white, with a brownish subcentral exuvia (Fig. 6a). The scale cover of male has an oval shape and white color with brownish subcentral (Fig. 6b). The developmental stage of *E. foeminea* does not affect the development of *D. ephedrarum*, but its infestation damages the host plant by sucking the sap and drying the shoots (Fig. 6).

Natural enemies of the scales

Regarding the presence and action of natural enemies, larvae of an endoparasitoid *Metaphycus* sp. (Hymenoptera: Encyrtidae) (Fig. 2d) were found in 1st and 2nd instar nymphs of *S. ephedrae*. The percentage of parasitism in the total population of the scale is presented in the Figure 7. As it is shown in this Figure, the parasitism fluctuated from 1.1 to 14%. The majority of the parasitized nymphs (up to 80%) were mainly found near to the internal basal side of the leaves. Smal number of individuals of the coccophagus predators *Chilocorus bipustulatus* (L.) and *Exochomus quadripustulatus* (L.) (Coleoptera: Coccinellidae) were observed sporadically in the colonies of *S. ephedrae* during the period of the study: three adults and two larvae of *C. bipustulatus* (10/6/2021), 2 adults *C. bipustulatus* (16/7/2022), and 2 adults of *E. quadripustutatus* (20/9/2023).

In the case of *D. ephedrarum*, predated scales by three adults of the predator *Cybocephalus fodori* Endrödy-Younga (Coleoptera: Cybocephalidae) were recorded on 10/6/2022, as well as one larva and two adults on 27/3/2023. Also, parasitism of female adults was recorded by an unidentified endoparasitoid species. Encapsulated eggs (1-2 per female adult), and parasitoid exit holes (1-3 per female adult scale cover) were found (Fig. 6).

Regarding *L. riccae,* an unidentified ectoparasitoid was found to parasitize the female adults of the scale (Fig. 8).
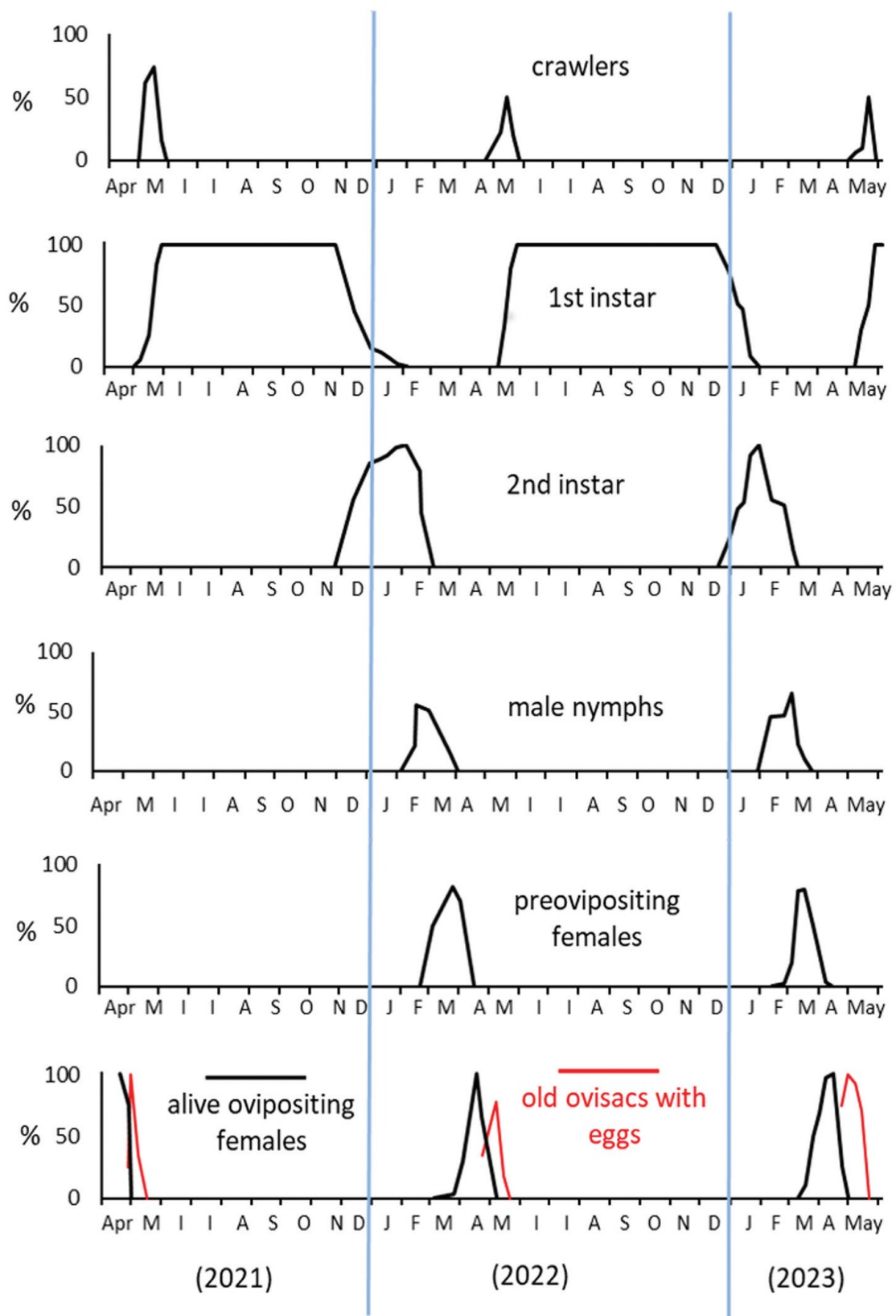
Figure 5. Percentage (%) of the developmental stages of *Stotzia ephedrae* on *Ephedra foeminea* from 20 April 2021 to 5 June 2023, Athens (Lecabettus Hill), Greece.

Table 2. Dimensions (mm) of the developmental stages of *Dynaspidiotus ephedrarum* (n=12).

Developmental stage	Length Mean ± SE (min-max)	Width Mean ± SE (min-max)
2nd instar nymph	1.14 ± 0.02 (1.05-1.22)	0.83 ± 0.04 (0.62-0.97)
Ovipositing female (with scale cover)	1.88 ± 0.07 (1.56-2.30)	1.18 ± 0.01 (1.13-1.21)
Scale cover of male adult	1.31 ± 0.04 (1.14-1.49)	0.62 ± 0.01 (0.60-0.66)
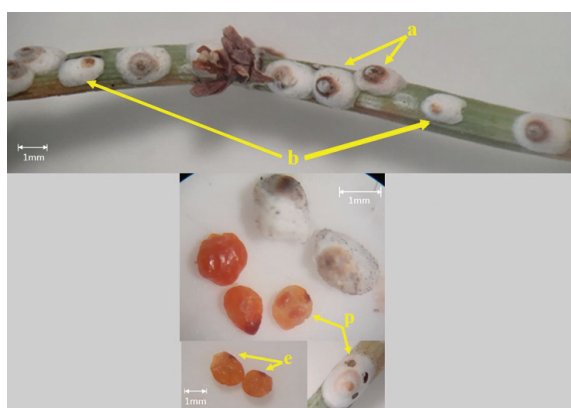
Figure 6. *Dynaspidiotus ephedrarum* infestation on *Ephedra foeminea*: (a) preovipositing female adults, (b) male nymphs, (e) encapsulated eggs by endoparasitoid in the body of female adults, (p) larvae (up) and exit holes (down) of endoparasitoid of the scale.

Figure 7. Percentage (%) of parasitism of 1st and 2nd instar nymphs of *S. ephedrae* by *Metaphycus* sp. during the period of the study.

Figure 8. *Leucaspis riccae* on *Ephedra foeminea*: (a) female nymph, (b) larva of ectoparasitoid under the scale cover of female scale nymph.

Discussion

The average number of eggs laid by *S. ephedrae* females was 493.13 ± 15.90 eggs per female. This number could be considered as indicative of the fecundity of the scale and cannot represent its total fecundity, as more eggs could be laid by the examined females after the date of the sampling (23 April 2022). *Stotzia ephedrae* completes one annual generation in the area of the present study. From the beginning of May to the end of January

was recorded as settled 1st instar nymph, although the weather conditions of this period are suitable for the development of scale insects to next instars in Greece. This could indicate that this instar enters diapause. Similar behavior with a long period of diapause of the 1st instar is also recorded to other scale insect species in Greece, e.g. *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccomorpha: Coccidae) infesting *Citrus sinensis* from the beginning of June to the beginning of the next April in Athens (Stathas and Karipidis, 2020), as well as *Kermes echinatus* Balachowsky (Hemiptera, Coccomorpha: Kermesidae) on *Quercus ilex* from the end of June to mid-April of the following year in Peloponnese (Stathas *et al.,* 2018). Some minor differences recorded on the presence of the several developmental stages of *S. ephedrae* could be related with differences of climatic conditions of each year during the period of the study.

The size of female (1.88 mm length, 1.18 mm width) and male covers (1.31 mm length, 0.62 mm width) of *D. ephedrarum* are similar to those found in other studies, e.g. according to Lindiger female scales are 1.5 mm in diameter (García Morales *et al.,* 2016) while according to Balachowsky the female scale has a diameter of 2 – 2.2 mm and the scale cover has a length 1.4 - 1.5 mm (García Morales *et al.,* 2016).

The *Metaphycus* endoparasitoid of *S. ephedrae* could not be identified in species level. Nevertheless, two *Metathycus* endoparasite species of *S. ephedrae* are known: *M. stephaniae* sp. nov. in Israel (Guerrieri, 2006) and *M. hodzhevanishvilii* in Iran (Fallahzadeh and Japoshvili, 2017). The coccinellid predators *C. bipustulatus* and *E. quadripustulatus* are common coccophagus species worldwide, e.g. *Chilocorus bipustulatus* has been recorded as a natural enemy of 76 scale insect species and *E. quadripustulatus* on 40 species (García Morales *et al.,* 2016).

In the present study, parasitism of female adults of *D. ephedrarum* and *L. riccae* was found by an unidentified endoparasitoid and ectoparasitoid, respectively. Reports for parasitoid species of *D. ephedrarum* include *Coccobius sybariticus* Pedata (Hymenoptera: Aphelinidae) (Pedata, 1999), while for *L. riccae,* the aphelinids *Aphytis libanicus* Traboulsi and *A. mytilaspidis* (Le Baron) (Hymenoptera: Aphelinidae) (Rosen and DeBach, 1979). The coccophagus predator *C. fodor,* found in low populations in the colonies of *D. ephedrarum*, could also be considered as a natural enemy of the scale. In fact, *C. fodori* is referred as predator of five scale insect species of the family Diaspididae in Turkey and Greece: *Comstockaspis perniciosa* (Comstock) in Antalya, Turkey (Erler and Tunç, 2001), *Diaspis echinocacti* (Bouché) in Kalamata, Greece (Stathas *et al.,* 2021), *Parlatoria pergandii* Comstock in Leonidion, Peloponnese, Greece (Stathas, 2001), *Parlatoria ziziphi* (Lucas) in Tyros, Peloponnese, Greece (Stathas *et al.,* 2007) and *Targionia vitis* (Signoret) in Kalamata, Greece (Stathas *et al.,* 2021).

The results on the phenology of *S. ephedrae* which was the scale species found in higher numbers on *E. foeminea* contribute to the knowledge of the critical period for the pest control, i.e. from May to January during which the scale remains in the sensitive instar of crawlers. In addition, the records of parasitoids and predators in the colonies of the scale insects infesting *E. foeminea* provide information on the available natural enemies for potential use in biological control schemes of these pests.

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Νέα δεδομένα σχετικά με τα κοκκοειδή που προσβάλλουν το φυτό *Ephedra foeminea* στην Ελλάδα

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Περίληψη Το δίοικο γυμνόσπερμο φυτό *Ephedra foeminea* Forssk (Ephedraceae: Gnetales), είναι ένα αυτοφυές καλλωπιστικό φυτό που έχει ιδιαίτερο ενδιαφέρον. Στην παρούσα εργασία παρουσιάζονται στοιχεία για τη φαινολογία του φυτού στην Αττική, και για τα επιβλαβή για το φυτό κοκκοειδή έντομα (Hemiptera: Coccomorpha): *Stotzia ephedrae* (Newstead) (Coccidae) (μορφολογία, φαινολογία, βιολογία, φυσικοί εχθροί), *Dynaspidiotus ephedrarum* (Lindinger) (μορφολογία, φυσικοί εχθροί) and *Leucaspis riccae* Targioni Tozzetti (Diaspididae) (φυσικοί εχθροί), που εξασφαλίστηκαν από παρατηρήσεις που έγιναν από τον Απρίλιο του 2021 έως τον Ιούνιο του 2023. Το είδος *Stotzia ephedrae* σε σχέση με τα άλλα δύο είδη παρατηρήθηκε σε μεγαλύτερους πληθυσμούς επί του *E. foeminea*. Συμπληρώνει μία γενεά το έτος, είναι είδος ωοτόκο και αμφιγονικό και παραμένει εγκατεστημένο στο στάδιο της νύμφης $1^{ης}$ ηλικίας στους βλαστούς του *E. foeminea*, από τον Μάιο έως τον Ιανουάριο. Η γονιμότητά του κυμάνθηκε από 370 έως 598 ωά ανά θηλυκό. Από τα αποτελέσματα της μελέτης της φαινολογίας του *S. ephedrae* φαίνεται ότι η καταλληλότερη περίοδος για την καταπολέμηση του εντόμου είναι κατά το διάστημα Μαΐου – Ιανουαρίου, καθώς το έντομο βρίσκεται στο ευαίσθητο στάδιο της νύμφης $1^{ης}$ ηλικίας. Επίσης, οι καταγραφές της δράσης παρασιτοειδών και αρπακτικών εναντίον των κοκκοειδών που προσβάλλουν το *E. foeminea*, παρέχουν πληροφορίες για τους διαθέσιμους φυσικούς εχθρούς που δυνητικά θα μπορούσαν να χρησιμοποιηθούν ως παράγοντες βιολογικού ελέγχου αυτών των προσβολών.

Pollination and fruit set of the Protected Designation of Origin apple cv. 'Delicious Pilafa Tripoleos' depends on insect pollinators

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Summary   Insect dependency of apple crop for pollination vary in different cultivars. The cv. 'Delicious Pilafa Tripoleos', is a Protected Designation of Origin apple of Greece, which lacks information on self-compatibility and needs for insect pollination for commercial fruit production. Here, the effect of wind, free (wind and insects), honeybee, free with at least one visit from a bumblebee and hand pollination was examined on fruit set and fruit characteristics. Also, the effect of flowering patches as a practice to attract pollinators in the apple orchards, on fruit quality characteristics was studied. A pollinizer apple variety and insect pollinators are necessary for successful pollination of 'Delicious Pilafa Tripoleos' since hand pollination with pollen of the same cv., and wind pollination resulted in very low fruit set. Single flower visits by honeybees could give fruit set, however, free pollination with at least one visit of *B. terrestris* resulted in higher fruit set compared to the other pollination treatments. Free pollination resulted in more fruits with higher number of seeds than wind pollination (only one fruit obtained). Apples produced from flowers adjacent to the flowering mixture patches had significantly higher skin firmness and lower total soluble solids at harvest (both desirable traits for 'Delicious Pilafa Tripoleos'), compared to fruits from trees in naturally occurring groundcover.

Additional keywords: apple, bees, Delicious Pilafa Tripoleos, fruit characteristics, fruit set, pollination

Introduction

Apple (*Malus domestica* Borkh., Rosaceae) is one of the most economically important fruit crops globally (FAO, 2022). Apple crop is highly dependent on insects for its pollination as the majority of apple cultivars are self-incompatible and insect pollinators act as vectors for pollen movement among cultivars (Jahed and Hirst, 2017; Nunes-Silva *et al.,* 2020). Therefore, enhancing pollinator management in apple crop may result in large financial benefits (Garratt *et al.*, 2014). However, when discussing the economic benefits and management of pollinators in apple orchards, a basic concern is how pollinator dependency and potential yield deficiencies vary throughout different apple cultivars (Garratt *et al.*, 2016).

In a recent global study in 177 commercial apple orchards across 33 countries, honey bees were the dominant pollinator across all biogeographical zones, while other managed and wild bee species (644 bee species/morphospecies collected) were recorded in lower numbers (Leclercq *et al.*, 2023). Apple growers utilize honey bees, *Bombus* spp., *Osmia* spp., and other bee species to ensure adequate levels of pollination and hence apple yields (Osterman *et al.,* 2021). Especially, bumblebees are considered excellent pollinators by farmers due to their ability to forage in low temperatures, common during apple's spring bloom (Brittain, 1935). In addition, conservation practices such as providing floral resources have been used to attract bees aiming to promote ecosystem stability and enhance food security in insect pollination dependent crops like apples (Park *et al.*, 2012; Campbell *et al.*, 2013).

Insect pollination is known to increase cross-pollination between cultivars and thus

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improve fruit set and yields of apples (Leonhardt *et al.*, 2013), which are self-incompatible to some extent depending on the cultivar (McGregor, 1976). However, the influence of insect pollination on apple fruit quality characteristics is not clear yet (Samnegard *et al.*, 2019). For example, no significant differences were observed between wind-pollination, wind + bee-pollination and hand-pollination treatments in fruit firmness, soluble solids content, titratable acidity, dry matter and starch content, while fruit weight increased moving from the first to the third abovementioned treatment (Vizzotto *et al.*, 2018). Moreover, insect pollination has been reported to improve apple fruit weight, length, diameter (Hünicken *et al.*, 2020). Also, better fruit quality (greater fruit symmetry) was recorded in apples produced in trees close to *Osmia lignaria* Say nests (Sheffield, 2014).

When valuing ecosystem services such as pollination to agricultural production, it is always important to consider the apple variety (Garratt *et al.*, 2014). The apple 'Delicious Pilafa Tripoleos', is a Protected Designation of Origin (PDO) cultivar which is produced in altitude higher than 600 m, mainly at the Tegea plateau, in the Prefecture of Arcadia Peloponnese, Greece (European Union 1997, Kizos and Vakoufaris, 2011). 'Delicious Pilafa Tripoleos' apples were recognized as PDO by the Greek Ministry of Agriculture in 1962 (225700/13362) (peliti.gr), but they were cultivated for the first time in Greece in 1920 as a sporophyte (Davidis, 1981). Their irregular shape, rough texture of their skin and short life of storage do not give the best first impression to the consumers, but they have excellent organoleptic characteristics for which are famous to apple connoisseurs (Mitropoulos and Lambrinos, 2005; Mitropoulos and Lambrinos, 2007; Thanopoulos *et al.*, 2021). 'Delicious Pilafa Tripoleos' flowering period is very short, usually less than two weeks from mid to late April (Barda *et al.*, 2023). Moreover, the weather in April is usually unstable in Tegea plateau, resulting in frost problems and limited fruit setting (Hatziiremia, 2021). Honey bees and wild bees, i.e., *Andrena*, *Anthohora*, *Bombus* (e.g.,

B. terrestris, *B. argilaceus*), *Xylocopa*, *Lasioglossum* (e.g., *L. marginatum*), Megachilidae have been recorded on flowers of 'Delicious Pilafa Tripoleos' (Barda *et al.*, 2023). Therefore, the study of 'Delicious Pilafa Tripoleos' pollination aiming to enhance pollination services and possibly improve fruit marketable characteristics is of great interest for promoting and preserving this local cv. In addition, there is no previous relevant study for the cv. 'Delicious Pilafa Tripoleos', which makes this study novel.

The aim of this study is to evaluate the effect of a) free pollination with contribution of insects and wind pollination on 'Delicious Pilafa Tripoleos' fruit set and fruit quality characteristics; b) different pollinator taxa (honey bees and bumblebees) to fruit set, and c) groundcover management practices (patches of flowering plants established to attract pollinators *vs* naturally occurring groundcover in the apple orchard) to fruit quality characteristics.

Materials and Methods

To study the effect of different pollination treatments and groundcover on 'Delicious Pilafa Tripoleos' fruit set and fruit quality, three field experiments were conducted during the years 2020, 2021, 2022.

Effect of free pollination (insects and wind) vs wind pollination on fruit set and fruit characteristics of the cv. 'Delicious Pilafa Tripoleos'

The study took place in an IPM apple orchard (palmette, 0.45 ha) located in Tegea plateau, Arcadia, Peloponnese, Greece (37.4415, 22.40492) in year 2020. To minimize variability effects due to different tree vigor, site and other factors that could affect pollination and fruit setting, the treatments were set in pairs on branches of the same tree. In particular, two branches of the same length, approximately 1m, on the same apple tree were selected at pink bud stage, one was marked as "free pollinated" and the second was closed in an insect-proof net (1.20m x

0.50m, 16/10) which allows pollen entry, and marked as "wind pollinated" (Figure 1a). After the end of flowering, the net was not removed from the "wind pollinated" branches aiming to protect them. For the same reason a net was also placed on the "free pollinated" branches. A total of 8 paired replicates per treatment were set. During flowering no plant protection products were applied.

To evaluate the effect on fruit setting, the following measurements were conducted in the field a) the number of buds on each selected branch at the pink bud stage (BBCH 57) (Meier et al., 2009), b) the number of fruits two weeks after petal fall (BBCH 71), c) 'initial fruit set' as the proportion of fruits on BBCH 71 to flowers on BBCH 57, d) the number of fruits after natural fruit drop and fruit thinning by hand, e) fruits on harvest (BBCH 87), f) 'final fruit set' as the proportion of fruits on BBCH 87 to flowers on BBCH 57. Apple fruits produced by free pollination, wind pollination and randomly selected apples from free pollination without net after petal fall (as a control), were harvested to study the effect of pollination treatment on fruit quality characteristics. 'Initial fruit set' is considered the best indicator of pollination success, as measurements are taken before any fruit loss due to diseases, pests, or natural abscission by the tree (Klein et al., 2012). The 'final fruit set', was also recorded in autumn as an important pollination success indicator for orchard managers (Campbell et al., 2017b).

The fruit quality characteristics examined in the laboratory were: a) fruit weight (g), b) maximum fruit length (mm), c) minimum fruit length (mm), d) maximum fruit width (mm), e) minimum fruit width (mm), f) seeds/fruit, g) seeds' weight/fruit (g), d) total soluble solids - TSS (°Bx) (Figure 1e). Fruit width and length were measured using a digital caliper (Figure 1f) and TSS using a hand refractometer calibrated with distilled water.

Effect of pollination by different taxa of pollinators on the cv. 'Delicious Pilafa Tripoleos' fruit set

The study was carried out in an organic apple orchard (palmette, 0.25 ha) located in Tegea plateau, Arcadia, Peloponnese, Greece (37.43705, 22.46942) in year 2022. Two bumblebee hives (Bioinsecta Bombox Fruit) of the species *Bombus terrestris* L. (Apidae) were established in the orchard, as an adopted practice by the farmers, to ensure visits of *B. terrestris* on the apple flowers. Visits of *Apis mellifera* L. (Apidae) on apple flowers were already guaranteed as beekeepers transfer their hives in Tegea plateau during apple flowering. The bumblebee hives were placed in the field on 13 April 2022 to acclimatize. The apple blossom started on 25 April 2022. Insect net cages (1.20m x 0.50m, 16/10) were placed in apple inflorescences (corymb) at the pink bud stage (BBCH 57), on 19 April 2022. Regarding plant protection applications, these included one application of a copper product before apple flowering, and one application of a *Beauveria bassiana* product after the end of flowering.

Apple inflorescences were caged (30 cm x 20cm, density type: 16/10) at the pink bud stage to be able to handle the following a, b and d treatments. The following treatments were implemented: a) wind pollination, using an insect-proof net (30 cm x 20cm, 16/10) which allows pollen but not insects entry, b) a single visit by *A. mellifera*. Inflorescences caged at the pink bud stage, were released on full bloom under supervision for observation of a single honey bee visit. The flower visited by a honey bee was caged again individually after destroying the rest of the flowers of the inflorescence, c) at least one visit by *B. terrestris*. Flowers that were free to pollination by wind and insects and received (recorded by observation) at least one visit by *B. terrestris*. Upon the bumblebee visit, one flower was kept in each cage (destruction of the flowers of the inflorescence not visited by the bumblebee), d) Hand pollination, was performed using a flower from a neighboring tree of the same variety (also a way to check whether self-incompatible or compatible), by tapering its anthers on the stigma of the examined flower, destruction of the rest of the rosette's flowers, then close the flower in an insect-proof cage (Fig-

ure 1b, 1c, 1d)

The measurements recorded were: a) duration of each pollinator visit to one flower (in seconds), to provide a measure of mean visitation period for each pollinator (Garantonakis *et al.,* 2016), b) fruit set, in each pollination treatment, two weeks after the end of anthesis.

Effect of patches of flowering plants on the apple fruit quality

To determine any possible differences of different groundcover management practices, the above mentioned IPM apple orchard in the 2020 experiment was used with the establishment and the plant species of the flowering mixture described in Barda *et al.* (2023). All fruits were free-pollinated and randomly harvested. In autumn 2021, 12 apple fruits were harvested from apple trees in rows which during spring had been surrounded by flowering mixture patches (main flowering by *Vicia sativa* L., *Vicia faba* L., *Eruca sativa* L. Cav. and *Coriandrum sativum* L.) and 12 fruits from trees in rows that had been surrounded by naturally occurring groundcover of the orchard (main flowering by *Ranunculus repens* L., *Veronica* persica Poir., *Veronica hederifolia* L., *Calepina irregularis* (Asso) Thell., *Cardaria draba* L.). The quality characteristics examined were the same as those examined in the year 2020 with the addition of three characteristics a) fruit skin firmness (kg), b) flesh firmness (kg), c) apple shape, measured as the difference between maximum and minimum fruit width. The skin firmness was measured using a penetrometer at two antidiametrical points, flesh firmness was measured using a penetrometer by cutting the apple in half and measured at two points of the flesh between the ovary and the skin of the fruit.
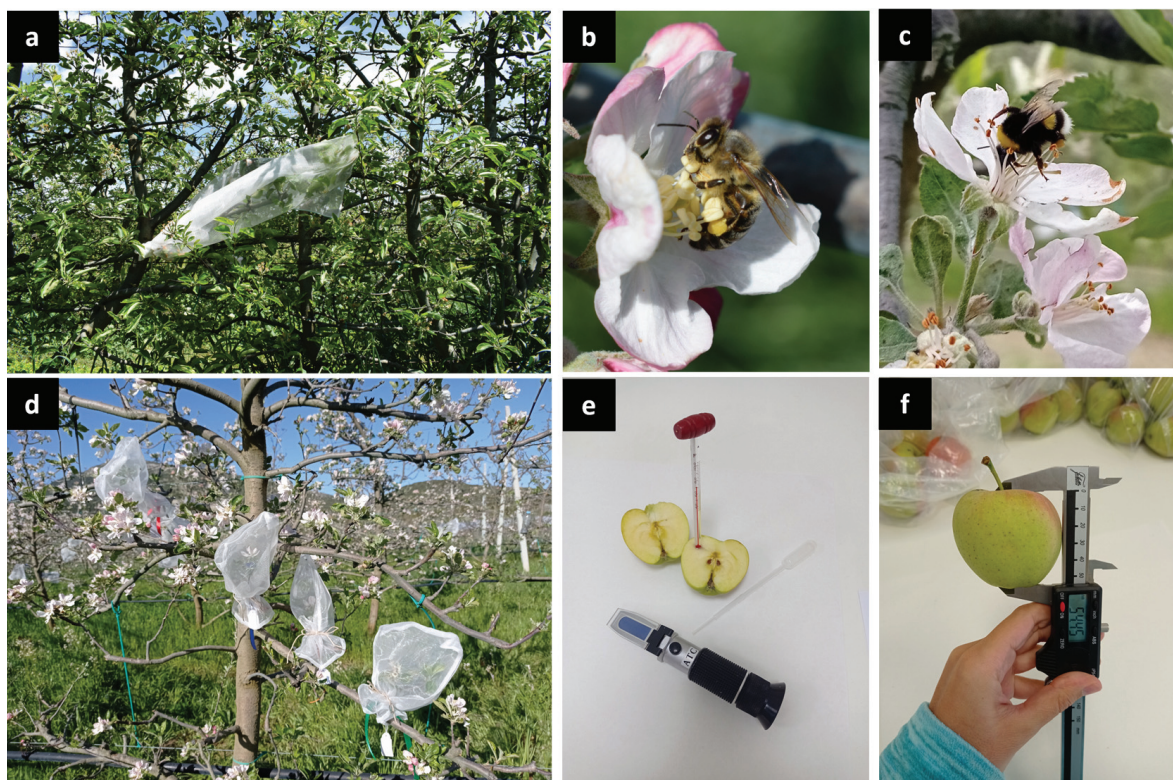
Figure 1. Investigation of the importance of different pollination treatments in fruit set and fruit characteristics of the "Delicious Pilafa Tripoleos" apple: a) branch with insect-proof net (wind pollination), experiment 2020, b) visit of *Apis mellifera* to an apple flower, c) visit of *Bombus terrestris* to an apple flower, d) different pollination treatments inside insect proof cages (*A. mellifera, B. terrestris*, wind pollination, hand pollination), experiment 2022, e) Measurement of total soluble solids by hand- refractometer, f) measurement of fruit length with a caliper.

Statistical analysis

T-test was applied to determine any statistically significant differences between: a) fruit quality measurements of fruits from free pollination, kept in the net after petal fall *vs* free pollination without the net after petal fall in 2020; b) free pollinated fruits adjacent to flowering patches *vs* the naturally occurring groundcover (control) in year 2021, when data followed the normal distribution (α=0.05); Kruskal-Wallis nonparametric test when data did not follow the normal distribution (α=0.05). Differences among pollination treatments on fruit set in the 2022 experiment were separated by Dunn's test, while visit duration differences between honey bees and bumblebees were determined by Kruskal-Wallis nonparametric test.

Results and Discussion

In year 2020, the bagged branches allowing only wind pollination, gave very low fruit set indicating that insect visits are necessary for apple flower pollination of the cv. 'Delicious Pilafa Tripoleos'. The positive effect of free pollination (wind and insects) is obvious even from the 'initial fruit set' and is clearly depicted on the 'final fruit set' (Table 1). Only one fruit from wind pollinated flowers was obtained on harvest. On the contrary, 36 fruits were obtained by free pollinated flowers indicating the importance of insect pollinators in fruit set. The importance of the insect mediated pollination has been underlined for diverse apple varieties (Garratt *et al.,* 2016; Pardo *et al.,* 2020; Burns, 2022) and is confirmed for 'Delicious Pilafa

Table 1. Fruit set of the cv. 'Delicious Pilafa Tripoleos' flowers after free pollination or wind pollination: number of flowers at pink bud stage (BBCH 57); number of fruits two weeks after petal fall (BBCH 71); 'initial fruit set' as proportion of fruits at BBCH 71 out of flowers at BBCH 57; fruits on harvest (BBCH 87); final fruit set as the proportion of harvested fruit out of flowers at BBCH 57, Tegea plateau, spring-autumn 2020.

Tree	Pollination treatment	Nr of flowers at BBCH 57	Nr of fruits at BBCH 71	Initial fruit set	Fruits after fruit thinning*	Fruits on harvest	Final fruit set
1	Wind	50	0	0.00	0	0	0.00
	Free	20	17	0.85	7	7	0.35
2	Wind	19	10	0.53	0	0	0.00
	Free	25	14	0.56	8	8	0.32
3	Wind	5	2	0.40	0	0	0.00
	Free	9	8	0.89	4	4	0.44
4	Wind	4	0	0.00	0	0	0.00
	Free	12	6	0.50	2	2	0.17
5	Wind	31	1	0.03	0	0	0.00
	Free	28	24	0.86	5	5	0.18
6	Wind	21	1	0.05	0	0	0.00
	Free	16	9	0.56	4	4	0.25
7	Wind	51	1	0.02	0	0	0.00
	Free	24	18	0.75	4	4	0.17
8	Wind	11	2	0.18	1	1	0.09
	Free	12	6	0.50	2	2	0.17

* Fruit thinning was applied as it is a common practice by apple producers and to have the same parameters that affect fruit quality between wind, free pollination and the control fruits (free pollinated fruits without net, randomly selected on harvest).

Tripoleos'.

In the experiment of 2022, the results of the wind pollination treatment confirmed the results of the 2020 experiment that insect pollinators are necessary for successful pollination of 'Delicious Pilafa Tripoleos' apples (Figure 2). Hand pollination gave only 3 fruits out of 17 hand pollinated flowers, highlighting the importance of a pollinizer apple variety on the pollination of the cv. 'Delicious Pilafa Tripoleos' (Figure 2).

Pollinator visit duration on a single apple flower differed significantly between honey bees and bumble bees (p < 0.001) with honey bees spending approximately twelve times longer time per flower than bumblebees. Specifically, the mean duration of a single honey bee visit on an apple flower was 48.1 ± 3.81 seconds and for bumblebees 4.09 ± 0.35 seconds. 'Free + *B. terrestris*' treatment resulted in significantly higher fruit set followed by honey bee and hand pollination, while wind pollination resulted in the lowest fruit set (Kruskal-Wallis, Dunn's test: p= 0.0047), (Figure 2). Pairwise multiple comparison Dunn's test did not show any differences (marginally, p= 0.055) between '*A. mellifera*' and 'Free + *B. terrestris*' treatments but Wilcoxon test showed that the latter resulted in significantly higher fruit set than single visits from honey bees (p= 0.0188). This demonstrates that a single

flower visit from a honey bee (although longer than the one of a bumblebee) does not guarantee successful pollination, supporting the results of previous studies that fruit set requires repeated pollinator visits (Bernauer *et al.*, 2022). Moreover, longer visit duration does not always equal better pollination than shorter visit durations as pollination effectiveness depends on a variety of factors such as pollinator hair density, body length and flower handling (Roquer-Beni *et al.*, 2022). Other factors such as pollen carryover and pollinator movement are also important. For instance, bumblebees have been reported to outperform honey bees in terms of outcross pollen delivery per flower visited in apple (Hung *et al.*, 2023), as *Bombus* spp. frequently contact stigmas while foraging thus deposit more pollen to apple stigmas than honey bees (Thomson and Goodell, 2001). This aligns with the higher fruit set of the 'Free + *B. terrestris*' treatment in the present study from flowers which had received at least one visit of *B. terrestris,* although previous visits by other pollinators cannot be excluded. Nevertheless, both honey bees and bumblebees store moistened pollen in the corbicula of their hind legs, making pollen generally unavailable for pollination (Westerkamp, 1991; Michener, 2007), hence further research with more wild bee taxa which possess different structures and ways to carry pollen should be conducted.

Considering possible effect of pollination treatments on fruit quality characteristics, analyses on the 2020 harvested fruits from free (insects and wind), wind pollinated flowers and free (insects and wind) pollination without net until harvest, revealed some differences. Given the low number of apples harvested from the insect exclusion (wind pollination) treatment, we could not assess its effect on fruit quality. However, the wind pollinated fruit had one seed, whereas the free pollinated in cages and the control (free pollination – randomly selected fruits) had more than 3 seeds/fruit. Seed set is an indicator of apple fruit quality parameters, with greater seed numbers result-

Figure 2. Percentage of fruit set two weeks after petal fall of flowers of the apple cv. 'Delicious Pilafa Tripoleos' after different pollination treatments: (a) a single visit of *A. mellifera,* (b) at least one visit of *Bombus terrestris,* (c) hand, (d) wind, Kruskal-Wallis: χ^2= 12.959, p= 0.0047, Tegea, plateau, Greece, spring 2022. Small letters indicate statistically significant differences between the pollination treatments.

ing in greater production of top commercial value fruits (Webber *et al.*, 2020). Statistically significant differences were found between the fruits from free pollination and the control which was also free pollinated but without the effect of the net-cage after petal fall (Table 2). The latter (control) showed significantly higher fruit weight, minimum, maximum fruit length and minimum fruit width (p= 0.0120, p= 0.0047, p= 0.0141, p= 0.0197 respectively). These differences could possibly be attributed to the effect of the net used only in the free pollination treatment from BBCH 71 to BBCH 87, as published studies mention that net covering can affect production and fruit quality of apple and other fruits (Manja *et al.*, 2019; Jordaan, 2023).

Regarding any possible effect of ground-cover management on fruit quality characteristics, no effect was found on most of the fruit characteristics examined in year 2021. Floral plantings, which attract wild bees and enhance overall pollinator abundance in apple orchards (Campbell *et al.,* 2017a; Barda *et al.,* 2023) have been reported to have positively affected apple size (Ratto *et al.,* 2021) whereas no differences were found in the current study. Also, others reported that apple shape measured as the difference between maximum and minimum fruit width was significantly affected by pollination with insect-excluded treatment fruits being more misshapen (Campbell *et al.,* 2017b). Here, only fruits derived from free pollinated flowers were studied. The fruit shape did not differ between fruits produced from free pollination close to the flowering mixture and fruits from trees in the natural occurring groundcover (p= 0.7175) (Table 3). However, fruits produced from flowers adjacent to the flowering mixture had significantly higher skin firmness (p= 0.0224) while fruits from the control showed significantly higher total soluble solids when harvested (p= 0.0004). As the fruit matures, total soluble solids readings increase. However, both fruit samples were obtained the same day from the same field. In general, it is recommended to harvest apples with readings around 12% to 14% total soluble solids, but this var-

ies by cultivar and target markets for the fruits (Farcuh, 2023). Lower total soluble solids at harvest may warrant longer storability period for 'Delicious Pilafa Tripoleos' apples. Generally, there is a positive trend of increasing consumer preference in apples with firmness (Harker *et al.,* 2008) which underlines the positive effect of the flowering mixture to the marketability of 'Delicious Pilafa Tripoleos' apples.

Conclusions

Overall, we can conclude that a pollinizer apple variety and insect pollinators are necessary for successful pollination and fruit set of 'Delicious Pilafa Tripoleos' apples. Wind pollination cannot result in fruit set. Single flower visits by honey bees can successfully pollinate flowers in a low percentage, which we assume could be increased with more visits. However, free pollination with at least one visit of *B. terrestris* (use of bumblebee hives is an increasingly applied farming practice) resulted in higher fruit set. Enhancement of pollinators using a groundcover management scheme of flowering patches in the apple orchard seems to have a further positive impact on pollination and fruit quality characteristics in 'Delicious Pilafa Tripoleos' since fruits produced from flowers neighboring the flowering mixture patches had significantly higher skin firmness (desirable by consumers) and significantly lower total soluble solids at harvest (may warrant longer storability period). Further research with diverse bee taxa is suggested to study the effect of different pollinators on fruit set and fruit quality.

Table 2. Fruit quality characteristics (mean ± SE) of 'Delicious Pilafa Tripoleos' apples obtained from: a) wind pollination (1 fruit), b) free pollination with insect proof net from petal fall until harvest (37 fruits), c) free pollination without insect proof net (control, 32 fruits), Tegea, plateau, Greece, spring 2020.

Pollination treatment	Fruit weight (g)	Maximum fruit length (mm)	Minimum fruit length (mm)	Maximum fruit width (mm)	Minimum fruit width (mm)	seeds/fruit	Seeds' weight/ fruit (g)	Total soluble solids (°Bx)
Free pollination (n=37)	115.35 ± 5.29 **b**	60.97 ± 1.14 **b**	55.05 ± 0.96 **b**	64.86 ± 1.13	60.47 ± 0.94 **b**	3.58 ± 0.24	0.19 ± 0.01	15.45 ± 0.20
Control (n=32)	129.50 ± 3.48 **a**	64.56 ± 0.81 **a**	58.37 ± 0.64 **a**	67.40 ± 0.67	63.22 ± 0.62 **a**	4.28 ± 0.34	0.23 ± 0.02	15.95 ± 0.12
*Wind pollination (n=1)	117.07	64.39	52.88	62.76	62.01	1.00	0.01	14.73

Small letters indicate statistically significant differences between 'free pollination' and 'control' treatments (T-test, Wilcoxon test, α = 0.05). Analysis was not possible for wind pollinated fruits since only one fruit was obtained; n = number of fruits.

Table 3. Means of fruit quality characteristics of 'Delicious Pilafa Tripoleos' apples obtained from free pollination on trees close to the flowering mixture (FM) and trees close to naturally occurring groundcover (control), Tegea plateau, Greece, year 2021.

Groundcover	Fruit weight (g)	Maximum fruit length (mm)	Minimum fruit length (mm)	Maximum fruit width (mm)	Minimum fruit width (mm)	seeds/fruit	Seeds' weight/ fruit(g)	Total soluble solids (°Bx)	Skin firmness (kg)	Flesh firmness (kg)	Fruit shape
Free pollination FM (n= 12)	175.74 ± 6.35	69.63 ± 1.30	62.28 ± 1.30	77.04 ± 1.14	71.48 ± 1.11	6.42 ± 0.66	0.34 ± 0.04	14.62 ± 0.19 **b**	2.67 ± 0.08 **a**	2.24 ± 0.11 **b**	5.55 ± 0.77
Free pollination Control (n=12)	188.46 ± 11.41	71.31 ± 1.67	64.33 ± 2.00	77.99 ± 1.80	72.82 ± 1.38	5.58 ± 0.79	0.31 ± 0.04	15.94 ± 0.25 **a**	2.43 ± 0.10 **b**	1.99 ± 0.13 **b**	5.18 ± 0.70

Small letters indicate statistically significant differences between the treatments (T-test, Wilcoxon test, α = 0.05); n= number of fruits.

orchards and sharing their invaluable field experience, the Professor Emeritus Stavros Vemmos (Agricultural University of Athens) for his guidance regarding apple fruit characteristic measurements, the Researcher Kyriaki Varikou (ELGO-DEMETER) for her field advice regarding the bagging of the apple flowers, the Professor Ioannis Karapanos (Agricultural University of Athens) for providing the penetrometer and instructing the first author on the use of the equipment and the company Hellagro for donating the net used in the different pollination treatments.

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Η επικονίαση και καρπόδεση της Προστατευόμενης Ονομασίας Προέλευσης ποικιλίας μηλιάς «Ντελίσιους Πιλάφα Τριπόλεως» εξαρτάται από έντομα επικονιαστές

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Περίληψη   Η ανάγκη επικονίασης από τα έντομα στην καλλιέργεια της μηλιάς διαφέρει ανάλογα με την ποικιλία. Η «Ντελίσιους Πιλαφά Τριπόλεως» είναι καλλιεργούμενη ποικιλία μηλιάς Προστατευόμενης Ονομασίας Προέλευσης για την οποία δεν υπάρχουν στοιχεία σχετικά με τον βαθμό αυτοασυμβίβαστου που παρουσιάζει και τις ανάγκες για επικονίαση από τα έντομα για εμπορική παραγωγή. Στην παρούσα εργασία εξετάστηκε η επίδραση της επικονίασης από τον άνεμο (άνθη σε κλωβούς), άνεμο και έντομα (άνθη χωρίς κλωβούς), από τη μελιττοφόρο μέλισσα, από ελεύθερη επικονίαση με τουλάχιστον μία επίσκεψη βομβίνου, και με το χέρι, στην καρπόδεση και στα χαρακτηριστικά του καρπού. Επίσης, μελετήθηκε η επίδραση ανθοφόρων φυτικών μειγμάτων με τη μορφή νησίδων εντός του μηλεώνα ως πρακτική για την προσέλκυση επικονιαστών, στα ποιοτικά χαρακτηριστικά των καρπών. Για την επιτυχή επικονίαση της μηλιάς cv. «Ντελίσιους Πιλαφά Τριπόλεως» είναι απαραίτητη η παρουσία επικονιάστριας ποικιλίας και εντόμων επικονιαστών, καθώς η επικονίαση με το χέρι με γύρη ανθέων από την ίδια ποικιλία, και η επικονίαση με τον άνεμο οδήγησαν σε χαμηλά ποσοστά καρπόδεσης. Μία επίσκεψη *A. mellifera* ανά άνθος μηλιάς οδήγησε σε καρπόδεση, ωστόσο, η ελεύθερη επικονίαση με τουλάχιστον μία επίσκεψη από *B. terrestris* είχε ως αποτέλεσμα υψηλότερο ποσοστό καρπόδεσης σε σύγκριση με τις άλλες μεταχειρίσεις επικονίασης. Η «ελεύθερη» επικονίαση οδήγησε σε περισσότερους καρπούς με μεγαλύτερο αριθμό σπερμάτων από ότι η επικονίαση με τον άνεμο (η οποία έδωσε μόνο έναν καρπό). Τα μήλα που προέρχονται από άνθη που βρίσκονταν κοντά σε νησίδες ανθοφόρου μείγματος είχαν σημαντικά μεγαλύτερη συνεκτικότητα φλοιού και χαμηλότερα ολικά διαλυτά στερεά συστατικά κατά τη συγκομιδή (και τα δύο επιθυμητά χαρακτηριστικά για την cv. «Ντελίσιους Πιλαφά Τριπόλεως»), σε σύγκριση με καρπούς δένδρων που γειτνίαζαν με νησίδες αυτοφυούς χλωρίδας.

Περιεχόμενα

Contents

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