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## Collection of data and information on biology and control of vectors of *Xylella fastidiosa*

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### Abstract

The scope of this project was the collection of data and information on biology, phenology and control of vectors and potential vectors of *Xylella fastidiosa* (referred to, from now on, as Xf vectors). Systematic literature searches covering the different topics were conducted on authoritative databases indexing peer-reviewed scientific publications as well as on grey literature repositories. An extraction table on the ecology and phenology of Xf vectors was generated, together with a draft protocol for field data collection targeted to *Philaenus spumarius*. Efficacy tables on different control methods were also produced and crosschecked with data on plant protection products currently applied in the EU with effect on Xf vectors, producing a good agricultural practices table. With the help of questionnaires, we inventoried: i) the ongoing integrated pest management programmes on stone fruits, citrus, grapevine and olive crops in the Mediterranean Member States (MedMSs), and the monitoring activity on *Xylella* vectors currently conducted in all the EU MSs. Finally, we collected new experimental data on phenology and ecology of *P. spumarius* and other spittlebug species for three consecutive years (2016-2018). The data have been collected via field surveys in olive orchards, vineyards and insect rearing at the macrocosm (field), mesocosm (screenhouse) and microcosm (small rearing cage) level. All observations in olive groves were conducted in parallel in the Apulia and Liguria regions, while those in vineyard were carried out in the Piedmont region (Italy). The results are provided in the body of the report as metanalysis and in Excel tables as supporting material and concern i) phenology, host-plant associations and population dynamics of nymphs and adults of *P. spumarius* and other spittlebugs under different environmental conditions, ii) fecundity and reproductive biology from microcosms and field-collected females. Field and mesocosm data are supported by agronomic and meteorological data.

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**Key words:** control, phenology, population dynamics, sharpshooters, spittlebugs, vectors, *Xylella fastidiosa*

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## Summary

*Xylella fastidiosa*, a quarantine organism, has been first detected in Europe in 2013 in the Salento area of Italy, where it is severely affecting olive crops in the Apulia region. Soon after the first discovery, the bacterium has been identified in Corse and in the PACA Region of France, where so far it is limited to ornamental plants and some landscape trees, and in Spain, where the bacterium has been detected in the Balearic Islands and in mainland (provinces of Alicante and Madrid). Very recently, *X. fastidiosa* has been found in Tuscany Region of Italy and in Portugal. *X. fastidiosa* is a major risk to the EU territory because it has the potential to cause severe diseases once it establishes, as hosts are present and the environmental conditions are favourable. *X. fastidiosa* may affect several crops in Europe, such as citrus, grapevine and stone fruits (almond, peach, plum), but also several tree and ornamental plants, for example oak, sycamore and oleander. *X. fastidiosa* has a very broad host range, including many cultivated and wild plants common in Europe and is transmitted by Hemipteran insects that feed in the xylem sap. All xylem fluid-feeding insects in Europe have to be considered as potential vectors. Members of the families Cicadellidae, Aphrophoridae and Cercopidae are vectors in the Americas and, hence, should be considered potential vectors in Europe. The species of Cicadidae and Tibicinidae should also be considered potential vectors, although their role in *X. fastidiosa* transmission is not proven yet. The Aphrophoridae spittlebug *Philaenus spumarius* has been identified as a vector in Apulia, Italy.

Following the invitation of EFSA within the call "Collection of data and information on biology and control of vectors of *Xylella fastidiosa*", the IPSP-CNR carried out several activities with the aim to provide EFSA with a comprehensive overview on biology and phenology of the vectors/potential vectors (referred to, from now on, as Xf vectors) and on available control methods. This general objective is achieved by the integration of literature-based data, questionnaire-based data and experimental data in order to allow EFSA for the analysis of different scenarios based on combination of vector species/control option/environmental conditions. This document is the final report of these activities and deals with the results of

- a) data collection from literature on ecology and phenology of Xf vectors (Task 1)
- b) data collection from literature on control options against Xf vectors for integrated and for organic farming (Task 2)
- c) data collection on plant protection products allowed in the EU with effect on *X. fastidiosa* vectors applied on stone fruits, citrus, grapevine and olive plants in the MedMSs (Task 3)
- d) inventory of the ongoing integrated pest management (IPM) programmes on stone fruits, citrus, grapevine and olive plants in the MedMSs (Task 4)
- e) inventory of national and regional competent organization in MedMS with access to monitoring data on Xf vectors (Task 5)
- f) field observations on phenological phases (supported also by agronomic and meteorological data) of *P. spumarius* in olive orchards in 2016, 2017 and 2018 (Tasks 6 and 7 and activities 1 and 2 of the Amendment)
- g) study of *P. spumarius* fecundity and development under microcosm conditions (activity 3 of the Amendment)
- h) data on reproductive biology of *P. spumarius* (maturation of eggs over time) (activity 4 of the Amendment)
- i) data managing and analysis of *P. spumarius* in vineyard 2016 and 2017 (activity 5 of the Amendment)
- j) training activity for knowledge transfer to the new Art 36 EFSA funded project on vectors of *Xylella fastidiosa* in Balearic Islands (activity 7 of the Amendment)

The points g), h), i), j) as well as the continuation in 2018 of some activities described in f), are established in the Annex 1 of the Amendment to the Procurement.

For the systematic literature search (Tasks 1 and 2) the adopted protocol was based on the following milestones:

- Definition of research questions
- Establishment of search terms and their arrangement into search strings
- Definition and access to information sources
- Screening of the search results
- Reference management
- Quality assessment

The following authoritative databases indexing peer-reviewed scientific publication have been used as primary sources of information: AGRICOLA, CABI-Abstracts (CAB), EPPO Global Database, JSTOR, MEDLINE, Scopus and Web of Science. The databases were interrogated using the same strategy in two consecutive years (2016 and 2017).

Besides authoritative databases, the following grey literature repositories were interrogated in 2016: GreyNet International, [www.greynet.org](http://www.greynet.org), Google Scholar, <http://www.scholar.google.it>, Internet Archive, <http://www.archive.org>, OCLC WorldCat, <http://www.worldcat.org>. In addition, the recursive agents DEVONagentPro ([www.devontechnologies.com](http://www.devontechnologies.com)) and GrandReporter (<http://www.tri-edre.com>) were used for recursive research in INTERNET.

For phenology and ecology of vectors/potential vectors (Task 1), about 4,000 records were deduplicated and screened for relevance based on title and the abstract, allowing to the identification of 607 potential interesting papers, 267 of which were rated relevant and used for generating the extraction summary table on *X. fastidiosa* vectors and potential vectors phenology and ecology. This summary table shows that the most well-known species is the American sharpshooter *Homalodisca vitripennis*, whose biology/ecology/phenology is very well characterized with respect to host plant preference, life cycle and population dynamics, fecundity, flight dispersal, feeding behaviour and symbionts. However, there is a good amount of available knowledge on the ecology and phenology of the two main species of Cicadellinae present in Europe: *Cicadella viridis* and *Graphocephala fennahi*. Few information about the habitat/ecology of the other European Cicadellinae, *Evacanthus interruptus* and *E. acuminatus* and *Cicadella lasiocarpae*, is available, while almost no information on their phenology is available. Among spittlebugs, *Philaenus spumarius* is the most studied species and data on host plants of nymphs and adults, life cycle and population dynamics are available, although detailed knowledge on the abundance and phenology of this species on selected crops is not available. Ecology and phenology of other European spittlebugs is poorly known, and information is scattered. However, some interesting data on the host plants and life cycle are available for species of the genera *Aphrophora*, *Cercopis* and *Neophilaenus*. The main available information on cicadas concerns their host plants, the characteristics of environments colonized by cicadas and the period of adult presence. Few data about egg laying and prolificacy are also available.

Based on data retrieved from the literature and on our own experience, a draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius* was generated. An inventory of sampling methods for xylem-sap feeder insects has also been compiled. Visual inspection and counting of spittlebug nymphs is the only practical method for the study of the nymph population of *P. spumarius* (and of the other spittlebugs), while sweep net is the most widely applied sampling method of *P. spumarius* adults. Yellow sticky traps can be used to collect adult of the spittlebug, although their efficiency is debated.

For potentially effective control options against all the vectors and potential vectors of *X. fastidiosa* all around the world (Task 2), more than 2700 records were deduplicated and screened for relevance on the base of the abstract (reports dealing with insecticides not registered in the EU anymore were

discarded), allowing to identify more than 800 potential interesting papers, 395 of which were rated relevant and used for generating efficacy tables on chemical, biological and agronomic/physical control methods. Literature on insecticides activity against xylem-sap feeder insects is mainly restricted to sharpshooter vectors of *X. fastidiosa* in North and South America. Neonicotinoids appear to be effective and persistent on both adults and nymphs. Pyrethroids are effective against both adults and nymphs of sharpshooters but by far less persistent than neonicotinoids. Data on activity of organophosphates against sharpshooters are sometimes conflicting and, when tested weeks after treatment, these molecules were poorly persistent. The application of Insect Growth Regulators (IGR) against the spittlebug nymphs seems not to be promising, also because of the possibility of suppressing nymph population with soil tilling. There is a major gap of knowledge on the activity of insecticides allowed in organic farming, although preliminary data suggest that essential citrus oil is more active than pyrethrins. Unfortunately, citrus oil, like pyrethrins, is not persistent at all. In spite of the very limited data available on insecticide activity against spittlebugs, it is likely that neonicotinoids show an active and persistent activity against all *X. fastidiosa* vectors/potential vectors while pyrethroids, although active, are not persistent.

Literature on biological control of *X. fastidiosa* vectors/potential vectors is restricted to the description of natural enemies, mainly represented by egg parasitoids of the genus *Gonatocerus* (family Mymaridae) and *Oligosita* (family Trichogrammatidae), the endoparasitoid *Verrallia aucta* (family Pipunculidae) and by the entomopathogenic fungus *Metarhizium anisopliae*. However, in our large-scale mesocosm rearing, a fungal epizootic killed all *P. spumarius* nymphs. From these, we isolated two fungi, *Beauveria bassiana* and *Fusarium oxysporum*, that are known to be entomopathogenic, though not noticed for *P. spumarius*. Their actual role as causal agent of the lethal disease is still to be demonstrated, still the finding is interesting. The analysis of literature shows that so far only "classic" biological control programs (inoculative introduction of natural enemies from the original area of distribution of the insect vector) have been applied and this application was targeted against the invasive sharpshooter vector *Homalodisca vitripennis*. Since *P. spumarius* is a native species in the EU, inoculative biological control is not feasible, and inundative biological control, based on mass-reared beneficials, has never been experienced and is unlikely to be effective because the spittlebug is ubiquitous and polyphagous. The most well-known natural enemy of *P. spumarius* seems to be a pipunculid fly, *Verrallia aucta*, a parasitoid of nymphs and adults that can parasitize up to one third of the adults under field conditions. Apart for some generalist predators, very few information is available on other natural enemies. This is a major research gap that hampers the possibility of implementing a biological control based on a conservation approach, which might contribute to the partial suppression of *P. spumarius* populations.

There is very little literature on control options other than insecticides and biological control. However, some interesting information were found. For example, removal of straw in pastures seems to significantly reduce the populations of spittlebugs. Spraying with "Surround WP", that forms a particle film on the vegetation, strongly reduced the population of sharpshooters, both in choice and no choice tests. Recent experiences in the Apulia Region pointed out that soil tilling resulted in a very strong reduction of spittlebug adults on the ground vegetation, although the reduction of adults collected on the olive canopy can be less important. Finally, it is worth to remember that protecting nurseries with netting is an effective method to keep nursery stocks free of insect-transmitted pathogens, including *X. fastidiosa*. A prolonged flooding can be effective in killing cicada nymphs but this technique is not applicable in the vast majority of cases (no water available in dry environments where cicadas live, sloped crop plots).

Based on the result of Task 2, a list of plant protection products allowed in the EU with effect on *X. fastidiosa* vectors were identified and used for the construction of a GAP table (Task 3). Information were retrieved from national official databases (most of the links were gathered from the EPPO website) and organized in an excel table that included columns on: a.i., mode of action (MoA) category, crop, MedMs, target pest. One entry was prepared for each DAR/crop/state. The table can be filtered by active ingredient (a.i.), crop, MedMS and mode of action. According to the table, neonicotinoids have the widest application on stone fruits, citrus, grapevine and olive in the MedMSs, followed by organophosphates, the phenoxy derivative etofenprox and pyrethroids.



The aim of Task 4 was to provide an updated inventory of the Integrated Pest Management (IPM) programmes in place in the MedMSs for stone fruits, citrus, grapevine and olive, and the pests targeted by these IPM programmes. To obtain information on the ongoing IPM programmes, a questionnaire was prepared and sent to contact points of all MedMSs. The recipients of the questionnaire were the official websites of the Ministry of Agriculture (or equivalent ones). Further information was retrieved by websites of the Ministries of Agriculture of the MedMSs. The results are summarized in an excel table, which provides information for each crop and each country. IPM programs are ongoing in all/almost all the MedMSs on most of the stone fruit and citrus orchards as well as on grapevine and olive. None of these IPM programs mentions xylem-sap feeder insects among the target pests and, thus, current control programs do not consider *X. fastidiosa* vectors as pests.

For Task 5, a list of public national and regional as well of private organisations has been compiled based on contacts proposed by CNR-IPSP, EFSA, EPPO and EUPHRESKO. These organisations are located in all the MedMs, in the rest of the EU and in several non-EU countries and might have access to monitoring data on vectors and potential vectors of *X. fastidiosa*. The questionnaire has been sent to all these organisations, including all the Italian Regional Phytosanitary services. We obtained 30 answers from outside Italy, coming from 19 different countries and 18 answers from Italy, coming from 15 regions. All the answers have been included in a single excel file, each sheet corresponding to an answer from an organisation, ordered in country alphabetic order. In order to comply with the request of the procurement, the information obtained from the survey (questionnaire), have been combined with those obtained from literature (Task 1, Extraction Table Ecology and phenology) and a new version of this Table has been produced. Moreover, we summarized the main results (monitoring campaign ongoing or planned for 2017, no monitoring campaign ongoing or planned) in two maps of Europe and Italy, respectively. Based on the questionnaires, monitoring programs on *X. fastidiosa* vectors have been enforced in most of the European countries and in all the Italian regions. Few programs started in 2014, most of them started in 2015 or 2016. Monitoring programs are focused mainly on adults of spittlebugs and of *C. viridis*. Sweep net and yellow sticky traps are the most commonly applied sampling methods.

The activities of tasks 6 and 7 provided a big amount of data on biology, ecology, phenology and population dynamics of *P. spumarius* nymphs and adults in olive agroecosystems of the Mediterranean area. Although the nymphs were polyphagous, they showed a strong host-preference for herbaceous plants of the Asteraceae and Fabaceae families both in Liguria and in Apulia regions of Italy. The nymphs found on the plants of these families accounted for 72-88% of the total nymphs. Nymphs of *Aphrophora* showed a similar host-preference, while those of *Neophilaenus* were strongly associated with Poaceae (85-100% of the nymphs were found on gramineous plants). These two spittlebug species, although present in the olive agroecosystems, had a very low population density compared to *P. spumarius*. In this latter, the average nymphs population density varied from 13 to 30 individuals/m<sup>2</sup> in Liguria (min value=0, max value= 68) according to the olive grove and the year, and from 5 to 19 individuals/m<sup>2</sup> in Apulia (min value=0, max value= 39), although in 2018 population density in the same olive groves of Apulia never exceeded 5 nymphs/m<sup>2</sup>. The reasons for this lower density, besides annual variation due to environmental variables, can be soil and olive canopy disturbance at Locorotondo (where soil has been tilled in order to fertilize and a mild pruning of olive has been done). As for the olive grove of Valenzano no special disturbance has been provided to the field, still the population density was lower than previous years.

Phenological data based on physiological time revealed that in Liguria the peak of abundance of *P. spumarius* nymph population was between 150 and 210 degree day (DD) while in Apulia the same peak was between 100 and 270 DD. This difference among locations could be explained by a non-linear component in the temperature-dependent development rate function of *P. spumarius*. The phenological pattern in the two regions is more similar if referred to chronological time. In fact, nymphs developed in Liguria between early March and end of May and in Apulia between the end of February and mid-May.

The pattern of adult population abundance varied with the site, the plant compartment and the habitat. The maximum adult density on olive was recorded in summer in Liguria and in late spring/early summer in Apulia. In addition, the number of individuals found tends to drop more abruptly in July/August in Apulia compared to the same period in Liguria, likely due to the complete drying up of the herbaceous cover in this region. Regarding the alternative woody host compartments, *P. spumarius* adults preferred *Quercus* spp. (*Q. ilex*, *Q. petraea* and *Q. crenata*) and *Pistacia* spp. (*P. lentiscus* and *P. terebinthus*) in the Ligurian sites. In the Apulian sites, the spittlebug adults were more frequently collected on *Myrtus communis*, *P. lentiscus* and *Phillyrea angustifolia*, although in 2018 *Quercus* was the most important alternative woody host also in Apulia. It is worth noting that woody host preference is driven by the plant species composition of the investigated sites. However, evergreen and deciduous oaks seem to be among the preferred hosts of adults. A clear host-shifting of adults that move from the herbaceous cover to woody hosts and back to herbaceous plants at the end of summer-autumn was observed in three out of four sites.

In the mesocosm, following two releases of 200 (100 males and 100 females) adults, in 2016 only few adults have been found on olive trees. Many more adults were found on olive trees in 2017 (between 15-50% of all adults counted in the mesocosm in June-July, according to the sampling date). This result can be explained by referring to the strong component of artificiality introduced in the mesocosm: the establishment of a forced environment through a selection of plant species, the higher relative humidity due to the confined environment, and the small size of olive trees in the mesocosm. In 2017, the highest number of adults sampled was between June and early August, decreasing from August to October, and confirming the phenological trend of adults observed in the field. Nymph population increased gradually until reaching the maximum peak of abundance around 250 DD and then decreasing. Pre-imaginal stages followed a phenological trend with respect to physiological time similar to that found in the open field. The development of pre-imaginal stages in the mesocosm occurs from 23 DD to 357 DD, following the general trend that has been observed in open field.

Data on *P. spumarius* prolificacy obtained in the microcosm studies indicated that a single female produced on average about 90-110 and 18-20 eggs in the experiments carried out in Torino and Bari, respectively. The reasons for the very important difference in prolificacy recorded in Piedmont and Apulia must be investigated, but are beyond the scope of this work. The analysis of the ovaries of *P. spumarius* females over the year revealed that they mature eggs after a very long ovaric diapause. The first mature eggs occur at mid-September in Northern and at the beginning of October in Southern Italy. In colder areas, ovaric diapause is shorter and mature eggs are found since August.

Data analysis of *P. spumarius* in vineyard in 2016 and 2017 in Piedmont Region show that phenology and host-plant association of the spittlebug are very similar to those recorded in olive groves of Liguria Region, with a slightly higher density of population. As for the habitat selection of adults, the adult density on grapevine is quite low and constant from the end of May to the end of September. The host-shifting of adults from the herbaceous cover to woody hosts and back to herbaceous plants at the end of summer-autumn, observed in olive groves, has been confirmed also in the vineyard.

The present work represents a substantial improvement on the knowledge on *P. spumarius*, namely on its phenology in the Mediterranean area, host-shifting of adults over the season, prolificacy and development in relation to temperature. The different results obtained in Liguria and Apulia regions in term of DD can be explained by a strong non-linear component of the development rate function of *P. spumarius*. The collected data can be used to identify the nonlinear functions that are appropriate for modelling the spittlebug phenology.

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# 1. Introduction

## 1.1. Background and Terms of Reference as provided by EFSA

### 1.1.1. Background

*Xylella fastidiosa* (Wells and Raju) is a vector-transmitted bacterial pathogen associated with important diseases in a wide range of plants. Following its first outbreak under field conditions in the European Union (in Lecce province in Apulia, Italy, in October 2013) the European Commission asked EFSA to provide scientific and technical assistance on this subject. Since the first mandate (received from DG SANCO in November 2013) EFSA published four outputs on *X. fastidiosa*:

- Statement of EFSA on host plants, entry and spread pathways and risk reduction options for *Xylella fastidiosa* Wells et al. (26 November 2013 – Statement of EFSA)
- Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options (06 January 2015 – Opinion of the Scientific Panel)
- Categorisation of plants for planting, excluding seeds, according to the risk of introduction of *Xylella fastidiosa* (20 March 2015 – Scientific Report)
- Response to scientific and technical information provided by an NGO on *Xylella fastidiosa* (17 April 2015 – Statement of EFSA)

*X. fastidiosa* is exclusively transmitted by xylem sap-feeding insects of the order Hemiptera, sub-order Auchenorrhyncha, infraorder Cicadomorpha, superfamilies Cercopoidea (spittlebugs, froghoppers), Cicadoidea (cicadas) and Membracoidea (of this latter only the subfamily Cicadellinae (sharpshooters) is relevant). Spittlebugs, froghoppers and sharpshooters are proven vectors of *X. fastidiosa*, while for cicadas, final evidences are still missing.

As infectious insect vectors play a key role in the epidemics and spread of *X. fastidiosa*, EFSA needs to collect further information on their control. That information, combined with data on the seasonal development and life cycle of the vectors, would allow EFSA a more quantitative approach in the risk assessment of *X. fastidiosa*. This information can also be of use for identifying best options to control the vectors in the field both in conventional and in organic farming agriculture, that may affect the rate of the spread and the impact of *X. fastidiosa*.

The lists of the putative European vectors and of the known American vectors of *X. fastidiosa* were provided in the EFSA Plant Health Panel Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory, with the identification and evaluation of risk reduction options and are reproduced in Tables 1 and 2 of the technical specification of the EFSA contract.

The preparatory work for EFSA scientific advice needs to follow a systematic approach using a defined methodology to conduct the literature search and select the relevant studies, as described in EFSA (2010), in particular sections 3.2 and 3.3.

The objectives of the Specific Contract RC/EFSA/ALPHA/2015/01 – CT1 as specified by EFSA are as follows:

**Objective 1:** collect data from literature and field observation on biology and life cycle of vectors and potential vectors of *X. fastidiosa* (referred to, from now on, as Xf vectors) in the EU.

**Objective 2:** collect data from literature and databases on control options against Xf vectors for conventional and for organic farming agriculture.

These outputs will provide EFSA with a useful overview on the control methods currently available in the EU against *X. fastidiosa* vectors and on alternative ones for which evidence on effectiveness has been proved outside the EU. The combination of this information with data on biology and phenology

of the vectors will allow EFSA for the analysis of different scenarios based on combination of vector species/control option/environmental conditions.

The steps envisaged for completing objective 1 (collection of data on biology of Xf vectors in the EU) are:

- A systematic literature search on the seasonal development and life cycle of the Xf vectors in the EU. This search should cover scientific literature and grey/technical literature (e.g. technical bulletins, conference proceedings etc.) published in English or in, at least, the languages spoken in the EU Member States with Mediterranean climates (hereinafter referred to as Mediterranean Member States, MedMSs: Croatia, Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia and Spain). As some of the European Xf vectors are also present outside the EU territory, the search should not have any geographical limitations. Considering the results of the searches above, the contractor should propose a draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius* (which is at the moment the only proven vector of *X. fastidiosa* in the EU), which should list the minimum data requirements and could be used also in other studies (Task 1).
- An inventory of national and regional competent organisations (including public national and regional organisations as well as private, cooperative or non-governmental organisations) covering at least the EU MedMS in the areas of monitoring insect plant pests, with access to monitoring data on Xf vectors. A survey by questionnaire and search on websites, addressed to the above mentioned national and/or regional competent organisations (e.g. organisations competent for plant health such as the national and regional plant protection organisations but also public and/or private organisations competent at national or regional level for extension and advice on integrated crop management, organic farming or sustainable agriculture) of the MedMSs, with the aim to identify monitoring programmes and surveys conducted and/or planned on Xf vectors. The list of all found monitoring and survey programmes on Xf vectors should be presented by the contractor in Excel in a tabular form summarising all key information (such as contact details and links of the organisations as well as details on the monitoring activities conducted for these insects, including spatial and temporal aspects). When results of these monitoring programmes are available, these should be collected, analysed and, when applicable, integrated with other data on vectors biology collected by the contractor in a summary table that combine information gathered from literature (Task 1) with those obtained with this task (5).
- Two years or three years (only in Apulia region) observations on phenological phases (supported also by agronomic and meteorological data), and ecology of the vector *P. spumarius* in olive orchards (Tasks 6 and 7): the contractor should propose a good balance between number of observation sites and temporal resolution of the observations. IPSP-CNR has designed observations at three levels: macrocosm (open field surveys in Liguria and Apulia regions), mesocosm (two screen houses made of insect-proof net settled in Piedmont and Apulia regions), microcosm (small cages for the study of the fecundity of single individual females, caged together with a male) settled in Piedmont and Apulia (Tasks 6 and 7). The field surveys were conducted in two olive groves of the Liguria and two of the Apulia Regions, in order to represent a large variation in climatic conditions within the warm-summer Mediterranean climate. Experimental data obtained from the field, the mesocosm and the microcosm are included in an inventory table that combine collected data from literature (Task 1), surveys enforced by national and regional organisations (Task 5) and data from the field, mesocosm and microcosm surveys (Tasks 6 and 7).
- studies on reproductive biology of *P. spumarius* (maturation of eggs over time) (activity 4 of the Amendment)
- data managing and analysis of two years observations on phenological phases and ecology of the vector *P. spumarius* in vineyard in Piedmont (activity 5 of the Amendment)

- training activity for knowledge transfer to the EFSA funded project on vectors of *X. fastidiosa* in Balearic islands (prof. Miguel Miranda, University of Balearic islands) (activity 7 of the Amendment)

The steps envisaged for completing objective 2 (collection of data on control of Xf vectors for conventional and organic farming agriculture) are:

- A list of potentially effective control options against Xf vectors both for conventional agriculture and for organic farming (such as active ingredients, biocontrol agents, agricultural practices etc.): in order to obtain it, the contractor shall conduct a systematic literature search on control of all species of known (both American and European) and potential (European) vectors of *X. fastidiosa*. The search should address, without any geographical limitations, all control options against Xf vectors for use in conventional agriculture and organic farming (including active insecticide ingredients, biocontrol agents, agriculture practices such as for example soil and weed management, integrated pest management strategies etc.). This search should cover scientific literature and grey/technical literature (e.g. technical bulletins, conference proceedings etc.) published in English or in, at least, the languages spoken in the EU Member States with Mediterranean climates (hereinafter referred to as Mediterranean Member States, MedMSS: Croatia, Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia and Spain). All the references screened and selected need to then be compiled in EndNote files from which the user should be able to filter those studies relevant for the EU. A data extraction table resulting from the literature screening should be built and compiled in such a way that users will be able to filter the information by control method (e.g. active ingredient, biocontrol agent, etc), by affected pest (i.e. by insect Xf vectors), by approval for use in Europe, by farming system (i.e. conventional agriculture, organic farming). The efficacy of the control option should be indicated as in the example provided by EFSA (i.e. extraction table provided in Annex V of the procurement; please note that the table should include information on pest species on which the substance has been tested) (Task 2).
- A review of the 71 Draft Assessment Reports (DARs) on insecticides, that EFSA Pesticides (PRAS) Unit has received till now (which are freely accessible online at EFSA website) and in which information on ecotoxicology of the active ingredient is available, with the aim to identify reports where Xf vectors have been studied as target or as non-target species and to extract data when relevant (Task 2).
- An inventory table of GAPs (Good Agricultural Practices) applicable to those active ingredients which have been identified by the above mentioned literature search and/or review of DARs and are allowed for use in the EU. GAPs should be extracted from national databases at least of MedMSS. The GAP table should be built in such a way that users will be able to filter the information by crop or by country<sup>6</sup> (Task 3).
- An inventory of the Integrated Pest Management (IPM) programmes for woody crops (in particular almond, citrus, grapevine, olive, stone fruits) at least for the EU MedMSS (Task 4).

### 1.1.2. Terms of Reference

This contract was awarded by EFSA to:

Contractor: Istituto per la Protezione Sostenibile delle Piante del Consiglio Nazionale delle Ricerche (IPSP – CNR)

Contract title: 'Collection of data and information on biology and control of vectors of *Xylella fastidiosa*'

Contract number: Specific Contract RC/EFSA/ALPHA/2015/01 – CT1

Implementing Framework Contract OC/EFSA/AMU/2014/01 Lot 6 – FWC 1 Plant Health

## 2. Introduction

This document is the final report in which the outputs of Tasks 1 to 7, specified in the contract RC/EFSA/ALPHA/2015/01 – CT1 (implemented in the Annex 1 of the Amendment), and the methodologies adopted to generate them, are shown. Therefore, this report includes information regarding:

- the literature searches on the phenology and ecology of *X. fastidiosa* vectors and potential vectors, and on the potentially effective control options against *X. fastidiosa* vectors and potential vectors, together with the requested deliverables including i) the extraction summary table on *X. fastidiosa* vectors and potential vectors phenology and ecology (Task 1), ii) the draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius* (Task 1), and the efficacy tables on control options against *X. fastidiosa* vectors and potential vectors (Task 2).
- the inventory tables of i) Good Agricultural Practices (GAPs) and ii) Integrated Pest Management (IPM) programmes for woody crops (Tasks 3 and 4, respectively).
- the list of all found monitoring and survey programmes on *X. fastidiosa* vectors and potential vectors in the EU MS summarized in an Excel file (Task 5)
- the data collected during the 3-year observations (2016-2018) on phenology and ecology of the vector *P. spumarius* at the macrocosm (field), mesocosm and microcosm levels in Apulia, Liguria and Piedmont regions of Italy (Tasks 6 and 7, Annex 1 of Amendment)
- the summary/inventory table combining data gathered from literature (Task 1), surveys enforced by national and regional organisations (Task 5) and from the field, mesocosm and microcosm surveys (Tasks 6 and 7, Annex 1 of Amendment )
- the data collected in 2018 on reproductive biology (maturation of eggs over time) of *P. spumarius* (Annex 1 of Amendment)
- the data collected during the 2-year observations (2016-2017) on phenology and ecology of the vector *P. spumarius* in vineyard (Annex 1 of Amendment)

## 3. Systematic literature review on phenology and ecology of *Xylella fastidiosa* vectors and potential vectors (Task 1)

### 3.1. Objectives

The overall aim of this chapter is to provide an overview of the scientific literature regarding *X. fastidiosa* vectors and potential vectors phenology and ecology. The general strategy adopted for the literature search, which is consistent with the EFSA guidance (EFSA, 2010), has been agreed with EFSA during the kick-off meeting and further meetings. The searched literature has been used to generate i) an extraction summary table of *X. fastidiosa* vectors phenology and ecology and ii) a draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius*, which are the two deliverables of Task 1, specified in the EFSA contract.

### 3.2. Materials and Methods

The adopted protocol is based on the following milestones:

- Definition of search questions
- Establishment of search terms and their arrangement into search strings



- Definition and access to information sources
- Screening of the search results
- Reference management
- Quality assessment

### 3.2.1. Definition of search questions

Preliminary meeting between members of the systematic review (SR) team and members of other project tasks identified the following specific questions to be answered by the searches related to the Task 1:

1. Which is the phenology of *X. fastidiosa* vectors and potential vectors?
2. Which is the ecology of *X. fastidiosa* vectors and potential vectors?

According to guidance of EFSA on SR (EFSA, 2010), both questions were considered descriptive questions of populations (PO) in which the population (P) and the outcome of interest (O) are specified as reported in Table 1 (PO questions).

**Table 1:** Definition of terms within the objective of the systematic literature review.

<b>Population (P)</b>	European potential vector species and some relevant American vector species
<b>Outcomes (O)</b>	parameters of ecology and phenology

### 3.2.2. Specification of search terms

Based on the questions and the definition terms, search terms that could ensure identification of as wide relevant literature as possible, excluding most of the irrelevant literature, were identified by the SR team and agreed with the other researchers involved in other project tasks and with EFSA.

The terms used for defining the population include all European xylem-sap feeder species (listed in Annex C of the opinion published by EFSA PLH Panel, 2015) and only the main American vectors (listed in the technical offer contained in the specific contract) as shown in Table 2. These latter were identified, among the species listed in Annex D of the above-mentioned opinion, as the ones most common and abundant in diverse crops/ecosystems and associated with disease epidemics based on literature.

**Table 2:** List of European xylem-sap feeder species listed in Annex C of the opinion published by EFSA PLH Panel (2015) and of the American vectors included in the systematic literature searches

<b>EU xylem-sap feeder species</b>	<i>Anoterostemma ivanoffi</i> , <i>Aphrophora alni</i> , <i>Aphrophora corticea</i> , <i>Aphrophora major</i> , <i>Aphrophora pectoralis</i> , <i>Aphrophora salicina</i> , <i>Aphrophora similis</i> , <i>Aphrophora willemis</i> , <i>Cercopis arcuata</i> , <i>Cercopis intermedia</i> , <i>Cercopis sabaudiana</i> , <i>Cercopis sanguinolenta</i> , <i>Cercopis vulnerata</i> , <i>Cicada barbara</i> , <i>Cicada barbara lusitanica</i> , <i>Cicada mordoganensis</i> , <i>Cicada orni</i> , <i>Cicadatra alhageos</i> , <i>Cicadatra atra</i> , <i>Cicadatra hyalina</i> , <i>Cicadatra hyalinata</i> , <i>Cicadatra persica</i> , <i>Cicadatra querula</i> , <i>Cicadella lasiocarpae</i> , <i>Cicadella viridis</i> , <i>Cicadetta albipennis</i> , <i>Cicadetta caucasica</i> , <i>Cicadetta concinna</i> , <i>Cicadetta dubia</i> , <i>Cicadetta fangoana</i> , <i>Cicadetta flaveola</i> , <i>Cicadetta hageni</i> , <i>Cicadetta lobulata</i> , <i>Cicadetta mediterranea</i> , <i>Cicadetta montana</i> , <i>Cicadetta petryi</i> , <i>Cicadetta podolica</i> , <i>Cicadetta undulata</i> , <i>Cicadivetta tibialis</i> , <i>Errhomenus brachypterus</i> , <i>Evacanthus acuminatus</i> , <i>Evacanthus interruptus</i> , <i>Evacanthus rostragnoi</i> , <i>Graphocephala fennahi</i> , <i>Haematoloma dorsata</i> , <i>Hilaphura varipes</i> , <i>Lepyronia coleoptrata</i> , <i>Lyristes plebejus</i> , <i>Macugonalia</i>
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	<i>Iecomelas</i> , <i>Neophilaenus albipennis</i> , <i>Neophilaenus campestris</i> , <i>Neophilaenus exclamationis</i> , <i>Neophilaenus infumatus</i> , <i>Neophilaenus limpidus</i> , <i>Neophilaenus lineatus</i> , <i>Neophilaenus longiceps</i> , <i>Neophilaenus minor</i> , <i>Neophilaenus modestus</i> , <i>Neophilaenus pallidus</i> , <i>Pagiphora annulata</i> , <i>Pagiphora aschei</i> , <i>Paraphilaenus notatus</i> , <i>Peuceptylus coriaceus</i> , <i>Philaenus italosignus</i> , <i>Philaenus lukasi</i> , <i>Philaenus maghresignus</i> , <i>Philaenus signatus</i> , <i>Philaenus spumarius</i> , <i>Philaenus tarifa</i> , <i>Philaenus tessellatus</i> , <i>Tettigetta aneabi</i> , <i>Tettigetta argentata</i> , <i>Tettigetta atra</i> , <i>Tettigetta baenai</i> , <i>Tettigetta brullei</i> , <i>Tettigetta carayoni</i> , <i>Tettigetta dimissa</i> , <i>Tettigetta estrellae</i> , <i>Tettigetta josei</i> , <i>Tettigetta leunani</i> , <i>Tettigetta manuli</i> , <i>Tettigetta mariae</i> , <i>Tettigetta musiva</i> , <i>Tettigetta prasina</i> , <i>Tettigetta pygmaea</i> , <i>Tibicina cisticola</i> , <i>Tibicina contentei</i> , <i>Tibicina corsica</i> , <i>Tibicina fairmairei</i> , <i>Tibicina garricola</i> , <i>Tibicina haematodes</i> , <i>Tibicina luctuosa</i> , <i>Tibicina nigronevosa</i> , <i>Tibicina picta</i> , <i>Tibicina quadrisignata</i> , <i>Tibicina tomentosa</i> , <i>Trieophorella geniculata</i> , <i>Tympanistalna distincta</i> , <i>Tympanistalna gastrica</i>
<b>American vectors</b>	<i>Acrogonia citrina</i> , <i>Bucephalogonia xanthophis</i> , <i>Dilobopterus costalimai</i> , <i>Draeculacephala minerva</i> , <i>Graphocephala atropunctata</i> , <i>Homalodisca vitripennis</i> , <i>Oncometopia facialis</i> , <i>Oncometopia nigricans</i>

Synonyms of the xylem-sap feeder species were also considered as search terms. They were defined based on searching in <http://www.fauna-eu.org/> and/or <http://www.catalogueoflife.org/> and are reported in Table 3.

**Table 3:** Synonyms of the xylem-sap feeder species included in the systematic literature search

Species	Synonym	Second Synonym	Reported in
<i>Anoterostemma ivanoffi</i> (Lethierry 1876)	<i>Doratura ivanoffi</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Aphrophora alni</i> (Fallen 1805)	<i>Cercopis alni</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Aphrophora corticea</i> (Germar 1821)	<i>Cercopis corticea</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Aphrophora salicina</i> (Goeze 1778)	<i>Cicada salicina</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Bucephalogonia xanthophis</i> (Berg, 1879)	<i>Bucephalogonia xanthopis</i>	<i>Tettigonia xanthophis</i>	<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Cercopis sanguinolenta</i> (Scopoli 1763)	<i>Cicada sanguinolenta</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicada barbara</i> (Stål)	<i>Tettigia barbara</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadatra alhageos</i> (Kolenati)	<i>Cicada alhageos</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadatra atra</i> (Olivier)	<i>Cicada atra</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadatra hyalina</i> (Fabricius)	<i>Tettigonia hyalina</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadatra hyalinata</i> (Brullé)	<i>Tibicen hyalinata</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadatra querula</i> (Pallas, 1773)	<i>Cicada querula</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadella viridis</i> (Linnaeus 1758)	<i>Cicada viridis</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta caucasica</i> (Kolenati, 1857)	<i>Cicada caucasica</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta concinna</i> (Germar)	<i>Cicada concinna</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta dubia</i> (Rambur)	<i>Cicada dubia</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta flaveola</i> (Brullé)	<i>Tibicen flaveola</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta montana</i> (Scopoli)	<i>Cicada montana</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta podolica</i> (Eichwald)	<i>Cicada podolica</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadetta undulata</i> (Waltl)	<i>Cicada undulata</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Cicadivetta tibialis</i> (Panzer)	<i>Tettigonia tibialis</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Draeculacephala minerva</i> Ball, 1927	<i>Tettigonia atropunctata</i>	<i>Tettigonia circellata</i>	<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Evacanthus acuminatus</i> (Fabricius 1794)	<i>Cicada acuminatus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Evacanthus interruptus</i> (Linnaeus)	<i>Cicada interruptus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>

1758)			
<i>Evacanthus rostragnoi</i> (Picco 1921)	<i>Euacanthus rostragnoi</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Graphocephala atropunctata</i> (Signoret)	<i>Graphocephalus atropunctata</i>		<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Haematoloma dorsata</i> (Ahrens 1812)	<i>Cercopis dorsata</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Hilaphura varipes</i> (Waltl)	<i>Cicada varipes</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Homalodisca vitripennis</i> (Germar)	<i>Homalodisca coagulata</i>	<i>Tettigonia coagulata</i>	<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Lepyronia coleoptrata</i> (Linnaeus 1758)	<i>Cicada coleoptrata</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Lyrstes plebejus</i> (Scopoli)	<i>Cicada plebejus</i>		<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Macugonalia lecomelas</i> (Walker)	<i>Tettigonia albopunctulata</i>	<i>Tettigonia leucomelas</i>	<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Neophilaenus albipennis</i> (Fabricius 1798)	<i>Cercopis albipennis</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus campestris</i> (Fallen 1805)	<i>Cercopis campestris</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus exclamtionis</i> (Thunberg 1784)	<i>Cicada exclamtionis</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus infumatus</i> (Haupt 1917)	<i>Philaenus infumatus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus limpidus</i> (Wagner 1935)	<i>Philaenus limpidus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus lineatus</i> (Linnaeus 1758)	<i>Cicada lineatus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus longiceps</i> (Puton 1895)	<i>Ptyelus longiceps</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus minor</i> (Kirschbaum 1868)	<i>Ptyelus minor</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus modestus</i> (Haupt 1922)	<i>Philaenus modestus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Neophilaenus pallidus</i> (Haupt 1917)	<i>Philaenus pallidus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Oncometopia facialis</i> (Signoret)	<i>Tettigonia facialis</i>		<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Oncometopia nigricans</i> (Walker)	<i>Proconia marginata</i>	<i>Proconia nigricans</i>	<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Oncometopia nigricans</i> (Walker)	<i>Proconia scutellata</i>	<i>Proconia tenebrosa</i>	<a href="http://www.catalogueoflife.org/">http://www.catalogueoflife.org/</a>
<i>Pagiphora annulata</i> (Brullé)	<i>Tibicen annulata</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Paraphilaenus notatus</i> (Mulsant & Rey 1855)	<i>Ptyelus notatus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Peuceptyelus coriaceus</i> (Fallen 1826)	<i>Cercopis coriaceus</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Philaenus spumarius</i> (L.)	<i>Cicada spumarius</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta argentata</i> (Olivier)	<i>Cicada argentata</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta atra</i> (Gómez-Menor Ortega)	<i>Melampsalta atra</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta brullei</i> Fieber	<i>Cicadetta brullei</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta dimissa</i> (Hagen)	<i>Cicada dimissa</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta musiva</i> (Germar)	<i>Cicada musiva</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta prasina</i> (Pallas, 1773)	<i>Cicada prasina</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tettigetta pygmaea</i> (Olivier)	<i>Cicada pygmaea</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina cisticola</i> (Hagen)	<i>Cicada cisticola</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina contentei</i> (Boulard)	<i>Euryphara contentei</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina corsica</i> (Rambur)	<i>Cicada corsica</i>		<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>

<i>Tibicina haematodes</i> (Scopoli)	<i>Cicada haematodes</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina luctuosa</i> (Costa)	<i>Tibicen luctuosa</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina picta</i> (Fabricius)	<i>Tettigonia picta</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina quadrisignata</i> (Hagen)	<i>Cicada quadrisignata</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tibicina tomentosa</i> (Olivier)	<i>Cicada tomentosa</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Triecphorella geniculata</i> (Horvath 1881)	<i>Triecphora geniculata</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tympanistalna distincta</i> (Rambur)	<i>Cicada distincta</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>
<i>Tympanistalna gastrica</i> (Stal)	<i>Cicada gastrica</i>	<a href="http://www.fauna-eu.org/">http://www.fauna-eu.org/</a>

Initial testing with broader search terms, including specific behaviours (xylem–sap feeder), common names (spittlebug, froghopper, sharpshooter, cicada) and suprageneric taxa names (i.e. families Aphrophoridae, Cercopidae, Cicadellidae, subfamilies Cicadellinae, Cicadidae and Tibicinidae, and others), located many irrelevant results, pertaining to non-relevant species. In addition, phenology and ecology are generally referred to insect species and, according to the EFSA contract, data obtained with the systematic literature review must be used to generate an extraction summary table built in such a way that users will be able to filter the information by vector species, which must be defined by their scientific name. Since scientific literature databases include always the scientific name of the species of interest, the common names and suprageneric taxa names were not included in the population of interest for the literature searches regarding Task 1.

The terms selected for defining the outcomes of phenology and ecology are specified in Table 4.

**Table 4:** List of terms (strings) selected for performing the systematic literature review on phenology and ecology of *Xylella fastidiosa* vectors and potential vectors

<b>Phenology</b>	add, adult, biology, brood, developmental time, dispersal, egg, flight, generation, juvenile, life cycle, longevity, movement, naiad, nymph, oviposition, phenology, population abundance, population dynamic, seasonal, temperature, voltinism
<b>Ecology</b>	antagonist, diapause, ecology, ectosymbiont, endocytobiont, endosymbiont, fecundity feeding, natural enemy, parasite, parasitoid, plant, predator, symbiont

### 3.2.3. Literature search strategy

Based on the selected terms, two strings of terms, including the scientific names (Table 2) and synonyms (Table 3) of *X. fastidiosa* insect-vectors, defined the population of interest, and two strings of terms regarding their ecology and phenology specified the searched outcomes (Table 4). Whenever allowed by the database, these strings were combined adopting the Boolean operators AND/OR according to the following strategy.

[Insect species names, separated by OR] OR [Insect species synonyms, separated by OR] AND [(add OR adult\* OR biology OR brood\* OR developmental time OR dispersal OR egg\* OR flight OR generation\* OR juvenile\* OR life cycle OR longevity OR movement OR naiad\* OR nymph\* OR oviposition\* OR phenology OR population abundance OR population\* dynamic\* OR seasonal OR temperature\* OR voltinism) OR (antagonist\* OR diapause OR ecology OR ectosymbiont\* OR endocytobiont\* OR endosymbiont\* OR fecundity OR feeding OR natural enem\* OR parasite\* OR parasitoid\* OR plant\* OR predator\* OR symbiont\*)]

In alternative, when the database did not allow searches based on strings, the scientific names included in the population of interest were used and the records were screened for relevance to the Task in a following step. The searches did not have geographical and language restrictions.

The searches were performed without limitation regarding the quality of the items, thus reports, bulletins, proceedings, etc, (grey literature) were taken into consideration when available in the interrogated databases.

The literature search was carried out in 2016 (March/April) and updated in 2017 (April/June).

### 3.2.4. Specification of information sources

Authoritative databases, indexing peer-reviewed scientific publication, and other similar data sources (i.e. EPPO Global Database) have been used as primary sources of information. The methods used for retrieving information from each database are detailed below.

#### 3.2.4.1. Web of Science

The search was performed in the ISI Web of Science-All databases (WoS), which incorporates Web of Science, Core Collection, BIOSIS Citation Index, Chinese Science Citation Database, Data Citation Index, KCI-Korean Journal Database, Derwent Innovations Index, MEDLINE, Russian Science Citation Index, SciELO Citation Index, Zoological Record. The database was interrogated using the field tag "Topic" (TS, which searches within titles, abstracts, keywords and indexing fields such as systematics, taxonomic terms and descriptors). The Boolean operators AND/OR were adopted to combine the searches performed using, separately, the population (Tables 2 and 3) and outcome (Tables 4) strings. No restriction was adopted in the searches concerning language and time (years from 1864 to the search date).

#### 3.2.4.2. Scopus

Scopus is a database of peer-reviewed literature covering over 21,500 peer-reviewed journals, conference papers, 360 trade publications and more than 113,000 books. In the first search, performed in 2016, Scopus allowed only the use of limited string length (256 characters). Therefore the strings of terms defined in Tables 2-4 were broken into several independent blocks and combined with the Boolean operators AND/OR to develop an appropriate search strategy. In 2017, Scopus allowed the use of strings without the previous length limitation, therefore the search was performed using the Boolean operators AND/OR to combine the items identified using, separately, the entire population (Tables 2 and 3) and outcome (Tables 4) strings.

#### 3.2.4.3. MEDLINE

MEDLINE records journal citations and abstracts for biomedical literature from around the world. The MEDLINE database was searched through the OVID platform according to a string-based search strategy in which Boolean operators AND/OR were used to link the strings of terms (Tables 2-4). The covered period ranged from 1946 to the search date.

#### 3.2.4.4. AGRICOLA

The National Agricultural Library is one of four national libraries of the United States and houses one of the world's largest collections devoted to agriculture and its related sciences. The NAL Catalog (AGRICOLA) provides citations to agricultural literature. Since AGRICOLA admits strings composed of maximum 383 characters, the search was performed combining each name or synonym of vectors (Tables 2-3) with the strings for phenology and ecology (Table 4), separately, according to the following strategies:

- i) search for phenology:

[Insect species name OR synonym] AND [add OR adh OR adult\* OR biology OR brood\* OR developmental time OR dispersal OR egg\* OR flight OR generation\* OR juvenile\* OR life cycle OR longevity OR movement OR naiad\* OR nymph\* OR oviposition\* OR phenology OR population abundance OR population\* dynamic\* OR seasonal OR temperature\* OR voltinism]



ii) search for ecology:

[Insect species name OR synonym] AND [antagonist\* OR diapause OR ecology OR ectosymbiont\* OR endocytobiont\* OR endosymbiont\* OR fecundity OR feeding OR natural enem\* OR parasite\* OR parasitoid\* OR plant\* OR predator\* OR symbiont\*]

Items found by these searches were joined and de-duplicated.

### 3.2.4.5. CAB-Abstracts

CAB Abstracts Archive and CAB Abstracts (CAB) are databases from CABI (Centre for Agriculture and Biosciences International) that, together, cover applied life sciences records. These databases were searched by the OVID platform according to a string-based search strategy in which Boolean operators AND/OR were adopted to combine the population (Tables 2-3) and outcome strings (Tables 4) and covering all the available data on the database (records from 1910 to the search date).

### 3.2.4.6. JSTOR

JSTOR is a digital library of academic journals, books, and primary sources. Despite the database does accept searches based on strings of terms, the results are paged and, thus, difficult to verify in case of multiple species search. Because of this, JSTOR was searched separately by each Latin binomial name listed in Tables 2 and 3.

### 3.2.4.7. EPPO Global Database

EPPO Global Database is aimed to include all pest-specific information that has been produced or collected by EPPO. It contains:

- basic information (scientific names, common names in different languages, taxonomic position) for more than 72,000 pest species of interest to agriculture, forestry and plant protection;
- detailed information for more than 1,600 pest species that are of regulatory interest (EPPO and EU listed pests, as well as pests regulated in other parts of the world).

This database does not allow searches based on strings of terms. Therefore, each name of selected vector species (Tables 2 and 3) was searched separately.

## 3.2.5. Reference Management

Records from each database were loaded into an EndNote library. Preliminary tests showed that searches performed in the interrogated databases were redundant, generating a large number of duplicated items. However, when files from the different searches were merged, a number of duplicated records escaped the EndNote-based de-duplication (likely because they were tagged differently); therefore, a further de-duplication was carried out in a database developed *ad hoc* using the software FileMaker, that was also used in the step of screening for relevance (see below). Only few records (nineteen) were found in the EPPO Global Database (Appendix A) that were immediately evaluated and considered not relevant for Task 1 and, therefore, not loaded into the EndNote or FileMaker libraries for further evaluation. When the records were loaded into FileMaker, each item was tagged to highlight that it was obtained by Task 1 search strategy, excluding those obtained from the search in JSTOR that were searched based only on Latin binomial names. In fact, the potential relevance for Task 1 of JSTOR records was not known at this stage because they were imported in the EndNote library without any selection for relevance to the Task. The relevance for Task 1, Task 2 or the non-relevance of JSTOR records was established later during the step of screening for relevance based on title and abstract (see below).

To update the search performed in 2016 (March/April), a second search was carried out in 2017 (April/June) interrogating the databases Web of Science, Scopus, MEDLINE, AGRICOLA and CAB-Abstract. The items retrieved in 2017 in all databases were joined and deduplicated and, finally, were searched in the FileMaker database (generated in 2016) to exclude those records already evaluated in

the previous year. Finally, the remaining items, corresponding to those retrieved in 2017 and not evaluated previously, were further screened for relevance.

### 3.2.6. Screening the search results for relevance

Searched records were stored in the FileMaker database and shared online between six independent reviewers that screened the results to remove those that were irrelevant. The process to exclude an irrelevant item required the sequential examination of i) the title and the abstract and ii) the full-text, according to the following protocol.

Each publication was screened by at least two reviewers that inserted their independent opinion in FileMaker. In case of double concordant opinions, the publication was directly preserved or removed, accordingly. In case of discordant opinions, the publication was evaluated by a third reviewer and, if needed, the case discussed among the three reviewers before taking the final decision. Items considered to be relevant according to their title and abstract were further evaluated in a second screening step based on the full-text, which was considered into details also to extract the data for generating the extraction table and the draft protocol for field data collection (see below).

#### 3.2.6.1. Criteria for exclusion based on titles and abstracts

To minimise the chance of incorrectly rejecting relevant literature during the step of screening for relevance for Task 1, the screening criteria were established in advance and they are given below.

Based on title and abstract, items were removed from FileMaker:

- when the item was identified as a duplicate (due to redundancy among the databases). Duplicated items in Filemaker were identified automatically by searching items with the same title (the first 50 characters) and removed after ascertaining manually that the authors and publication year were identical to the items maintained in FileMaker. Further deduplication was performed manually when needed.
- when the abstract was not reported and all the attempts to identify the abstract and/or the full-text were unsuccessful.
- when the described species was not included in the terms of the procurement (Tables 2 and 3);

Only few publications lacked the abstract. Additional attempts to identify a source containing the abstract were successful in few occasions and when this happened the article was screened according to the process reported above.

Since the searches were not limited to articles published in peer reviewed journals, references regarding reports, bulletin, proceedings, newsletters, national and local journals, etc. (grey literature) were also considered. Collection of the full text of some of these records was presumed to be difficult and in several occasions was unsuccessful. However, to provide EFSA with summary tables including as wide relevant literature as possible, in the absence of the full text, data were extracted from the available abstract, whenever considered to be informative (containing data of interest and pertinent to the summary table). These records were specifically marked in the summary tables.

#### 3.2.6.2. Additional criteria for exclusion based on full-texts

Items selected as relevant to Task 1 were further screened before extracting data useful to fill the extraction table.

In particular, criteria for exclusion based on full-text were:

- When the full text was not found and the available abstract was not considered informative

- The article did not contain data useful to fill the extraction table (data on phenology and ecology were lacking, they were review articles without experimental data, information was not referred to the target species, data were referred to ecology and phenology of exotic species not covered by the mandate).

### 3.3. Testing the quality of the search protocol (Task 1)

Before running the full searches, the search strings were tested in each database, thus obtaining preliminary information on the volume of items expected and whether items of interest for Task 1 were actually reached.

A check list based on PRISMA statement (Moher et al., 2009) was adopted and the flow of information through the different phases of the systematic review reported.

In addition, a test ex post was carried out to validate the search strategy and the selected terms for their sensitivity and specificity. The test was performed searching in WoS all the records regarding the species *P. spumarius*. The resulting WoS items were ordered by relevance and the first 50 items were screened for relevance to Task 1. The selected items were then searched in FileMaker to confirm that they had been identified by the search strategy previously reported.

Comparison between the two independent searches performed in 2016 and 2017 allowed to further validate the obtained results.

### 3.4. Collected literature on phenology and ecology of *X. fastidiosa* vectors and potential vectors

Only 19 name species and 2 synonyms among those listed in Tables 2 and 3 were found in the in the EPPO Global Database searched on March 2016. The information associated with these species included four documents regarding three species (Appendix A). The documents were downloaded and further evaluated for their relevance to the literature search. Due to the limited number of documents, selection for relevance according to the established criteria was performed immediately. No one of the retrieved items was considered relevant to Task 1.

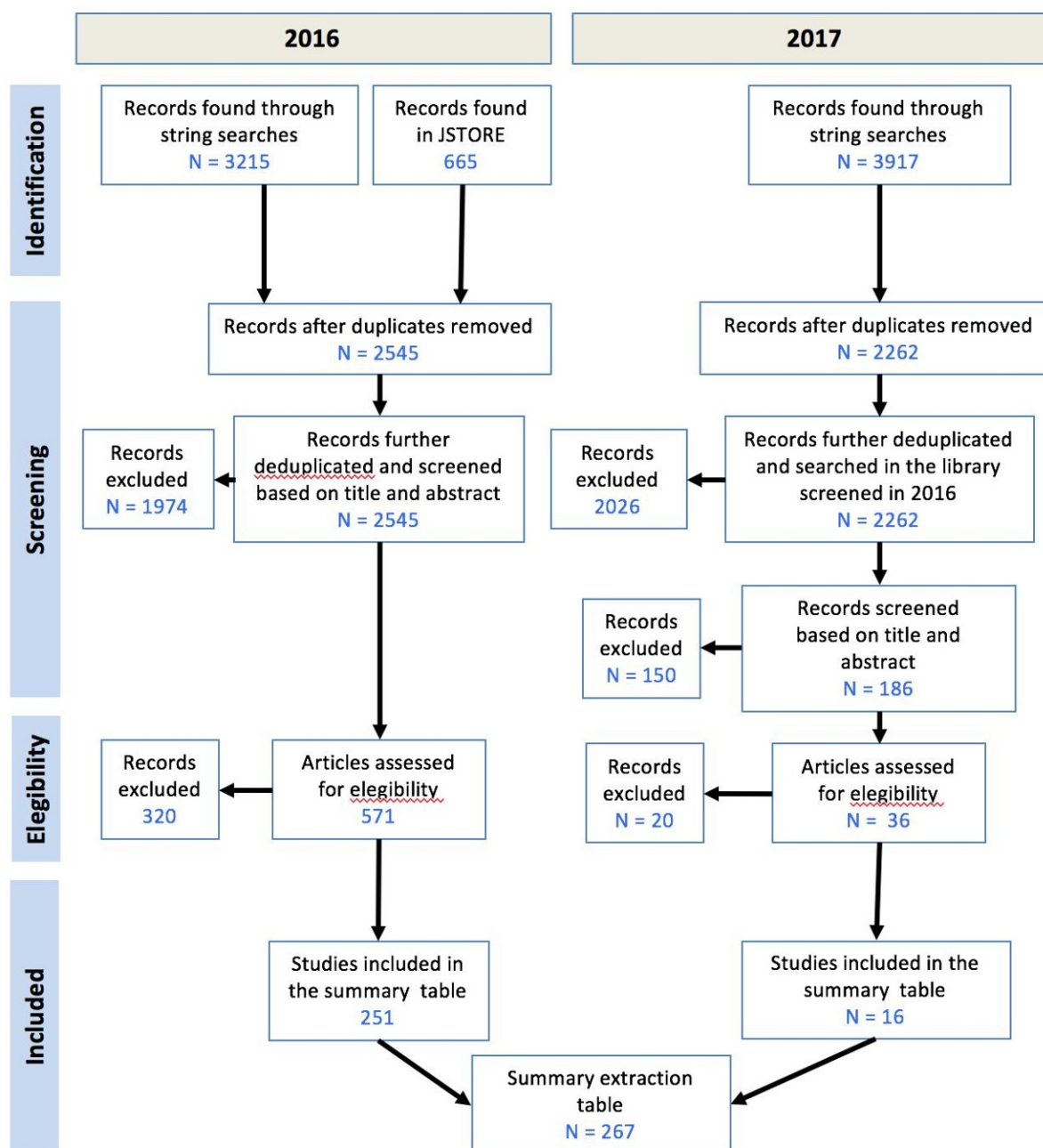
The literature searches in the databases AGRICOLA, Cab-Abstracts Scopus, JSTOR, MEDLINE, and Web of Science were conducted on March/April 2016 (Table 5). Records identified in each database were merged in an EndNote file and de-duplicated. Since several records were not successfully de-duplicated in EndNote, a further step of de-duplication was performed in our *ad hoc* developed database FileMaker, thus providing a final number of 2,545 records in 2016 that were screened for relevance based on title and abstract. After this first screening, 571 records were further evaluated based on full-text and 251 records were used for generating the extraction table. Figure 1 resumes the main steps of this de-duplication and screening process.

In May 2017, the searches in the databases AGRICOLA, Cab-Abstracts, MEDLINE, Scopus and Web of Science were repeated generating 2,262 references (Table 5), most of which (2,076) were already found in 2016, with only additional 186 records not evaluated previously. A total of 36 of these references were selected as relevant based on title and abstract, 16 of which were used to implement the summary extraction table (Figure 1). Finally, a total of 267 references from the searches performed in 2016 and 2017 were included in the summary extraction table.

**Table 5:** Number of references identified by the systematic literature searches on phenology and ecology of *X. fastidiosa* vectors and potential vectors performed in 2016 and 2017.

Database	Number of References Year 2016	Number of References Year 2017
Web of Science	1,653	1,901
Scopus	490	499
MEDLINE	143	152

Cab-Abstract	1,155	1,190
AGRICOLA	237	249
JSTOR	1,023	/
Total joined and deduplicated records	2,545	2,262



**Figure 1:** Flow of information through the different phases of the systematic literature review on phenology and ecology of *Xylella fastidiosa* vectors and potential vectors (Task 1)

## 4. Systematic literature review on the potentially effective control options against vectors and potential vectors of *X. fastidiosa* (Task 2)

### 4.1. Objectives

The objective of the literature review reported below has been to identify relevant references for generating efficacy tables regarding chemical, biological and other alternative potentially effective control options against vectors and potential vectors of *X. fastidiosa*, according to the specifications reported in the EFSA contract (Task 2)

### 4.2. Materials and Methods

The adopted search strategy and the information sources were substantially the same as reported in the previous paragraphs 1.2 and 1.3. However, the search questions and terms to be used to find information on the potentially effective control options against all the vectors and potential vectors of *X. fastidiosa* were specifically determined according to the protocol reported below.

#### 4.2.1. Definition of search questions

Preliminary meeting between members of the SR team and members of the other project tasks, and in consultation with EFSA Project Steering Committee, the following primary questions were identified for the systematic literature search of the Task 2:

Which are the effects of active ingredients in the control of vectors and potential vectors of *X. fastidiosa* all around the world?

Which are the effects of biocontrol agents in the control of vectors and potential vectors of *X. fastidiosa* all around the world?

Which are the effects of mechanical and physical control tools in the control of vectors and potential vectors of *X. fastidiosa* all around the world?

Which are the effects of agricultural practices in the control of vectors and potential vectors of *X. fastidiosa* all around the world?

The key elements identified in these questions include a population of interest (P), an intervention or exposure (I), a comparator (C), and outcomes (O) according to Table 6 definitions and can be indicated with the acronym PICO (or PECO) (EFSA, 2010).

**Table 6:** Definition of terms within the objective of the systematic literature review

<b>Population (P)</b>	Xylem-sap feeders (including American vector species and European potential ones)
<b>Intervention (I) or Exposure (E)</b>	Active ingredients (synthetic and natural insecticides), biocontrol agents, physical and mechanical control tools, agricultural practices
<b>Comparator (C)</b>	Untreated populations as a control or lack of exposure to the control factor
<b>Outcomes (O)</b>	Mortality (or survival) following the application of the intervention or exposure with respect to control (efficacy of the intervention or exposure on the reduction of insect population)



#### 4.2.2. Specification of terms, sources and strategy of the literature search

Based on the questions and the definition terms, search terms that could ensure identification of as wide relevant literature as possible, excluding most of the irrelevant literature, were identified by the SR team and discussed with the other researchers involved in other project tasks and with EFSA.

The terms used for defining the population must include all xylem-sap feeder species (including American vector species and European potential ones) without any geographic limitation. Therefore, a string of term specified in Table 7, composed of suprageneric taxa of xylem-sap feeder species, was used to define the population of interest.

**Table 7:** List of suprageneric taxa included in the systematic literature search for Task 2.

<b>Family</b>	Aphrophoridae, Cercopidae, Cicadidae, Clastopteridae, Epipygidae, Machaerotidae, Tibicinidae
<b>Subfamily</b>	Cicadellinae

To make sure the search was complete, we included in the population (P) strings composed by species names of vectors and potential vectors (reported in Tables 2-3).

To find publications containing relevant data related to the Intervention (I) and Exposure (E) options of interest two strings of terms were combined. One included generic terms for control options of plant pests, the other one contained more specific terms regarding the method of control (Table 8).

**Table 8:** List of terms (strings) selected for performing the literature searches on the potentially effective control options against *X. fastidiosa* vectors and potential vectors.

<b>Control generic terms</b>	Application, control, management, treatment
<b>Control specific terms</b>	active ingredient, biocontrol, biological, chemical, insecticide, integrated, mechanical, natural enemy, organic, parasitoid, pesticide, physical, predator

Therefore, in this search, when it was possible, the strings defining the populations (P, including terms in Table 7 and Tables 2 and 3) were used in combination with the strings composed of the terms selected for defining the outcomes of the control options (Table 8).

Regarding the languages for the search, considering that i) the population (P) is defined by scientific names of pests, which are in Latin, ii) in the scientific literature databases, scientific names of species are expected to be provided in Latin, iii) the efficacy tables required as deliverables of Task 2 must include a column on pest species that are defined by the scientific name in Latin, the use of common names and their translation to MedMS languages was not considered needed at this stage. In agreement with EFSA, it was established to perform the searches without any language restriction and, when needed, to translate the full-text records of interest published in MedMs languages different from English in the step of data extraction to generate the efficacy table, whenever this was necessary to finalize the data extraction.

The searches were performed including also reports, bulletins, proceedings (grey literature), that were taken into consideration when available in the interrogated databases. The sources used have been specified in the par. 3.2.4. The literature search was carried out in 2016 (March) and updated in 2017 (May).

#### 4.2.3. Reference management and exclusion criteria

The records from the different databases (AGRICOLA, Cab-Abstracts, JSTOR, MEDLINE, Scopus, and Web of Science) were joined in a single EndNote library and loaded in the FileMaker database where they were further de-duplicated. After de-duplication, records of potential interest for Task 2 were screened for relevance as reported in the paragraph 3.2.5.

The same criteria reported in par. 3.2.5.1. were adopted to exclude items based on titles and abstract. However, in this specific case, based on the abstract, it was also taken into account the presence/absence of experimental data on the efficacy of the active ingredient/biological control/other control methods (agronomic, mechanical, physical...). In particular, for chemicals (insecticides) not allowed in the EU (= not included in the 71 Draft Assessment Reports on pesticides, hereinafter DARs insecticides), the publications were excluded. To prepare the table, all the DARs insecticides allowed for use in the EU were considered, regardless of their pest target indications (Annex 1). The list of DARs insecticides tested against *X. fastidiosa* vectors according to the literature is attached (Annex 1).

Records not excluded by the previous steps were further checked by searching in the full-text data of relevance for generating the efficacy tables requested by EFSA. In this step, records were excluded according to the following exclusion criteria list:

- When the full text was not found, and the available abstract was not considered informative
- The full-text is a review paper without relevant primary experimental data on control of *X. fastidiosa* vectors and potential vectors.
- It does not contain data on insecticide included in the DARs insecticide list (according to Annex 1: approved insecticides).
- It contains data on insecticide(s) registered in Europe but sprayed in combination with not registered ones.
- Data on insecticide activity and/or insect mortality and/or target insect control are not reported.
- Data comes from field observation under uncontrolled conditions.
- No experimental data is provided, and conclusions are theoretical and/or speculative.

Especially for some relevant articles not published in peer reviewed journals (i.e. reports, bulletin, proceedings, newsletters, etc.) and for some very old publication, it was not possible to find the full text. To provide EFSA with summary tables including as wide relevant literature as possible, in these cases extraction of data was based on the available abstract, whenever the latter was considered informative. The references rated as relevant on the base of the abstract only are specifically marked in their respective extraction tables.

#### 4.3. Collected literature on control options against vectors and potential vectors of *X. fastidiosa*

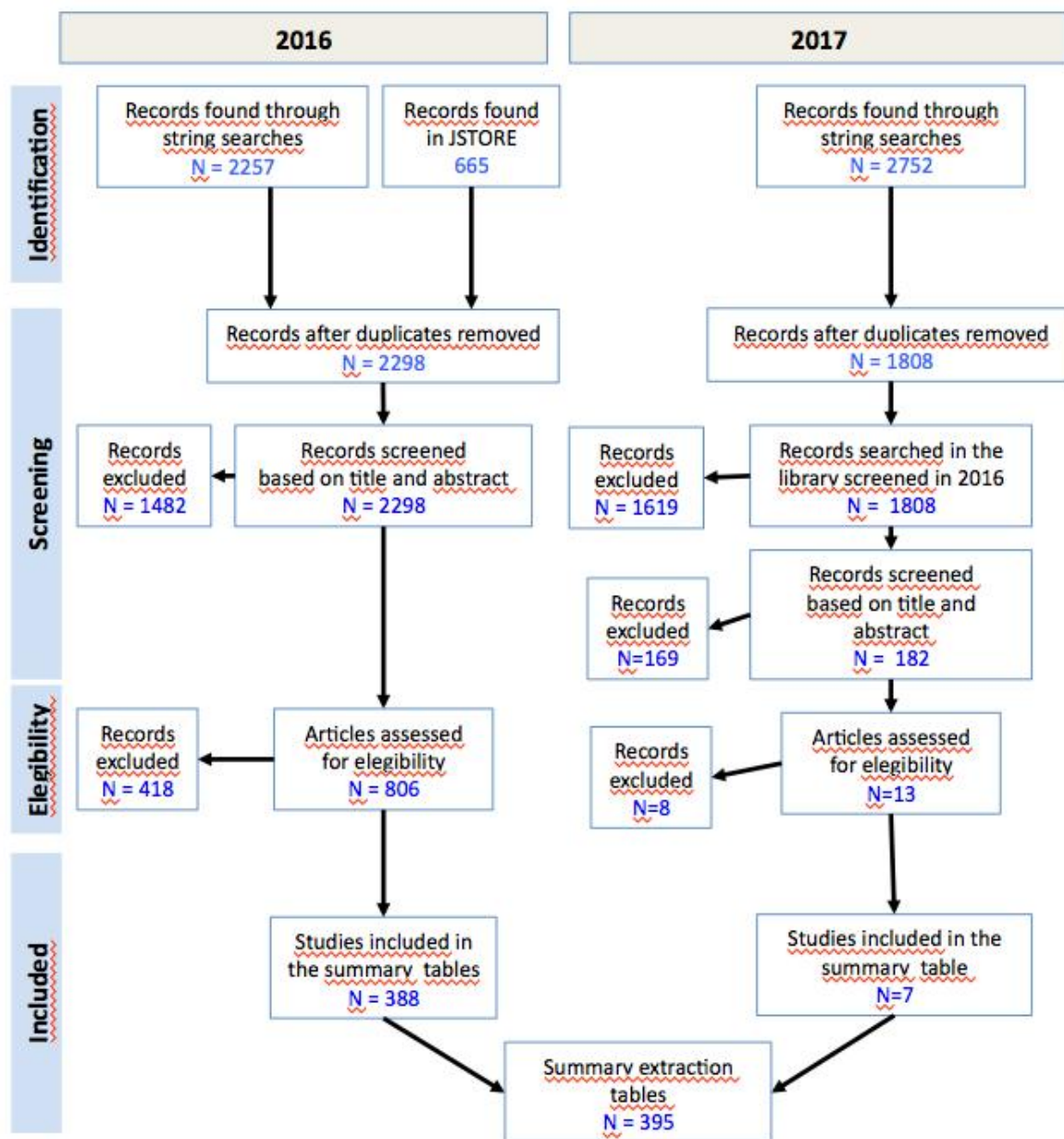
The search, carried out on March/April 2016 in the databases Web of Science, Scopus, MEDLINE, AGRICOLA, Cab-Abstracts and JSTOR, generated a total of 2298 references (Table 9) that after screening for relevance were reduced to 806, 388 of which were finally used for generating the extraction tables. Figure 2 resumes main steps of this de-duplication and screening process.

The searches performed in May 2017 was carried out in the databases Web of Science Scopus, MEDLINE, AGRICOLA, Cab-Abstracts and did not included JSTOR database. It generated 1808 references (Table 9), most of which (1619) were already found in 2016, with only additional 186 records not evaluated previously. Thirteen of these references were selected for relevance based on

title and abstract for further screening based on full text (Figure 2). Finally, a total of 7 additional references identified in the search performed in 2017 were retained to implement the extraction tables.

**Table 9:** Number of references identified by the systematic literature search on control options against vectors and potential vectors of *X. fastidiosa* performed in 2016 and 2017.

Database	Number of References Year 2016	Number of References Year 2017
Web of Science	648	1,124
Scopus	214	216
MEDLINE	63	71
Cab-Abstract	1,277	1,292
AGRICOLA	159	168
JSTOR	665	/
<b>Total joined and deduplicated records</b>	<b>2,298</b>	<b>1,808</b>



**Figure 2:** Flow of information through the different phases of the systematic literature review on all the potentially effective control options against vectors and potential vectors of *Xylella fastidiosa* all around the world (Task 2).

## 5. Grey literature search on phenology and ecology of and potentially effective control options against *X. fastidiosa* vectors and potential vectors

### 5.1. Specification of grey literature sources

To extend the search to grey literature not included in the previous searches the following grey literature repositories were interrogated:

Google Scholar, <http://www.scholar.google.it>. Broadly searching engine for scholarly literature, targeting articles, theses, books, abstracts and court opinions, from academic publishers, professional societies, online repositories, universities and other web sites. Google Scholar ranks the documents by weighing the full-text, place of publication, author(s), citations, actuality and so on.

OCLC WorldCat, <http://www.worldcat.org>. The world's largest library-based content and services network. Searching WorldCat put in touch with thousands of other libraries around the world to locate a reference into a library near you that owns the item. Libraries in your community and elsewhere will provide to online access or loaning the item.

GreyNet International, [www.greynet.org](http://www.greynet.org). The Grey Literature Network Service was founded in 1992. The goal of GreyNet is to facilitate dialog, research, and communication between persons and organisations in the field of grey literature. GreyNet further seeks to identify and distribute information on and about grey literature in networked environments. Its main activities include the creation and maintenance of web-based resources, a moderated Listserv and combined Distribution List, The Grey Journal (TGJ), and curriculum development.

International Archive, <http://www.archive.org>. The Internet Archive is a San Francisco-based non-profit Digital library with the stated mission of Universal access to all knowledge. It provides free public access to collections of digitized materials, including web sites, software applications/games, music, movies/videos, moving images, and nearly three million public-domain books. As of October 2012, its collection topped 10 Petabyte. The Internet Archive allows the public to upload and download digital material to its data cluster, but the bulk of its data is collected automatically by its web crawlers, which work to preserve as much of the public web as possible. Its web archive, The Wayback Machine, contains over 150 billion web captures. The Archive also oversees one of the world's largest book digitization projects.

Armed Forces Pest Management Board (AFPMB, <https://www.acq.osd.mil/eie/afpmb/>) is a repository that provides data on pest management issues.

In addition, the recursive agents GrandReporter (<http://www.tri-edre.com>), and DEVONagentPro ([www.devontechnologies.com](http://www.devontechnologies.com)) were used for recursive research in Internet. The CABI Crop Protection Compendium, that collects data from national plant protection organizations (NPPO) and bibliography to set specific contents focused on particularly concerning pest species, was also interrogated.

Different strategies were followed for searching in non-recursive (Armed Forces Pest Management Board, CABI, Google Scholar, GreyNet International Archive, OCLC WorldCat) and recursive (DEVONagentPro and GrandReporter) databases.

### 5.2. Strategy for searching in AFPMB, Google Scholar, GreyNet International, Internet Archive and OCLC WorldCat

The same approach was adopted for these databases. Actually, any of them contain grey literature alone, intended as matters published or made formally available by no-profit (educational) Institutions. In the best cases, we found repositories offering a mix of white and grey literature. Because of this, reference types described as: Book, Book Section, Generic, Journal Article, Patent, Report and Thesis were recognized, but the found results were sub-searched for Report and Thesis



only, because the remaining categories were considered to be targeted in the white literature searches. The checked records were already found in the white literature search, with the exception of about twenty reports that were included in the evaluation process of the white literature for Task 1 and 2.

Each grey literature database was searched for the binomial strings composed of the genus/species names combination. Genus and species names listed in tables 2-3 were searched as string included into primary quotation marks to avoid over findings of not consistent references, i.e. "*Philaenus spumarius*". Then the following actions were carried out:

- a) Results were either extracted as .doc/.docx or searchable .pdf files.
- b) All results were exported as searchable .pdf files and bound in single .pdf file each pertaining to one search engine result.
- c) Binders were sub-searched a first time for strings as: "report" or "thesis" (not case-sensitive) to understand the way terms were placed into the references.
- d) Binders were sub-searched a second time for strings as: "report" or "thesis". Note the string "thesis" is actually "spacebar+thesis" to avoid findings of the term "hypothesis" that was found in first sub-search
- e) Lists of sub-searched records were sorted and scored for relevance by at least two independent members of the SR team according to the available information.

### 5.3. Strategies for searching in recursive databases

#### 5.3.1. GrandReporter

GrandReporter performs synchronous multiple scans over the Internet by creating single or combined searches and adding them to a search list. We set the software (SW) to search and re-search every 30' the same strings. The SW replies writing a list of all encountered URLs that include the search string. URL can be visited by internal or external web browser by double click. The following strategy was adopted for this search:

1. The binomial strings composed of the genus/species names combination in the previous paragraph was adopted.
2. We inputted all the binomial strings and launched the searches on 1<sup>st</sup> April 2016. The SW has been running for two months but new results were found during the first 34 days. Later the searched pages number remained stable.
3. Each found link was examined and distributed among species-related folders.
4. Moreover, all the downloadable contents were stored in species-related folders.
5. All the downloaded (DLd) files were re-named.
6. All the files were put in one folder and sorted by name to eliminate duplicates.
7. Files were: 1) de-duplicated placing duplicates in a proper folder (25 files); 2) scrutinized according to criteria reported above (par. 3.2.5 and 4.2.3) and sorted into two folders, namely "grey Task 1" (33 files), "grey Task 2" (3 files). However, the records obtained with Grand Reporter were not included in the extraction/efficacy tables because they were represented by books (too long to be searched for relevant data), or they were taxonomic lists without relevant biological data.

#### 5.3.2. DEVONagent Pro

DEVONagentPro performs single searches with not cumulative results. As results it needs an operator to refresh the searches continuously. Moreover, the SW searches also for linked terms making the

evaluation of results rather confusing. After some attempts to stabilize the searches and the consequent results we abandoned this SW because we recognized that it does not fit our needs.

#### **5.4. Results of grey Literature search in AFPMB, Google Scholar, GreyNet International, Internet Archive and OCLC WorldCat**

The search in Google Scholar, generated the highest number of records (more than 15,500), while low number of records (from 1 to 75) was found in AFPMB, Internet Archive, GreyNet International and OCLC WorldCat (Annex 2).

##### **5.4.1. Results of search in Google Scholar**

A total of 15,537 records were found in Google Scholar (<http://www.scholar.google.it>; search performed from 24th to 30th June 2016) when this database was searched using the binomial strings composed of the genus/species names combination listed in Tables 2 and 3. They corresponded to a total of 1,765 pages that were bound into a single .pdf file and searched for "report" and "thesis". Found references were copy-pasted into a word document as a list of reports and a list of theses. Lists were sorted and scored for relevance by at least two independent members of the SR team according to the available information. A total of 26 and 44 theses and reports were found, respectively. The following records were considered potentially relevant: two theses (one for Task 1 and one for Task 2, Annex 2.1) and nine reports (seven potentially relevant to Task 1 and two to Task 2, Annex 2.3). The full text of one thesis and four reports were found, further evaluated and finally considered not relevant to our tasks (the thesis and three reports) or relevant, but already identified by other searches (one report). The other non relevant theses and records are reported in Annexes 2.2 and 2.4, respectively.

##### **5.4.2. Results of search in WorldCat**

Searching into WorldCat (search performed on 8th June 2016) resulted in 35 pages bound .pdf file. Scoring as reported in the previous paragraph led to select six theses, the first of which was considered of potential interest for Task 2. However, after full text evaluation the thesis was considered not relevant to task 2 (Annexes 2.5 and 2.6). No report was gathered.

##### **5.4.3. Results of search in GreyNet International**

Searching into this repository (search performed on 24th June 2016) resulted in two records, none of which corresponded to a thesis of possible interest (Annex 2.7).

##### **5.4.4. Results of search in AFPMB**

Searching into AFPMB repository (search performed on 9th June 2016) resulted in one reference that was already retrieved with other searches.

##### **5.4.5. Results of search in Internet Archive**

The search in Internet Archive (search performed on 9th June 2016) resulted in 10 items all belonging to white literature (Annexes 2.8 and 2.9). Two of these items, rated relevant to Task 1 (Annex 2.8), were independently identified by the systematic literature search.

#### **5.5. Results of grey literature search in recursive search agents**

##### **5.5.1. Results of search in GrandReporter**

A total of 2,027 (Annex 2.10: Grand Reporter) files were found (search from 20th February 2016 to 20th June 2016, at this date any new Uniform Resource Locator (url) was found), of which only 26 were of grey literature and considered of potential interest to Task 1 (26) and one to both Tasks 1

and 2. Sixteen full text were identified, four of which were considered relevant to Task 1. All these items were found also by other searches.

## 6. Deliverables

### 6.1. Deliverables of Task 1

After screening the references based on title and abstracts, a total of 607 records (571 in 2016 + 36 in 2017) were considered relevant and 267 of them selected for generating the extraction summary table and the draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius*. All the references selected for the extraction table were compiled in an EndNote file (References Task 1 Ecology Phenology).

#### 6.1.1. Extraction summary table on *X. fastidiosa* vectors and potential vectors phenology and ecology

Data collected by the systematic literature search were used for generating the extraction summary table on *X. fastidiosa* vectors and potential vectors phenology and ecology (Annex 3). There is great evidence that the most well-known species is the sharpshooter *Homalodisca vitripennis*, whose biology/ecology/phenology is very well characterized with respect to host plant preference, life cycle and population dynamics, fecundity, flight dispersal, feeding behaviour and symbionts. However, there is a good amount of available knowledge on the ecology and phenology of the two main species of Cicadellinae present in Europe: *Cicadella viridis* and *Graphocephala fennahi*. For these two species, host plants, life cycle and population dynamics are well known. Few information about the habitat/ecology of the other European Cicadellinae, *Evacanthus interruptus*, *E. acuminatus* and *Cicadella lasiocarpae*, is available, while almost no information on their phenology is available. Among spittlebugs, *Philaenus spumarius* is the most studied species and data on host plants of nymphs and adults, life cycle and population dynamics are available. However, due to its wide geographical range (from Northern to Southern Europe and North America) information on ecology and phenology of the species in the different areas is not always available. There is a consensus on the fact that *P. spumarius* has a univoltine life cycle and overwinters as egg, although a second generation has been hypothesized in Greece. Temperature-dependent development has been studied in the US, where a good amount of data has been published. As for the dispersal, several observations, but few scientific evidences, are available on the actual spread capability. However, a minimum of 80-90 m active dispersal has been recorded. Moreover, detailed knowledge on the abundance and phenology of this species on selected crops is not available. Data on fecundity of this species are conflicting, ranging from few tens (according to two authors) to few hundreds of eggs per females (according to a third author) (experiments aimed at determining fecundity are also outputs of this procurement). Several authors confirm the polyphagy of this species and lists of host plants are available for both nymphs and adults, although the most detailed information comes from the US. Plant host shifting during the lifecycle of *P. spumarius* is reported by several authors in different environments. Ecology and phenology of other European spittlebugs is poorly known, and information is scattered. However, some interesting data on the host plants and life cycle are available for species of the genera *Aphrophora*, *Cercopis* and *Neophilaenus*. The main available information on cicadas concerns their host plants, the characteristics of environments colonized by cicadas and the period of adult presence. Few data about egg laying and prolificacy are also available.

### 6.1.2. Draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius*

Based on data in the extraction table on *X. fastidiosa* vectors and potential vectors phenology and ecology and on our own experience, a draft protocol for field data collection on phenology and ecology of the vector *P. spumarius* was generated. An inventory of sampling methods for xylem-sap feeder insects has also been compiled (Annex 4, Draft Protocol for field data collection). Visual inspection and counting of spittlebug nymphs is the only practical method for the study of the nymph population of *P. spumarius* (and of the other spittlebugs), while sweep net is the most widely applied sampling method of *P. spumarius* adults. Yellow sticky traps can be used to collect adult of the spittlebug, although their efficiency is debated. As for the sampling methods, it is worthy to note that sharpshooter and spittlebug nymphs require different sampling methods. Sharpshooter and spittlebug adults require similar sampling methods, which might vary depending on the host plant (woody or herbaceous).

## 6.2. Deliverables of Task 2

After screening the reference based on title and abstracts, a total of 819 records (806 in 2016 + 13 in 2017) were considered relevant, and 395 were finally selected for generating efficacy tables of control options against vectors and potential vectors of *X. fastidiosa*.

### 6.2.1. Efficacy tables of control options against vectors and potential vectors of *X. fastidiosa*

To facilitate table consultation three different tables were generated: one regarding chemical control (Annex 5, Efficacy table chemicals), another one containing data on efficacy of biological control (Annex 6, Efficacy table natural enemies) and the third one with data on efficacy of other methods (agronomic, physical, etc) (Annex 7, Efficacy table other control methods).

A total of 51 records were the source of data used for generating the extraction summary table on chemicals (Annex 5). Most of the reports on the activity of insecticides towards xylem-sap feeders are about sharpshooter vectors of *X. fastidiosa* in North and South America. Neonicotinoids appear to be effective and persistent on both adults and nymphs. The same insecticides also appear effective in preventing transmission of *X. fastidiosa* when evaluating their activity in terms of transmission prevention. Both foliar and soil drench treatments of neonicotinoids are active and persistent, although soil treatments appear more persistent. Few data on pymetrozine suggest that this insecticide is not very effective on grapevine against sharpshooters. Pyrethroids are effective against both adults and nymphs of sharpshooters but by far less persistent than neonicotinoids. Data on activity of organophosphates against sharpshooters are sometimes conflicting and, when tested weeks after treatment, these molecules were poorly persistent. Insect growth regulators (IGR) seem not to be promising, as their LC50 are high compared to other insecticides; moreover, the possibility of suppressing nymph population with soil tilling, may replace the use of IGR in many situations. Therefore, the most interesting insecticides are those targeting adults. As for spittlebugs, few data on insecticide activity are available. The activity of neonicotinoids has been evaluated in sugarcane with foliar sprays against *Mahanarva fimbriolata* and the efficacy seems comparable to that observed against sharpshooters. Preliminary trials of neonicotinoids against *P. spumarius* confirmed that this class of insecticides is the most active. Organophosphates show a good activity against some spittlebugs (although data on *P. spumarius* indicate a limited efficacy) but are not persistent (except when granular chlorpyrifos was applied to the soil). Few data are available for pyrethroids, but they confirm a high efficacy with a very low persistence. Pymetrozine and Spirotetramat seem to be ineffective against *P. spumarius*. Some activity of azadirachtin (70% mortality) against spittlebug nymphs (*M. fimbriolata*) is recorded together with some reduction of female fertility, but this insecticide lacks activity against adults (including those of *P. spumarius*). A high dose (1%) of aqueous extract of *Anacardium humile* kills about 50% of *M. fimbriolata* nymphs. As for cicadas, neonicotinoids may reduce the number of oviposition scars, but data on mortality are not clear and consistent. Organophosphates have no activity, neither in terms of mortality nor in the reduction of the number of laid eggs. Pyrethroids can kill cicadas in high percentages but they are not useful in preventing oviposition, due to their limited persistence. In conclusion: despite the very limited data available on insecticide activity against spittlebugs, it is likely that neonicotinoids show an active and persistent activity against *X. fastidiosa* vectors while pyrethroids, although active, are not persistent. There is a major gap of knowledge on the activity of insecticides allowed in organic farming, although preliminary data suggest that essential citrus oil is more active than pyrethrins. Unfortunately, citrus oil, like pyrethrins, is not persistent at all.



A total number of 329 records were considered for the efficacy table of natural enemies/biological control methods (Annex 6). There is great evidence that the most effective natural enemies of *X. fastidiosa* vectors are the egg parasitoids (*Gonatocerus* - Mymaridae, *Oligosita* - Trichogrammatidae), an endoparasitoid pipunculid fly (*Verrallia aucta*, Pipunculidae) and the entomopathogenic fungus (particularly *Metarhizium anisopliae*). For these antagonists several observations are available from both laboratory and field experiments. However, in the introduction of exotic parasitoid species in countries where they are needed, some constraints can be encountered because of their possible polyphagia. This gap should be filled to have effective tools readily available to contrast the main vectors of *X. fastidiosa*. Conversely, the entomopathogenic fungus *M. anisopliae* is worldwide distributed and there seem to be no apparent obstacles to its use for reducing the populations of *X. fastidiosa* vectors. It is also evident from the literature that so far only "classic" biological control programs (inoculative introduction of natural enemies from the original area of distribution of the insect vector) have been applied against the invasive sharpshooter vector *H. vitripennis*. As *Philaelus spumarius* is a native species in the EU, inoculative biological control is not feasible, and inundative biological control, based on mass-reared beneficials, periodically released in large quantities to obtain an immediate control of pest, is unlikely to be effective because the spittlebug is ubiquitous and polyphagous.

So far, the most well-known natural enemy of *P. spumarius* seems to be a pipunculid fly, *V. aucta*, attacking both nymphs and adults, that can parasitize one third of the adults under field conditions (Harper and Whittaker, 1976). Apart from some generalist predators, very few information is available on other natural enemies. This is a major research gap that hampers the possibility of implementing a biological control based on a conservation approach (an indirect method including measures to preserve beneficials, i.e. increase of alternative hosts in agroecosystems, correction of unfavourable agronomic techniques, use of selective insecticides), that might contribute to the partial suppression of *P. spumarius* populations. Therefore, to achieve some results of practical importance, research is needed to identify and evaluate the most important natural enemies of *P. spumarius* and of the other spittlebug species in the different environments.

Data in the efficacy table on other control options (Annex 7) were extracted from 15 records. Although few papers are available on the application of agronomic, mechanical and physical methods for the control of xylem-sap feeder insects, some interesting approaches are envisaged. For example, removal of straw in pastures or in sugarcane fields, allowed to significantly reduce the populations of spittlebugs in Brazil. This finding is consistent with the one of Weaver and King (1954) who suggested that the presence of straw was apparently responsible for an increase of 65% in egg deposition by *P. spumarius* females. Spraying with "Surround WP", that forms a particle film on the vegetation, strongly reduced the population of sharpshooters, both in choice and no choice tests. No information is available on the efficacy of such a technique on spittlebugs. Cuttings of grasslands resulted in a population increase of *Neophilaenus campestris*, but no information is available for *P. spumarius*. Recent experiences in the Apulia Region pointed out that soil tilling resulted in a very strong reduction of spittlebug adults on the ground vegetation, although the reduction of adults collected on the olive canopy can be less important. Although not specifically reported in publications, it is worth to remember that protecting nurseries with netting is an effective method to keep nursery stocks free from insect-transmitted pathogens, including *X. fastidiosa*. A prolonged flooding can be effective in killing cicada nymphs, but this technique is not applicable in the vast majority of cases (no water available in dry environments where cicadas live, sloped crop plots).

All the references selected for the efficacy tables are compiled in three EndNote files (References Task 2 Control Biological/Chemicals/Other Control Options).

## 7. Identification of national databases and/or official contact points of MedMSs for the extraction of GAPs (Task 3)

### 7.1. Objectives

The overall aim of this activity is to provide EFSA with updated information on the insecticides that are currently registered for use and applied in the Mediterranean Member States (Croatia, Cyprus, France, Greece, Italy, Malta, Portugal, Slovenia and Spain; referred to, from now on, as MedMS) on stone fruits, citrus, grapevine and olive with potential activity against *X. fastidiosa* vectors/potential vectors.

### 7.2. Materials and Methods

For the identification of national databases the EPPO website page [www.eppo.int/PPPRODUCTS/information/information\\_ppp.htm](http://www.eppo.int/PPPRODUCTS/information/information_ppp.htm) was chosen as a starting point.

Based on the result of Task 2, a list of plant protection products allowed in the EU with effect on *X. fastidiosa* vectors was generated. In particular, all the DARs insecticides (based on Annex 1) for which sucking insects were indicated among target species were considered. Moreover, other insecticides included in the DARs list that, based on the data collected in the efficacy table of chemicals (Annex 5), showed some activity against *X. fastidiosa* vectors were also considered for the GAP table.

The first attempts to use the available links from the EPPO website revealed that Portugal and Malta connections were not working. In the meantime, available information for the requested crops and DAR(s) were gathered from National official pages as detailed in attached file (Annex 8). Time by time, Countries made either downloadable updated .pdf files or accessible database searchable by crop and insecticide active ingredient (a.i.) at least. Email messages were addressed to Portugal and Malta NPPO contact persons in order to ask for webpage missing access/information, however, at the time of performing the task, on June 2016, the links were not active. Gathered information were saved as .doc/.docx/.pdf/.xls/.xlsx files. Each file was searched for each a.i. (DARs) to spot useful control information for candidate *X. fastidiosa* vectors on all the above-mentioned crops.

The excel table (Annex 9) was build including columns on: a.i., mode of action (MoA) category, crop, MedMs, Target pest, notes. One entry (= Excel table line) was prepared for each DAR+Crop compliant useful information found.

### 7.3. Results and deliverables

A table summarizing applications of neonicotinoids (Acetamiprid, Clothianidin, Imidacloprid, Thiacloprid and Thiamethoxam), pyrethrins, pyrethroids (cyhalothrin, tau-fluvalinate, Bifenthrin, Cyfluthrin, Cypermethrin, Deltamethrin), phenoxy-derivatives (etofenprox), organophosphates (dimethoate, phosmet), carbamates (formetanate), lipid biosynthesis inhibitors (Spirodiclofen), phenyl pyrazoles (fipronil), fatty acid potassium salts and *Beauveria bassiana* on stone fruits, citrus, grapevine and olive in the MedMSs have been prepared (Annex 9: GAPs-DARs). The table can be filtered by a.i., crop, MedMS and mode of action. It is worthy to note that, although some of the a.i. are active also against *X. fastidiosa* vectors, they are actually targeted against pests other than the xylem-sap feeders, and therefore timing of application is likely to be inappropriate for the control of spittlebugs and sharpshooters. According to the table, neonicotinoids have the widest application on the considered crops in the MedMSs, followed by organophosphates, the phenoxy derivative etofenprox and pyrethroids. However, it is worth noting that three neonicotinoid active ingredients, clothianidin, imidacloprid and thiamethoxam, have been recently banned and an almost complete prohibition on the use of neonicotinoid insecticides across the EU is on the way.

## 8. Inventory of the Integrated Pest Management programmes (IPM programmes) in place in the MedMSs for woody crops (Task 4)

### 8.1. Objectives

The aim of this activity is to provide EFSA with updated information on the IPM programmes in place in the MedMSs for stone fruits, citrus, grapevine and olive, and the pests targeted by these IPM programmes.

### 8.2. Materials and Methods

To obtain information on the ongoing IPM programmes, a questionnaire was prepared and sent to contact points of all MedMSs. The questionnaire raised the following points:

- cultivated hectares of each crop considered in the survey (almond, apricot, cherry, grapevine, lemon, olive, orange, peach and plum)
- whether an IPM programme was currently ongoing in the above-mentioned crops
- the main pest/s targeted in the ongoing IPM programs

The aim was to produce a questionnaire informative and user-friendly, at the same time. For this reason, we built a questionnaire possible to complete in 5 minutes.

The main source of recipients for the compilation of the questionnaire were the official websites of the Ministry of Agriculture (or equivalent ones). In most cases, there was a clear indication of the contacts for questions. We sent the questionnaire a maximum of three times to the indicated contacts. In the event of no reply after the third attempt, we contacted a number of qualified colleagues in the same Country that were either able to compile the questionnaire or to re-direct it to more competent people. When both ways failed and it was not possible to identify the adequate recipient for the questionnaire, we asked EFSA personnel to provide a list of alternative candidates. Further information was retrieved by websites of the Ministries of Agriculture of the MedMSs.

### 8.3. Results and deliverables of Task 4

The summary excel table is built with one sheet per MedMSs and a column for each crop. Therefore, available information is provided for each crop and each country (Annex 10, IPM inventory). The quality of the obtained data varies a lot from country to country and we didn't receive reply from Malta. However, it is clear that IPM programs are ongoing in all/most of MedMSs on most of the stone fruit and citrus orchards as well as on grapevine and olive. None of these IPM programs mentions xylem-sap feeder insects among the target pests and, thus, current control programs do not consider *X. fastidiosa* vectors as pests. Therefore, in case of *X. fastidiosa* introduction in new EU areas, a revision of these IPM programs will be mandatory in order to include xylem-sap feeders' control.

## 9. Survey of the monitoring programs on vectors and potential vectors of *X. fastidiosa* in place in the MedMSs (Task 5)

### 9.1. Objectives

The aim of this activity is to provide EFSA with updated information on the vector monitoring programmes enforced in the MedMSs (and in other European countries).

### 9.2. Materials and Methods

A list of public national and regional as well of private organisations has been compiled based on contact proposed by CNR-IPSP, EFSA, EUPHRESKO and EPPO to which a questionnaire (Annex 11) including questions on the enforced monitoring programs was sent. The contractor provided a coverage larger than requested, involving in the questionnaire not only MedMSs, but all EU MSs and third countries represented in the EUPHRESKO network. EFSA also participated to the sending of the questionnaire by contacting directly the Chief Officers of Plant Health (COPHS). Given the larger number of national languages needed to extend the geographic area of the questionnaire, it was then sent to all the contact persons in English language. All these interim changes were previously agreed with EFSA.

The questionnaire (Annex 11) has been sent via email to all these organisations at the beginning of December 2016 with an accompanying message, also in English. Only in the case of Italy, the message has been sent first to the National coordinator, who forwarded the questionnaire to all the Regional Phytosanitary Services.

On 13<sup>th</sup> January 2017 a reminder with the deadline of 1st February was sent to the organizations that did not provide a feedback. A late answer, obtained from the Swiss Plant Protection Service (13<sup>th</sup> February), has been included in the revised version of the report.

All the answers have been included in a single excel file, each sheet corresponding to an answer from an organisation (Annex 12).

Finally, in order to comply with the request of the procurement, the information obtained from the survey (questionnaire), has been combined with that obtained from literature (Task 1, Extraction Table Ecology and phenology) and from the macrocosm, mesocosm and microcosm surveys (Tasks 6 and 7) to produce a summary table (Annex 13).

### 9.3. Results and deliverables of Task 5

We obtained 30 answers from outside Italy, coming from 19 different countries and 18 answers from Italy, coming from 15 regions (NUTS2). The European countries from which we obtained replies are represented in the maps reported in the Annex 14 (Figure 1), where different colours indicate countries in which monitoring campaigns are in place, not enforced, or that did not answer to the survey. The same Annex 14 (Figure 2) provides details of the Italian regions where monitoring campaigns are in place or that did not answer to the survey. Actually, all the Italian regions that answered the questionnaire noticed that some monitoring campaigns for *Xylella* vectors/potential vectors were ongoing. Few programs started in 2014, while most of them started in 2015 or 2016. All the answers have been included in a single excel file, with each sheet containing the answers from an organisation, ordered in country alphabetic order (Annex 12). Based on all the answers we received, we can say that monitoring programs are focused mainly on adults of spittlebugs and of the sharpshooter *C. viridis*. The number and quality of target insect species reflect the taxonomic skills in the different organisations and the different environmental/climatic conditions that determine faunistic composition of this xylem-sap feeder group under different environments. It is worth noting that in some monitoring programs also some phloem- or mesophyll-sucking insects, such as Cicadellidae belonging to the subfamilies Deltocephalinae and Typhlocybinae, are targeted. This is probably due to the fact that in one of the early papers on potential vectors in Apulia the Deltocephalinae leafhopper *Euscelis lineolatus*, a phloem sucking insect, was found positive for the presence of *X. fastidiosa* in

PCR assays. Sweep net and yellow sticky traps are the most commonly applied sampling methods, although in some monitoring programs other tools are used, such as beat tray, stem tapping. The inventory of museum collections has been used as an extra source of information in Belgium only. The majority of the organisations involved in monitoring programs chose to focus on adults only; all those programs that also monitor nymphs use visual inspection of foams/nymphs to spot and count the immatures. As for the crops or the non-cultivated species where monitoring campaigns are ongoing, olive is the most targeted crop. Grapevine is targeted both in Mediterranean and non-Mediterranean countries. Meadows, areas with natural vegetation, nurseries, gardens, main roads, ports and their surroundings are also included in monitoring programs by some organisations.

## 10. Field observations on phenological phases (supported also by agronomic and meteorological data) of *Philaenus spumarius* in olive orchards (Tasks 6 and 7), including field activities in 2018, as established in the Amendment (activities 1 and 2 of the amendment)

### 10.1. Objectives

Results on the development and ecology of individuals and populations of *P. spumarius* as well as information on the abundance of other potential vector species of *X. fastidiosa* are presented.

### 10.2. Materials and methods

As required in Task 6 and 7 of the project, elements of the phenological phases and the population ecology of the vector *P. spumarius* and other potential vector species of *X. fastidiosa* in olive orchards have been investigated. Reliable estimates of the density of a population of a species must be based on the collection of samples that are representative of the range of species abundance in the area under evaluation. Therefore, a long-term survey program with standard sampling methods for monitoring of *P. spumarius* and other potential vectors of *X. fastidiosa* has been established for selected sample site locations distributed in Northern Italy (Liguria and Piedmont) and Southern Italy (Apulia). This monitoring campaign lasted for two consecutive years, 2016 and 2017, in Liguria and Piedmont and three consecutive years, 2016-2018, in Apulia. The use of sampling sites at specific geographical locations with different climatic and agro-ecological conditions has allowed to monitor the variability encountered by populations of *P. spumarius* and other potential vectors of *X. fastidiosa* in olive orchards of MedMSs throughout time, within year and between years.

IPSP-CNR has designed observations at three levels:

- Macrocosm: open field surveys in olive orchards of Liguria and Apulia regions
- Mesocosm: two screen houses made of insect-proof net settled in Piedmont and Apulia regions
- Microcosm: small cages for the study of the fecundity of single individual female (observing a male and a female of *P. spumarius*) settled in Piedmont and Apulia regions.

Field studies have been based on visual inspection and counting of nymphs, since this is the only practical method for the study of nymphal population, while sweep net is the most widely applied sampling method of adults of *P. spumarius* (as for other xylem-sap feeder insects).

Three specific protocols have been developed for the three levels as described below.



### 10.2.1. Sampling protocol for the macrocosm

Data collection has been carried out in four olive orchards, two in Northern Italy (Liguria) and two in Southern Italy (Apulia). In each region, one olive orchard is located in warmer climate (coastal area) and one in cooler climate due to different elevation or meso-climatic conditions (inland area). Orchards of one-hectare (ha) size have been selected based on a series of common characteristics: (i) homogeneous field, (ii) same olive cultivar within a field, (iii) presence of weed cover, (iv) no application of synthetic insecticides.

The main purpose of field observations is to describe the patterns of population phenology and dynamics. To guarantee reliability of the estimated patterns and reduce the errors in population parameters estimates, field population abundance of *P. spumarius* and other potential vectors should be sufficiently high in selected fields. Sampling effort (number of sampled units) has to be controlled and to result effective. For this reason, at the beginning of the survey more olive orchards have been inspected obtaining a preliminary estimation of population abundance in order to select fields with suitable populations where a long-term survey program can be carried out in a standardized way. After a first screening, four fields have been selected in the following locations: Arnasco (260 m a.s.l.) and Finale Ligure (260 m a.s.l.) in Liguria, and Locorotondo (400 m a.s.l.) and Valenzano (85 m a.s.l.) in Apulia (Figure 3). The climatic conditions of the four sites represent a large variation within the warm-summer Mediterranean climate. Variability in population abundance is expected in the four fields.



**Figure 3:** Localization of the four olive orchards for the phenology-ecology of *Philaenus spumarius* field surveys.

#### 10.2.1.1. Sampling design

Sampling units and variables have been described based on how data collection has been performed in the field. Sampling program and methods were standardized to allow working in different locations and to enable the comparability of results. Therefore, operational definitions of sampling units and variables are illustrated.

The primary sampling unit (PSU) is represented by the olive field, whereas the minimal spatial unit where sampling variables have been recorded represents the secondary sampling unit (SSU). Four SSU have been considered in the macrocosm:

- SSUp: minimum area of herbaceous vegetation for the sampling of nymphs and first adults (**p**reimaginal stages);
- SSUa: minimum area of herbaceous vegetation for the sampling of **a**dults;

- SSUs: single shrub/tree sampled in the spontaneous woody plant compartment for the sampling of adults;
- SSUo: single plant of olive sampled in the olive tree compartment for the sampling of adults.

Seven sampling variables have been considered in the macrocosm for *P. spumarius* and other xylem-sap feeder species (Cercopoidea and Cicadellinae):

- First instar: number of first nymph instars found inside foam and pooled together with eggs for practical purposes during sampling in SSUp;
- Second instar: number of second nymph instars found inside foam in SSUp;
- Third instar: number of third nymph instars found inside foam in SSUp;
- Fourth instar: number of fourth nymph instars found inside foam in SSUp and characterized by the presence of wing pads;
- Fifth instar: number of fifth nymph instars found inside foam in SSUp and characterized by the presence of wing pads;
- First adults: number of first adults detected in SSUp;
- Adults: number of total females and males detected in SSUa, SSUs and SSUo.

The sampling variables for SSUp and SSUa have been reported in results respectively as number of individuals per square meter (SSUp) and per single swept (SSUa), whereas sampling variables for SSUs and SSUo have been reported as single plant sampled.

At each sampling date, *P. spumarius* and other potential vectors nymphal stages and first adults have been counted on herbaceous vegetation in 30 SSUp randomly selected within the PSU. Herbaceous vegetation has been screened to identify pre-imaginal stages appearance by visually detecting the presence of *P. spumarius* and other potential vectors typical foams on host-plants within the SSUp. Once the foam has been recorded, individuals have been counted in each foam found on plants within the SSUp. First adults have been also reported if detected. Pre-imaginal stages and first adults have been respectively recorded as total number of individuals per foam and total number of individuals per SSUp, however, the number of nymphs and first adults per m<sup>2</sup> has been estimated, considering the total investigated surface per sampling date.

At each sampling date, *P. spumarius* and other potential vectors adult stage has been investigated in three habitats or compartments: herbaceous vegetation (SSUa), shrubs, trees and other spontaneous woody plants (SSUs) and olive trees (SSUo). In herbaceous vegetation 30 SSUa have been randomly selected within the PSU. Individuals have been reported as total number per SSUa, however, the number of adults per single sweeping net swept has been estimated and used to represent results. In the shrub/tree compartment, 10 SSUs have been randomly selected within the PSU. Individuals have been recorded as total number per SSUs and reported as such in the results. In the olive tree compartment, 10 SSUo have been randomly selected within the PSU. Individuals have been recorded as total number per SSUo and reported as such in the results.

Sampling surveys have been carried out every 10/15 days (in 2016) and 7/10 days (in 2017 and 2018) during the 10/12 weeks of pre-imaginal stages in SSUp, from the beginning-end of March until beginning-end of May, depending on the region and year. In SSUa, SSUs and SSUo sampling surveys have been carried out every 7/14 days, from mid-April (Southern Italy) – beginning of May (Northern Italy) until mid or end of October (Southern Italy) - October/November (Northern Italy). In SSUo sampling surveys have been extended up to one sampling every two weeks, but in pre-flowering and fruit set, sampling surveys have been intensified again returning to one sampling every week, given that these olive phenological phases represent the period of *P. spumarius* and other vectors potential visit to the olive tree.

During sampling surveys, weather data (air temperature and relative humidity) have been recorded from data loggers that have been located within each field. Weather data have been also gathered from the closest weather station and have replaced data in case the data logger has been found out of order or with missing data. Data logger with probes for air temperature and humidity have been hanged at the centre of the four PSU from the beginning of the sampling program to the end of the year in 2016 and for the whole year in 2017. Probes have been shaded with shield and a support has been used to place every data logger at 40-50 cm from the ground in the four olive orchards.

Other events or characteristics that may have been significant for population dynamics and vector biology such as vegetation cutting, treatments and anything else that could have affected insect populations of interest have been reported.

#### 10.2.1.2. Sampling techniques

In SSUp, herbaceous host-plant community has been defined based on the most dominant species and their relative abundance in terms of percentage of abundance. Plants have been identified directly in the field when possible, otherwise plant samples have been brought to the laboratory and/or digital pictures of the plant have been taken to classify it in a second moment. The individual herbaceous plants, on which individuals have been found, have been classified in terms of genus/species (if specific recognition has not been possible, a higher systematic category has been used) and according to the following criteria: (i) three phenological classes have been defined for the herbaceous plants at the time of insect sampling: pre-flowering, flowering phase, post-flowering phase, (ii) two phases of water stress have been defined: presence or absence of water stress.

All plants within the SSUp have been visually inspected for foams and nymphs and each nymph has been assigned to a phenological class. The position of the nymphs on the host-plant has been recorded (bottom third, medium third and upper third) and all the nymphs in each class have been counted, but not collected (conservative sampling). First adults have been also reported in the SSUp. Since the sampling is disturbing the sampled area, SSUp is always different within and between sampling dates. The randomly selected area sample for direct visual observations is rectangular and has a dimension of 1.00 x 0.25 (0.25 m<sup>2</sup>). To measure the area sample, a portable wooden frame has been used as shown in Figure 4.



**Figure 4:** Wooden frame of 1.0 x 0.25 m used for counting the spittlebug nymphs in the herbaceous vegetation

At each selected point within the SSUa, four steps have been made and a swept with the sweeping net (38 cm in diameter) of about 70 cm has been made for each step for a total of 4 swept for each point. Adults have been counted, sexed, identified and immediately released (conservative sampling). Herbaceous plant community within the SSUa has been defined based on the most dominant species and their relative abundance in terms of percentage of abundance. Since sampling is partially destructive, SSUa is always different within and between sampling dates. The counting of adults has been made through one sweeping net (38 cm in diameter) swept every 70 cm for four times. Therefore, the portion of vegetation investigated corresponds to a linear transect of defined and standard length (70 cm x 4 swept).



Ten swept per selected shrub/tree have been made around the crown of each SSUs. Adults have been counted, sexed, identified and immediately released (conservative sampling). All the examined spontaneous woody plants have been classified in terms of genus/species. Since sampling is partially destructive, SSUs is always different within and between sampling dates. The counting of adults has been made through one sweeping net (38 cm in diameter) built on a 2 m handle that has been swept ten times around the shrub/tree crown.

Ten swept per selected olive tree have been made around the crown of each SSUo. Adults have been counted, sexed, identified and immediately released (conservative sampling). Phenology of olive trees at the time of insect sampling has been determined according to "Growth stages of mono- and dicotyledonous plants" BBCH Monograph (2001) and crown size has been classified based on three classes: Small, Medium, Big. Since sampling is partially destructive, SSUo is always different within and between sampling dates. The counting of adults has been made through one sweeping net (38 cm in diameter) built on a 2 m handle that has been swept ten times around the olive tree crown.

For other xylem-sap feeder species (Cercopoidea and Cicadellinae) the same technique is applied except that sampling of adults is not conservative and specimen are brought to the lab under ethanol for species identification, if needed.

Data collected in the macrocosm have been used to define *P. spumarius* host-plant preference, stage structure, phenological events and spatial distribution (in compartments or habitats).

### 10.2.2. Sampling protocol for the mesocosm

Two mesocosms have been established, one in Turin (Piedmont region, in the IPSP-CNR campus; Figure 5) and one in Bari (Apulia region, in the University/IPSP-CNR campus; Figure 6). The two mesocosms are slightly different, although they have a very similar size. The characteristics of the two mesocosms are summarized below.

#### 10.2.2.1. Turin mesocosm

The mesocosm has been built on a reinforced concrete base on which a layer of soil has been placed. A tunnel made of insect-proof net of 7.5 x 6.0 m, max height of 3.6 m, with double entrance doors, hosts the *P. spumarius* rearing. Inside, two areas of 5.5 x 2.5 m (in total 27.5 m<sup>2</sup>) divided by a passage of 1 m have been sown with a blend of dicotyledonous (*Medicago*, *Sonchus*, *Taraxacum*, etc.) and of gramineous (*Sorghum halepense*) plants. Ten 2-year-old potted olive plants (5 Leccino and 5 Frantoio cultivars) with 20-25 sprouts have been placed in tight continuity with the areas covered by herbaceous plants. Irrigation is manual and applied when needed. A data logger with probes for air temperature and humidity has been hanged at 40-50 cm from the ground.



**Figure 5:** Outside and inside view of the mesocosm settled in Turin

### 10.2.2.2. Bari mesocosm

A screen house made of insect-proof net of 4 x 8 m, 3.6 m high, with triple entrance doors, hosts the *P. spumarius* rearing. Inside, there is a single area with herbaceous plants (natural grass cover plus other plant species that have been sown: dicotyledonous (*Cardus*, *Fumaria*, *Trifolium*, etc.) and gramineous (*Cynodon*, *Lolium*) plants. Twenty-eight 2-year-old olive plants (1.5 m high) have been interspersed with the grass cover. The mesocosm has been equipped with a drop irrigation system and a sprinkler system for supplemental irrigation in the warmest period. Two data loggers with probes for air temperature and humidity have been hanged at 40-50 cm from the ground.



**Figure 6:** Outside and inside view of the mesocosm settled in Bari

### 10.2.2.3. Sampling design

Before illustrating sampling units and variables, two points have to be underlined concerning the mesocosm microclimate characteristics and the inoculum of *P. spumarius* that has been used in the two mesocosms. The two mesocosms have reported hotter and more humid conditions compared to open field and this could have produced effects on the phenology of plants and insects within the mesocosm. Some effects could have been caused by irrigation that has been seldomly used to prevent drying of the herbaceous cover. *P. spumarius* inoculum could have also produced effects on population development. Three weeks before the inoculum, treatment with a knockdown insecticide has been done to eliminate all possible spittlebugs that may have been present. A non-persistent insecticide, pyrethrin, has been used. *P. spumarius* adults have been collected close to the mesocosm site and two inocula have been realized to establish the starting population of adults:

- First inoculum: 200 *P. spumarius* (sex ratio 1:1) at the beginning of June 2016 in both mesocosms
- Second inoculum: 200 *P. spumarius* (sex ratio 1:1) at mid-July 2016 in Turin and in the second half of July in Bari

The starting population has been monitored in 2016 and has produced nymphs and new adults monitored in 2017 and 2018. The population of *P. spumarius* could have been affected by stress due to the displacement of adults, used for the inoculum, from a natural habitat to an artificial one.

Sampling units and variables have been described based on how data collection has been performed in the mesocosm. As for open field surveys, sampling program and methods have to be standardized to allow working in different locations and to enable the comparability of results. Therefore, operational definitions of sampling units and variables are illustrated.



The PSU is represented in this case by the mesocosm, whereas the minimal spatial units where sampling variables have been recorded (SSU) correspond to three of the four applied in the macrocosm (section 10.2.1.1.):

- SSUp: minimum area of herbaceous vegetation for the sampling of nymphs and first adults (preimaginal);
- SSUa: minimum area of herbaceous vegetation for the sampling of adults;
- SSUo: single plant of olive sampled for adults.
- The same seven sampling variables proposed for the macrocosm (section 10.2.1.1.) have been considered in the mesocosm, with the only difference that adults number doesn't include detection on SSUs as this SSU category does not apply to mesocosm. The sampling variables for SSUp and SSUa have been reported in results as number of *P. spumarius* individuals per mesocosm.

The study in the mesocosm, even if based on sampling, has to produce abundance estimates with high precision in order to describe the pattern of development and survival of a cohort population. It also allows to evaluate the aggregative behaviour of *P. spumarius*. This implies an adequate sampling intensity. *P. spumarius* nymphal stages and first adults have been counted in herbaceous vegetation through 10 SSUp randomly selected within the PSU in 2017 and 2018. One "enhanced" survey during the fourth-fifth instar stages, consisting in 40 random samples in 2017 and 30 in 2018 has been carried out at the beginning of May in Turin. Herbaceous vegetation has been screened to identify pre-imaginal stages appearance by visually detecting the presence of *P. spumarius* typical foams on host-plants within the SSUp. Once the foam has been recorded, individuals have been counted in each foam found on plants within the SSUp. First adults have been also reported if detected. Pre-imaginal stages and first adults have been respectively recorded as total number of individuals per SSUp, however, the number of nymphs and first adults per mesocosm has been estimated, considering the total investigated surface per sampling date.

*P. spumarius* adult stage has been investigated in two habitats or compartments: herbaceous vegetation (SSUa) and olive trees (SSUo). In herbaceous vegetation 10 SSUa have been randomly selected within the PSU. Individuals have been reported as total number per SSUa, however, the number of adults per mesocosm has been estimated and used to represent results. In the olive tree compartment, all the olive trees have been checked within the PSU at each visit of the mesocosm. Individuals have been recorded as total number per SSUo and reported as total number per mesocosm in the results.

Unfortunately, the mesocosm settled in Bari reported technical issues due to planting of herbaceous species in the first year and subsequent delay with the *P. spumarius* inoculum that resulted to be fatal for oviposition and subsequent development of pre-imaginal stages in the second year, therefore all data refer to the Torino mesocosm only.

Sampling surveys have been carried out every 7/10 days (in 2017 and 2018) during the 9/10 weeks of pre-imaginal stages critical phases in SSUp, from the beginning of March until mid-May. In SSUa and SSUo sampling surveys have been carried out every 7/10 days, from mid-June (in 2016) - beginning of June (in 2017) until mid-November (2016) - beginning of October (2017). Samplings have been conducted until beginning of June 2018 in Torino when, due to the spread of entomopathogenic fungi, population collapsed. In SSUa and SSUo sampling surveys have been carried out every week for all the period of insect life, except later in autumn-winter when samplings have been carried out every two weeks.

During sampling surveys, weather data (air temperature and relative humidity) have been recorded from data loggers located within each mesocosm. Weather data have been also gathered from the closest weather station and have replaced data in case the data logger has been found out of order or with missing data. Data logger with probes for air temperature and humidity have been hanged inside the mesocosm from the beginning of the sampling program to June 2018. Probes have been placed at 40-50 cm from the ground.

Other events or characteristics (inside or outside the mesocosm) that may be significant for population dynamics and vector biology were noted.

#### 10.2.2.4. Sampling techniques

In the SSUp, the individual herbaceous plants, on which individuals have been found, have been classified with the same system applied in the macrocosm (section 10.2.1.2.): (i) pre-flowering, flowering phase, post-flowering phase, (ii) presence or absence of water stress.

All plants within the SSUp have been visually inspected for foams and nymphs and each nymph has been assigned to a phenological class. The position of the nymphs on the host-plant has been recorded (bottom third, medium third and upper third) and all the nymphs in each class have been counted. First adults in the SSUp have been also reported. Since sampling is disturbing the inspected area, SSUp is always different within and between sampling dates. The randomly selected area is 0.25 m<sup>2</sup> (section 10.2.1.2. and Figure 4).

The use of sweep net within the mesocosm has been avoided due to the stress that can occur in both the sampled vegetation and insect population, including an increase in mortality. Therefore, at each selected point within the SSUp, adults have been counted in an area of 0.16 m<sup>2</sup> delimited by a 40 x 40 x 40 cm transparent Plexiglas cage ("minicage", Figure 7) opened in two points (bottom and glove port) and randomly placed on the vegetation. Adults have been counted, sexed, identified and immediately released (conservative sampling). Since sampling is partially destructive, SSUp is always different within and between sampling dates.

Counting of adults on all the young olive trees inside the mesocosm (SSUp), by direct observation as shown in Figure 8, has allowed to directly summarize the total number for the whole mesocosm (the fraction of population visiting a single plant in a given time can be easily derived). Adults have been counted, sexed, identified and immediately released (conservative sampling). Phenology of olive trees at the time of insect sampling has been determined according to "Growth stages of mono- and dicotyledonous plants" BBCH Monograph (2001). Since sampling is partially destructive, SSUp is always different within and between sampling dates.

Data collected in the mesocosm have been used to define *P. spumarius* stage structure, phenological events and spatial distribution (in compartments or habitats).



**Figure 7:** Minicage for nymph and adult counting in the herbaceous cover inside the mesocosm



**Figure 8:** An adult of *Philaenus spumarius* observed on an olive stem inside the mesocosm

### 10.2.3. Protocol for the study in the microcosm

Since the counting of eggs of *P. spumarius* has not been feasible in the field or in the mesocosm for operational reasons, an experiment in microcosm has been set to measure the number of eggs per female in order to estimate female fecundity (number of eggs per female). 20 cages have been established in Turin and 20 in Bari in 2016, whereas in 2017 and in 2018, 40 cages have been established in Turin and 30 cages in Bari in order to increase data collection, avoiding technical problems due to misidentification of dead females. Cages made of fine nylon net with a cylindrical shape (45 cm in diameter and 50 cm height) have been chosen (Figure 9). Inside the cages one pot (24 cm in diameter) has been placed with one plant of *Polygala myrtifolia*, a tuft of alfalfa (*Medicago sativa*) and some straw to favour oviposition. The microcosms have been maintained in open-air conditions and periodically inspected to check the survival of the insects.

A male and a female of *P. spumarius* have been introduced in each microcosm at the beginning of September in Turin and at the end of September in Bari in 2016, whereas in 2017 inocula have been made in mid-September both in Turin and in Bari. Morphotypes have been recorded in 2017 before being introduced in the microcosm. In 2016, the inocula have been observed for 2-3 weeks to check if *P. spumarius* (in particular females) were alive, dead or missing. If the female has not been found or has been found dead, another female has been added. After 2-3 weeks, survival checks continued until late autumn (beginning of November) without replacement of dead or not found females. In 2017, a higher number of microcosm was set up, in order to avoid dead females replacement that, in 2016, produced few mistaken identification of missing females.

Microcosms have been observed in winter 2016 and 2017, for the counting of eggs, and in spring 2017 and 2018 for the counting of offspring.

In 2016, 10 microcosms (random choice: called subset A) have been considered for counting of eggs in winter (January). Straw has been accurately observed with a magnification lens (2-3 x), together with plants, pots and cage structure in order to detect the presence of egg masses. Once an egg mass has been identified, individual eggs have been counted under a stereomicroscope, using tweezers and needles to open the sheaths leaning against the culms and those rolled up. The following data were recorded: i) number of egg masses, ii) number of eggs per egg mass, iii) number of damaged eggs, iv) number of dead/dark coloured eggs, and v) location of the egg mass (hale, dry leaves, green leaves, pot). After counting of eggs, the straw has been placed back inside the pot in each microcosm to allow the development of the juvenile stages. All microcosms have been inspected again after *P. spumarius* pre-imaginal stages development, collecting all the straw from each microcosm, and hatched/unhatched eggs have been counted.

In 2017, 20 microcosms (random choice: called subset A) have been considered for counting of eggs in winter (January), with the same procedure described above. The same 20 microcosms have been inspected again for *P. spumarius* pre-imaginal stages development, by identifying and counting nymphal instars every week. Immediately after the first appearance of adults, all the microcosms (40 in Torino and 30 in Bari) were inspected weekly and newly emerged adults counted. The counting of adults ended at the beginning of June 2018 both in Torino and Bari.

Data loggers have not been set in 2016, whereas in 2017 and 2018, one or more data loggers (to control different microcosms exposure to sun or shade) with probes for air temperature and humidity have been hanged outside the microcosms at the beginning of the experiment. Probes have been placed at 40-50 cm from the ground.

Other events or characteristics (inside or outside the microcosms) that may be significant for population dynamics and vector biology have been noted.

Based on the analysis of literature on phenology and ecology field data collection (Task 1) and our own experience (Tasks 6 and 7), a draft protocol for *Philaenus spumarius* field data collection has been produced (Annex 4).



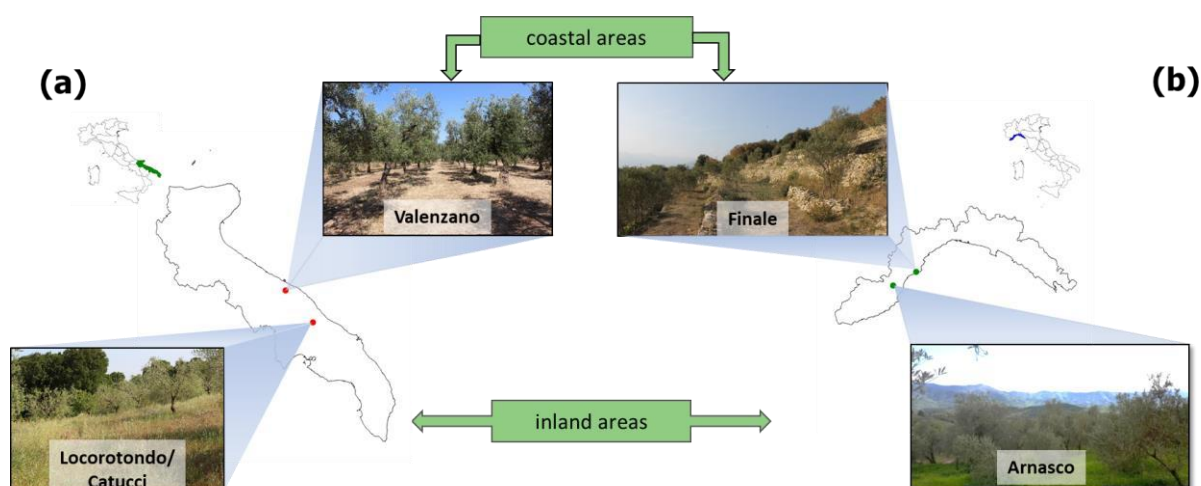


**Figure 9:** Outside and inside view of the oviposition cages (microcosms)

### 10.3. Results and deliverables of Tasks 6 and 7

#### 10.3.1. Macrocosm

Open field surveys have been carried out in four olive orchards in Italy (two in Liguria and two in Apulia regions) from March 2016 to October 2017. For each region, one experimental site in coastal area (Finale in Liguria and Valenzano in Apulia) and one in inland area (Arnasco in Liguria and Locorotondo in Apulia) have been selected, in order to better represent the influence of different habitats and climate conditions on populations of *P. spumarius* (Figure 10). In the second year of sampling, the field of Locorotondo has been replaced due to agricultural practices that have made the field selected in 2016 incompatible with the study. Another field has been identified in the same area in Apulia (Locorotondo – Catucci), about 3 Km far from the previous one. A third year of sampling (2018) has been added for the two fields in Apulia. Host-plant preference, population phenology and dynamics, and seasonal movement between habitats, have been investigated for *P. spumarius* and other potential vectors of *X. fastidiosa*, both within year and between years.



**Figure 10:** Locations of olive orchards used in open field surveys in Apulia (a) and Liguria (b).

### 10.3.1.1. Host-plant preference

The sampling of pre-imaginal stages of *P. spumarius* and other potential vectors of *X. fastidiosa* allowed to detect the host-plant preference for specific plant families for instar nymphs. Other potential vectors of *X. fastidiosa* detected in open field are members of the genera *Aphrophora*, *Neophilaenus* and *Cercopis* in Liguria and members of the genus *Neophilaenus* in Apulia. The survey has provided the plant genus or, where possible, the plant species on which insects have been recorded. Afterwards, plant family has been chosen to better represent the overall preference. Data have been analysed at the regional level combining data, in each year, of the two sites in each region in order to obtain an overall view of plant preference.

Host-plant preference is represented by the percentage of *P. spumarius* or other potential vectors found on a specific plant family over the total of vectors found. Host-plant preference is reported for all pre-imaginal stages and for the first adults detected in the field. It should be noted that in 2016 the beginning of first instars appearance in all four sites has been difficult to be precisely identified, therefore the sampling reports low values in Liguria (sampling began on 02/03/2016 in the two fields) or no values in Apulia (sampling began on 31/03/2016 at Locorotondo and on 01/04/2016 at Valenzano) concerning the first instar stage. Moreover, in 2016 in Apulia, the second and third instars were counted together, as well as the third and fourth, therefore data have been reported as such. To fill this information gap, in 2017 and in 2018 the monitoring of first instars in the two sites in Apulia has begun in March and in February respectively, in order to detect the presence of first instars of *P. spumarius* as soon as they appeared.

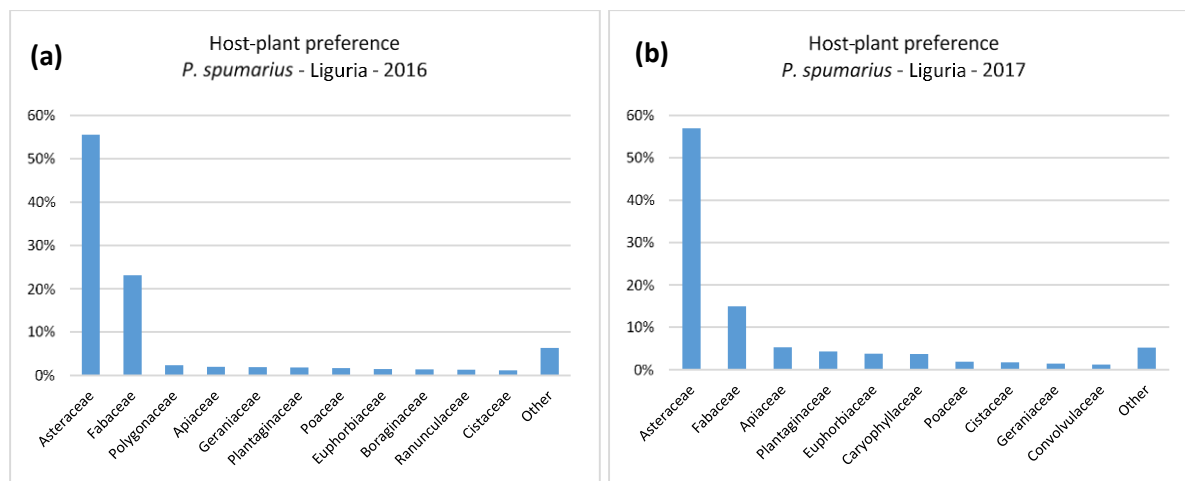
The number of plant families is different in the two regions. Considering *P. spumarius*, in Liguria, 24 and 29 families were registered in 2016 and 2017, respectively, while in Apulia the families recorded were 9 in 2016, 12 in 2017 and 8 in 2018. The comparison is not significant for the other potential vectors as the species are different in the two regions. For each considered region, also the most preferred genera have been reported in order to indicate what host-plants genera are mostly chosen.

All the graphs of this section (Figures 11-20) reported only plant families with the highest percentage of host-plant preference. In category "other" are grouped plant families with less than 1% of host-plant preference, unknown species, dry grass or cases in which the insect has not been found on a plant.

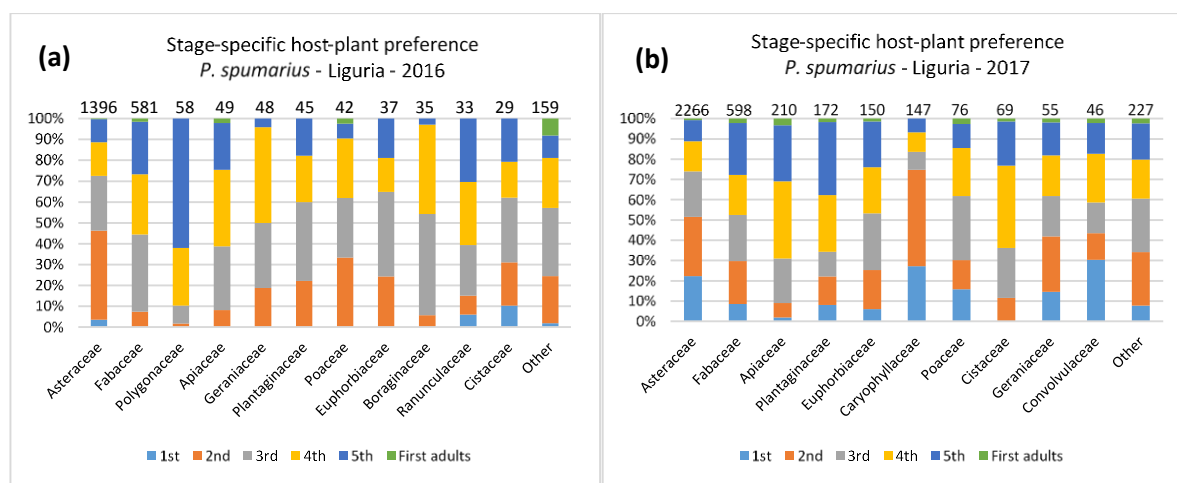
#### 10.3.1.1.1 *Philaenus spumarius* host-plant preference in Liguria

The samplings in Liguria identified as predominant the Asteraceae and Fabaceae families both for the year 2016 and 2017. Jointly, these two families represent 78.7% (2016) and 71.9% (2017) of the total plants on which *P. spumarius* was found. The Asteraceae family predominates in both 2016 and 2017 with a host-plant preference of 55.6% and 57% respectively (Figure 11). In particular, the following five genera have been found to be the most preferred by *P. spumarius* in 2016: *Picris* (Asteraceae) > *Sonchus* (Asteraceae) > *Medicago* (Fabaceae) > *Bellis* (Asteraceae) > *Hyoseris* (Asteraceae), while in 2017 the most preferred genera, all belonging to Asteraceae, have been: *Crepis* > *Cichorium* > *Picris* > *Bellis* > *Hyoseris*. Concerning the host-plant preference of each stage of development there is not a clear preference of a single stage for a specific family (Figure 12). However, first instar stage nymphs have been found only on few families in 2016, but this could be linked to observation issues at the beginning of the survey. Therefore, the complete development of *P. spumarius* can generally occur on plants of all the families that have been identified in Ligurian fields. Total absolute values of individuals per host-plant family have been reported (Figure 12).





**Figure 11:** Host-plant preference of *P. spumarius* in Liguria in 2016 (a) and in 2017 (b).

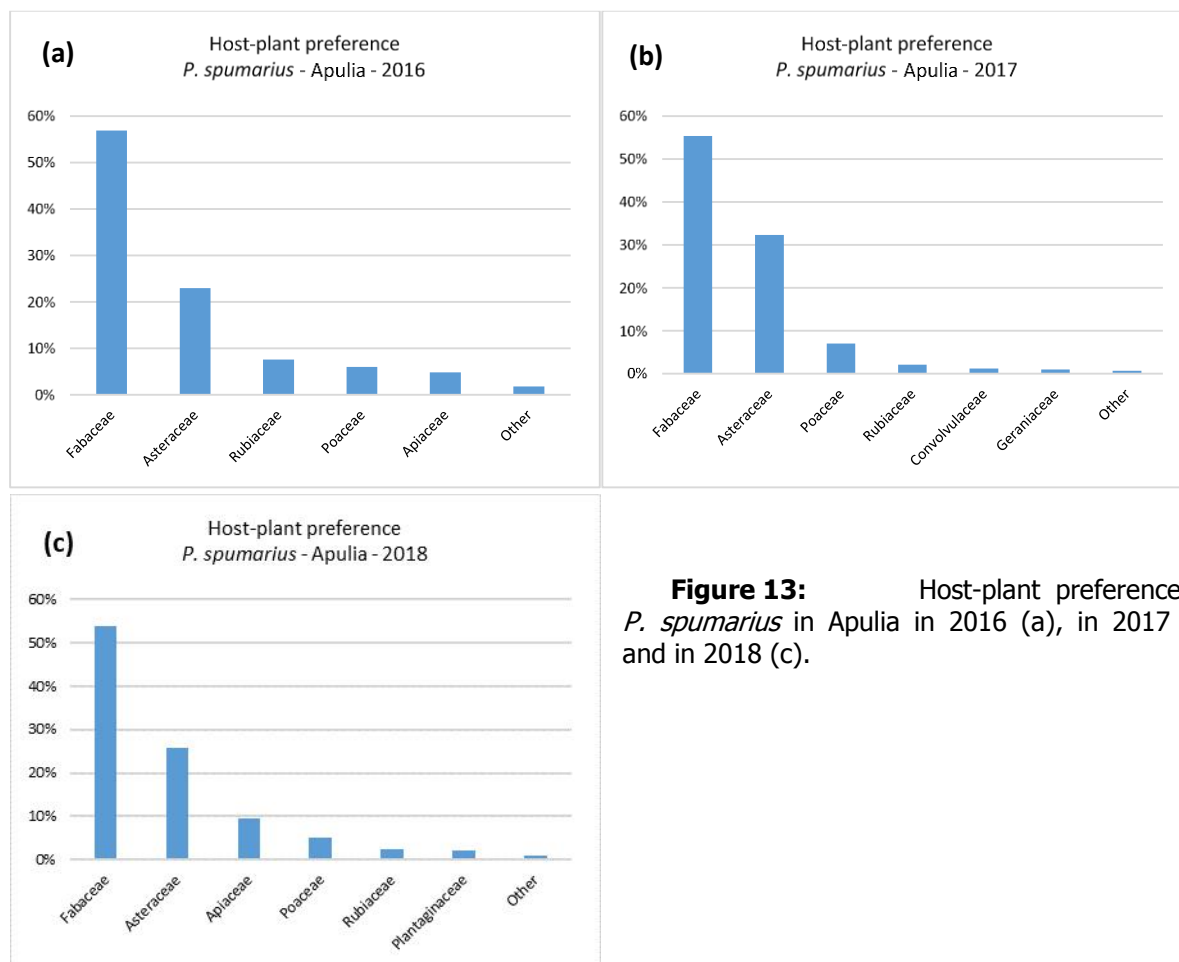


**Figure 12:** Stage-specific host-plant preference of *P. spumarius* in Liguria in 2016 (a) and in 2017 (b).

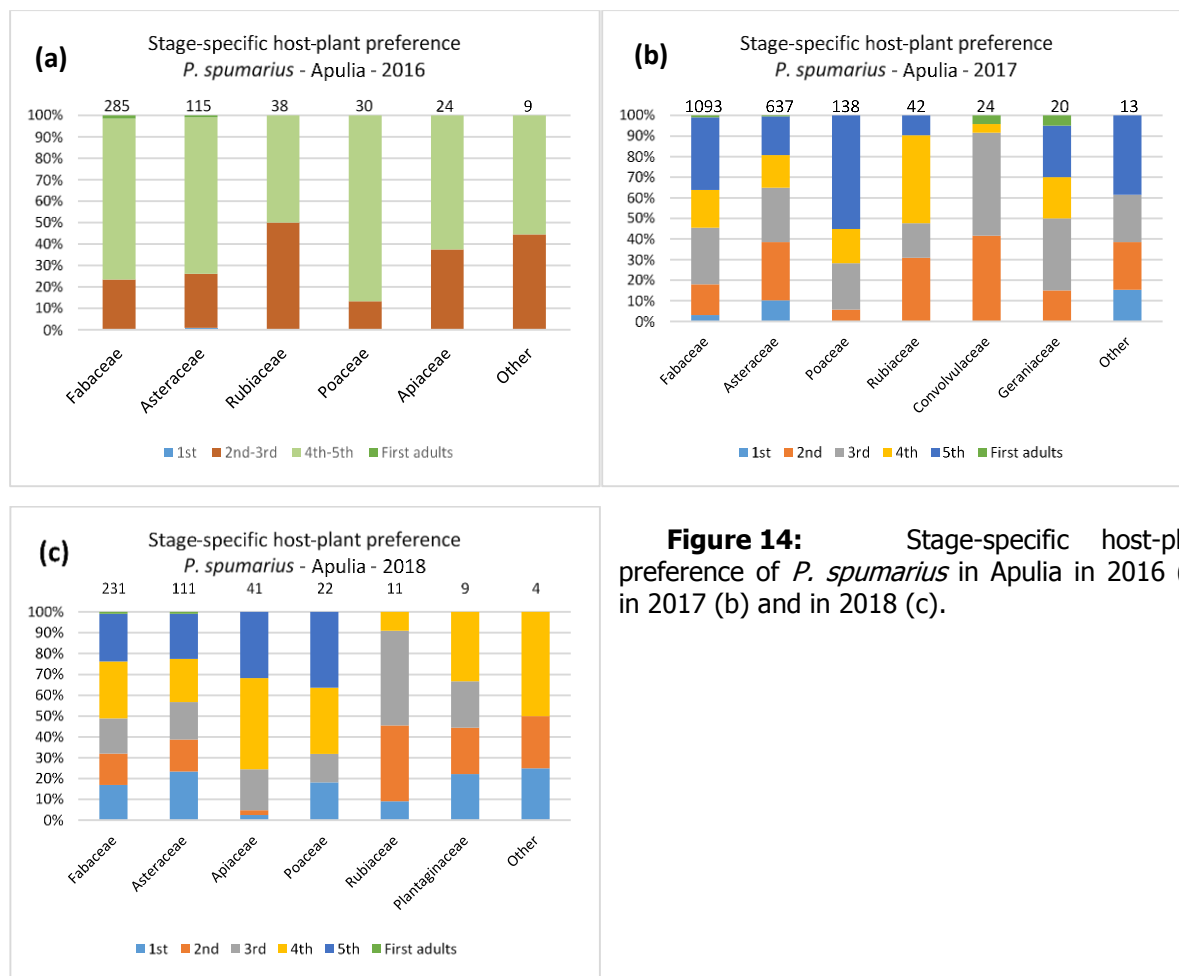
#### 10.3.1.1.2 *Philaenus spumarius* host-plant preference in Apulia

The host-plant preferences of the two study sites in Apulia report the Fabaceae family and the Asteraceae family as preferred by *P. spumarius*, reaching 79.8% of the preferences in 2016, 87.7% of the preferences in 2017 and 79.7% of the preferences in 2018 (Figure 13). Unlike the Ligurian situation, in this case the most chosen family is the Fabaceae (56.9% in 2016, 55.4% in 2017 and 53.8% in 2018). In particular, in 2016 *P. spumarius* has been identified mainly in the following four genera and one family, for which no distinction in genera has been made: *Medicago* (Fabaceae) > *Trifolium* (Fabaceae) > *Sonchus* (Asteraceae) > *Sherardia* (Rubiaceae) and plants of the Poaceae family. In 2017 the most preferred genera have been: *Medicago* > *Picris* (Asteraceae) > *Trifolium* > *Sonchus* and plants of the Poaceae family (without reporting genera distinction as for 2016). In 2018, *P. spumarius* has been mainly observed in the following two genera and one species: *Medicago* > *Sonchus* > *Foeniculum vulgare* (Apiaceae). As previously described, sampling in 2016 started too late

(end of March/beginning of April) so the first instar was not adequately sampled, in fact only one case was reported. By concentrating on the results of observations from 2017 and 2018 (more accurate), it seems that some plant families are specific to some pre-imaginal stages only (e.g. Convolvulaceae family), although this could be the consequence of the low number of insects found in these families. The development of *P. spumarius* can be hosted on all the families identified, with a preference for Asteraceae and Fabaceae.



**Figure 13:** Host-plant preference of *P. spumarius* in Apulia in 2016 (a), in 2017 (b) and in 2018 (c).

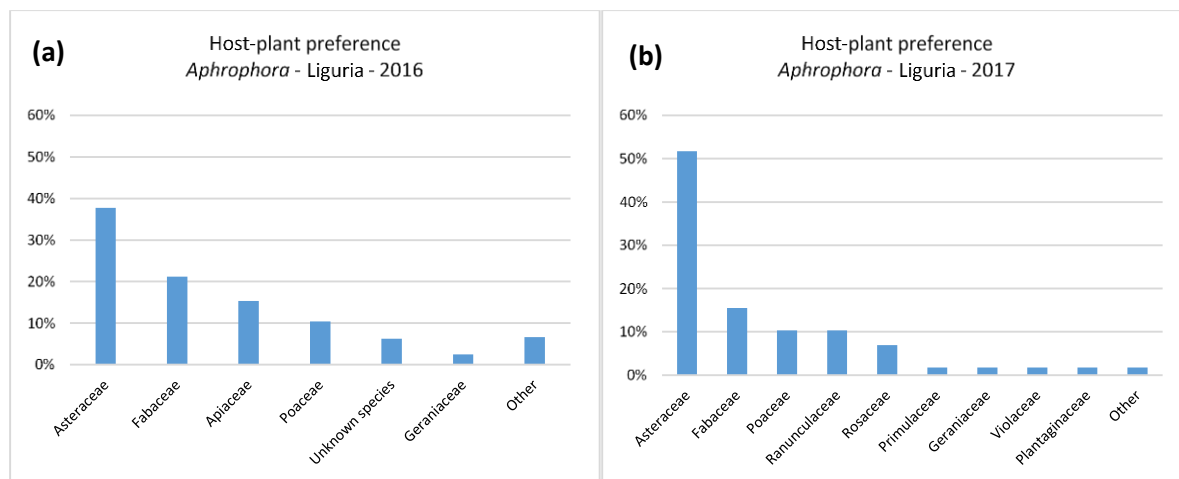


**Figure 14:** Stage-specific host-plant preference of *P. spumarius* in Apulia in 2016 (a), in 2017 (b) and in 2018 (c).

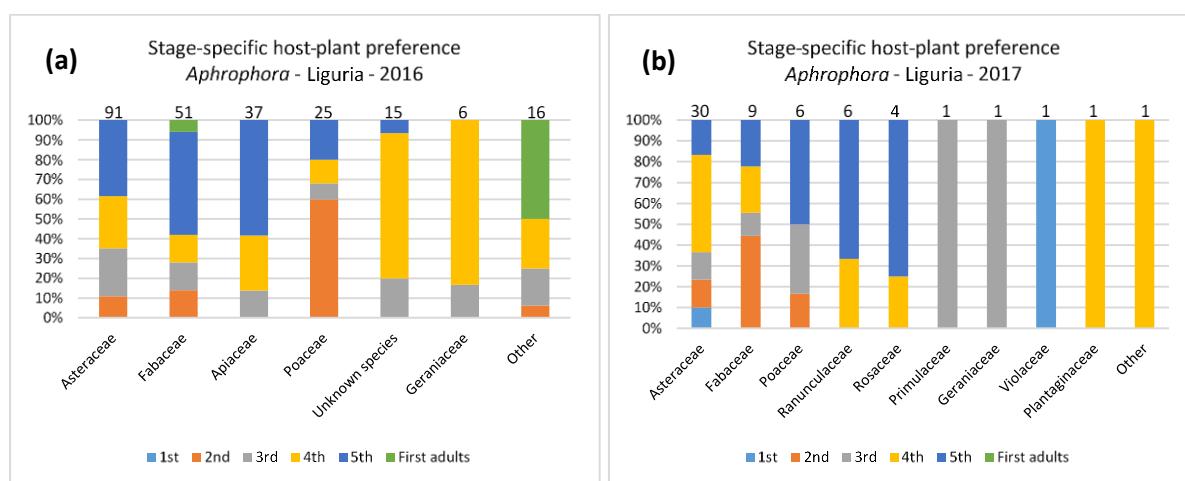
#### 10.3.1.1.3 Host-plant preference of other potential vectors of *X. fastidiosa* in Liguria

Only the host-plant preferences of potential vectors belonging to the genera *Aphrophora* e *Neophilaenus* in Liguria have been considered, because too few individuals of the genus *Cercopis* have been recorded in the stage of adult both in 2016 and 2017.

Taking into account the genus *Aphrophora* (Figure 15), the host-plant preferences of the two study sites in Liguria fall mainly within Asteraceae and Fabaceae families (58.9% in 2016 and 67,2% in 2017), as for *P. spumarius*. In 2016, the category “unknown species” has been considered apart from the category “other” due to high percentage of individuals found in the former category in Liguria. Concerning the stage-specific host-plant preference (Figure 16), some plant families only present specific pre-imaginal stages, but total absolute values of individuals per plant family is too low to conclude that nymphs have preferences for specific families. Although the nymphs cannot be identified at the species level, all the adults of *Aphrophora* collected in the Ligurian sites belonged to the species *A. alni*.

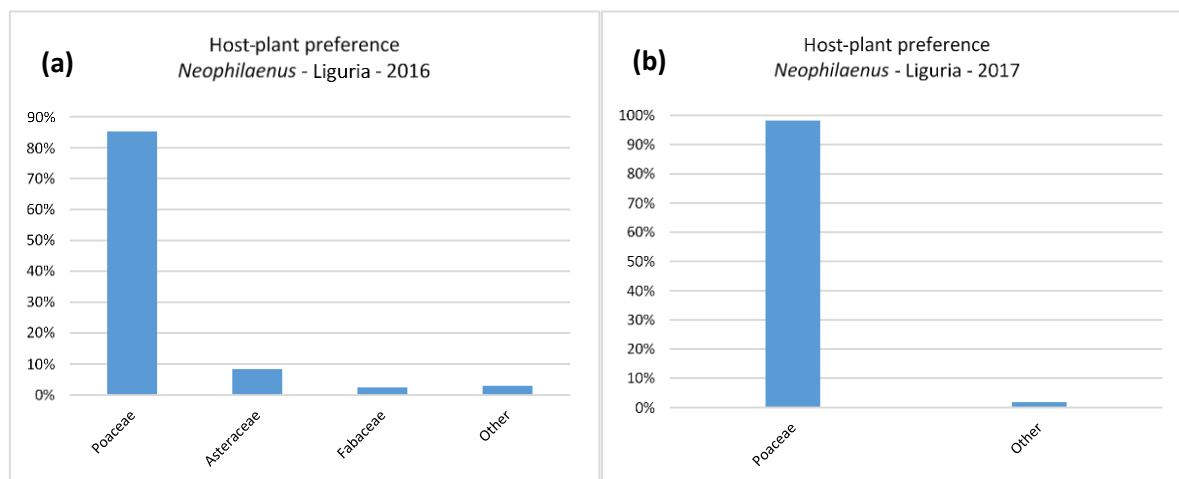


**Figure 15:** Host-plant preference of *Aphrophora* in Liguria in 2016 (a) and in 2017 (b).

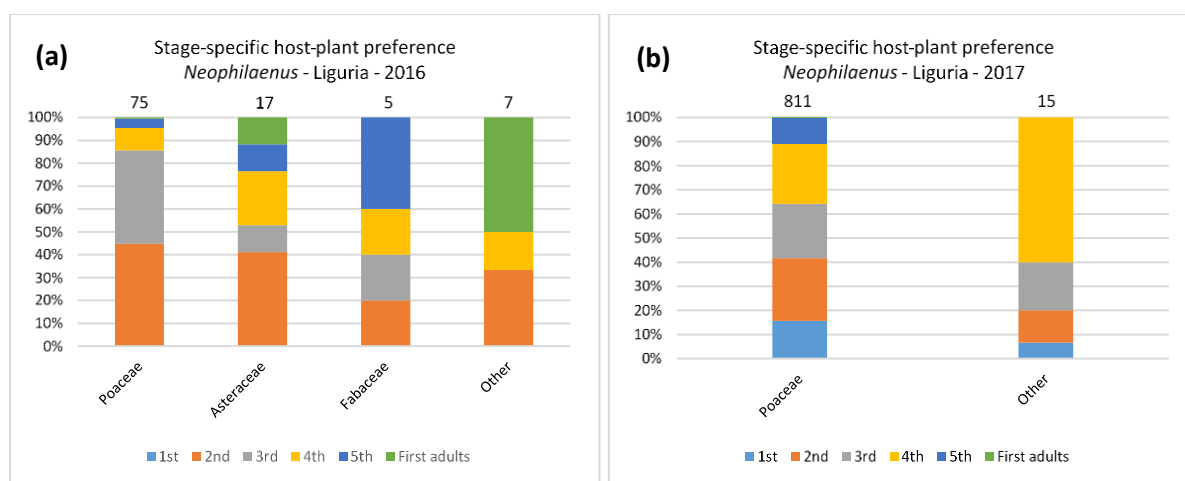


**Figure 16:** Stage-specific host-plant preference of *Aphrophora* in Liguria in 2016 (a) and in 2017 (b).

Genus *Neophilaenus* presents a clear preference for the Poaceae family with a host-preference of 85.3% in 2016 and 98.3% in 2017 (Figure 17). Concerning the stage-specific host-plant preference (Figure 18), there is not a clear preference of a single stage for a specific family. Although the nymphs cannot be identified at the species level, all the adults of *Neophilaenus* collected in the Ligurian sites belonged to the species *N. campestris*.



**Figure 17:** Host-plant preference of *Neophilaenus* in Liguria in 2016 (a) and in 2017 (b).

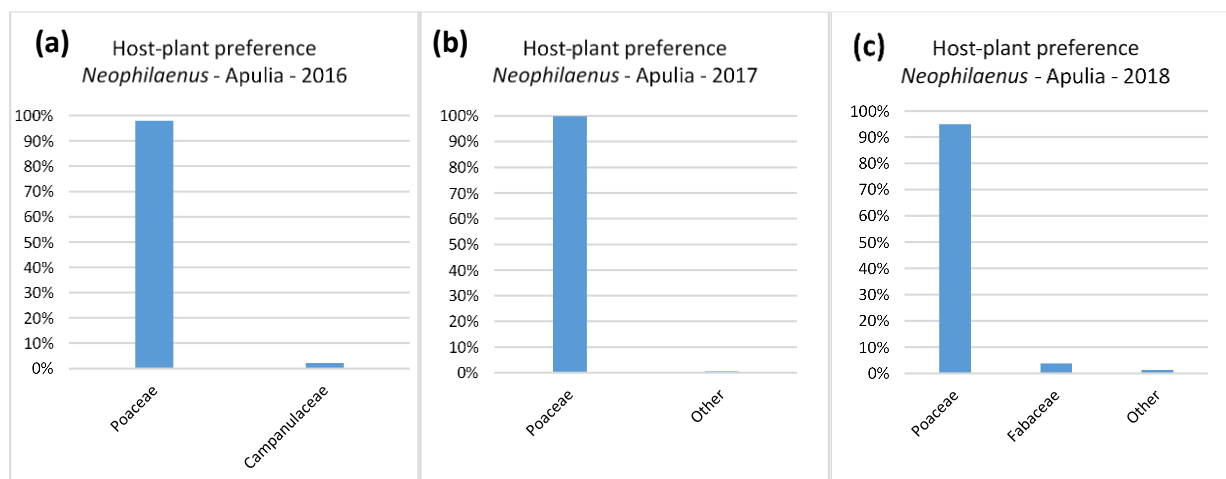


**Figure 18:** Stage-specific host-plant preference of *Neophilaenus* in Liguria in 2016 (a) and in 2017 (b).

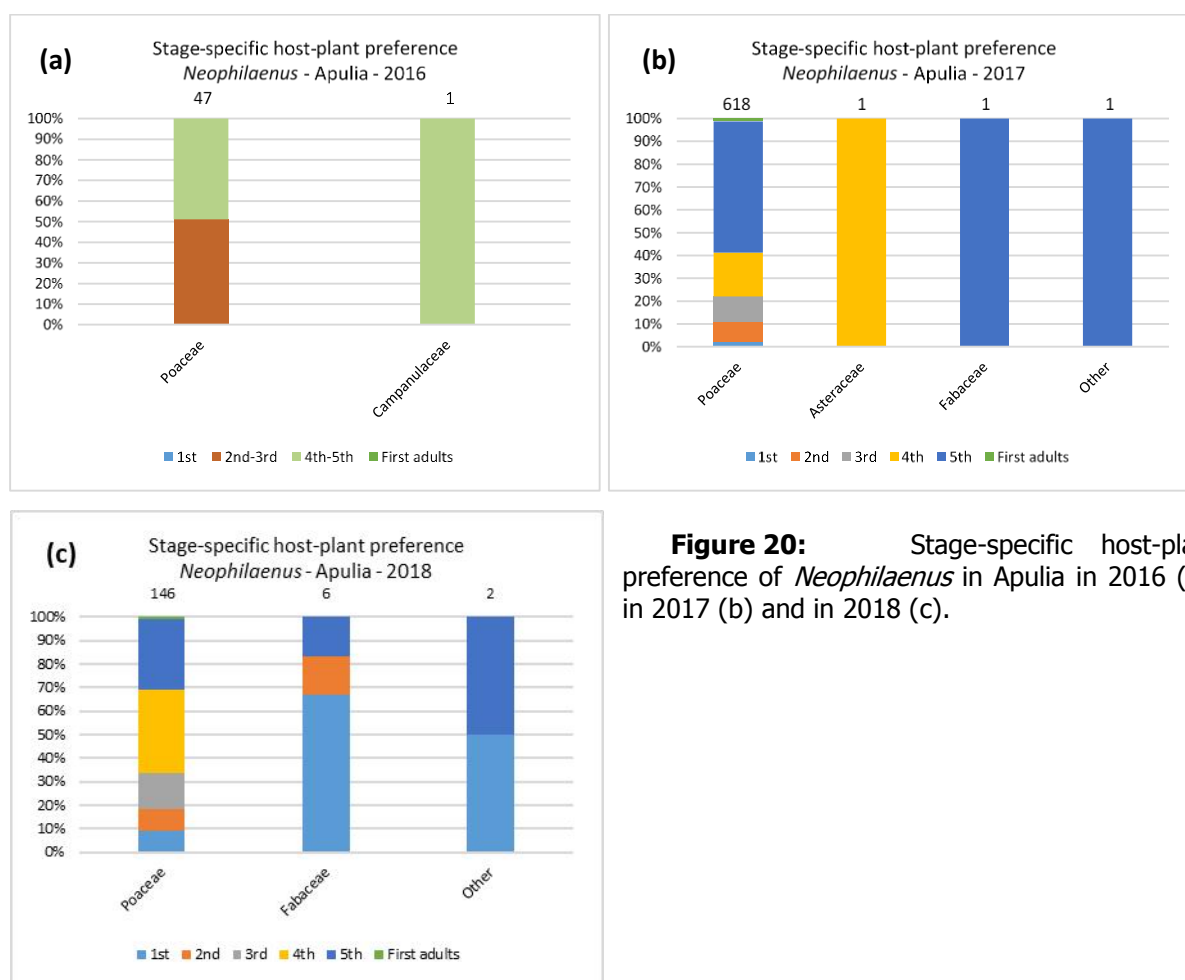
#### 10.3.1.1.4 Host-plant preference of other potential vectors of *X. fastidiosa* in Apulia

Figure 19 shows the host-plant preference of the potential vector belonging to the genus *Neophilaenus*, the only one recorded in Apulia. As for results in Liguria, there is a clear preference of *Neophilaenus* for the Poaceae family 97.9% in 2016, 99.8% in 2017 and 95% in 2018. Concerning the stage-specific host-plant preference (Figure 20), some plant families only present specific pre-imaginal stages, but total absolute values of individuals per plant family is too low to conclude that nymphs have preferences for specific families. Although the nymphs cannot be identified at the species level, all the adults of *Neophilaenus* collected in the Apulian sites belonged to *N. campestris* species.





**Figure 19:** Host-plant preference of *Neophilaenus* in Apulia in 2016 (a), in 2017 (b) and in 2018 (c).



**Figure 20:** Stage-specific host-plant preference of *Neophilaenus* in Apulia in 2016 (a), in 2017 (b) and in 2018 (c).

### 10.3.1.2. Population dynamics and phenology

The study of population dynamics and phenology, with respect to chronological and physiological time, has mainly focused on *P. spumarius*, which is the proven vector of the CoDiRO strain of *X. fastidiosa*. However, the population dynamics for nymphs and first adults with respect to chronological time has been reported also for other potential vectors in order to present some general patterns of the population ecology of other potential vectors in each studied location.

To improve the interpretability of the results, data are reported per area unit ( $\text{m}^2$ ), calculated based on the field sampling units of  $0.25 \text{ m}^2$ .

In some cases (Arnasco e Finale), it was necessary to integrate some missing hourly data, due to lack of data recording, with those of the nearest weather station. Due to technical problems with the data logger in the field, in 2016 the hourly temperatures of Locorotondo were not available, so the daily minimum and maximum temperatures, provided by the Apulia regional system have been used. To calculate the physiological time, degree-days (DD) were used, based on hourly temperatures or on min/max temperatures with a low threshold temperature of  $10^\circ\text{C}$ .

#### 10.3.1.2.1 Population dynamics with respect to chronological time

##### *Philaenus spumarius*

In 2016, the field sampling in Apulia started too late to adequately detect the first stages of *P. spumarius*. The delay in the beginning of the sampling was due to a difficulty in the determination of the appearance of the first stages of *P. spumarius* in that region. This gap has affected the quality of data to study the pre-imaginal stages and first adult in 2016 in Apulia. Compared to the data of the two sites in Liguria (Arnasco e Finale) in 2016, data of the two sites in Apulia (Locorotondo e Valenzano) do not present a curve with a peak of abundance but a purely decreasing trend (Figure 21a). In 2017 and in 2018, the sampling procedure was indeed anticipated in Apulia, thus succeeding in detecting the first stages of development of *P. spumarius* (Figure 21c and d).

Regarding the abundance, in 2016 in Liguria the peak of nymphs and first adults sampled was 49 individuals/ $\text{m}^2$  at Arnasco and 45 individuals/ $\text{m}^2$  at Finale, with an average of nymphs and first adults close to 21 individuals/ $\text{m}^2$  in both sites. In both sites of Apulia individuals never exceed 20 individuals/ $\text{m}^2$ , probably due to sampling delay.

The reported situation is quite different in 2017. Finale (Liguria) has the highest number of individuals per square meter, (mean 30 individuals/ $\text{m}^2$ ; maximum 68 individuals/ $\text{m}^2$  in mid-April), followed by Locorotondo (Apulia) (mean 19 individuals/ $\text{m}^2$ ; maximum 39 individuals/ $\text{m}^2$ ). At Arnasco (Liguria) the mean is of 13 individuals/ $\text{m}^2$  (maximum 31 individuals/ $\text{m}^2$ ), and at Valenzano 5 individuals/ $\text{m}^2$  does (with a maximum not reaching the 20 individuals/ $\text{m}^2$ ).

In 2018, in both Apulian sites, individuals never exceed 10 individuals/ $\text{m}^2$ : the highest peak of abundance is 9 individuals/ $\text{m}^2$  in Locorotondo (mean 3 individuals/ $\text{m}^2$ ) and while 7 individuals/ $\text{m}^2$  in Valenzano (mean of 3 individuals/ $\text{m}^2$ ).

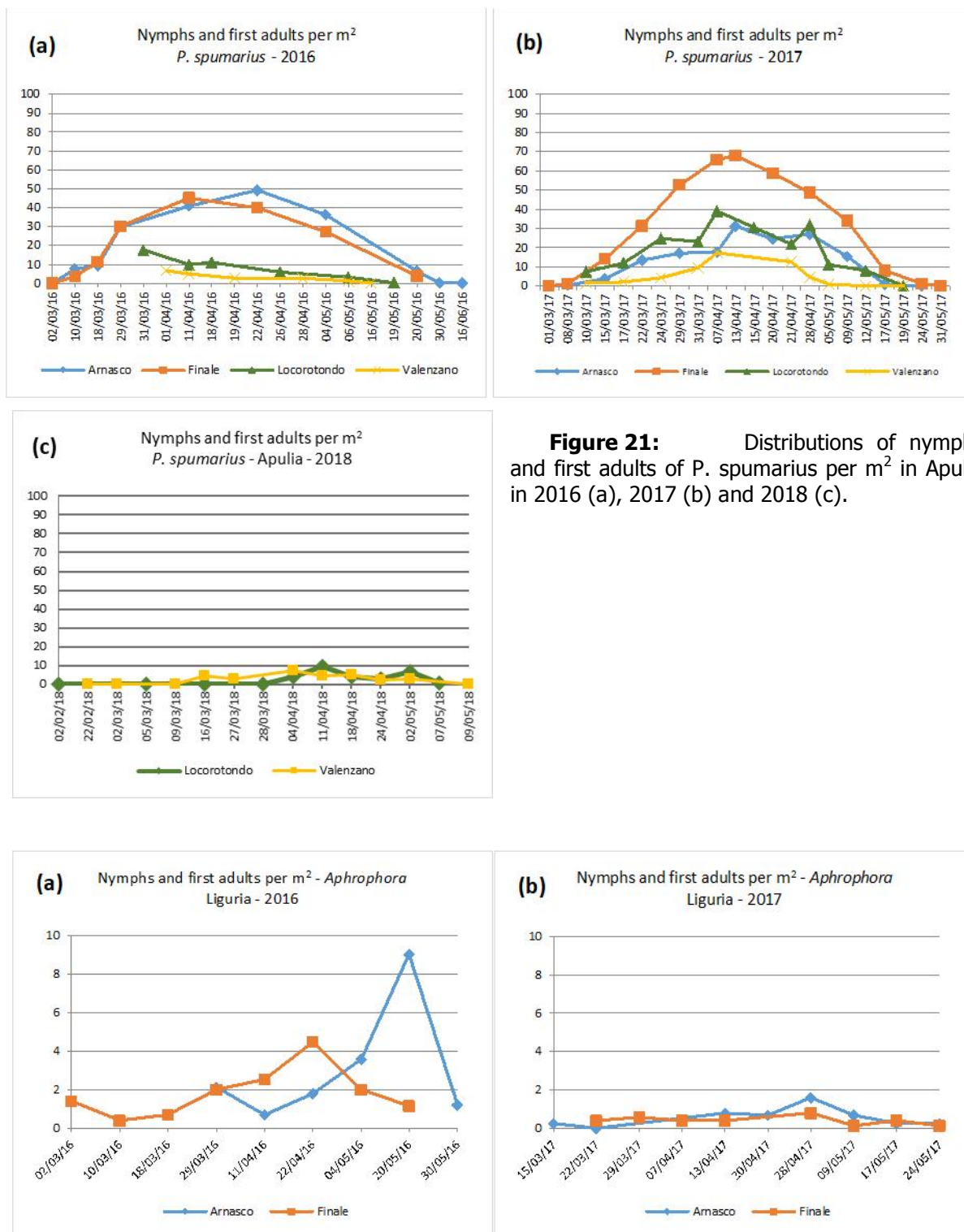
In 2016, the peak of the population density appears to be immediately before mid-April at Finale and immediately after mid-April at Arnasco, whereas it is not possible to indicate the highest population density for the two Apulia sites, presumably it could be towards the end of March. In 2017, the peak falls shortly before mid-April for all sites and in 2018, the peak of the population density appears to be at the beginning of April in Valenzano and the week after in Locorotondo (Figure 21).

##### Other potential vectors

Besides *P. spumarius*, the other potential vectors of *X. fastidiosa* identified in Liguria and Apulia belong to the genera *Aphrophora* and *Neophilaenus*. However, the number of detected individuals of these two species is always considerably lower than that of *P. spumarius*.

The genus *Aphrophora*, detected only in Liguria, reaches the maximum population density (9 individuals/ $\text{m}^2$ ) in Arnasco after mid-May in 2016. In 2017 the number of detected individuals is

considerably lower than the previous year (with less than 2 individuals/m<sup>2</sup>) and the maximum density is recorded around the end of April in both places (Figure 22).



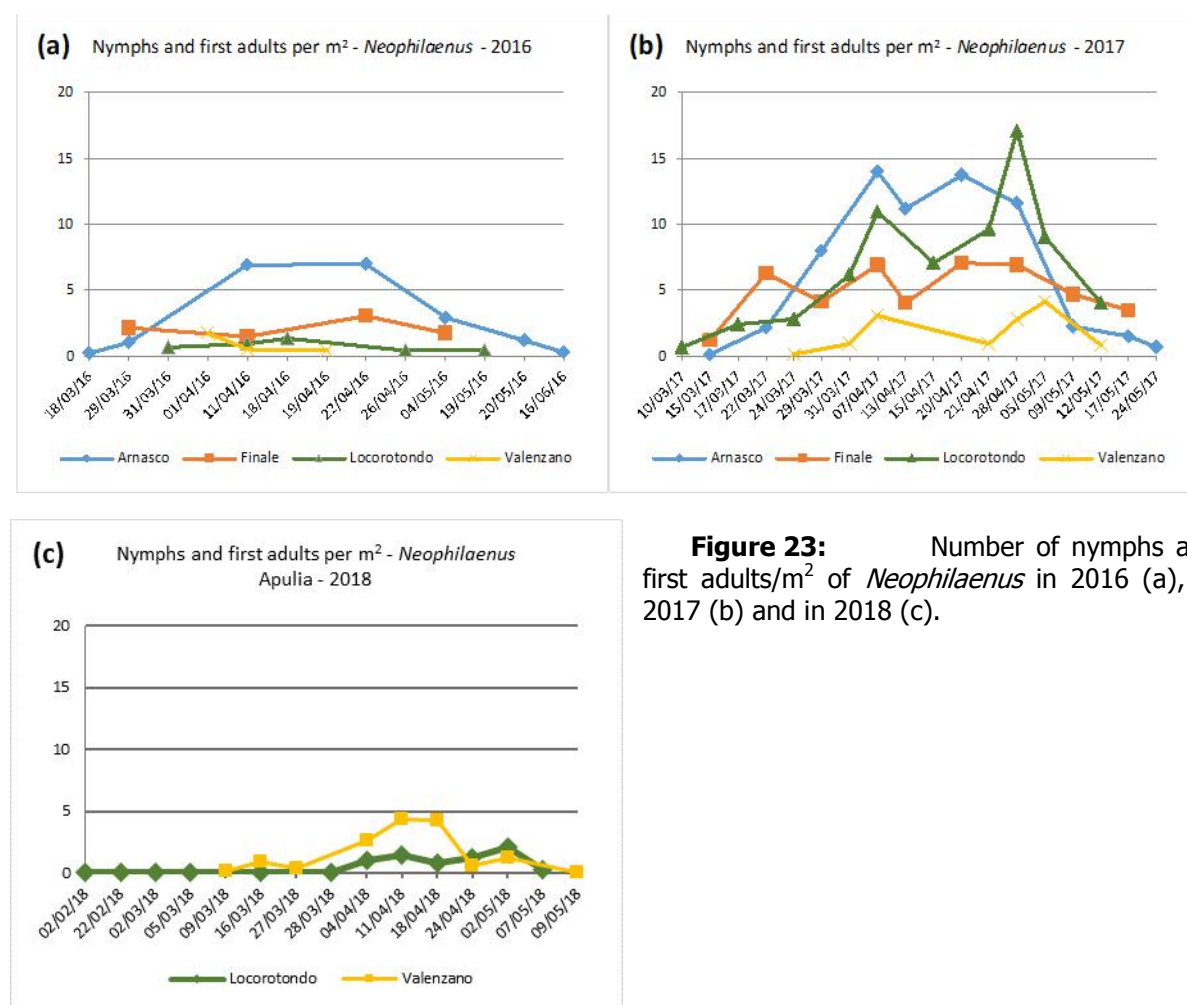
**Figure 22:** Number of nymphs and first adults/m<sup>2</sup> of *Aphrophora* in Liguria in 2016 (a) and in 2017 (b).

For the individuals of the genus *Neophilaenus*, the data collected in 2016, 2017 and 2018 show the presence of this potential vector both in Liguria and in Apulia.

In 2016, Arnasco presents the highest number of sampled individuals, with a maximum of 7 individuals/m<sup>2</sup> in April, followed by Finale with a maximum of 3 individuals/m<sup>2</sup> after mid-April. In Apulia in 2016 the density of *Neophilaenus* never exceeds 2 individuals/m<sup>2</sup>, with higher values towards the beginning of the year till April.

In 2017 the maximum abundance of individuals belonging to the genus *Neophilaenus* is higher than in 2016. Locorotondo has the highest number (17 individuals/m<sup>2</sup> at the end of April), followed by Arnasco (14 individuals/m<sup>2</sup> in early April), Finale (7 individuals/m<sup>2</sup> after the middle of April) and Valenzano (4 individuals/m<sup>2</sup> at the beginning of May).

In 2018, Valenzano presents the highest number of sampled individuals, with a maximum peak of approximately 4 individuals/m<sup>2</sup> recorded in mid-April (mean 1 individual/m<sup>2</sup>). In Locorotondo, the density of *Neophilaenus* reach higher values at the beginning of May (2 individual/m<sup>2</sup>) (mean 1 individual/m<sup>2</sup>) (Figure 23).



**Figure 23:** Number of nymphs and first adults/m<sup>2</sup> of *Neophilaenus* in 2016 (a), in 2017 (b) and in 2018 (c).

A marked seasonality in the development of the pre-imaginal and early adult stages of *P. spumarius* and other potential vectors has been observed from the end of February/beginning of March to the end of May/beginning of June.

#### 10.3.1.2.2 Population dynamics with respect to physiological time

To identify the threshold values of *P. spumarius* development, degree-days (DD) have been used.

Distributions of individuals/m<sup>2</sup> of *P. spumarius* according to the physiological time, estimated with the DD, differ from what has been previously observed using chronological time. Comparing the values of the two distributions (based on chronological and physiological time) within the same date, it is indeed observed that DD in Apulia in 2016 are higher than in Liguria. In 2017 this happens only in the Valenzano field, while the Locorotondo field reports a lower thermal summation.

More in details, in Liguria in 2016 the peak of abundance of the population of the pre-imaginal *P. spumarius* stages is recorded at 212 DD at Arnasco and 147 DD at Finale, while in 2017 the peak of abundance is respectively at 173 DD and 160 DD. The development of the pre-imaginal stages of *P. spumarius* in 2016 ends around 563 DD at Arnasco and around 357 DD at Finale, while in 2017 the end is recorded around 399 DD at Arnasco and around 451 DD at Finale (table 10).

Concerning Apulia, in 2016 no peaks of abundance are present due to missing information, while in 2017 peaks of abundance have been recorded around 267 DD, both for Locorotondo and for Valenzano. In 2016, the end of the pre-imaginal stages of *P. spumarius* is around 439 DD at Locorotondo and 591 DD in Valenzano, while in 2017 the end of the pre-imaginal stages of *P. spumarius* is around 290 DD for Locorotondo and 611 DD in Valenzano (table 10).

In Apulia in 2018 the peak of abundance of the population of the pre-imaginal *P. spumarius* stages is recorded at 122 DD in Locorotondo and at 217 DD in Valenzano. The development of the pre-imaginal stages of *P. spumarius* in 2018 ends around 306 DD in Locorotondo and around 521 DD in Valenzano (table 10).

It is interesting to note that the response of population of *P. spumarius* to temperature variations (measured by degree-days) is not linear.

**Table 10.** Degree days (DD) of main events related to *P. spumarius* population dynamics in the olive groves. N/A: data not available. In 2018 only the two Apulian sites were monitored.

	Year	DD			
		Arnasco	Finale	Locorotondo	Valenzano
Peak of abundance of the population of the pre-imaginal stages of <i>P. spumarius</i>	2016	212	147	N/A	N/A
	2017	173	160	102	267
	2018			122	217
End of development of the population of the pre-imaginal stages of <i>P. spumarius</i> (first adults included)	2016	563	357	439	591
	2017	399	451	290	611
	2018			306	521

#### 10.3.1.2.3 Pre-imaginal stages population dynamics with respect to chronological time

The pre-imaginal data collected in 2017 and 2018 have a higher quality than those of 2016, thanks to a refinement of the sampling technique. Looking at the 2017 and 2018 data, all the stages from first to fifth instars to early adults are clearly distinguishable in all locations, unlike in 2016 where in Apulia the totals for the second-third instars and fourth-fifth instars were recorded together.

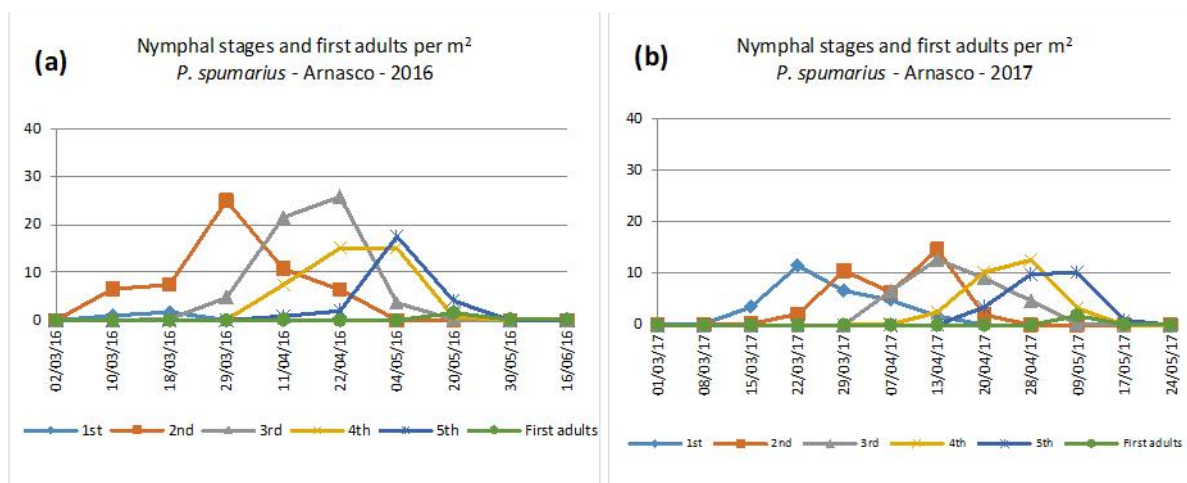


In 2017, in Liguria the first *P. spumarius* nymphs are recorded in early March and the pre-imaginal stages end in late May, while in Apulia the first instar nymphs appear around the end of February/beginning of March and the pre-imaginal stages end in mid-May. In Liguria, in conjunction with the peak of population abundance previously described and recorded in mid-April, there is a simultaneous presence of the second, third and fourth instars, while in Apulia this concurrence is recorded in a minor way around the end of March/beginning of April.

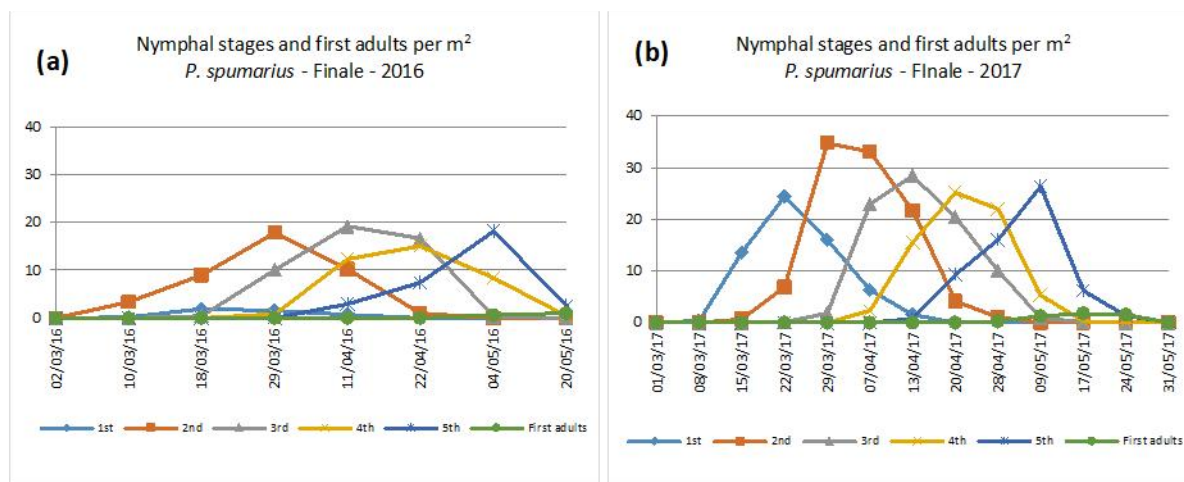
In 2018, the first *P. spumarius* nymphs are recorded in late March/beginning of April in Locorotondo and in early March in Valenzano. The pre-imaginal stages end in early May in both locations in Apulia. In Locorotondo, in conjunction with the peak of population abundance previously described and recorded immediately before mid-April, there is a simultaneous presence of the first, second, third and fourth instars, while in Valenzano there is a simultaneous presence of the second, third and fourth instars in conjunction with the peak of population abundance recorded at the beginning of April.

Considering the abundance of *P. spumarius* stages in Liguria in 2016, in Arnasco no more than 30 individuals/m<sup>2</sup> are reported, with the greatest abundance of second and third instars (Figure 24). The peak of the third instars follows the peak of the second one of about a month, while the third, fourth and fifth instars peaks are closer in time. At Finale, the peaks of the second and third instars appear earlier than in Arnasco and with a maximum of 20 individuals/m<sup>2</sup> (Figure 25).

In 2017 the values collected in Arnasco are lower than in the previous year for all the stages, with a maximum of 15 individuals/m<sup>2</sup>. The situation is different at Finale, where during the second year of observation there is an increase in the abundances of the *P. spumarius*, with a peak of 35 individuals/m<sup>2</sup> for second instar individuals, and the peaks of the other instars slightly under 30 individuals/m<sup>2</sup>. The second and fourth instars appear in Finale earlier than in Arnasco, while the peaks of the other stages appeared at the same time.



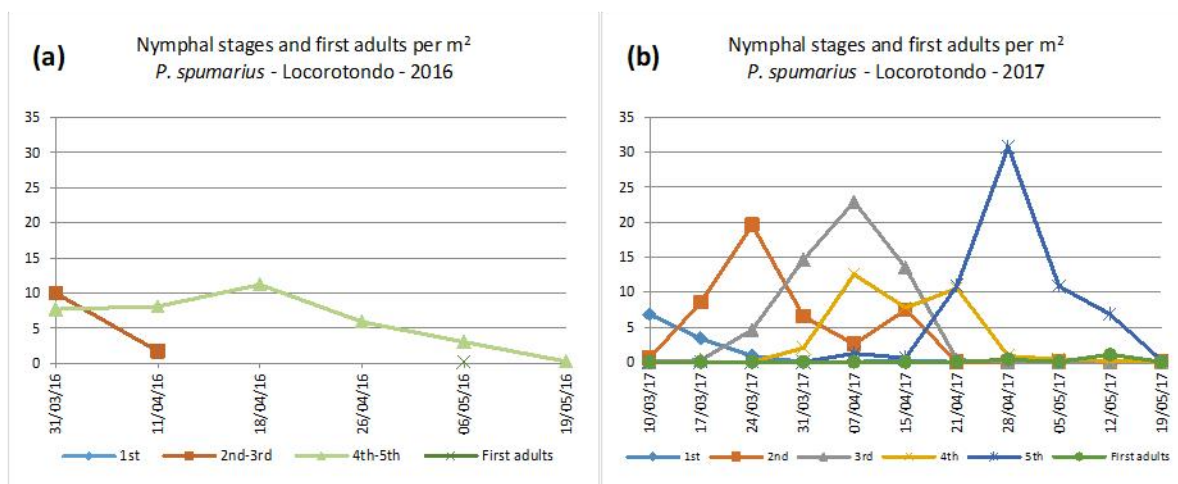
**Figure 24:** Nymphal stages and first adults per m<sup>2</sup> of *P. spumarius* in Arnasco in 2016 (a) and in 2017 (b).



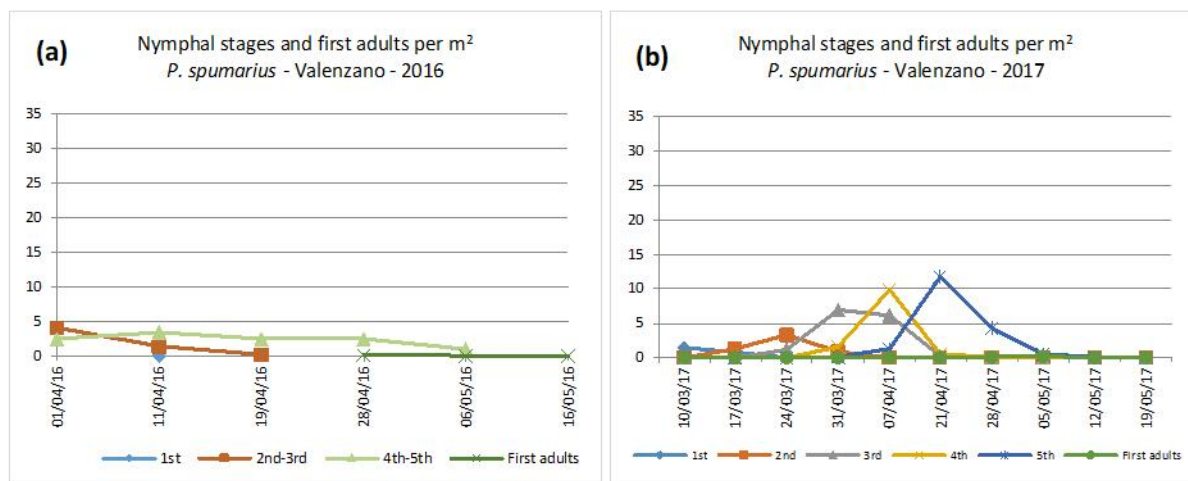
**Figure 25:** Nymphal stages and first adults per m<sup>2</sup> of *P. spumarius* in Finale in 2016 (a) and in 2017 (b).

Considering the abundance of the different stages of *P. spumarius* in Apulia, it is not possible to determine any peak of abundance in 2016 for the two locations of Locorotondo and Valenzano. The registered maximum abundances slightly exceed 10 individuals/m<sup>2</sup> in Locorotondo while in Valenzano are lower than 10 individuals/m<sup>2</sup> (Figure 26 and Figure 27).

In 2017 data on the abundance of *P. spumarius* preimaginal stages register a higher abundance in Locorotondo compared to Valenzano, with a peak of fifth instar individuals that slightly exceeds 30 individuals/m<sup>2</sup>. Second and third instars are around 20 individuals/m<sup>2</sup>, while fourth stage individuals are slightly more than 10 individuals/m<sup>2</sup>. Also, at Valenzano, the fifth stage individuals reach a greater maximum abundance compared to the previous stages. This does not occur in Liguria. In Valenzano the third and fifth stages also reach their maximum earlier than in Locorotondo field.

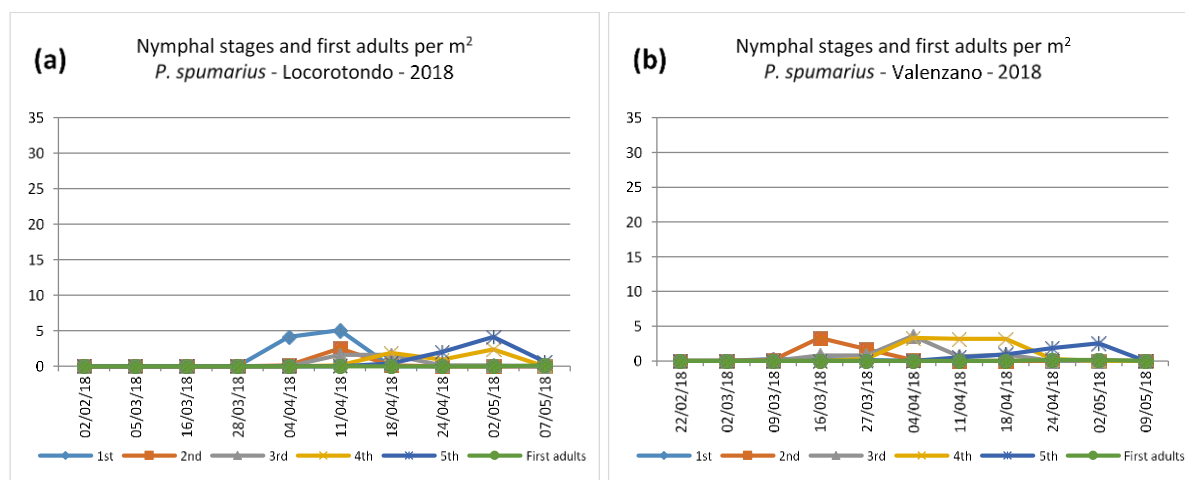


**Figure 26:** Nymphal stages and first adults per m<sup>2</sup> of *P. spumarius* in Locorotondo in 2016 (a) and in 2017 (b).



**Figure 27:** Nymphal stages and first adults per m<sup>2</sup> of *P. spumarius* in Valenzano in 2016 (a) and in 2017 (b).

In 2018, no more than 5 individuals/m<sup>2</sup> of *P. spumarius* are reported in Locorotondo, with first and fifth instars as the most represented stages. The peak of the fourth and fifth instars follows the peak of the first instars of about 20 days, while the third instar immediately follows the first instar stages. In Valenzano, the abundance peaks of the first, second, third and fourth stages are earlier than in Locorotondo with a maximum abundance of 4 individuals/m<sup>2</sup>. The peak of the fourth stage is earlier in Valenzano than in Locorotondo, where fourth and fifth stages have the pick at the same time (Figure 28).



**Figure 28:** Nymphal stages and first adults per m<sup>2</sup> of *P. spumarius* in Locorotondo (a) and in Valenzano (b) in 2018.

#### 10.3.1.2.4 Pre-imaginal stage phenology with respect to physiological time

Considering the two sites in Liguria for the year 2016 and 2017, the development of all the *P. spumarius* stages is generally between 30/60 DD and 390/420 DD. Since in 2016 the beginning of first instar stage has not been recorded, only data of the second year have been reported for this stage. In 2017, the first instar stage is found between 30 and 150/180 DD. Considering both years and sites, in Liguria the second instar stage develops between 60 DD and 210/220 DD, the third instar stage between 90 DD and 280 DD, the fourth instar stage between 100 DD and 360 DD, the fifth instar stage between 120 DD and 390/420 DD. The maximum abundance of the first instar stage is between 60/90 DD, for the second instar stage it varies from 90 to 180 DD, for the third instar stage the maximum is experienced between 150/210 DD, for the fourth instar stage between 210/270 DD and for the fifth stage the peak of abundance is recorded at about 270 DD (table 11).

Considering the study sites in Apulia, the variability between the locations is higher compared to the variability between the Ligurian sites. Taking into account 2016 and 2017 and both sites in Apulia, Locorotondo seems to have a development of *P. spumarius* pre-imaginal stages concentrated between 30 DD and 300/450 DD while in Valenzano this development takes place between 120 and 510/530 DD. It should be noted, however, that the first instar stage could not be well represented due to the lack of data at the beginning of the development of this stage in 2016. The values of early development reported, and exclusively based on 2017 data, can therefore vary. Moreover, since data in 2016 have been aggregated for second-third and fourth-fifth instars and due to difference in DD in the two locations, results have been presented separately. Considering the 2016 data in Apulia, the development of the second-third instars ends at 227 DD in Locorotondo and at 403 DD at Valenzano, that of fourth-fifth instars ends around 440 DD in Locorotondo and around 500 DD in Valenzano. In 2017, in the field of Locorotondo, the second instar stage presents a development between 30 and 140 DD, the third stage between 40 and 145 DD, the fourth stage between 90 and 200 DD while the fifth stage between 100 and 300 DD. At Valenzano in 2017 the second stage develops between 140 and 230 DD, the third stage between 190 and 340 DD, the fourth stage between 230 and 390 DD, while the fifth stage between 230 and 530 DD. The maximum abundance of the second instar stage in 2017 is recorded at Locorotondo around 60 DD, the third and fourth instar stages at about 100 DD, the fifth stage around 170 DD, while in Valenzano the maximum abundance of the second instar stage is reached at 190 DD, the third instar stage around 230 DD, the fourth instar stage at about 270 DD and the fifth instar stage at about 350 DD. In 2018, the development of all the *P. spumarius* stages is generally between 97 DD and 306 DD in Locorotondo and between 122 DD and 451 DD in Valenzano. The first instar stage is found between 97 and 122 DD in Locorotondo and between 122 and 179 DD in Valenzano. The second instar stage develops between 97 and 158 DD in Locorotondo and between 122 and 217 DD in Valenzano, the third and fourth instar stages develop between 122 DD and 272 DD in Locorotondo, the third and fourth instar stages develop respectively between 154 and 305 DD and between 179 and 360 DD in Valenzano, the fifth instar stage develops between 158 and 306 DD in Locorotondo and between 256 and 451 DD in Valenzano. The maximum abundance of the first and second instar stages is at 122 DD in Locorotondo and at 154 DD in Valenzano, for the third instar stage the maximum is experienced between 122/158 DD in Locorotondo and at 217 DD in Valenzano, for the fourth and fifth instar stages at 272 DD in Locorotondo and respectively at 217 DD and at 451 DD in Valenzano (table 11).



**Table 11.** Degree days (DD) of main events related to *P. spumarius* pre-imaginal stage phenology in the olive groves. N/A: data not available. In 2018 only the two Apulian sites were monitored.

	Year	Stage	DD			
			Arnasco	Finale	Locorotondo	Valenzano
Beginning of development of the pre-imaginal stages of <i>P. spumarius</i>	2016	1st	N/A	N/A	N/A	N/A
		2nd	64	45	N/A	N/A
		3rd	76	60		
		4th	100	85	N/A	N/A
		5th	159	147		
	2017	1st	41	40	N/A	N/A
		2nd	60	61	36	144
		3rd	141	79	40	191
		4th	141	133	86	227
		5th	203	160	102	227
	2018	1st			97	122
		2nd			97	122
		3rd			122	154
		4th			122	179
		5th			158	256
Peak of abundance of the pre-imaginal stages of <i>P. spumarius</i>	2016	1st	76	60	N/A	N/A
		2nd	159	85	N/A	N/A
		3rd	212	147		
		4th	212	206	N/A	N/A
		5th	264	262		
	2017	1st	81	79	N/A	N/A
		2nd	173	96	65	191
		3rd	173	160	102	227
		4th	228	190	102	267
		5th	271	253	168	344
	2018	1st			122	154
		2nd			122	154
		3rd			122/158	217
		4th			272	217

		5th			272	451
End of development of the pre-imaginal stages of <i>P. spumarius</i>	2016	1st	76	147	N/A	N/A
		2nd	212	206	227	403
		3rd	352	262		
		4th	352	357		
		5th	352	357	440	502
	2017	1st	173	160		
		2nd	203	215		
		3rd	271	310		
		4th	271	310		
		5th	333	451		
	2018	1st			122	179
		2nd			158	217
		3rd			272	305
		4th			272	360
		5th			306	451

#### 10.3.1.2.5 Habitat selection in the adult stage

Adult sampling was carried out on three different habitats: herbaceous, shrubs/trees and olive trees. All the habitats have been investigated in every field except for Locorotondo in 2016 where the shrub habitat is missing because not present in the selected field. In 2017, the field of Locorotondo has been substituted by Locorotondo – Catucci at a distance of about 3 km (section 10.3.1.) *P. spumarius* close to the previous one, allowing the comparison of data between the two locations.

The habitat of the shrubs/trees bushes in Liguria consists mainly of plant species belonging to the families of the Anacardiaceae and Fagaceae, with a prevalence of Anacardiaceae in the field of Arnasco and Fagaceae in the field of Finale. In Apulia, the Fagaceae family totally prevails in Locorotondo in 2017 and in 2018, while in the field of Valenzano there are exclusively Myrtaceae, Anacardiaceae and Oleaceae in 2016 and in 2017 and only Myrtaceae and Anacardiaceae in 2018, as bushes of *Phillyrea angustifolia* were cut in 2017.

Since the sampling methods used in the three habitats are different (see protocols for data collection, section 10.3.1.1), data were analysed with reference to the specific SSU: for the herbaceous habitat individuals are calculated by swept, while for shrubs and olive trees the individuals are counted by single plant.

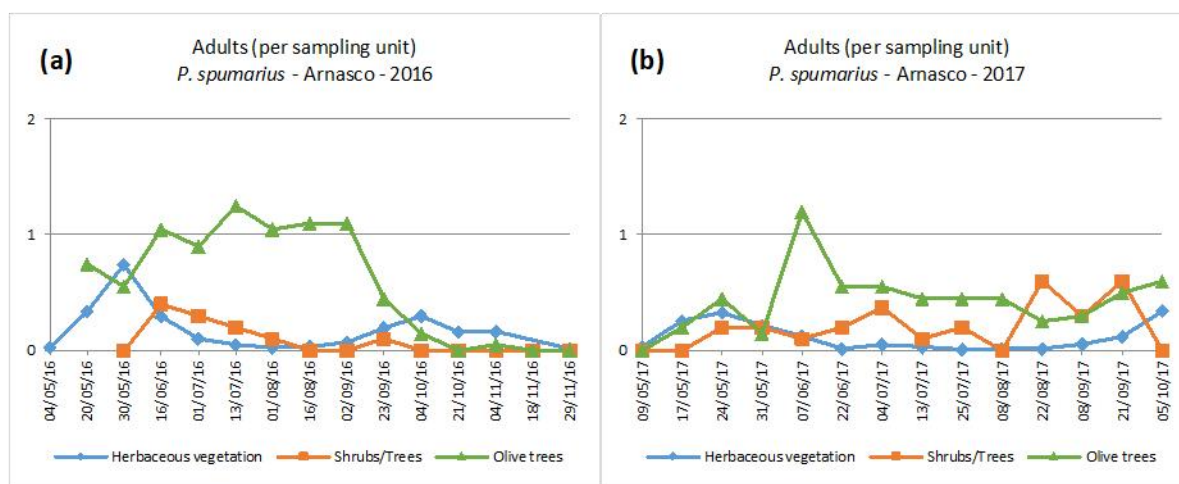
Observing the population dynamics of adults detected in the herbaceous habitat (Figures 29-33), two peaks of abundance can be found, one before and one after summer, as *P. spumarius* migrates from the herbaceous compartment into other compartments (shrub and olive) during the summer. In 2016 and in 2017, this trend is present in both locations in Liguria and in Apulia at Locorotondo (although the second peak is of modest intensity), while in Valenzano there is only one peak of density in late spring. In 2018, the trend is present in both locations in Apulia, although the peaks do not present high values and the second peak is not well defined.

Regarding the olive tree compartments in 2016 and in 2017, the maximum adult density of *P. spumarius* is recorded in summer in Liguria and in late spring/early summer in Apulia. In addition, the number of individuals found tends to drop more abruptly in July/August in Apulia compared to the same period in Liguria. In 2018, there are two peaks of density of *P. spumarius* in Locorotondo in late spring/early summer and in late summer/early autumn, while in Valenzano there is one peak in early summer.

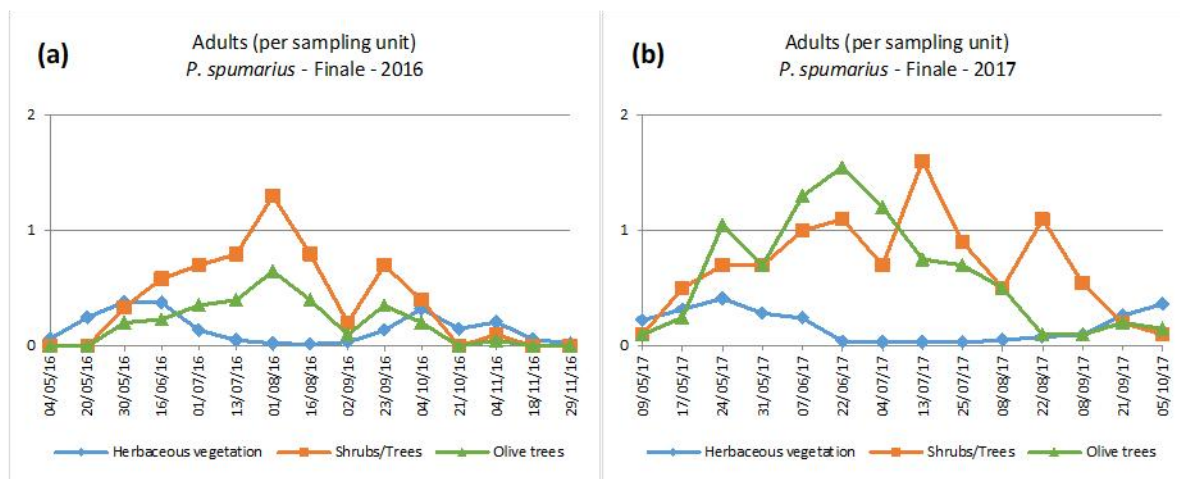
Regarding the alternative woody host compartments, *P. spumarius* adults preferred *Quercus* spp. (*Q. ilex* > *Q. crenata* > *Q. petraea*) and *Pistacia* spp. (*P. lentiscus* > *P. terebinthus*) in the Ligurian sites. In the Apulian sites, the spittlebug adults were more frequently collected on *Myrtus communis* > *P. lentiscus* > *Phillyrea angustifolia* in 2016, while in 2017 and 2018, *P. spumarius* adults preferred *Quercus* spp. > *Myrtus communis* > *Pistacia* spp. (*P. lentiscus*).

Evaluating the number of *P. spumarius* individuals found, Finale is the site with more adults found by swept/plant, while Valenzano results to be the site with the lowest number. The difference is mainly due to the shrubs/trees and the olive tree compartments, the number of *P. spumarius* individuals found in the herbaceous sector is similar in all sampling fields in 2016 and in 2017, but in 2018 the number of *P. spumarius* individuals found in the herbaceous sector in both fields is lower than the number in the two other compartments. At Valenzano, the low nymph population level in 2018 may be due to a winter manuring that involved soil tilling.

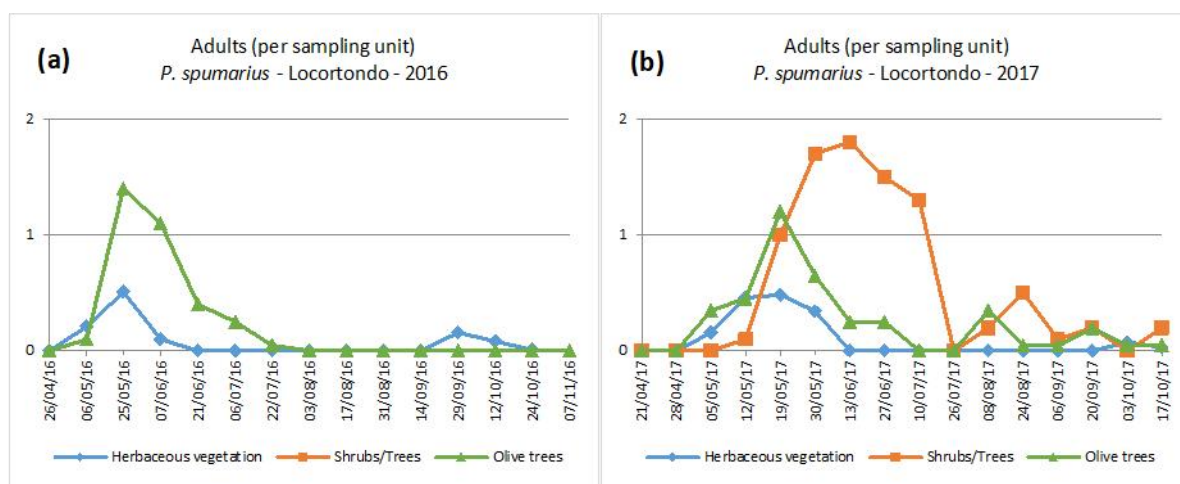
Taking into account degree-days in 2016 and in 2017, the peak of adults in spring generally occurs for all sites and years between 200 and 800 DD while in autumn the second peak in the herbaceous habitat is recorded between 1800 and 2200 DD, except in the fields in Valenzano, where the second peak in autumn is not present. In 2018, the peak of adults in spring generally occurs between 270 and 370 DD in Locorotondo and between 360 and 610 DD in Valenzano while in autumn the second peak in the herbaceous habitat is recorded between 1620 and 2200 DD in Locorotondo and between 2600 and 2890 DD in Valenzano.



**Figure 29:** Adults per swept (herbaceous vegetation) or per plant (shrubs/trees and olive trees) of *P. spumarius* in Arnasco in 2016 (a) and in 2017 (b).

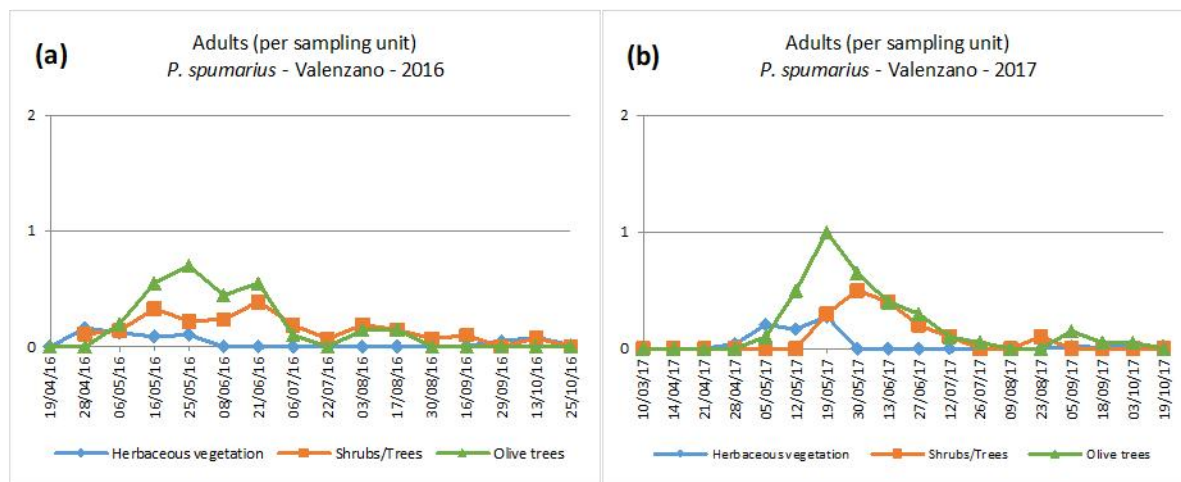


**Figure 30:** Adults per swept (herbaceous vegetation) or per plant (shrubs/trees and olive trees) of *P. spumarius* in Finale in 2016 (a) and in 2017 (b).

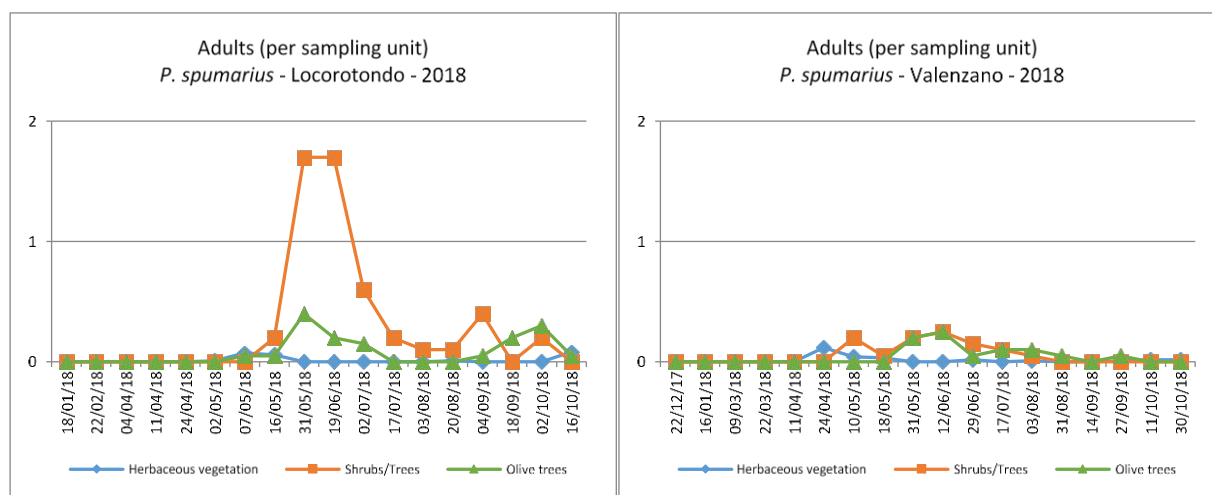


**Figure 31:** Adults per swept (herbaceous vegetation) or per plant (shrubs/trees and olive trees) of *P. spumarius* in Locorotondo in 2016 (a) and in 2017 (b).





**Figure 32:** Adults per swept (herbaceous vegetation) or per plant (shrubs/trees and olive trees) of *P. spumarius* in Valenzano in 2016 (a) and in 2017 (b).



**Figure 33:** Adults per swept (herbaceous vegetation) or per plant (shrubs/trees and olive trees) of *P. spumarius* in Locorotondo (a) and in Valenzano (b) in 2018.

Concerning other potential *X. fastidiosa* vectors identified in Liguria and Apulia, adults of *Aphrophora alni* and *Neophilaenus campestris* were detected in both fields in Liguria in 2016 and in 2017, together with some individuals of the families of the Cicadellidae and Issidae. In all years, in Apulia, adults of *Neophilaenus* were detected in both locations, together with individuals of the families of the Cicadellidae and Issidae.

### 10.3.2. Mesocosm

Data collection has been initially carried out in two different screen houses made of insect-proof net settled in Apulia (Bari) and Piedmont (Turin). However, the mesocosm settled in Bari reported technical issues due to planting of herbaceous species in the first year and subsequent delay with the *P. spumarius* inoculum that resulted to be fatal for oviposition and subsequent development of pre-imaginal stages in the second year. Therefore, only results of the mesocosm settled in Turin are presented. Mesocosm surveys have been performed from June 2016 to June 2018. Three different cycles have been considered within this period: from June to November 2016, from February to August 2017 and from March to May 2018. Population dynamics and phenology in both the herbaceous and olive tree compartments are investigated for *P. spumarius*.

The counting of individuals in the mesocosm includes only adults in 2016, pre-imaginal stages and adults in 2017 and only pre-imaginal stages in 2018 since the adult population has been decimated by entomopathogenic fungi. Isolations of fungi from dead insects allowed to culture four fungal species, of which two were identified as *Beauveria bassiana* and *Fusarium oxysporum*, well known as insect pathogens. The identification of the other two is still ongoing. Herbaceous vegetation has been investigated for adults and pre-imaginal stages and olive trees for adults only.

The sampling technique applied in the mesocosm is described at section 10.2.2.4.

Data are shown based on the sampling date which represents the chronological time, while for the calculation of the physiological time, mesocosm hourly temperatures from data loggers or closest weather station have been used to calculate degree-days (DD) with a low threshold temperature of 10°C.

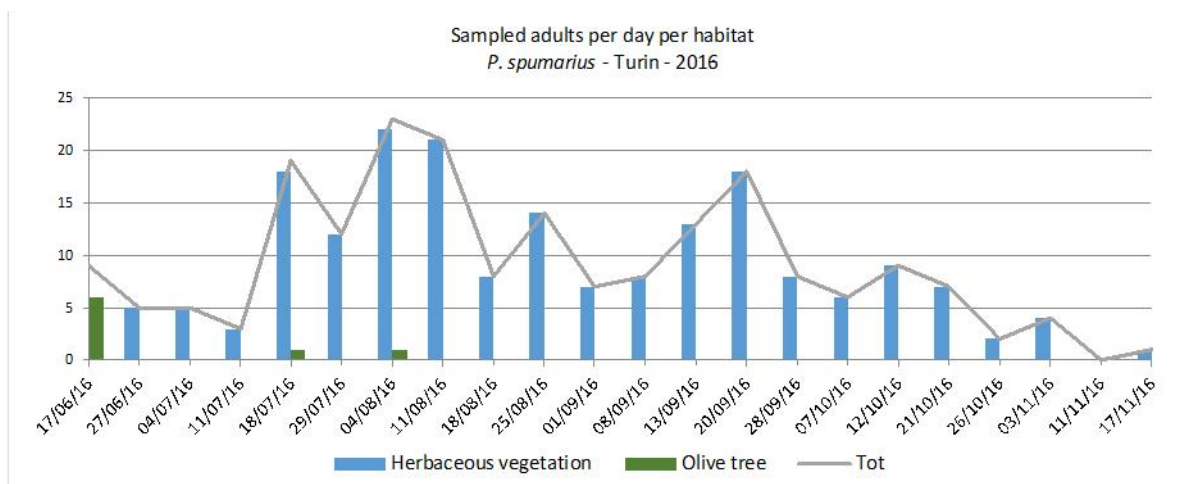
#### 10.3.2.1. Population dynamics with respect to chronological time

##### 10.3.2.1.1 Adults in 2016

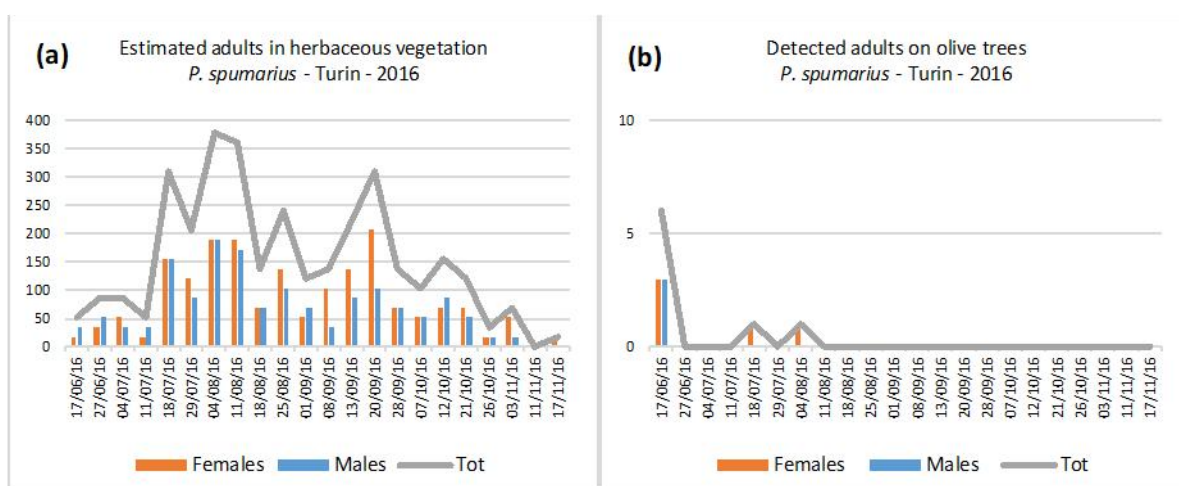
Adults have been introduced in the mesocosm in two distinct moments, 200 insects (sex ratio 1:1) at the beginning of June 2016 (09/06/16) and 200 insects (sex ratio 1:1) in mid-July (14/07/2016) (Figure 34). In 2016, in the whole mesocosm, only few adults have been found on olive trees. This result can be explained by the artificial conditions of the mesocosm: the establishment of a forced environment through a selection of plant species, the higher relative humidity due to the confined environment, and the small size of olive trees (that could not provide enough shelter) might have affected the spittlebug preference for the herbaceous habitat in summer.

In Figure 34 the total number of sampled adults of *P. spumarius* in herbaceous vegetation and the number of adults detected on all trees of the mesocosm are plotted. The highest absolute value of adults sampled in the herbaceous vegetation is 22 individuals/day, recorded at the beginning of August, whereas the highest value of adults found on olive trees is 6 individuals/day, recorded in mid-June in 2016.

In Figure 35 the estimated values of adult males and females in the entire mesocosm are shown, inferring the sampled values to the universe. For olive trees habitat, considering that the visual analysis was performed on all the trees, the total of males and females corresponds to the number of adults shown in the Figure 34. The estimated population of the entire mesocosm does not exceed 400 individuals which is the total amount of the inoculum of *P. spumarius*. The number of adult females is higher than the number of adult males and they are generally balanced to each other.



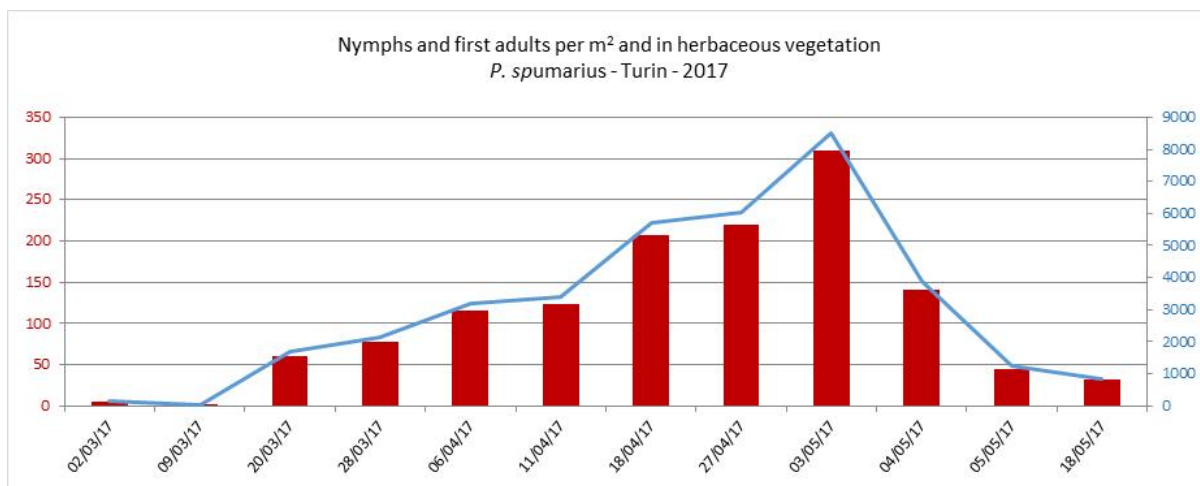
**Figure 34:** Adults of *P. spumarius* sampled in the herbaceous vegetation and counted on olive trees in the mesocosm of Turin in 2016.



**Figure 35:** Number of adults (females and males) of *P. spumarius* in the mesocosm of Turin in 2016: (a) estimated number of adults in the entire herbaceous vegetation and (b) detected individuals on olive trees.

#### 10.3.2.1.2 Nymphal stages and first adults in 2017

The appearance of nymphal stages and early adults from the adult population of the first year has been studied in 2017 (Figure 36). Counting started at the beginning of March, coinciding with counting of the larval stages in the field. A particularly intensive sampling has been carried out from 3rd to 5th May 2017, to examine a larger portion of the herbaceous cover, providing a maximum of 40 surveys for a total of 6.75 m<sup>2</sup> sampled compared to the 2.5 m<sup>2</sup> commonly sampled. Early May has been selected for the prediction of sampling a larger number of nymphal stages of *P. spumarius*. The estimated number of nymphal stages in the mesocosm has then been calculated, making the proportion between the sampled surface and the total surface of the mesocosm. The highest estimation of larval stages is 8,504 individuals on 03/05/2017.

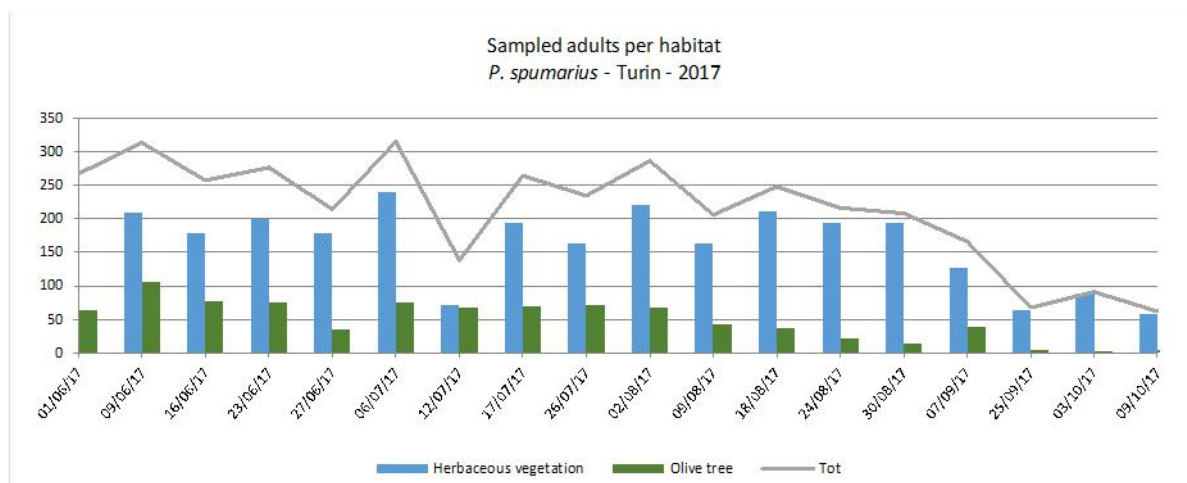


**Figure 36:** Number of nymphs and first adults of *P. spumarius* per m<sup>2</sup> (red bar) or estimated in the entire mesocosm (blue line) of Turin in 2017.

#### 10.3.2.1.3. Adults in 2017

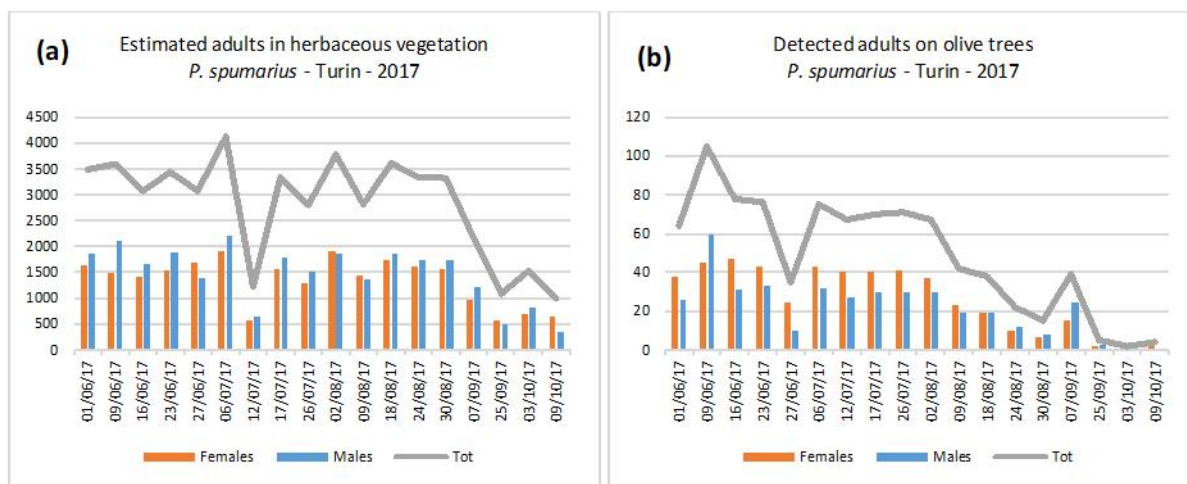
Compared to 2016, *P. spumarius* adult population in 2017 has reported a higher number of individuals on olive trees, however the herbaceous vegetation remains the main compartment occupied (Figure 37). Adult sampling in the mesocosm begins in June 2017. The highest number of adults sampled is between June and early August, decreasing from August to October. The highest value of adults sampled in the herbaceous vegetation is 240, recorded at the beginning of July, whereas the highest value of adults on olive trees is 105, recorded at the beginning of June.

Data at the level of the entire mesocosm are reported in Figure 38. The estimated population of adults of *P. spumarius* does not exceed 4,150 individuals.



**Figure 37:** Adults of *P. spumarius* sampled in the herbaceous vegetation and counted on olive trees in the mesocosm of Turin in 2017.

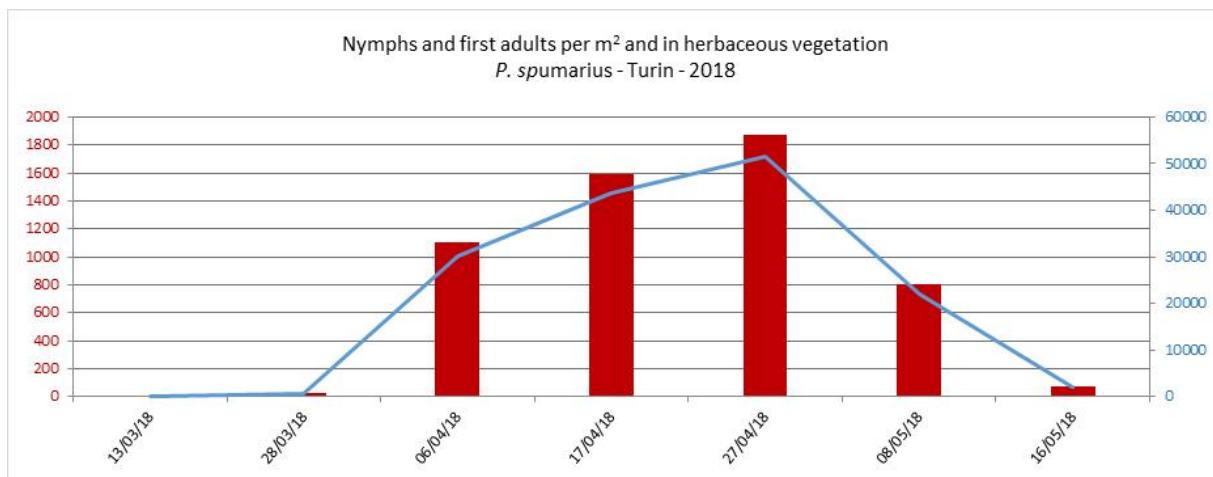




**Figure 38:** Number of adults (females and males) of *P. spumarius* in the mesocosm of Turin in 2017: (a) estimated number of adults in the entire herbaceous vegetation and (b) counted individuals on olive trees.

#### 10.3.2.1.4. Nymphal stages and first adults in 2018

The appearance of nymphal stages and early adults from the adult population of 2017 has been studied in 2018 (Figure 39). Counting started at the beginning of March, as in 2017, and coinciding with counting of the larval stages in the field. A particularly intensive sampling has been carried out on 8th May 2018, to examine a larger portion of the herbaceous cover, providing a maximum of 30 surveys for a total of 7.5 m<sup>2</sup> sampled. Early May has been selected for the prediction of sampling a larger number of nymphal stages of *P. spumarius*. The estimated number of nymphal stages in the mesocosm has then been calculated, making the proportion between the sampled surface and the total surface of the mesocosm. The highest value of estimated nymphal stages is equal to 51,553 individuals, on 27/04/2018.

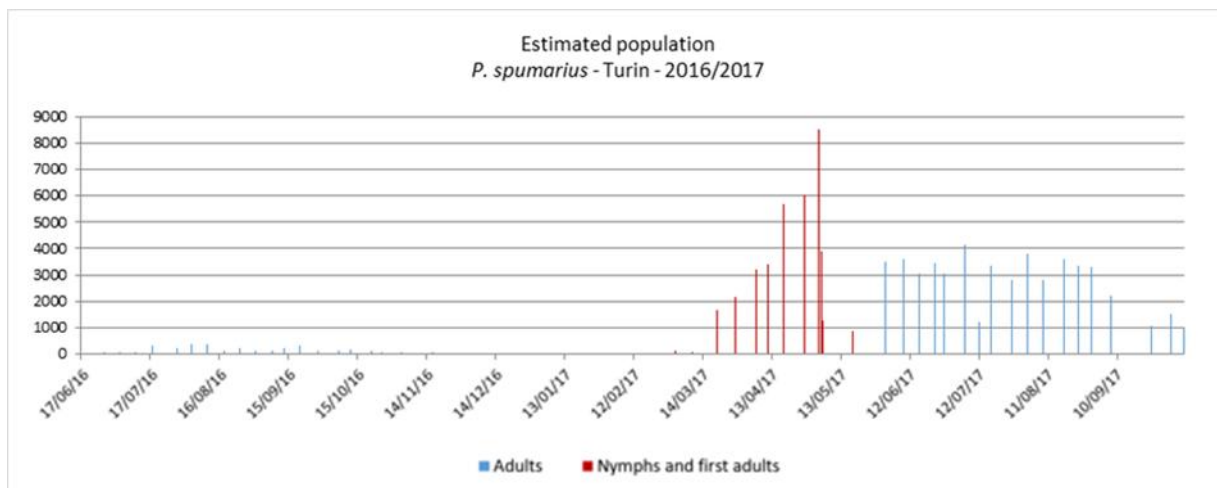


**Figure 39:** Number of nymphs and first adults of *P. spumarius* per m<sup>2</sup> (red bar) or estimated in the entire mesocosm (blue line) of Turin in 2018.

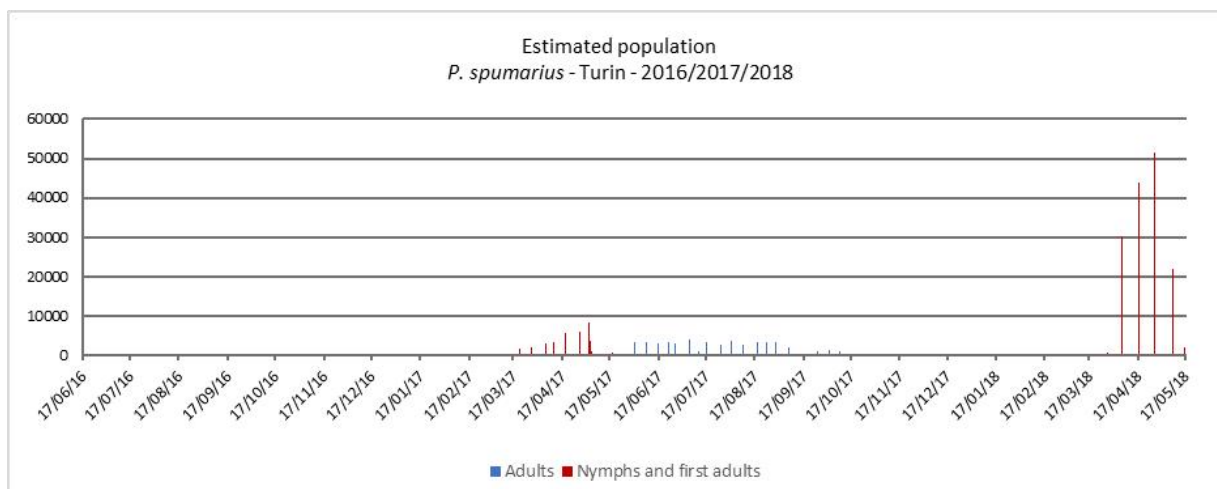
A general overview of *P. spumarius* population in 2016 and 2017 is presented in Figure 40, while a general overview of the two years together with 2018 is presented in Figure 41. Adult population has



been reported in blue, while pre-imaginal population in red. In the first year the population of adults is apparently affected by the inoculum, also reporting a certain stress due to the adaptation that has been faced passing from a natural environment to the mesocosm. In 2017 the population seems to explode, with a large number of nymphs and early adults that then decreases when adults properly start developing at the beginning of June. In 2018 the estimated population of nymphs and first adults reaches even higher values compared to 2017, decreasing around mid-May. Unfortunately, in 2018, the population of adults has been decimated by a fungus as previously described, therefore it was not possible to make a comparison of *P. spumarius* adults with previous years.



**Figure 40:** Estimated adults (blue bar) and nymphs and first adults (red bar) population of *P. spumarius* in the mesocosm of Turin from June 2016 to October 2017.



**Figure 41:** Estimated adults (blue bar) and nymphs and first adults (red bar) population of *P. spumarius* in the mesocosm of Turin from June 2016 to May 2018.

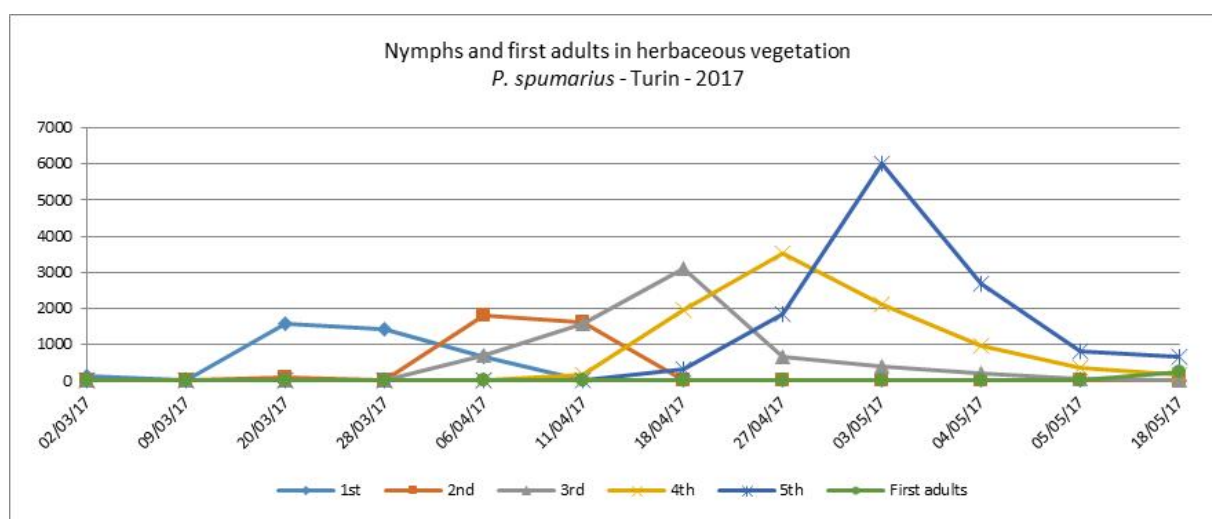
### 10.3.2.2. Total population dynamics with respect to physiological time

Considering physiological time, the population of adults in 2016 reports higher abundances between 900 and 1400 DD, whereas in 2017, higher abundances have been recorded between 540 and 1500 DD. In 2017, pre-imaginal population presents a gradual increase until reaching the maximum peak of abundance around 250 DD and then decreasing. In 2018, the maximum peak of pre-imaginal population abundance is recorded at 178 DD.

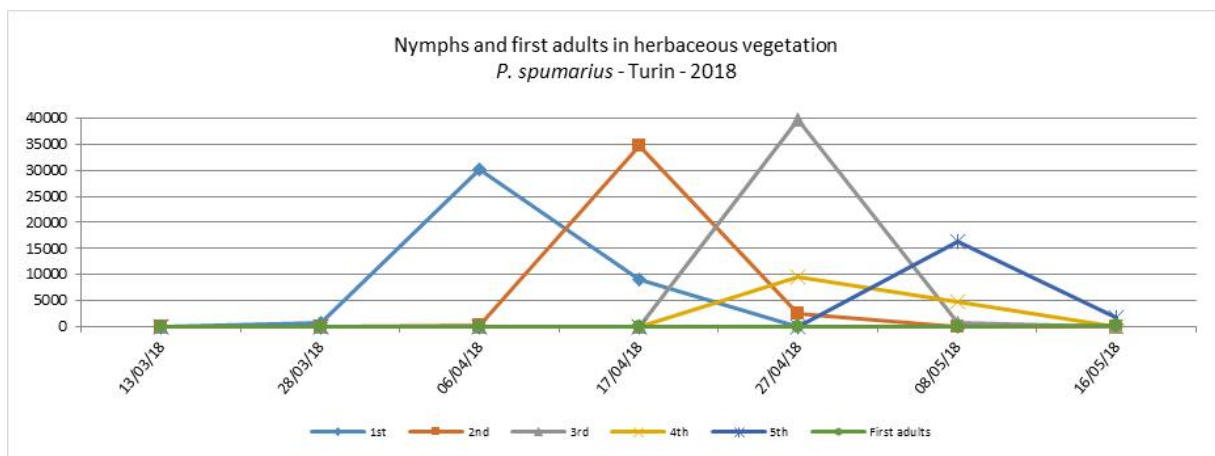
Unlike open field, the population of adults in herbaceous vegetation in the mesocosm does not present distinct peaks of abundance and the seasonality of *P. spumarius* adults is not represented. Pre-imaginal stages instead follow a trend similar to that found in the open field, presenting a distinct peak in abundance.

### 10.3.2.3. Pre-imaginal stage population dynamics with respect to chronological time

In the mesocosm all the pre-imaginal stages of *P. spumarius* are well represented and they develop from the beginning of March to mid-May as reported in Figure 42. The previously observed peak in the total pre-imaginal population recorded on May 3rd presents the third, fourth and fifth instar stages at the same time, with a clear prevalence of individuals belonging to the fifth instar. As for 2017, also in 2018 all the pre-imaginal stages of *P. spumarius* are well represented and they develop from the end of March to mid-May as reported in Figure 43. The previously observed peak in the total pre-imaginal population recorded on 27th April 2018 presents all five instar stages at the same time, with a clear prevalence of individuals belonging to the third instar stage. In the case of nymphs and first adults in the mesocosm, it is interesting to note that the development of the pre-imaginal stages takes place respecting the seasonality and the relative peaks found in open field, unlike what has been observed for adults in mesocosm.



**Figure 42:** Estimated nymphs and first adults of *P. spumarius* in herbaceous vegetation in the mesocosm of Turin in 2017.



**Figure 43:** Estimated nymphs and first adults of *P. spumarius* in herbaceous vegetation in the mesocosm of Turin in 2018.

#### 10.3.2.4. Pre-imaginal stage phenology with respect to physiological time

The development of pre-imaginal stages in the mesocosm in 2017 occurs from 15 DD to 357 DD, following the general trend that has been observed in open field. Considering the single stages, first instars develop between 15 and 152 DD, second instars between 15 and 258 DD, third instars between 121 and 357 DD, fourth instars between 152 and 357 DD, fifth instars between 198 and 357 DD and from 254 DD first adults appears. The peak in which a higher number of first instars is detected is recorded at 61 DD, while for second instars the peak of abundance is at 121 DD, whereas, the highest abundance in third, fourth and fifth instars has been found at 198, 233 and 251 DD respectively (table 12).

The development of pre-imaginal stages in the mesocosm in 2018 occurs from 32 DD to 312 DD. Considering the single stages, first instars develop between 32 and 178 DD, second instars between 52 and 254 DD, third instars between 87 and 254 DD, fourth and fifth instars between 178 and 312 DD and from 312 DD first adults appears. The peak in which a higher number of first instars is detected is recorded at 52 DD, while for second instars the peak of abundance is at 87 DD, whereas, the highest abundance in third and fourth instars has been found at 178 DD and the peak of the fifth instar stage has been found at 254 DD (table 12).

**Table 12.** Degree days (DD) of main events related to *P. spumarius* pre-imaginal stage phenology in the mesocosm.

	Stage	DD	
		Turin 2017	Turin 2018
Beginning of development of the pre-imaginal stages of <i>P. spumarius</i>	1st	15	32
	2nd	15	52
	3rd	121	87
	4th	152	178
	5th	198	178

Peak of abundance of the pre-imaginal stages of <i>P. spumarius</i>	1st	61	52
	2nd	121	87
	3rd	198	178
	4th	233	178
	5th	251	254
End of development of the pre-imaginal stages of <i>P. spumarius</i>	1st	152	178
	2nd	258	254
	3rd	357	254
	4th	357	312
	5th	357	312

The population of adults has been decimated by a fungus, as previously explained, and a decrease in the population in the mesocosm in 2018 is already visible in the fourth and fifth stages as shown in Figure 43.

### 10.3.3. Microcosm

Considering the difficulty of sampling eggs within the mesocosm, an experiment has been set up through the installation of 40 small cages (microcosms), 20 in Turin and 20 in Bari, in September 2016. In 2017, the number of microcosms was increased from 20 to 40 in Turin and 30 in Bari to solve issues encountered in 2016/2017, where in some cases two females have been found in some microcosms during the experiments, affecting fecundity estimate.

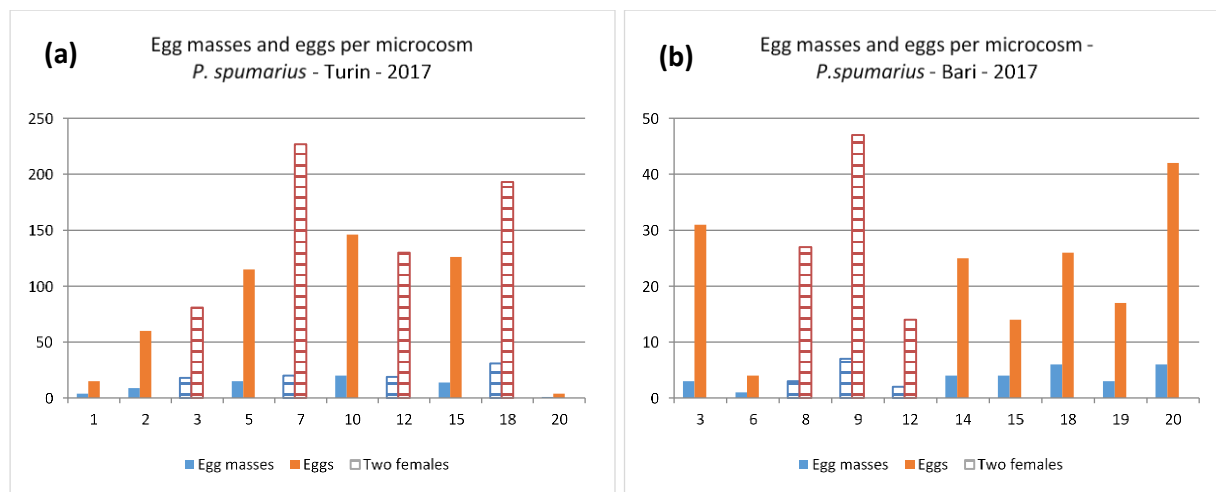
The study of the microcosms in 2016 has been performed in five distinct phases, dividing the microcosms into 2 subsets (A and B) of 10 microcosms randomly selected to avoid a possible disturbance due to the sampling method to all microcosms preventing the development of *P. spumarius* stages. Checks have been carried out in January, March, April and May as shown in Table 13.

**Table 13.** Microcosm experimental setting.

	Month	Stage				
		Eggs	Foam appearance	Second instar appearance	Third-fourth instars appearance	Unhatched eggs
		January	February	March	April	May
Subset		<b>Invasive</b>	<b>Non invasive</b>	<b>Non invasive</b>	<b>Non invasive</b>	<b>Invasive</b>
	<b>A</b>	Eggs counting	2-3 microcosms observed	Total nymphal stages counting	Total nymphal stages counting	Unhatched eggs counting
			<b>Non invasive</b>	<b>Non invasive</b>	<b>Non invasive</b>	<b>Invasive</b>
	<b>B</b>	-	2-3 microcosms observed	Total nymphal stages counting	Total nymphal stages counting	Unhatched eggs counting

Total egg masses and number of eggs per egg mass in Turin and Bari has been reported in Figure 44. Microcosms have been numbered from 1 to 20 and the subset A is made by microcosm 1, 2, 3, 5, 7, 10, 12, 15, 18 and 20 in Turin and by microcosm 3, 6, 8, 9, 12, 14, 15, 18, 19 and 20 in Bari. Over the total number of microcosms considered for egg counting, four microcosms have revealed to host two *P. spumarius* females in Turin and they are microcosm number 3, 7, 12 and 18. Bari has reported three microcosms with two females and they are microcosm number 8, 9 and 12. These microcosms are therefore excluded from the analysis on the fecundity. Although a single female could lay more than 100 eggs (almost 150), the average fecundity was about 90 and 20 eggs in the experiments carried out in Torino and Bari, respectively.

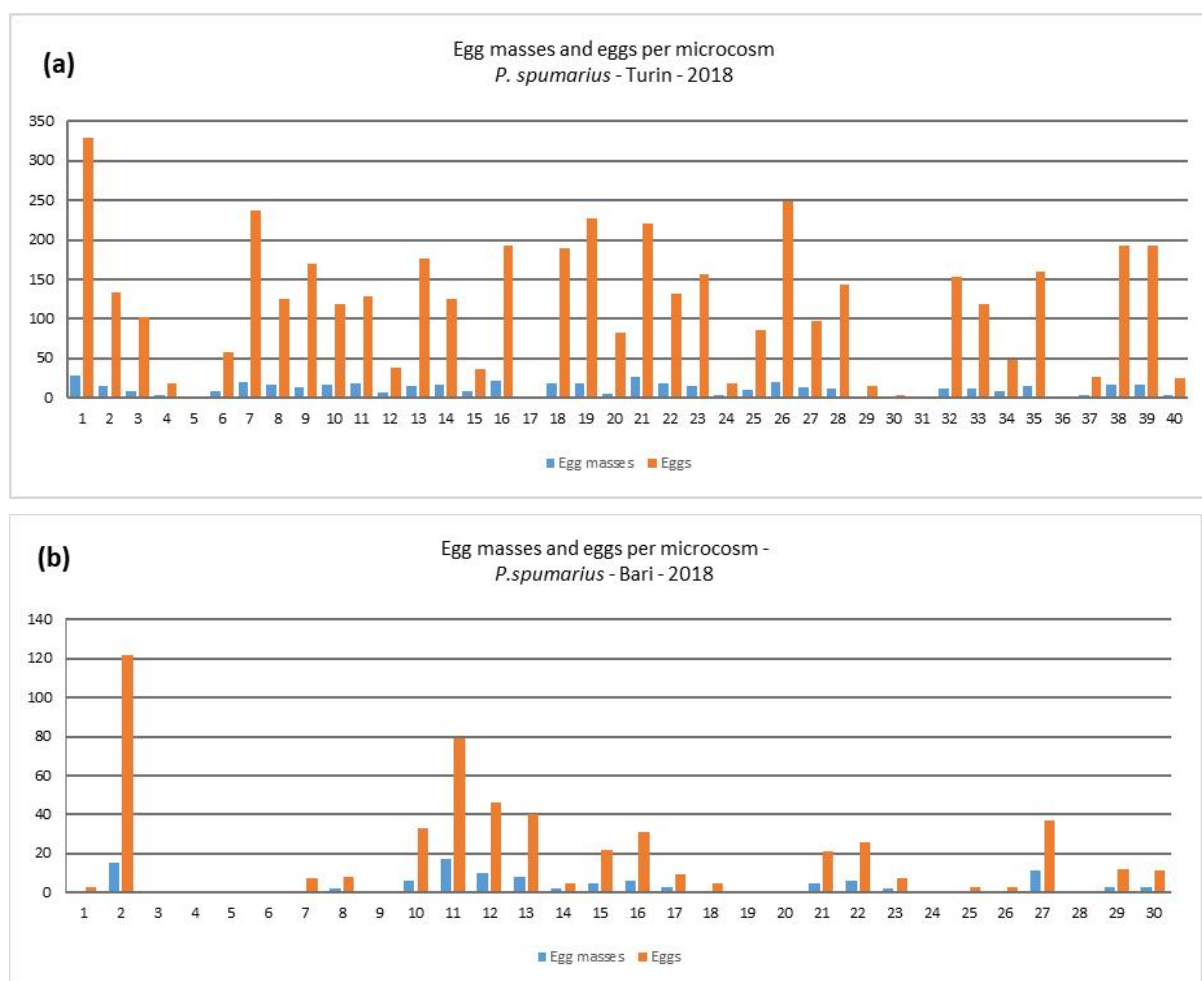




**Figure 44:** Egg masses and eggs per microcosm in Turin (a) and in Bari (b) in 2017. Microcosms where two females have been counted are reported.

The study of the microcosms in 2018 has been performed in five distinct periods: egg counting has been performed in January, and nymphal stages have been studied from February to May in Bari and from March to June in Turin. In 2018, 20 microcosms were randomly selected over a total of 40 in Turin and all 30 microcosms in Bari were sampled in order to investigate a higher number of microcosms compared to 2016/2017.

Total egg masses and number of eggs per egg mass in Turin and Bari has been reported in Figure 45. Microcosms have been numbered from 1 to 40 in Turin and from 1 to 30 in Bari. The total number of microcosms are considered for egg counting. In 2018, all microcosms presented one single female per microcosm. The average fecundity was about 113 and 18 eggs in the experiments carried out in Torino and Bari, respectively.



**Figure 45:** Egg masses and eggs per microcosm in Turin (a) and in Bari (b) in 2018.

## 11. Managing of data on phenological phases (supported also by agronomic and meteorological data) of *Philaenus spumarius* in vineyards in 2016 and 2017 (Activity 5 of the Amendment)

### 11.1. Objectives

Results on the development and ecology of individuals and populations of *P. spumarius* as well as information on the abundance of other potential vector species of *X. fastidiosa* in vineyard are presented.

### 11.2. Materials and methods

Data collection has been carried in a vineyard located in Asti (Piedmont Region) during 2016 and 2017. The main purpose of field observations is to describe the patterns of population phenology and dynamics in a vineyard agroecosystem.

This vineyard has been selected because of the ecological complexity of the site, that includes grapevine rows, an herbaceous cover within and around the rows and broadleaved trees/shrubs surrounding the vineyard, as alternative hosts for the spittlebug adults. The vineyard is 2.2 ha size, is located at about 180 m a.s.l. and is made of six different grapevine varieties, three white (Chardonnay, Cortese and Manzoni bianco) and three red (Albarossa, Barbera and Syrah). In the vineyard, no synthetic insecticides have been applied during the two years of investigations.

#### 11.2.1. Sampling design

Wherever it is not otherwise stated, sampling design is the same as the one described for olive groves (section 10.2.1.1.). Differences are logically that the PSU is the vineyard and that SSUo is substituted by SSUg, corresponding to the grapevine plant (grapevine canopy section).

The sampling variables for SSUp and SSUa have been reported in results respectively as number of individuals per square meter (SSUp) and per single swept (SSUa), whereas sampling variables for SSUs and SSUg have been reported as single SSU (10 sweeps on shrubs/trees and 4 on grapevine).

*P. spumarius* and other potential vectors nymphal stages and first adults have been counted in herbaceous vegetation through 30 secondary sampling units (SSUp) randomly selected within the PSU. *P. spumarius* and other potential vectors adult stage has been investigated in three habitats or compartments: herbaceous vegetation (SSUa), shrubs, trees and other spontaneous woody plants (SSUs) and grapevine plants (SSUg). In herbaceous vegetation, 30 secondary sampling units (SSUa) have been randomly selected inside and 30 outside the vineyard. Individuals have been reported as total number per SSUa, however, the number of adults per single sweeping net swept has been estimated and used to represent results. In the shrub/tree compartment, 30 shrubs/trees have been randomly selected within the PSU. Individuals have been recorded as total number per SSUs and reported as such in the results. In the grapevine plant compartment, 30 sections of grapevine canopy have been randomly selected within the PSU. Individuals have been recorded as total number per SSUg and reported as such in the results.

Sampling surveys have been carried out every 7-10 days for the nymphs and 10/15 days for the adults, from beginning of March until beginning of December in both years.

During sampling surveys, weather data (air temperature and relative humidity) have been recorded from the data logger located in the field. Weather data have been also gathered from the closest weather station and have replaced data in case the data logger has been found out of order or with missing data.

Other events or characteristics that may have been significant for population dynamics and vector biology such as vegetation cutting, treatments and anything else that could have affected insect populations of interest have been noted.

Ten sweeps per selected shrub/tree have been made around the crown of each SSUs. Adults have been counted, sexed, identified and immediately released (conservative sampling). All the examined spontaneous woody plants have been classified in terms of genus/species. Since sampling is partially destructive, SSUs is always different within and between sampling dates. The counting of adults has been made through one sweeping net (38 cm in diameter) built on a 2 m handle that has been swept ten times around the shrub/tree crown.

Four sweeps have been made along 1.5-2.0 m grapevine canopy section for each SSUg. Adults have been counted, sexed, identified and immediately released (conservative sampling).

### 11.3. Results and deliverables of Activity 5 of the Amendment

#### 11.3.1. Macrocosm in Piedmont

The vineyard in Asti has been divided into four headlands (A, B, C and D) and two inter-row areas, left (Sx) and right (Dx) as illustrated in Figure 46.



**Figure 46:** Vineyard in Asti divided into headlands and inter-row areas.

##### 11.3.1.1. Host-plant preference in Piedmont

The sampling of pre-imaginal stages of *P. spumarius* and other potential vectors of *X. fastidiosa* allowed to detect the host-plant preference for specific plant families for instar nymphs. Other potential vectors of *X. fastidiosa* that have been detected in open field are members of the genera *Aphrophora* and *Neophilaenus*. The survey has originally taken into account the plant genus on which insects have been recorded. Afterwards, plant family has been chosen to better represent the overall preference. Data have been analysed at the field level combining data of both headlands and inter-row areas in order to obtain an overall view of plant preference.

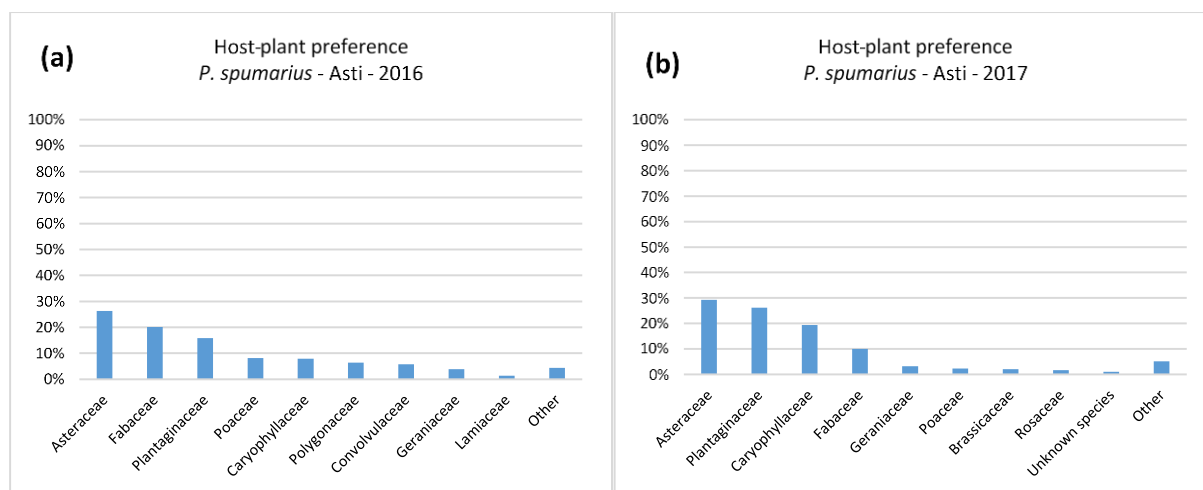
Host-plant preference is represented by the percentage of *P. spumarius* or other potential vectors found on a specific plant family over the total of vectors found. Host-plant preference is reported for all pre-imaginal stages and also for the first adults detected in the field after the fifth instar that have been found with the same sampling method. It should be noted that in 2016 the beginning of first instars appearance in vineyard has been difficult to precisely identify, therefore the sampling reports low values concerning the first instar stage.

The number of plant families is slightly different in the two years: for *P. spumarius*, 18 families were recorded in 2016 and 20 in 2017; for *Neophilaenus*, 10 families in 2016 and only 3 in 2017; for *Aphrophora*, 6 families in 2016 and 4 in 2017. For each year and vector, also the most preferred genera have been reported in order to indicate what host-plants genera are mostly chosen.

In all the graphs of this section, only plant families with the highest percentage of host-plant preference are reported. Plant families with less than 1% of host-plant preference, unknown species, dry grass or cases in which the insect has not been found on a plant are grouped in the category “other”.

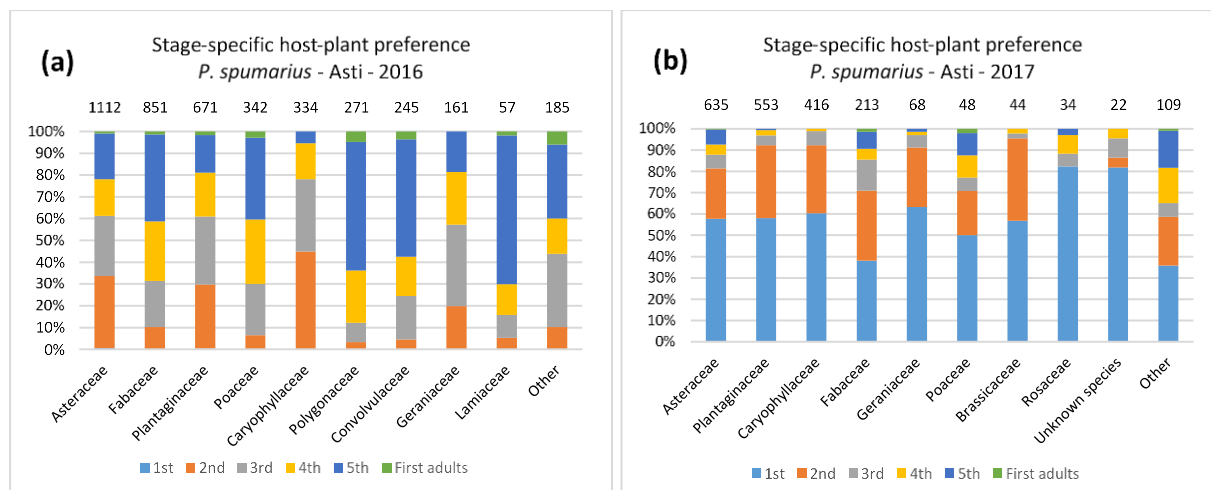
#### 11.3.1.1.1 *Philaenus spumarius* host-plant preference in Piedmont

The predominant plant families hosting *P. spumarius* are Asteraceae and Fabaceae in 2016, and Asteraceae and Plantaginaceae in 2017. Jointly, the Asteraceae and Fabaceae families represent 46.4% of the total plants preferred by *P. spumarius* in 2016 and the Asteraceae and Plantaginaceae the 55.5% *P. spumarius* in 2017. The Asteraceae family predominates in both 2016 and 2017 with a host-plant preference of 26.3% and 29.2% respectively (Figure 47). In particular, the following genera have been found to be the most preferred by *P. spumarius* in 2016: *Plantago* > *Trifolium* > *Cerastium* > *Medicago* > *Rumex*. In 2017 the most preferred genera have been *Plantago* > *Cerastium* > *Taraxacum* > *Trifolium* > *Veronica*. Regarding the host-plant preference of each stage of development there is not a clear preference of a single stage for a specific family (Figure 48). The limited number of families on which first instar stage nymphs have been found in 2016 could be due to observation issues at the beginning of the survey. Therefore, the complete development of *P. spumarius* can generally occur on plants of all the families that have been identified in the vineyard in Asti. Total absolute values of individuals per host-plant family have been reported (Figure 48).



**Figure 47:** Host-plant preference of *P. spumarius* in Piedmont in 2016 (a) and in 2017 (b).

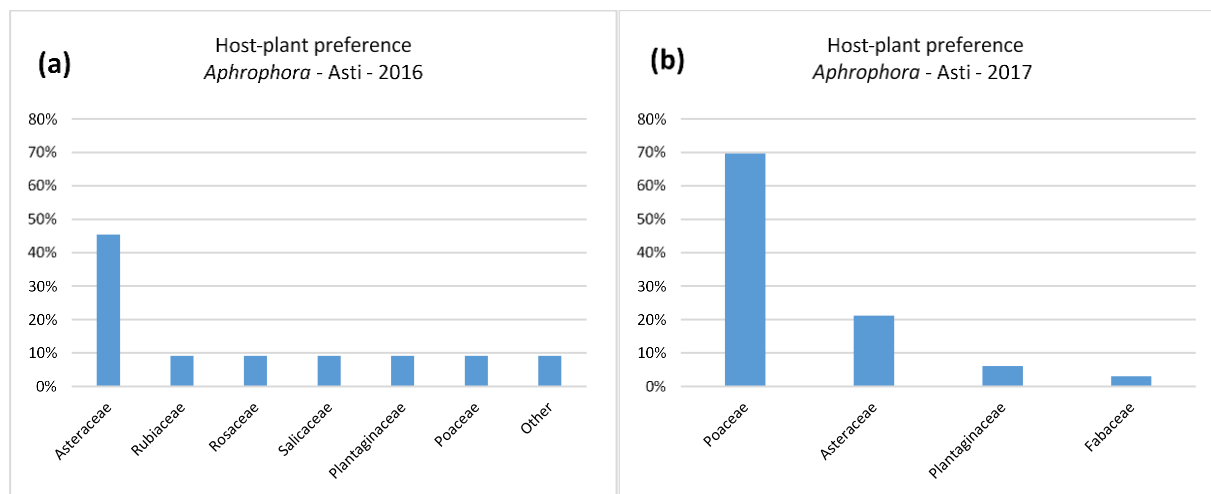




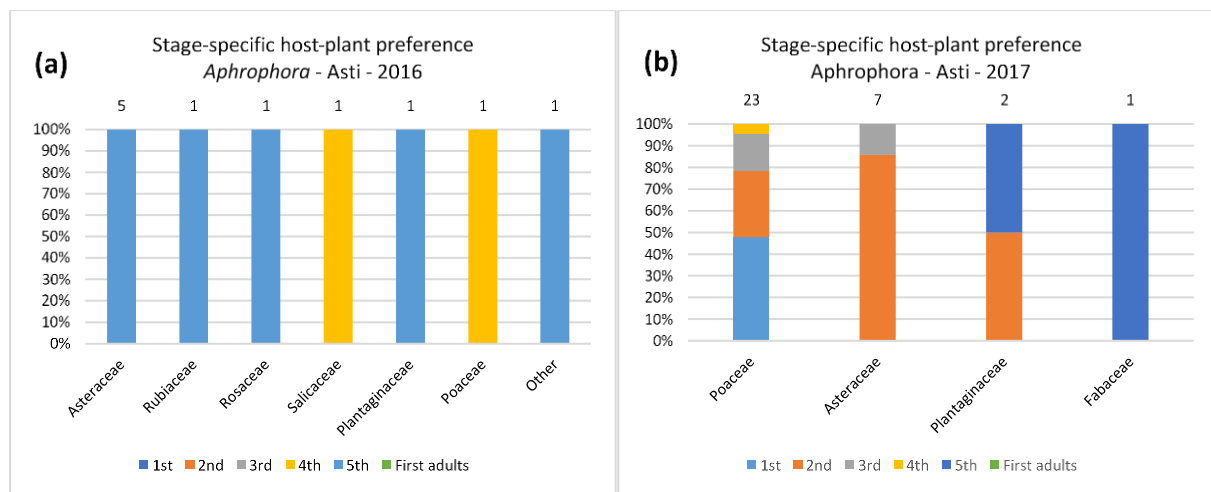
**Figure 48:** Stage-specific host-plant preference of *P. spumarius* in Piedmont in 2016 (a) and in 2017 (b).

#### 11.3.1.1.2 Host-plant preference of other potential vectors of *X. fastidiosa* in Piedmont

Considering the host-plant preferences of other potential vectors in Asti, only insects belonging to the genera *Aphrophora* and *Neophilaenus* have been found in 2016 and 2017. For the genus *Aphrophora* (Figure 49) the host-plant preference falls mainly within the Asteraceae family in 2016 (45.5%) and the Poaceae family in 2017 (69.7%). In particular, the following genera have been found to be the most preferred by *Aphrophora* in 2016: *Picris* > *Taraxacum*, while in 2017 there is not a distinction of genera within the Poaceae family for which data have been recorded as such. Concerning the stage-specific host-plant preference (Figure 50), some plant families only present specific pre-imaginal stages, but total absolute values of individuals per plant family is too low to conclude that nymphs have preferences for specific families.

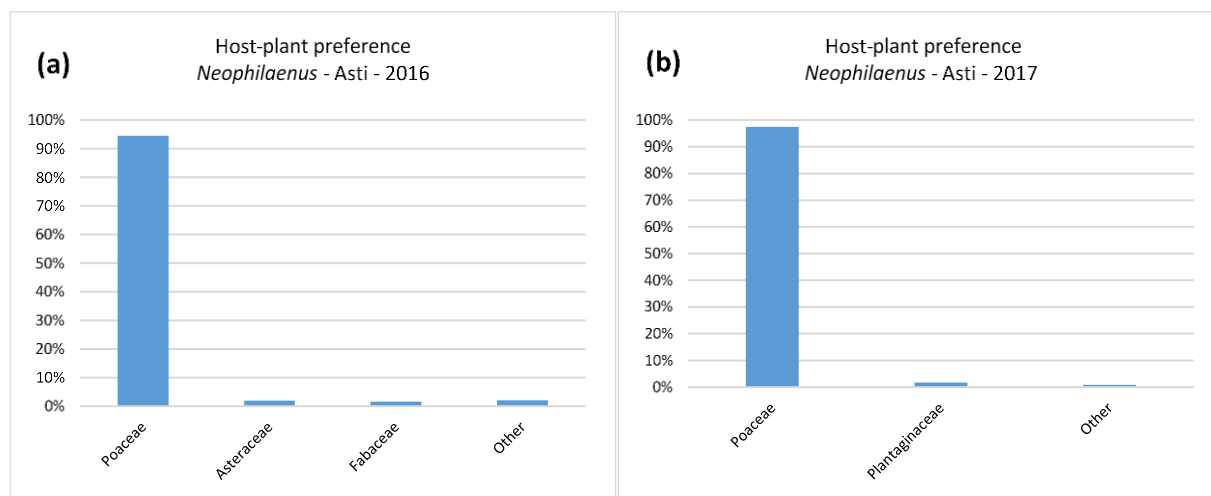


**Figure 49:** Host-plant preference of *Aphrophora* in Piedmont in 2016 (a) and in 2017 (b).

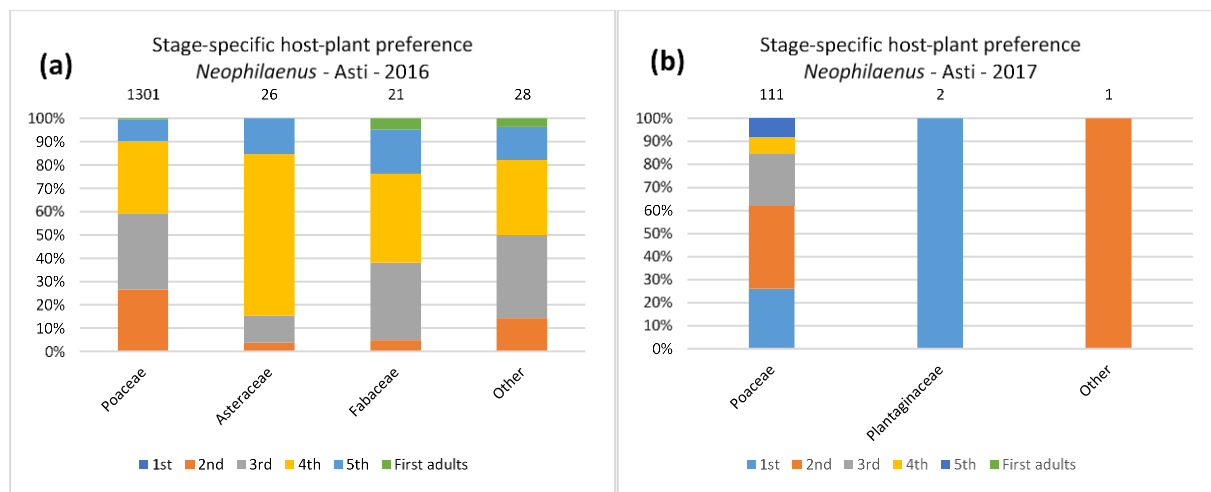


**Figure 50:** Stage-specific host-plant preference of *Aphrophora* in Piedmont in 2016 (a) and in 2017 (b).

Genus *Neophilaenus* presents a clear preference for the Poaceae family with a host-preference of 94.5% in 2016 and 97.4% in 2017 (Figure 51). In particular, the following genera have been found to be the most preferred by *Neophilaenus* in 2016: Poa > Bromus, while in 2017 there is not a distinction of genera within the Poaceae family for which data have been recorded as such. Concerning the stage-specific host-plant preference (Figure 52), there is not a clear preference of a single stage for a specific family, apart in 2017, but as in the case of *Aphrophora*, total absolute values of individuals per plant family is too low to conclude that nymphs have preferences for specific families.



**Figure 51:** Host-plant preference of *Neophilaenus* in Piedmont in 2016 (a) and in 2017 (b).



**Figure 52:** Stage-specific host-plant preference of *Neophilaenus* in Piedmont in 2016 (a) and in 2017 (b).

### 11.3.1.2. Population dynamics and phenology in Piedmont

The study of population dynamics and phenology, with respect to chronological and physiological time, has mainly focused on *P. spumarius*, which is the proven vector of the CoDiRO strain of *X. fastidiosa*. However, the population dynamics for nymphs and first adults with respect to chronological time has been reported also for other potential vectors in order to present some general patterns of the population ecology of other potential vectors in vineyard.

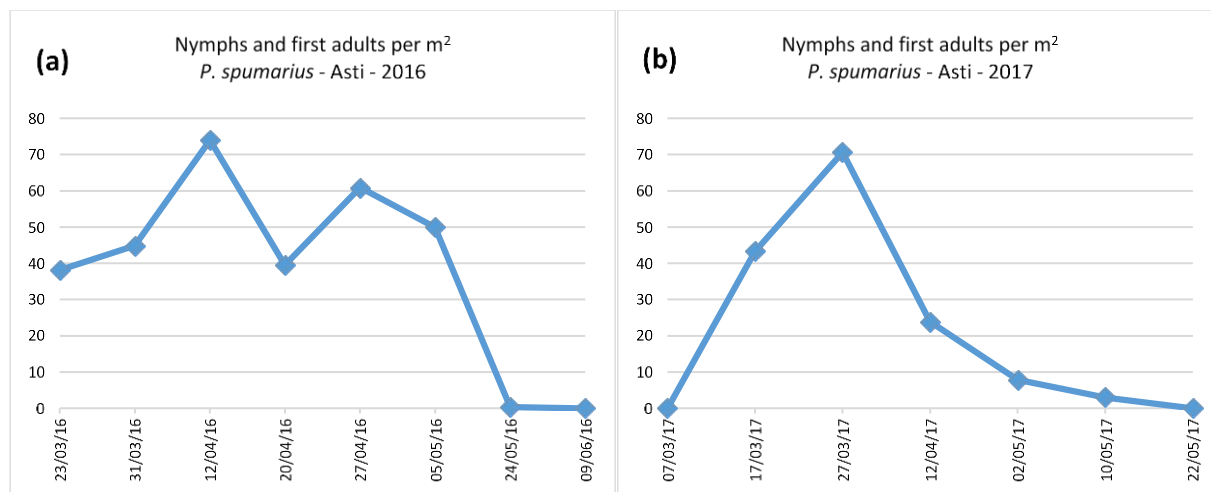
To improve the interpretability of the results, data are reported per area unit ( $\text{m}^2$ ), calculated based on the field sampling units of  $0.25 \text{ m}^2$ . Due to lack of data recording in the first months of 2016, it was necessary to integrate some missing hourly data with those of the nearest weather station. To calculate the physiological time, degree-days (DD) were used, based on hourly temperatures with a low threshold temperature of  $10^\circ\text{C}$ .

#### 11.3.1.2.1 Population dynamics with respect to chronological time

##### *Philaenus spumarius*

In 2016, the field sampling in Asti started too late to adequately detect the first stages of *P. spumarius*. This gap has affected the number of first instars recorded in Asti in 2016. Therefore, in 2017, the sampling procedure was anticipated in early March, thus succeeding in detecting the first stages of development of *P. spumarius*.

Regarding the abundance, in 2016 the peak of nymphs and first adults sampled is equal to 74 individuals/ $\text{m}^2$ , with an average of nymphs and first adults close to 39 individuals/ $\text{m}^2$ . The abundance reported in 2017 is slightly lower compared to 2016, with a peak of nymphs and first adults sampled equal to 71 individuals/ $\text{m}^2$  and a mean number of individuals/ $\text{m}^2$  equal to 21 individuals/ $\text{m}^2$ . In 2016, the peak of the population density appears to be immediately before mid-April and at the end of March in 2017 (Figure 53).

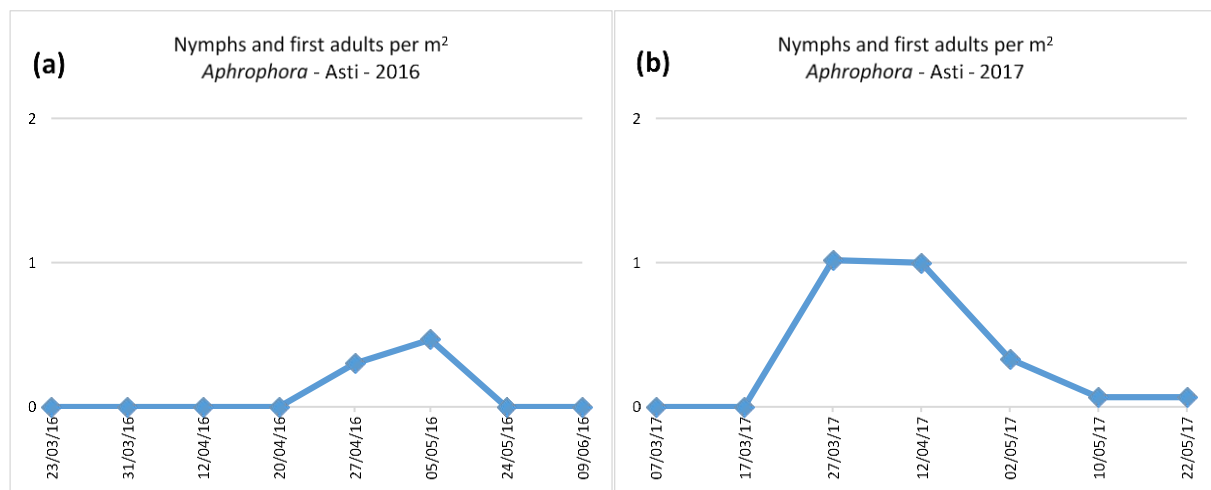


**Figure 53:** Number of nymphs and first adults of *P. spumarius* per m<sup>2</sup> in 2016 (a) and in 2017 (b).

#### Other potential vectors

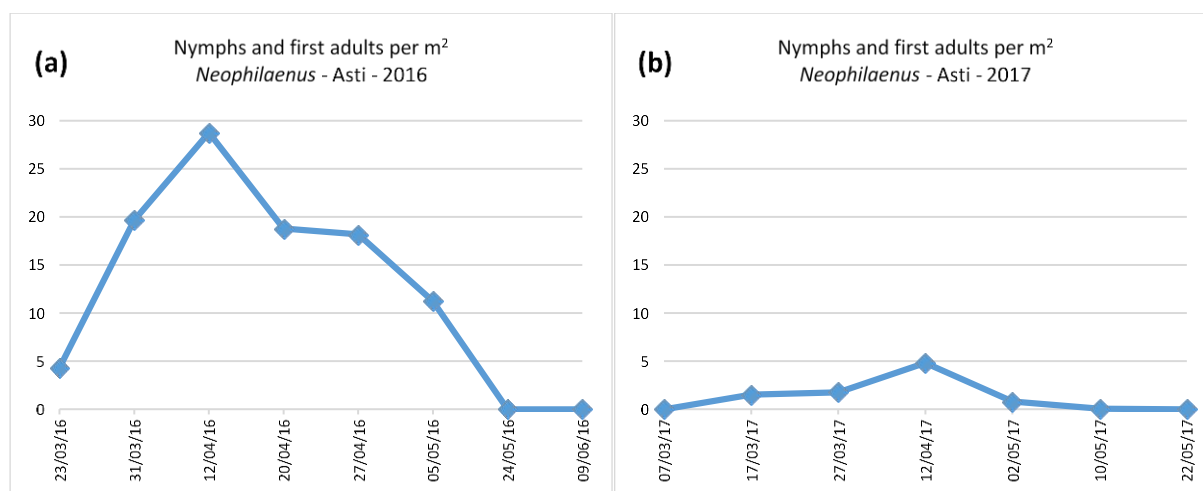
Besides *P. spumarius*, the other potential vectors of *X. fastidiosa* identified in Asti belong to the genera *Aphrophora* and *Neophilaenus*. However, the number of detected individuals of these two species is considerably lower than those of individuals of *P. spumarius* for both 2016 and 2017.

The genus *Aphrophora* reaches the maximum population density (less than 1 individuals/m<sup>2</sup>) at the beginning of May in 2016. In 2017 the number of detected individuals is slightly higher than the previous year (1 individuals/m<sup>2</sup>) and the maximum density is recorded around the end of March and beginning of April (Figure 54).



**Figure 54:** Number of nymphs and first adults of *Aphrophora* per m<sup>2</sup> in 2016 (a) and in 2017 (b).

Regarding the genus *Neophilaenus*, in 2016, the vineyard in Asti presents the highest number of sampled individuals, with a maximum of 29 individuals/m<sup>2</sup> before mid-April, while in 2017 the density of *Neophilaenus* reaches 5 individuals/m<sup>2</sup> before mid-April (Figure 55). The abundance of the *Neophilaenus* genus is higher in 2016 than in 2017.



**Figure 55:** Number of nymphs and first adults of *Neophilaenus* per m<sup>2</sup> in 2016 (a) and in 2017 (b).

A marked seasonality in the development of the pre-imaginal and early adult stages of *P. spumarius* and other potential vectors in the vineyard in Asti has been observed from the beginning of March to the end of April/beginning of May.

#### 11.3.1.2.2 Population dynamics with respect to physiological time

To identify the threshold values of *P. spumarius* development, degree-days (DD) have been used.

Distributions of individuals/m<sup>2</sup> of *P. spumarius* according to the physiological time, estimated with the DD, differ from what has been previously observed using chronological time. Comparing the values of the two distributions (based on chronological and physiological time) within the same date, it is indeed observed that DD in Asti in 2017 are higher than in 2016.

More in details, in the vineyard in 2016 the peak of abundance of the population of the pre-imaginal *P. spumarius* stages is recorded at 106 DD, while in 2017 the peak of abundance is at 84 DD. The development of the pre-imaginal stages of *P. spumarius* in 2016 ends around 314 DD, while in 2017 the end is recorded around 271 DD.

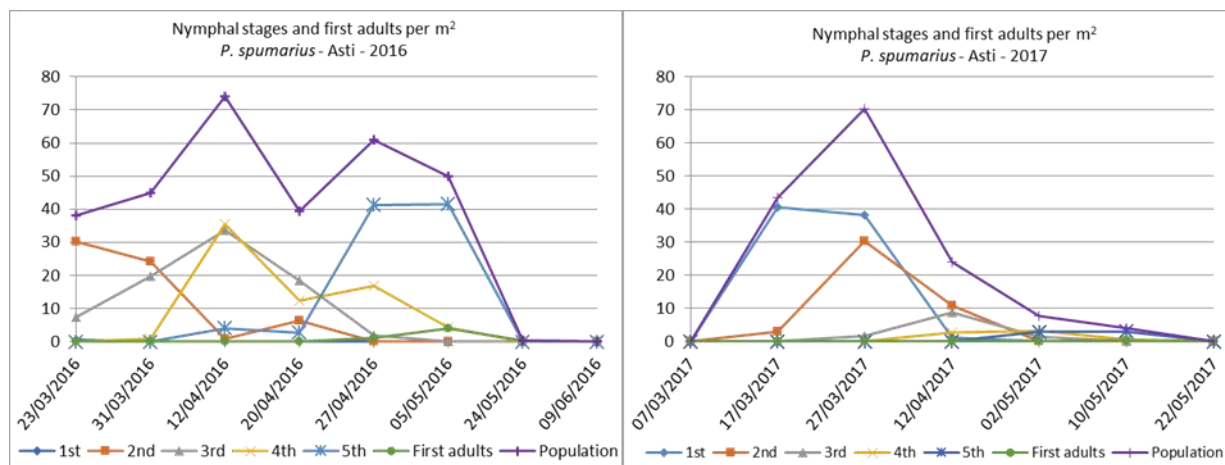
#### 11.3.1.2.3 Pre-imaginal stages population dynamics with respect to chronological time

Looking at the 2017 data, all the stages from first to fifth instars to early adults are clearly distinguishable (Figure 56). In 2016 the survey started the last decade of March and the occurrence of the first three instars were not monitored.

The first *P. spumarius* nymphs are recorded in early March in 2017 and the pre-imaginal stages end in late May in both years. In 2016, in conjunction with the peak of population abundance previously described and recorded around mid-April, there is a simultaneous presence of the second, third, fourth and fifth instars, while in 2017, in conjunction with the peak of population there is a simultaneous presence of the first, second and third instars around the end of March.

In 2016 a maximum of 74 *P. spumarius* individuals/m<sup>2</sup> is observed for the pre-imaginal stages (Figure 56). The peak of the fifth instars follows the peak of the third and fourth instars of 15 days. In 2017, the maximum density of *P. spumarius* population is 70 individuals/m<sup>2</sup>, with first and second stages as the most represented (Figure 56). In 2017, the third, the fourth and the fifth stages report a lower population abundance compared to the previous year.





**Figure 56:** Nymphal stages and first adults of *P. spumarius* per m<sup>2</sup> in Piedmont in 2016 (a) and in 2017 (b).

#### 11.3.1.2.4 Pre-imaginal stage phenology with respect to physiological time

Considering the two years of field observations in Asti, the development of all the *P. spumarius* stages is generally between 22 DD and 365/459 DD. Since in 2016 the beginning of first instar stage has not been recorded, only data of the end of development in 2016 and data of the second year have been reported for this stage. In 2017, the first instar stage is found between 52 DD and 163 DD, while in 2016 it develops until 37 DD. The second instar stage develops between 52 DD and 239 DD in 2017, while in 2016 it develops until 146 DD. As for the first stage, the beginning of the second stage has not been recorded in 2016. Taking into account both years, the third instar stage develops between 37/84 DD and 207/239 DD, the fourth instar stage between 58/163 DD and 207/271 DD, the fifth instar stage between 106/163 DD and 207/271 DD, respectively for 2016/2017. The maximum abundance of the first instar stage is between 52/84 DD in 2017, the maximum abundance of the second instar stage is at 84 DD in 2017, for the third instar stage the maximum is recorded at 106 DD in 2016 and 163 DD in 2017, for the fourth instar stage at 106 DD in 2016 and 239 DD in 2017 and for the fifth stage the peak of abundance is recorded at about 179/207 in 2016 and at 239/271 DD in 2017 (table 14).

**Table 14.** Degree days (DD) of main events related to *P. spumarius* pre-imaginal stage phenology in the vineyard.

	Stage	DD	
		Asti 2016	Asti 2017
Beginning of development of the pre-imaginal stages of <i>P. spumarius</i>	1st	N/A	52
	2nd	N/A	52
	3rd	37	84
	4th	58	163
	5th	106	163
Peak of abundance of the pre-imaginal stages of <i>P. spumarius</i>	1st	N/A	52
	2nd	N/A	84
	3rd	106	163

	4th	106	239
	5th	207	271
End of development of the pre-imaginal stages of <i>P. spumarius</i>	1st	37	163
	2nd	146	239
	3rd	207	239
	4th	207	271
	5th	207	271

### 11.3.1.3. Habitat selection in the adult stage

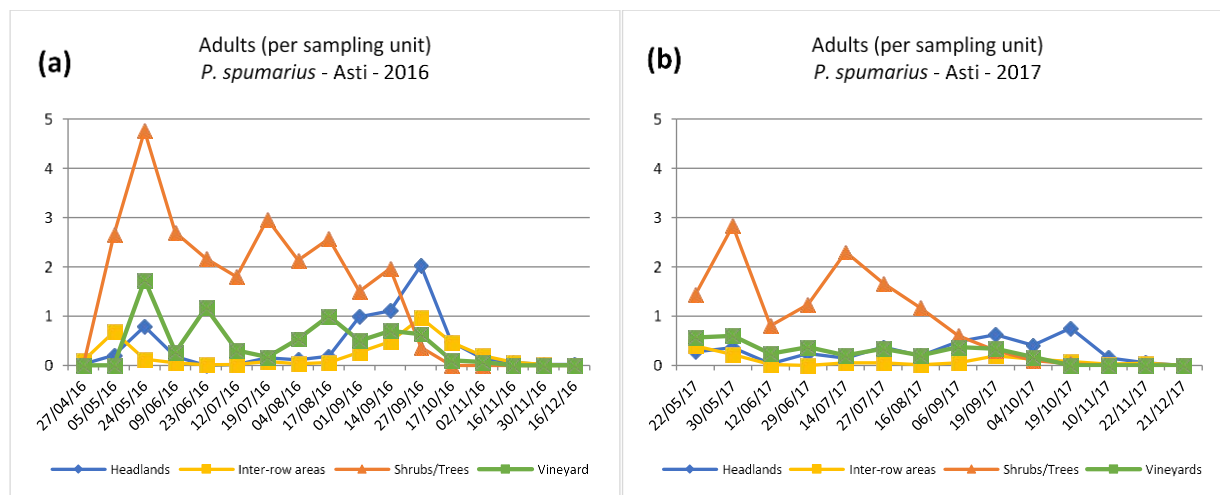
Adult sampling was carried out in three different habitats: herbaceous vegetation (headlands and inter-row areas), shrubs/trees and vineyard. All the habitats have been investigated to detect movements of *P. spumarius* population from one habitat to another.

The habitat of the shrubs/trees bushes in Asti consists mainly of plants belonging to the species *Quercus robur* and to the genera *Cornus* > *Robinia* > *Pyrus* > *Ulmus* > *Prunus* > *Vitis* in 2016, while in 2017, the habitat of the shrubs/trees bushes consists mainly of plant species belonging to the species *Quercus robur* and to the genera *Quercus* > *Robinia* > *Crataegus* > *Ulmus* > *Vitis* > *Prunus*. The genus *Quercus* shows a prevalence in both years.

Since the sampling method used in the three habitats is different (see protocols for data collection, section 11.2.1), data were analysed with reference to the SSU. For the herbaceous habitat individuals are calculated by swept (mean length is equal to 70 cm), while the individuals sampled on shrubs and vineyard are considered for each sampling unit (plant or portion of vineyard, respectively).

Observing the population dynamics of adults detected in the herbaceous habitat (headlands and inter-row areas), two peaks of abundance can be found, one before and one after summer, as *P. spumarius* migrates from the herbaceous compartment into other compartments (shrubs/trees and vineyard) during summer (Figure 57). This trend is clearly present in 2016, while in 2017 there is only the peak of density after summer. Regarding the vineyard compartment, the maximum adult density of *P. spumarius* is recorded at the beginning of summer in 2016 and in late spring in 2017. Evaluating the number of *P. spumarius* individuals found on shrubs/trees, the maximum adult density of *P. spumarius* is recorded in late spring in both years. Overall, in 2016, the number of individuals per swept is higher compared to the number found in 2017 in all habitats. The highest value of adult presence is found in shrubs/trees in both years, followed by vineyard in 2016 and by headlands in 2017.

Taking into account degree-days, the peak of adults in spring occurs in all habitats between 179 and 606 DD in 2016 and it occurs until 615 DD in 2017, but the beginning of the spring peak has not been recorded in the second year of sampling. The second peak in late summer/beginning of autumn is recorded in the herbaceous habitat between 1588 and 1968 DD in 2016 and between 1863 and 2201 DD in 2017.



**Figure 57:** Adults of *P. spumarius* per swept (herbaceous vegetation), per plant (shrubs/trees) or per portion of vineyard in Piedmont in 2016 (a) and in 2017 (b).

Concerning other potential *X. fastidiosa* vectors identified in Asti, adults of *Aphrophora*, *Neophilaenus* and *Cicadella viridis* were detected in both years, together with individuals of the families of the Cicadellidae and Issidae.

## 12. Study of the reproductive biology (maturation of eggs over time) under field conditions in different areas of the UE (Amendment)

### 12.1. Objectives

Description of reproductive biology (ovaries and egg maturation periods of female *P. spumarius*) under different environmental conditions are presented.

### 12.2. Materials and methods

The scope of this activity was to collect from different sites adult females of *P. spumarius* in order to dissect and analyse them for the presence of mature eggs in their ovaries (Fig. 58). *P. spumarius* adults were collected in 2018 weekly, every fortnight or once a month from mid-April (Southern and Northern Italy) or mid-June (Alps), until mid-September (Alps), late December (Northern Italy), beginning of February (Southern Italy). Collection sites were representative of four macroregions, ranging from 41 to 50° latitude north and spanning from the sea level up to 2,200 m a.s.l. elevation:

- Alps: located between 1,000 and 2,200 m a.s.l. in western Alps (Piedmont Region, Torino province) and in the autonomous province of Bolzano.
- Northern Italy: located at 175-350 m a.s.l. in the provinces of Torino and Asti (Piedmont Region), Savona (Liguria Region) and Trento (autonomous province of Trento).
- Southern Italy: located at 3-266 m a.s.l. in the provinces of Lecce and Bari (Apulia Region) and in Tocco Caudio (Benevento province, Campania Region)
- Germany: extra samples were gently provided by dr Michael Maixner and Anna Markheiser (Julius Kuhn Institute); they were collected from two sites in Rhineland-Palatinate between 180 and 400 m a.s.l...

Additional data from Finland and Germany were extracted from the literature (Halkka et al., 1967; Witsack, 1973) and included in Table 11.

Adults were captured by sweep netting and sorted by sex. Females were immediately dissected in laboratory or preserved in 70% ethanol for later dissection. Dissection was carried out under a stereomicroscope and number of ovaries and of mature eggs were counted.

The presence of eggs was claimed when more than 50% of females of each sample analysed showed at least one mature egg (Figure 58).



**Figure 58:** Mature eggs in a female of *Philaenus spumarius* collected in Asti, North Italy, 38th week (September).

### 12.3. Results and deliverables of Activity 4 of the Amendment

A total of 565 females were captured and dissected for the presence of eggs during 106 different sampling events. Table 15 summarizes the sites of female collections, their geographic references and number of females captured.

**Table 15.** Samplings of *Philaenus spumarius* females for detecting mature eggs.

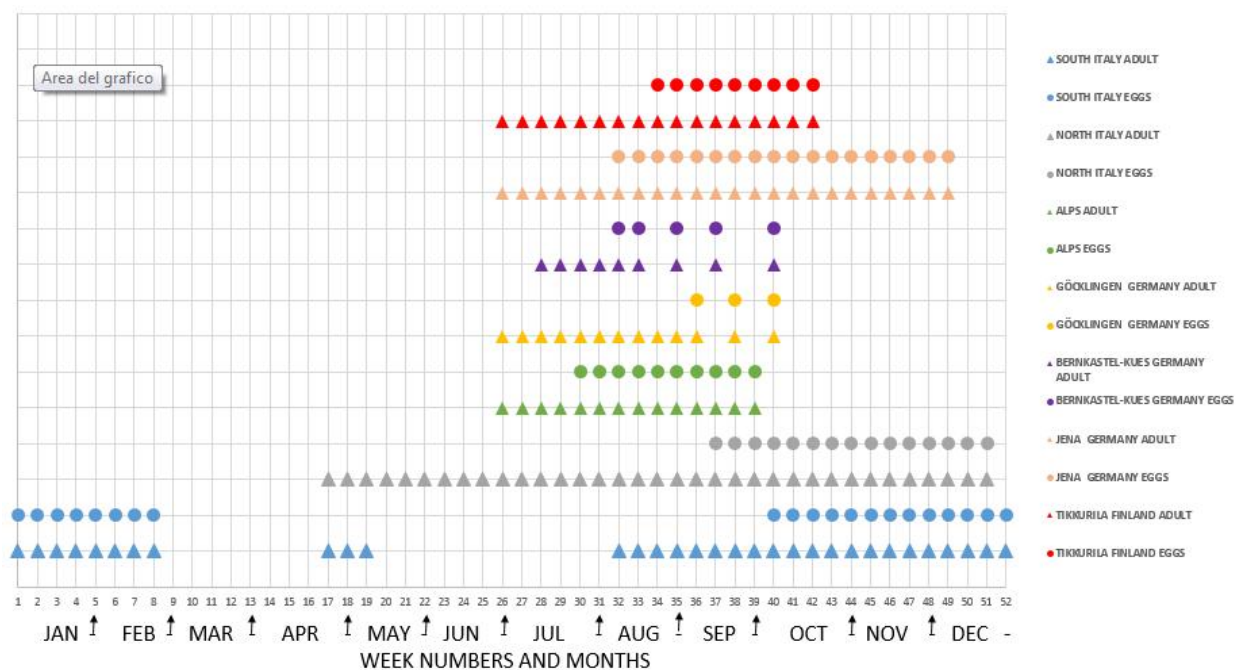
LOCALITY	COUNTRY OR MACROREGION	COUNTRY	REGION	LATITUDE	LONGITUDE	ALTITUDE (m asl)	MONTHS OF CAPTURE	NUMBER OF SAMPLINGS	NUMBER OF FEMALES CAPTURED
Gallipoli	South Italy	Italy	Apulia	40,0114472	18,0286694	3	Sep-Oct	4	23
Galatina	South Italy	Italy	Apulia	40,1742139	18,17257	75	Sep	1	4
Valenzano	South Italy	Italy	Apulia	41,0428611	16,8842027	85	Sep	1	10
Bari	South Italy	Italy	Apulia	41,1125722	16,883025	6	Jan-feb	3	4
Ruvo di Puglia	South Italy	Italy	Apulia	41,1168414	16,483773	266	Aug	2	16
Tocco Caudio	South Italy	Italy	Campania	41,1205889	14,6387	676	Oct	2	7
Arnasco	North Italy	Italy	Liguria	44,0766	8,1173	266	May-Dec	16	49
Finale	North Italy	Italy	Liguria	44,1811	8,3634	263	May-Dec	16	52
Paderna	North Italy	Italy	Piedmont	44,8260806	8,89468	230	Jun; Sep	2	7
Asti	North Italy	Italy	Piedmont	44,9194	8,1982	175	May-Dec	21	186
Chieri	North Italy	Italy	Piedmont	45,0154139	7,79305	345	Jun	2	8
Grugliasco	North Italy	Italy	Piedmont	45,073	7,5873861	293	Jul; Sep	2	2
Cocconato	North Italy	Italy	Piedmont	45,0825944	8,0594305	300	Jun; Sep	2	4
Druento	North Italy	Italy	Piedmont	45,1289611	7,5880194	265	Jun	1	2
Mezzocorona	North Italy	Italy	Trentino-South Tyrol	46,2131667	11,1455083	210	Jul	1	10
Prali	Alps	Italy	Piedmont	44,8759	7,0575	1350-1700	Jul-Sep	11	68
Sestriere	Alps	Italy	Piedmont	44,9472389	6,9054305	2210	Aug	1	5
Cesana	Alps	Italy	Piedmont	44,9529972	6,7936305	1350	Jul-Aug	2	4
Compaccio	Alps	Italy	Trentino-South Tyrol	46,5409944	11,6170472	1848	Sep	1	3
Castelrotto	Alps	Italy	Trentino-South Tyrol	46,56724	11,556	1000	Jul; Sep	2	10
Castelrotto San Michele	Alps	Italy	Trentino-South Tyrol	46,5786861	11,6021361	1283	Sep	1	5
Göcklingen	Germany	Germany	Rhineland-Palatinate	49,157321	8,027783	186	Jun; Oct	6	31
Bernkastel-Kues	Germany	Germany	Rhineland-Palatinate	49,911956	7,099129	409	Jul; Oct	6	55
Jena	Germany	Germany	Thuringia	50,9345	11,5807	194	Jun-Dec		Witsack (1973)
Tikkurila	Finland	Finland	Uusimaa	60,2933	25,0377	17	Jun; Oct		Halkka et al. (1967)

Details on time of appearance of mature eggs in the females collected at different times of the year in different sites are summarized in Table 16. The number of mature eggs in a single female ranged from 1 to more than 40. In spite of the fact that females of *P. spumarius* emerge as adults in spring (see section 10.3.1.2.3), they mature eggs only starting from mid-September in Northern Italy (Padana Valley) and beginning of October in Southern Italy. Interestingly, in the Alps, where the vegetative season is much shorter, maturation of the eggs is strongly anticipated and ovarian diapause is very short, as the females, that emerge by the very end of June, start developing eggs by the end of July. Consistently, in central-northern Europe, egg maturation is anticipated compared to Italy and females start to mature eggs in the first half of August. It is clear that female diapause ends sooner under colder climatic conditions and later under mild Mediterranean conditions. This can be explained as an evolutionary adaptation allowing the species to complete its life cycle and to optimize egg survival under different environmental conditions. The females collected in autumn-winter always show the presence of mature eggs, thus indicating that they are capable of maturing and laying eggs until death.



**Table 16.** Occurrence of first and last adults with mature eggs in localities with different sampling intensities.

LOCALITY	COUNTRY OR MACROREGION	FIRST ADULT SAMPLING WITH MATURE EGGS			LAST ADULT SAMPLING WITH MATURE EGGS			SINGLE SAMPLING OF ADULTS WITH MATURE EGGS		
		MONTH AND NUMBER	NUMBER OF EGGS PER ADULT (MINIMUM AND MAXIMUM)	NUMBER OF FEMALES DISSECTED	MONTH AND NUMBER	NUMBER OF EGGS PER ADULT (MINIMUM AND MAXIMUM)	NUMBER OF FEMALES DISSECTED	MONTH AND NUMBER	NUMBER OF EGGS PER ADULT (MINIMUM AND MAXIMUM)	NUMBER OF FEMALES DISSECTED
Gallipoli	South Italy	October - 40 <sup>th</sup> week	1-14	10	October - 43 <sup>th</sup> week	6-13	5			
Bari	South Italy				January - 4 <sup>th</sup> week	9	1			
Bari	South Italy				January - 5 <sup>th</sup> week	12-19	2			
Bari	South Italy				February - 8 <sup>th</sup> week	25	1			
Tocco Caudio	South Italy	October - 41 <sup>th</sup> week	22-43	3	October - 42 <sup>th</sup> week	2-24	4			
Arnasco	North Italy	September - 38 <sup>th</sup> week	1-19	6	December - 51 <sup>th</sup> week	2-41	2			
Finale	North Italy	September - 38 <sup>th</sup> week	1-17	4	December - 51 <sup>th</sup> week	15-28	4			
Asti	North Italy	September - 38 <sup>th</sup> week	7-30	8	December - 50 <sup>th</sup> week	18-24	3			
Grugliasco	North Italy							September - 39 <sup>th</sup> week	23	1
Coconato	North Italy							September - 37 <sup>th</sup> week	1-10	2
Prati	Alps	July - 30 <sup>th</sup> week	1-5	11	September - 38 <sup>th</sup> week	10-27	6			
Sestriere	Alps							August - 33 <sup>th</sup> week	11-21	5
Cesana	Alps							August - 33 <sup>th</sup> week	12	1
Compaccio	Alps							September - 38 <sup>th</sup> week	15-21	3
Castelfrotto	Alps							September - 39 <sup>th</sup> week	13-20	5
Castelfrotto San Michele	Alps							September - 38 <sup>th</sup> week	9-29	5
Göcklingen	Germany	September - 36 <sup>th</sup> week	18-27	5	October - 40 <sup>th</sup> week	11-21	5			
Bernkastel-Kues	Germany	August - 32 <sup>th</sup> week	4-11	5	October - 40 <sup>th</sup> week	10-22	10			
Jena	Germany		Witsack (1973)			Witsack (1973)				
Tikkurila	Finland		Haikka et al. (1967)			Haikka et al. (1967)				



**Figure 59:** Presence of *Philaenus spumarius* (L.) females (triangles) and of ovarian eggs (circles), in the different macroregions.

### 13. Knowledge transfer to the new EFSA Art 36 project on *X. fastidiosa* vectors in Balearic Islands to standardize sampling techniques of insect vectors

In 2017, EFSA launched a second project for data collection on Xf vectors, titled "Collection of data and information in Balearic Islands on biology of vectors and potential vectors of *Xylella fastidiosa*" (GP/EFSA/ALPHA/2017/01). The grant was awarded to UBI (Universitat de les Illes Balears), with Dr. Miguel Ángel Miranda Chueca as project leader. In the frame of the amendment of current EFSA Procurement "Collection of data and information on biology and control of vectors of *Xylella fastidiosa*" (RC/EFSA/ALPHA/2015/01), during the third year of activity, we were tasked to set up and validate a protocol for insect collection in the Balearic Islands in line with that applied in our project, during a mission for two scientists to Mallorca Island.

As part of this activity, we participated to field surveys for potential vectors of Xf in the island of Mallorca, together with prof. Miguel Angel Miranda. The scientific mission took place between 26 and 29 March 2018. A large almond orchard located in the municipality of Inca was inspected by applying the standard sampling method of the 0.25 m<sup>2</sup> quadrant. A low density of nymphs, mainly 3rd instar, in the range of 0.2 nymphs per quadrant, with a prevalence of *Neophilaenus* vs *Philaenus* was recorded. In an uncultivated area neighboring the orchard on one edge, where more dicotyledonous plants were present (wild fennel *Sonchus*, *Plantago*, and others), a higher population of *Philaenus* was recorded, together with some *Neophilaenus* (at least 2 nymphs/quadrant). In this almond orchard sweeping net was also applied through transects, but the adults captured were leafhoppers (Cicadellidae) and no spittlebug adults were found. The location of almond grove appears very suitable for field surveys of spittlebugs, as it includes many potential host plants/niches for nymphs and adults: a good herbaceous cover with gramineous and dicotyledonous like *Medicago* and *Sonchus*, a number of woody hosts (potential) for adults of both *Neophilaenus* (a long row of cypresses bordering the orchard) plus a good number of wild olive, lentisk, *Laurus* and other shrubs that proved to be good hosts of *P. spumarius* in Apulia. An olive grove in Montuiri municipality, about 0.5 ha in size, was then inspected. In this olive grove a very abundant herbaceous cover made of some gramineous (*Poa*, *Sorghum*) and many dicotyledonous (*Sonchus*, *Cardus*, *Calendula*, *Medicago*, *Lactuca*, *Daucus*) hosted a good number of *Neophilaenus* and *Philaenus* nymphs in an equivalent number. A systematic survey in this site was not applied but almost 50 nymphs have been collected in about one hour. Then, several surveys in different areas of the University of the Balearic Islands (UIB) campus were conducted. In a first site, characterized by herbaceous cover of wild fennel, Asteraceae (mainly *Lactuca* and *Deschampsia*) and gramineous interspersed with carob trees, a good number of nymphs was noted in about one hour (*Philaenus* and *Neophilaenus* in a similar proportion; the prevalent life stage was 4<sup>th</sup> instar at that time). In a second site that was heavily shaded we found almost no spittles, only very few *Neophilaenus*, in spite of the presence of good host-plants for nymphs. In a meadow characterized by the presence of *Glebionis coronaria*, *Lactuca*, *Sonchus* and gramineous plants, more than 30 *Philaenus* (mainly associated with *Glebionis* and *Lactuca*) and 10 *Neophilaenus* nymphs were recorded in about one hour. In a small experimental vineyard we failed to identify spittles, but the soil was tilled, as it is in all vineyards in the Balearic (however, to the edge of the small vineyard, in an uncultivated-abandoned small piece of land, two nymphs of *Philaenus* on Asteraceae were recorded). In another site, where the herbaceous cover was relatively poor and sparse, with a prevalence of gramineous, *Euphorbia*, *Daucus* and *Oxalis* few (5-6) nymphs of *Philaenus* (on *Euphorbia* and *Daucus*) and few (5-6) nymphs of *Neophilaenus* on gramineous plants were identified.

In conclusion we can state that i) the population of spittlebugs in the Balearic is low (much lower than the ones registered in several areas of Italy), but not extremely low, and should allow to complete the study of their phenology and host plant association ii) nymphs of *Neophilaenus* are associated with gramineous plants iii) *Philaenus* nymphs are mainly associated with dicotyledonous plants (mainly Asteraceae, Umbelliferae and few Fabaceae) iv) the ratio *Philaenus/Neophilaenus* is lower in Mallorca compared to Italy (*Neophilaenus* is possibly more abundant than *Philaenus* in the Balearic islands).

Although the same sampling techniques of Xf vectors applied in Italy within the frame of this Procurement can be applied in the Balearic, the experimental plan should differ because of the different environmental situation and the need to investigate different crops.

## 14. Conclusions

To achieve the results of tasks 1 to 7 of the Specific Contract RC/EFSA/ALPHA/2015/01 (Collection of data and information on biology and control of vectors of *Xylella fastidiosa*), integrated with the activities planned within the Amendment to the above mentioned Contract, the IPSP-CNR carried out several activities with the aim to provide EFSA with a comprehensive overview on biology and phenology of the vectors/potential vectors and on available control methods. This general objective is achieved by the integration of literature-based data, questionnaire-based data and experimental data in order to allow EFSA for the analysis of different scenarios based on combination of vector species/control option/environmental conditions. This document is the final report dealing with the results of

- a) data collection from literature on ecology and phenology of Xf vectors (Task 1)
- b) data collection from literature on control options against Xf vectors for integrated and for organic farming (Task 2)
- c) data collection on plant protection products allowed in the EU with effect on *X. fastidiosa* vectors applied on stone fruits, citrus, grapevine and olive plants in the MedMSs (Task 3)
- d) inventory of the ongoing integrated pest management (IPM) programmes on stone fruits, citrus, grapevine and olive plants in the MedMSs (Task 4)
- e) inventory of national and regional competent organization in MedMS with access to monitoring data on Xf vectors (Task 5)
- f) field observations on phenological phases (supported also by agronomic and meteorological data) of *P. spumarius* in olive orchards in 2016, 2017 and 2018 (Tasks 6 and 7 and activities 1 and 2 of the Amendment)
- g) study of *P. spumarius* fecundity and development under microcosm conditions (activity 3 of the Amendment)
- h) data on reproductive biology of *P. spumarius* (maturation of eggs over time) (activity 4 of the Amendment)
- i) data managing and analysis of *P. spumarius* in vineyard 2016 and 2017 (activity 5 of the Amendment)
- j) training activity for knowledge transfer to the new Art 36 EFSA funded project on vectors of *Xylella fastidiosa* in Balearic Islands (activity 7 of the Amendment)

For the systematic literature search (Tasks 1 and 2) the adopted protocol was based on the following milestones:

- Definition of research questions
- Establishment of search terms and their arrangement into search strings
- Definition and access to information sources
- Screening of the search results
- Reference management
- Quality assessment

The following authoritative databases indexing peer-reviewed scientific publication have been used as primary sources of information: Web of Science, Scopus, MEDLINE, AGRICOLA, CABI-Abstracts (CAB), JSTOR and EPPO Global Database. The databases were interrogated using the same strategy in two consecutive years (2016 and 2017).

Besides authoritative databases, the following grey literature repositories were interrogated in 2016: Google Scholar, <http://www.scholar.google.it>, OCLC WorldCat, <http://www.worldcat.org>, GreyNet International, [www.greynet.org](http://www.greynet.org), Internet Archive, <http://www.archive.org>. In addition, the recursive agents GrandReporter (<http://www.tri-edre.com>), and DEVONagentPro ([www.devontechnologies.com](http://www.devontechnologies.com)) were used for recursive research in INTERNET.

For phenology and ecology of vectors/potential vectors (Task 1), about 4,000 records were deduplicated and screened for relevance based on title and the abstract, allowing to the identification of 607 potential interesting papers, 267 of which were rated relevant and used for generating the extraction summary table on *X. fastidiosa* vectors and potential vectors phenology and ecology. This summary table shows that the most well-known species is the American sharpshooter *Homalodisca vitripennis*, whose biology/ecology/phenology is very well characterized with respect to host plant preference, life cycle and population dynamics, fecundity, flight dispersal, feeding behaviour and symbionts. However, there is a good amount of available knowledge on the ecology and phenology of the two main species of Cicadellinae present in Europe: *Cicadella viridis* and *Graphocephala fennahi*. Few information about the habitat/ecology of the other European Cicadellinae, *Evacanthus interruptus* and *E. acuminatus* and *Cicadella lasiocarpae*, is available, while almost no information on their phenology is available. Among spittlebugs, *Philaenus spumarius* is the most studied species and data on host plants of nymphs and adults, life cycle and population dynamics are available, although detailed knowledge on the abundance and phenology of this species on selected crops is not available. Ecology and phenology of other European spittlebugs is poorly known, and information is scattered. However, some interesting data on the host plants and life cycle are available for species of the genera *Aphrophora*, *Cercopis* and *Neophilaenus*. The main available information on cicadas concerns their host plants, the characteristics of environments colonized by cicadas and the period of adult presence. Few data about egg laying and prolificacy are also available.

Based on data retrieved from the literature and on our own experience, a draft protocol for field data collection on phenology and ecology of the vector *Philaenus spumarius* was generated. An inventory of sampling methods for xylem-sap feeder insects has also been compiled. Visual inspection and counting of spittlebug nymphs is the only practical method for the study of the nymph population of *P. spumarius* (and of the other spittlebugs), while sweep net is the most widely applied sampling method of *P. spumarius* adults. Yellow sticky traps can be used to collect adult of the spittlebug, although their efficiency is debated.

For potentially effective control options against all the vectors and potential vectors of *X. fastidiosa* all around the world (Task 2), more than 2700 records were deduplicated and screened for relevance on the base of the abstract (reports dealing with insecticides not registered in the EU anymore were discarded), allowing to identify more than 800 potential interesting papers, 395 of which were rated relevant and used for generating efficacy tables on chemical, biological and agronomic/physical control methods. Literature on insecticides activity against xylem-sap feeder insects is mainly restricted to sharpshooter vectors of *X. fastidiosa* in North and South America. Neonicotinoids appear to be effective and persistent on both adults and nymphs. Pyrethroids are effective against both adults and nymphs of sharpshooters but by far less persistent than neonicotinoids. Data on activity of organophosphates against sharpshooters are sometimes conflicting and, when tested weeks after treatment, these molecules were poorly persistent. The application of Insect Growth Regulators (IGR) against the spittlebug nymphs seems not to be promising, also because of the possibility of suppressing nymph population with soil tilling. There is a major gap of knowledge on the activity of insecticides allowed in organic farming, although preliminary data suggest that essential citrus oil is more active than pyrethrins. Unfortunately, citrus oil, like pyrethrins, is not persistent at all. In spite of the very limited data available on insecticide activity against spittlebugs, it is likely that neonicotinoids show an active and persistent activity against all *X. fastidiosa* vectors/potential vectors while pyrethroids, although active, are not persistent.

Literature on biological control of *X. fastidiosa* vectors/potential vectors is restricted to the description of natural enemies, mainly represented by egg parasitoids of the genus *Gonatocerus* (family Mymaridae) and *Oligosita* (family Trichogrammatidae), the endoparasitoid *Verrallia aucta* (family



Pipunculidae) and by the entomopathogenic fungus *Metarhizium anisopliae*. However, in our large-scale mesocosm rearing, a fungal epizootic killed all *P. spumarius* nymphs. From these, we isolated two fungi, *Beauveria bassiana* and *Fusarium oxysporum*, that are known to be entomopathogenic, though not noticed for *P. spumarius*. Their actual role as causal agent of the lethal disease is still to be demonstrated, still the finding is interesting. The analysis of literature shows that so far only “classic” biological control programs (inoculative introduction of natural enemies from the original area of distribution of the insect vector) have been applied and this application was targeted against the invasive sharpshooter vector *Homalodisca vitripennis*. Since *P. spumarius* is a native species in the EU, inoculative biological control is not feasible, and inundative biological control, based on mass-reared beneficials, has never been experienced and is unlikely to be effective because the spittlebug is ubiquitous and polyphagous. The most well-known natural enemy of *P. spumarius* seems to be a pipunculid fly, *Verrallia aucta*, a parasitoid of nymphs and adults that can parasitize up to one third of the adults under field conditions. Apart for some generalist predators, very few information is available on other natural enemies. This is a major research gap that hampers the possibility of implementing a biological control based on a conservation approach, which might contribute to the partial suppression of *P. spumarius* populations.

There is very little literature on control options other than insecticides and biological control. However, some interesting information were found. For example, removal of straw in pastures seems to significantly reduce the populations of spittlebugs. Spraying with “Surround WP”, that forms a particle film on the vegetation, strongly reduced the population of sharpshooters, both in choice and no choice tests. Recent experiences in the Apulia Region pointed out that soil tilling resulted in a very strong reduction of spittlebug adults on the ground vegetation, although the reduction of adults collected on the olive canopy can be less important. Finally, it is worth to remember that protecting nurseries with netting is an effective method to keep nursery stocks free of insect-transmitted pathogens, including *X. fastidiosa*. A prolonged flooding can be effective in killing cicada nymphs, but this technique is not applicable in the vast majority of cases (no water available in dry environments where cicadas live, sloped crop plots).

Based on the result of Task 2, a list of plant protection products allowed in the EU with effect on *X. fastidiosa* vectors were identified and used for the construction of a GAP table (Task 3). Information were retrieved from national official databases (most of the links were gathered from the EPPO website) and organized in an excel table that included columns on: a.i., mode of action (MoA) category, crop, MedMs, target pest. One entry was prepared for each DAR/crop/state. The table can be filtered by active ingredient (a.i.), crop, MedMS and mode of action. According to the table, neonicotinoids have the widest application on stone fruits, citrus, grapevine and olive in the MedMSs, followed by organophosphates, the phenoxy derivative etofenprox and pyrethroids.

The aim of Task 4 was to provide an updated inventory of the Integrated Pest Management (IPM) programmes in place in the MedMSs for stone fruits, citrus, grapevine and olive, and the pests targeted by these IPM programmes. To obtain information on the ongoing IPM programmes, a questionnaire was prepared and sent to contact points of all MedMSs. The recipients of the questionnaire were the official websites of the Ministry of Agriculture (or equivalent ones). Further information was retrieved by websites of the Ministries of Agriculture of the MedMSs. The results are summarized in an excel table, which provides information for each crop and each country. IPM programs are ongoing in all/almost all the MedMSs on most of the stone fruit and citrus orchards as well as on grapevine and olive. None of these IPM programs mentions xylem-sap feeder insects among the target pests and, thus, current control programs do not consider *X. fastidiosa* vectors as pests.

For Task 5, a list of public national and regional as well of private organisations has been compiled based on contacts proposed by CNR-IPSP, EFSA, EUPHRESCO and EPPO. These organisations are located in all the MedMs, in the rest of the EU and in several non-EU countries and might have access to monitoring data on vectors and potential vectors of *X. fastidiosa*. The questionnaire has been sent to all these organisations, including all the Italian Regional Phytosanitary services. We obtained 30 answers from outside Italy, coming from 19 different countries and 18 answers from Italy, coming

from 15 regions. All the answers have been included in a single excel file, each sheet corresponding to an answer from an organisation, ordered in country alphabetic order. In order to comply with the request of the procurement, the information obtained from the survey (questionnaire), have been combined with those obtained from literature (Task 1, Extraction Table Ecology and phenology) and a new version of this Table has been produced. Moreover, we summarized the main results (monitoring campaign ongoing or planned for 2017, no monitoring campaign ongoing or planned) in two maps of Europe and Italy, respectively. Based on the answers to the questionnaires, monitoring programs on *X. fastidiosa* vectors have been enforced in most of the European countries and in all the Italian regions. Few programs started in 2014, most of them started in 2015 or 2016. Monitoring programs are focused mainly on adults of spittlebugs and of *Cicadella viridis*. Sweep net and yellow sticky traps are the most commonly applied sampling methods.

The activities of tasks 6 and 7 provided a big amount of data on biology, ecology, phenology and population dynamics of *P. spumarius* nymphs and adults in olive agroecosystems of the Mediterranean area. Although the nymphs were polyphagous, they showed a strong host-preference for herbaceous plants of the Asteraceae and Fabaceae families both in Liguria and in Apulia regions of Italy. The nymphs found on the plants of these families accounted for 72-88% of the total nymphs. Nymphs of *Aphrophora* showed a similar host-preference, while those of *Neophilaenus* were strongly associated with Poaceae (85-100% of the nymphs were found on gramineous plants). These two spittlebug species, although present in the olive agroecosystems, had a very low population density compared to *P. spumarius*. In this latter, the average nymphs population density varied from 13 to 30 individuals/m<sup>2</sup> in Liguria (min value=0, max value= 68) according to the olive grove and the year, and from 5 to 19 individuals/m<sup>2</sup> in Apulia (min value=0, max value= 39), although in 2018 population density in the same olive groves of Apulia never exceeded 5 nymphs/m<sup>2</sup>. The reasons for this lower density, besides annual variation due to environmental variables, can be soil and olive canopy disturbance at Locorotondo (where soil has been tilled in order to fertilize and a mild pruning of olive has been done). As for the olive grove of Valenzano no special disturbance has been provided to the field, still the population density was lower than previous years.

Phenological data based on physiological time revealed that in Liguria the peak of abundance of *P. spumarius* nymph population was between 150 and 210 degree day (DD) while in Apulia the same peak was between 100 and 270 DD. This difference among locations could be explained by a non-linear component in the temperature-dependent development rate function of *P. spumarius*. The phenological pattern in the two regions is more similar if referred to chronological time. In fact, nymphs developed in Liguria between early March and end of May and in Apulia between the end of February and mid-May.

The pattern of adult population abundance varied with the site, the plant compartment and the habitat. The maximum adult density on olive was recorded in summer in Liguria and in late spring/early summer in Apulia. In addition, the number of individuals found tends to drop more abruptly in July/August in Apulia compared to the same period in Liguria, likely due to the complete drying up of the herbaceous cover in this region. Regarding the alternative woody host compartments, *P. spumarius* adults preferred *Quercus* spp. (*Q. ilex*, *Q. petraea* and *Q. crenata*) and *Pistacia* spp. (*P. lentiscus* and *P. terebinthus*) in the Ligurian sites. In the Apulian sites, the spittlebug adults were more frequently collected on *Myrtus communis*, *P. lentiscus* and *Phillyrea angustifolia*, although in 2018 *Quercus* was the most important alternative woody host also in Apulia. It is worth noting that woody host preference is driven by the plant species composition of the investigated sites. However, evergreen and deciduous oaks seem to be among the preferred hosts of adults. A clear host-shifting of adults that move from the herbaceous cover to woody hosts and back to herbaceous plants at the end of summer-autumn was observed in three out of four sites.

In the mesocosm, following two releases of 200 (100 males and 100 females) adults, in 2016 only few adults have been found on olive trees. Many more adults were found on olive trees in 2017 (between 15-50% of all adults counted in the mesocosm in June-July, according to the sampling date). This result can be explained by referring to the strong component of artificiality introduced in the mesocosm: the establishment of a forced environment through a selection of plant species, the higher

relative humidity due to the confined environment, and the small size of olive trees in the mesocosm. In 2017, the highest number of adults sampled was between June and early August, decreasing from August to October, and confirming the phenological trend of adults observed in the field. Nymph population increased gradually until reaching the maximum peak of abundance around 250 DD and then decreasing. Pre-imaginal stages followed a phenological trend with respect to physiological time similar to that found in the open field. The development of pre-imaginal stages in the mesocosm occurs from 23 DD to 357 DD, following the general trend that has been observed in open field.

Data on *P. spumarius* prolificacy obtained in the microcosm studies indicated that a single female produced on average about 90-110 and 18-20 eggs in the experiments carried out in Torino and Bari, respectively. The reasons for the very important difference in prolificacy recorded in Piedmont and Apulia must be investigated but are beyond the scope of this work. The analysis of the ovaries of *P. spumarius* females over the year revealed that they mature eggs after a very long ovaric diapause. The first mature eggs occur at mid-September in Northern and at the beginning of October in Southern Italy. In colder areas, ovaric diapause is shorter and mature eggs are found since August.

Data analysis of *P. spumarius* in vineyard in 2016 and 2017 in Piedmont Region show that phenology and host-plant association of the spittlebug are very similar to those recorded in olive groves of Liguria Region, with a slightly higher density of population. As for the habitat selection of adults, the adult density on grapevine is quite low and constant from the end of May to the end of September. The host-shifting of adults from the herbaceous cover to woody hosts and back to herbaceous plants at the end of summer-autumn, observed in olive groves, has been confirmed also in the vineyard.

The present work represents a substantial improvement on the knowledge on *P. spumarius*, namely on its phenology in the Mediterranean area, host-shifting of adults over the season, prolificacy and development in relation to temperature. The different results obtained in Liguria and Apulia regions in term of DD can be explained by a strong non-linear component of the development rate function of *P. spumarius*. The collected data can be used to identify the nonlinear functions that are appropriate for modelling the spittlebug phenology.

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## Abbreviations

a.i.	Active Ingredient
COPHS	Chief Officers of Plant Health
DARs	Draft Assessment Reports
DD	Degree-Days
GAP	Good Agricultural Practices
IGR	Insect Growth Regulators
IPM	Integrated Pest Management
MoA	Mode of Action
NPPO	National Plant Protection Organizations
PSU	Primary Sampling Unit
SSU	Secondary Sampling Unit
Xf	<i>Xylella fastidiosa</i>



## Appendix A – Results of search in EPPO GLOBAL database

Insect name	N. records	Title	Title
<i>Graphocephala atropunctata</i>	1	PM3/085(1) Inspection of places of production – Vitis plants for planting	
<i>Homalodisca vitripennis</i>	2	PM1/002(27): EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests (2018)	PM3/085(1): Inspection of places of production – Vitis plants for planting
<i>Philaenus spumarius</i>	1	PP2/009(1) Strawberry	