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Front cover: *Cicadella viridis* eggs in common rush. © Séverine Hasbroucq (ULB).

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Distribution, adult phenology and life history traits of potential insect vectors of *Xylella fastidiosa* in Belgium

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Abstract

The xylem-inhabiting phytopathogenic bacterium, *Xylella fastidiosa,* is mainly transmitted in Europe by the spittlebug (Aphrophoridae) *Philaenus spumarius.* In Belgium, other xylem-sap feeding Hemiptera (*Aphrophora alni*, *Aphrophora salicina*, *Cercopis vulnerata, Cicadella viridis*) are also present and considered as potential vectors. The distribution, adult phenology and host plants in Belgium of these five species were analysed, using information from the Royal Belgian Institute of Natural Sciences collections, the naturalist web site *Observations.be*, and our own field data collections in 2016-17. Adults of the highly polyphagous *A. alni*, *C. vulnerata, C. viridis,* and *P. spumarius* were found in all the ecological regions of Belgium; *A. salicina* was less widely distributed and, in particular, was absent from the Ardennes and Lorraine, probably due to its narrower specificity to *Salix* spp. The *C. vulnerata* adults were collected mostly in May, whilst the other species' adults were found mostly from April to October with a peak in July for *A. alni*, in August for *A. salicina* and *C. viridis*, in May and August for *P. spumarius*.

An egg winter diapause was observed in *P. spumarius* and *A. salicina*, whose eggs hatched only after overwintering under natural conditions. On the contrary, several successive generations of *C. viridis* could be reared under laboratory conditions. These last three species have five nymphal instars. In limited quantitative field samplings, *C. viridis* was found to be locally very abundant (up to 37,000 eggs/m^2), and egg parasitism by a Mymarid wasp, *Anagrus incarnatus*, reached nearly 12%.

Keywords: *Philaenus spumarius*, *Aphrophora alni*, *Aphrophora salicina*, *Cercopis vulnerata, Cicadella viridis*.

Introduction

Xylella fastidiosa Wells *et al.*, 1987 (henceforth Xf) is a xylem-inhabiting bacterium responsible for important plant diseases in Americas (e.g. PURCELL, 1997; AGUILAR *et al.,* 2005; COLETTA-FILHO *et al.,* 2013). Xf subsp. *pauca* has been identified officially for the first time in Europe in 2013, in the Italian region of Apulia. The disease spread, affecting several host plant species, including olives (*Olea europaea* L., 1753), which are massively killed (EFSA PLH Panel, 2015; EPPO, 2015). Since this first discovery, various Xf subspecies as well as possible recombinants have been found in Corsica, in mainland southern France (CRUAUD *et al*., 2018), in parts of the Spanish mainland (Alicante, Madrid: LANDA *et al*., 2017), as well as in the Balearic Islands (OLMO *et al*., 2017). Xf was also detected in Germany in 2016 on a potted *Nerium oleander* (EPPO, 2016), in 2018 on *Lavandula dentata* L., 1753 in a zoo in North Portugal (EPPO, 2019a), in another Italian region (Tuscany) (EPPO, 2019b) and in the same year intercepted in a nursery in Belgium on potted *Olea europaea* imported from Spain (AFSCA, 2018). In September 2019, two olive trees were found contaminated by Xf in Antibes and Menton (MINISTÈRE DE L'AGRICULTURE ET DE L'ALIMENTATION, 2019). Apart from clear introductions as in some of the examples above, SOUBEYRAND *et al*. (2018), inferring pathogens dynamics from temporal count data, suggest that the emergence of Xf in Europe is probably not recent, with at least one introduction event possibly between 1978 and 1993.

Xf is transmitted by xylem-sap feeding leafhoppers and spittlebugs (Hemiptera, Cicadomorpha. REDAK *et al*., 2004). In Europe, only *Philaenus spumarius* (Linnaeus, 1758)*, P. italosignus* Drosopoulos & Remane, 2000 and *Neophilaenus campestris* (Fallén, 1805) (Hemiptera, Aphrophoridae) have been identified so far as vectors (SAPONARI *et al.*, 2014; CORNARA *et al.,* 2016, 2017; CAVALIERI *et al*., 2019); while *Euscelis lineolatus* Brullé, 1832 (Hemiptera Cicadellidae) was found positive for the presence of Xf in PCR assays (BEN MOUSSA *et al*., 2016). EFSA (2015) identified five species, widespread in Europe and usually polyphagous, as particularly likely to be relevant candidates: the cercopid *Cercopis vulnerata* Rossi, 1807, the aphrophorid spittlebugs *Philaenus spumarius*, *Aphrophora salicina* (Goeze, 1778) and *A. alni* (Fallén, 1805), and one sharpshooter, the cicadellid *Cicadella viridis* (Linnaeus, 1758). The aim of the present study was to assess the distribution and adult phenology of these five species in Belgium.

Material and methods

Geographic distribution in Belgium and adult phenology

Three sources were used:

1. The collections of the Royal Belgian Institute of Natural Sciences (RBINS) were searched and the following data for each specimen found were registered in a database: family, species, location and date of the catch, name of the collector. 3958 insects were identified in the RBINS collection: 414 *Cercopis vulnerata*, 465 *Cicadella viridis,* 2,709 *Philaenus spumarius*, 30 *Aphrophora salicina* and 340 *Aphrophora alni*.
2. The records of the website *Observations.be* (https://observations.be/info.php), from 2005 to 2016 were collated. This site was founded by Aves-Natagora and Stichting Natuurinformatie, and is the French version of Waarnemingen.be, founded by Natuurpunt and Stichting Natuurinformatie. The two sites feed the same database, which gathers the observations of a network of amateur and professional naturalists over the whole country. Some observations are validated by the site's managers, others are not. We chose to take only the validated observations into account, plus those made by experienced observers as measured by their overall record on the site. For each observation, the location, time (month and year) were recorded. Overall, *Observations.be* yielded information about 15,073 insects, including 3,709 *Cercopis vulnerata*, 10,126 *Cicadella viridis*, 830 *Philaenus spumarius*, 346 *Aphrophora alni* and 62 *Aphrophora salicina*.
3. Our own field collections. The sites were selected based on the information retrieved from the RBINS's collections and from *Observations.be*, taking care to cover the whole country and all its natural regions (Table 1). In 2016, the sampling was made between June and October because, according to the literature (NICKEL & REMANE, 2002; ARZONE, 1972; CHAUVEL *et al.,* 2015) and to *Observations.be* (2010-2015), adults of all five species are present during this period. However, we caught a very small number

of *Cercopis vulnerata*, and therefore chose to run an additional sampling campaign in May 2017 to increase our data relative to this species. The samplings were made using a sweep net. In each site, ten samples were taken, each sample comprising ten sweeps, each sweep consisting in three lateral movements of the net (left-right-left). The insects collected were identified using the BIEDERMANN & NIEDRINGHAUS (2009) key. Various data were associated with a database with each species caught in each sample: identification code, species, number of individuals, collection date, commune, name of the site, latitude, longitude, elevation, developmental stage, plants on which the insects were caught (family or vegetation type), name of the collector. Our samplings yielded 820 *Philaenus spumarius*, 747 *Cicadella viridis*, 385 *Cercopis vulnerata*, 211 *Aphrophora alni*, and 156 *Aphrophora salicina*. Table 2 summarises the information above.

Table 1. Sampling sites visited during our own surveys for the distribution assessment of potential vectors of *X. fastidiosa* in Belgium.

N° site	Administrative entity	Site name	Latitude	Longitude
1	Auderghem	Jardin Massart /Rouge-Cloître	50.8112	4.443
2	Woluwe-Saint-Pierre	Parc de Woluwé	50.8334	4.4237
3	Kampenhout	Voort	50.92969	4.5685
4	Aarschot	Vorsdonkbos	50.97424	4.803
5	Meeuwengruitrode	Groote Heide	51.05782	5.5194
6	Ranst	Vlostraat	51.2078	4.5988
7	Hérinnes	Léaucourt	50.6734	3.3624
8	Ieper	Palingbeek	50.8214	2.9136
9	Beloeil	Forêt de Beloeil	50.5387	3.7027
10	Zwienkerke	Polders	51.2626	3.1437
11	Brugge	Lisseweg (sand dune)	51.3267	3.1695
12	Aalter	Gazeplas	51.0679	3.4624
13	Landen	C. Gregoirestraat	50.7295	5.093
14	Herve	Abbaye de Val Dieu	50.6988	5.8029
15	Bullange	Baraque Michel	50.5192	6.0647
16	Gouvy	Limerlé	50.1599	5.9479
17	Durbuy	Rue des Aguesses 60	50.3391	5.4566
18	Hamois	Frisée	50.3777	5.1194
19	Gembloux	Rue du Monceau	50.5141	4.6845
20	Virton	Rue de Latour	49.5777	5.5849
21	Rouvroy	Chemin de la montagne	49.5116	5.4788
22	Rouvroy	Rue des Pâquis	49.5282	5.4766
23	Chiny	La Noue	49.7463	5.3495
24	Saint-Hubert	Bois de Tellin	50.0072	5.293
25	Libin	Air des Nutons	49.9996	5.2559
26	Chimay	Bois de Chimay	49.9762	4.3833

N° site	Administrative entity	Site name	Latitude	Longitude
27	Chimay	Rue Arthur Masson	49.9583	4.3615
28	Chimay	Rue des Juifs	50.0293	4.3419
29	Chimay	Rue Gustave Joaris	50.0624	4.3751
30	Chimay	Virelles N 978	50.0753	4.3713
31	Feneffe	Massif de Saint-Martin	50.2345	4.5045
32	Pont-à-Celle	Rue des Ronquières	50.5082	4.3831
33	Hensies	Marais d'Harchies	50.4515	3.6788
34	Merelbeke	Klarenhof	51.0061	3.7782
35	Ath	Pidebec	50.6763	3.7791
36	La Hulpe	Château de la Hulpe	50.7376	4.467
37	Herent	Schotstraat	50.899	4.663
38	Jemappe-sur-Sambre	N912	50.4806	4.6593

Table 2. Total numbers of insects reported by the three sources of information used in our study.

Source	*Philaenus spumarius*	*Cicadella viridis*	*Cercopis vulnerata*	*Aphrophora alni*	*Aphrophora salicina*	Total
RBINS collections	2,709	465	414	340	30	3,958
Observations.be (2005-2016)	830	10,126	3,709	346	62	15,073
Our surveys (2016-2017)	820	747	385	211	156	2,319
Total	4,359	11,338	4,508	897	248	21,350

Oviposition, egg winter diapause and voltinism

Field-collected adults of four of the five species (*A. alni*, *A. salicina, C. viridis* and *P. spumarius*) were reared from mid-July to mid-November 2016 on caged host plants in a glasshouse, under controlled conditions (photoperiod (L:D): 15:9; day temperature: 21°C; night temperature: 18°C; solar screen over 130w/m²). *C. vulnerata* was not collected in high enough numbers in 2016 and therefore was not included in the rearing. The *A. alni* adults did not survive more than one week and were thus also finally not reared. Clear PET cylinders (60 or 100 cm high and respectively 30 cm or 45 cm in diameter), closed on the top with mosquito mesh, were fitted to potted plants. The following host plant species were selected, according to literature information and complementary advice from Jean-Yves Baugnée (DEMNA, Gembloux).

- *A. alni*: *Alnus glutinosa* (L.) Gaertn., 1790**;** *Rosmarinus officinalis* L., 1753; *Salix babylonica* L., 1753;
- *A. salicina*: *Salix babylonica*; *S. purpurea* L., 1753; *S. alba* Thunb., 1784;
- *C. viridis*: *Juncus effusus* Pollich 1776; *Ranunculus acer* L., 1753; *Mentha spicata* L., 1753;

- *P. spumarius*: *Medicago lupulina* L., 1753; *Rosmarinus officinalis*; *Mentha spicata*; *Ocimum basilicum* L., 1753.

In our breeding conditions, oviposition was observed in September for *A. salicina*, early October for *P. spumarius* and late October /early November for the second generation of *C. viridis*.

The eggs were kept on the host plants until the end of the oviposition period. In mid-November, they were transferred to clear polystyrene Petri dishes (8 cm in diameter), with a moist layer of Paris plaster on the bottom, in which they were kept between two layers of moist filter paper. These Petri dishes were then split in two groups. One group was kept in the laboratory under controlled conditions (21.5°C; photoperiod: 15:9); the second group was kept outside under natural conditions, under a clear plastic tunnel greenhouse (local temperatures during this period: see Table 3).

Table 3. Temperature conditions to which the eggs were submitted during the outdoor overwintering period.

Month	**Mean temperature (°C)**	**Minimal temperature (°C)**	**Maximal temperature (°C)**
November 2016	6,2	-4,5	16,0
December 2016	4,6	-2,8	13,4
January 2017	1,3	-6,9	12,6
February 2017	6,5	-3,3	17,7
March 2017	10,7	-0,1	31,0
April 2017	9,6	1,5	26,6
May 2017	15,7	4,7	30,5

Larval phenology and development

Immediately after hatching, the larvae produced in the oviposition experiments were transferred to potted plants kept outdoors. The larvae were monitored from early April to mid-June 2017. They were delicately collected twice a week, measured with a Leica MZ6 stereomicroscope equipped with a Leica DFC295 camera and measuring device, and then brought back to the plants.

Complementary observations on *Cicadella viridis*

Field sampling was prepared in order to assess egg density in a natural population. As a first step, the size of oviposition slits on *J. effusus* stems was measured in the laboratory and related to the number of eggs within. Then, five samples of *J. effusus* covering each a 30 x 30 cm area were collected in La Hulpe. Two samples were taken in a humid meadow, and the other three were taken on the banks of a pond, also in humid conditions. The following measurements were then taken in the laboratory: number of *J. effusus* stems per sample; number of stems with one or more oviposition slits; length of each stem; position and length of the slits on each stem.

Egg parasitism was measured, also at La Hulpe, in January 2017, by randomly collecting three bunches of *J. effusus* stems presenting oviposition slits. These stems were kept at 21.5°C in the laboratory, on moist filter paper in Petri dishes with a moist plaster layer on the bottom. The parasitized eggs were visually identified and counted.

Table 4. Summary data relative to our field surveys.

Insect species	Total catches	Number of sites where found	Host plant species, genus or family
Philaenus spumarius	820	36/36	*Alnus* sp.; Asteraceae; *Carex* sp.; *Crataegus* sp.; Fabaceae; *Picea* sp.; Poaceae; *Prunus laurocerasus*; *Quercus* sp.; Renonculaceae; *Rubus* sp.; *Salix* sp.; *Urtica* sp.
Cicadella viridis	747	28/36	*Alnus* sp.; Asteraceae; *Carex* sp.; *Corylus avellana*; *Crataegus* sp.; Ericaceae; Fabaceae; *Juncus* sp.; *Pinus* sp.; Poaceae; *Primula* sp.; *Prunus Laurocerasus*; *Quercus* sp.; Renonculaceae; *Rubus* sp.; *Silene* sp.; *Urtica* sp.; *Urtica* sp.
Cercopis vulnerata	385	21/36	*Alnus* sp.; Asteraceae; *Carex* sp.; *Cornus mas*; *Corylus avellana*; *Crataegus* sp.; Ericaceae; Fabaceae; *Fagus sylvatica*; *Picea* sp.; Poaceae; *Prunus laurocerasus*; *Quercus* sp.; Renonculaceae; *Rubus* sp.; *Salix* sp.; *Sambucus nigra*; *Urtica* sp.
Aphrophora alni	211	28/36	*Alnus glutinosa*; *Alnus* sp.; Asteraceae; *Betula* spp.; *Carex* sp.; *Corylus avellana*; *Crataegus* sp.; Ericaceae; Fabaceae; *Fagus sylvatica*; *Juglans regia*; *Malus domestica*; *Pinus* sp.; Poaceae; *Populus* sp.; *Prunus laurocerasus*; *Quercus* sp.; Renonculaceae; *Salix* sp.; *Sambucus nigra*; *Urtica* sp.;
Aphrophora salicina	156	6/36	*Salix* sp.

Results

Philaenus spumarius

Distribution in Belgium

It is present in all the country's ecological regions (Fig. 1). During our field samplings, it was found in all 36 sites. The insects were caught in all environments, on ground vegetation (in meadows, dunes, bogs) as well as on trees and bushes (willows, oaks, birches…) (Table 4).

Adult phenology

The adults can be found from April to November, with higher population from June to September (Fig. 2).

Oviposition, egg winter diapause and voltinism

In the population observed in the laboratory, the eggs were observed at the base of grass blades, protected by a layer of hardened foam. In this univoltine species, the eggs overwinter and need a winter period to overcome diapause.

Immature development under outdoor conditions

It took place between early April and early June (Fig. 3). There are five instars, with spittle protection. Immature development lasted 53 to 63 days, with adults appearing between May 29 and June 6.

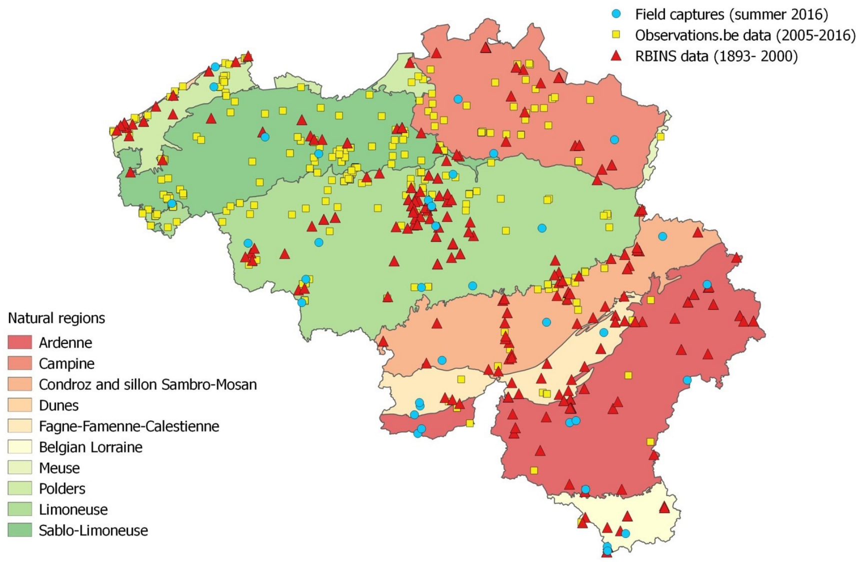

Fig. 1. Distribution map for *Philaenus spumarius* in Belgium. Data: RBINS; *Observations.be* (2010-2016); our own samplings (2016-2017).

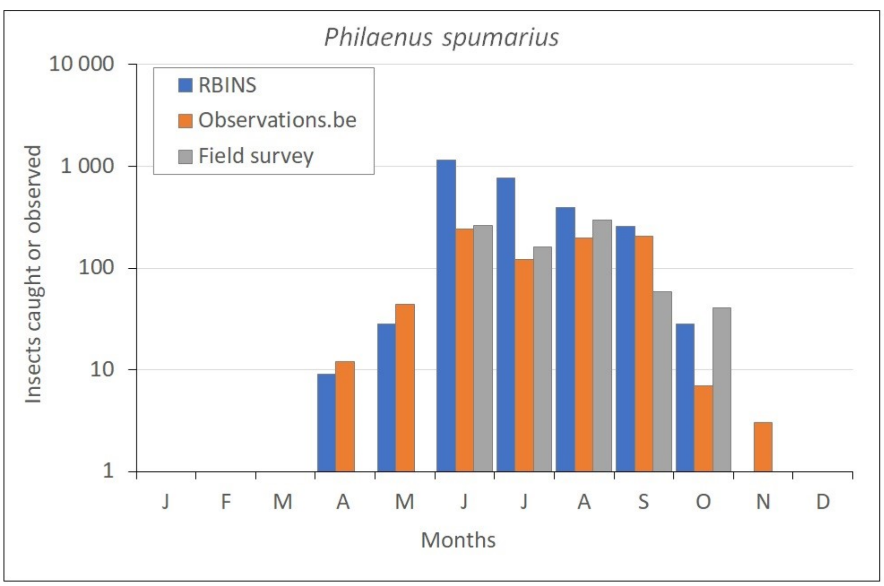

Fig. 2. Phenology of adult *Philaenus spumarius* in Belgium: Data: RBINS; *Observations.be* (2005-2017); our own samplings (2016-2017).

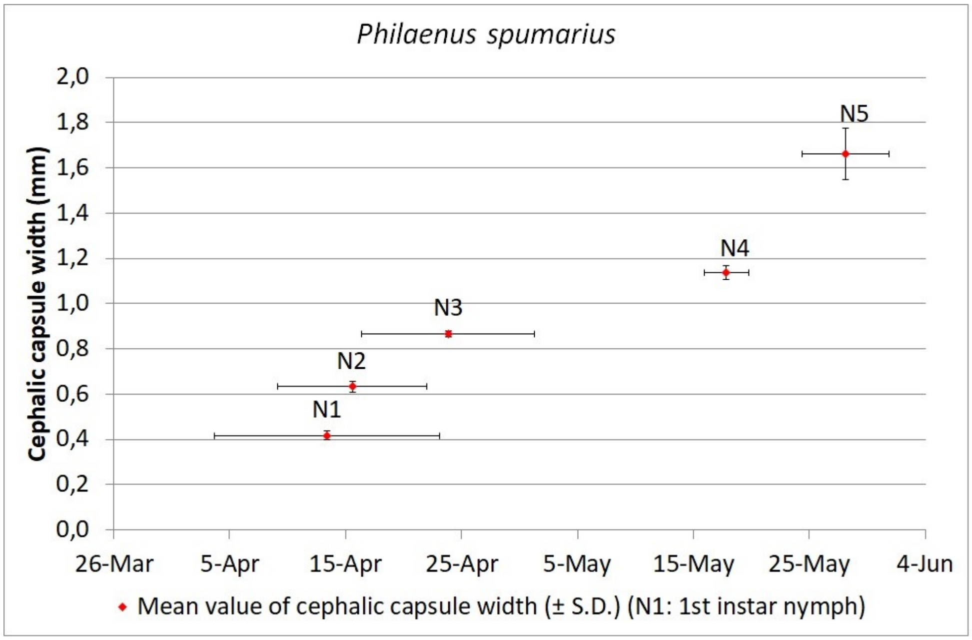

Fig. 3. Immature development of *Philaenus spumarius* under outdoor conditions (Ixelles, Belgium, 2016).

Cicadella viridis

Distribution in Belgium

It was found in all the ecological regions of the country (Fig. 4). We collected it in 28 sites out of 36.

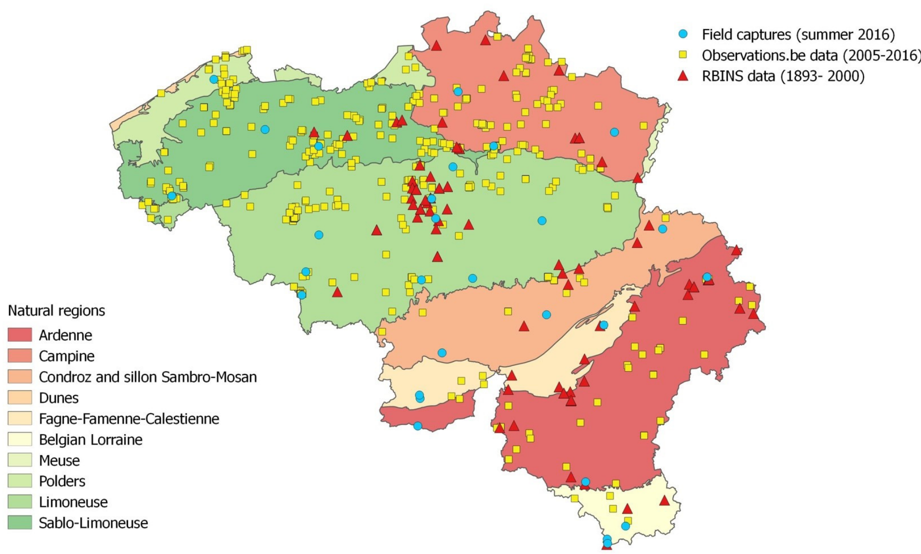

Fig. 4. Distribution map for *Cicadella viridis* in Belgium. Data: RBINS; *Observations.be* (2010-2016); our own samplings (2016-2017).

Adult phenology

Adults were found from May to December (Fig. 5). We collected them in higher number from June to September, mostly in humid meadows but also along roads and in dry meadows, on a large range of plants but mostly on *Juncus* spp. (Table 4).

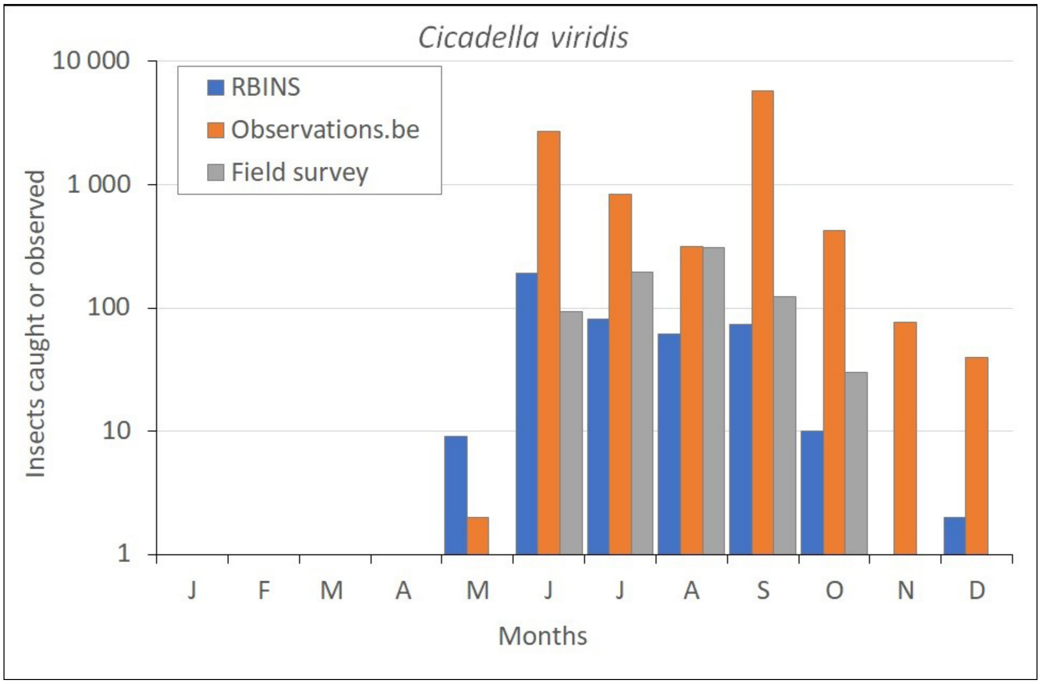

Fig. 5. Phenology of adult *Cicadella viridis* in Belgium. Data: RBINS; *Observations.be* (2005-2017); our own samplings (2016-2017).

Oviposition, egg winter diapause and voltinism

Oviposition was observed in slits inside *Juncus* stems. The eggs overwinter and there is no diapause.

Immature development

Under outdoor conditions, development of the first generation started in late April and ended in mid-June (Fig. 6A). There are five instars and nymphs are mobile, without spittle protection. The cephalic capsule measures between 0,5–1,6 mm according to nymphal instar. Immature development lasts from 49 to 62 days. The adults appeared between June 6 and 19 (Fig. 6A).

Under controlled conditions, immature development lasted 21–27 days. The last 5th instar nymphs appeared after 25 days (Fig. 6B).

Local abundance in the field

37 oviposition slits were measured and the eggs therein were counted. The slits were 3 to 8 mm long (mean: 5,9 ± 1,1 mm) and contained a mean number of 10,3 ± 2,4 eggs. There was a linear relationship between the slits' length and the eggs they contained (y=1,7662x; R^2=0,7177; p<<0.001), corresponding to 1.8 eggs/mm in the slits. 32% to 83% of the *Juncus* stems in the samples contained eggs. Extrapolating from the mean length of oviposition slits in each sample and from the linear relationship between slit length and egg load, we estimated that there were ca 500 to 3,300 eggs in the samples, corresponding to a density of 6,000 to 37,000 eggs per m^2 (Table 5).

Table 5. Results of the field assessment of oviposition by *C. viridis* (La Hulpe, Belgium).

Sample	Nº of *Juncus* stems	Nº stems with eggs	% stems with eggs	Length of oviposition slits (cm)	Calculated Nº eggs	Nº eggs/m²
1	106	54	50,9	2.6±2	969.6	10 773.7
2	151	48	31,8	3.3±3.2	823.0	9 144.9
3	35	29	82,9	5±4	1 564	17 377.3
4	105	28	26,7	4.6±3.8	531.6	5 906.9
5	91	50	54,9	6.8±5.7	3 326.5	36 960.3

Parasitism by the egg-parasitoid Anagrus incarnatus *(Hymenoptera, Mymaridae)*

Parasitism rates varied from 3.48% to 11.77% in the samples, with an average of 6.67% (Table 6).

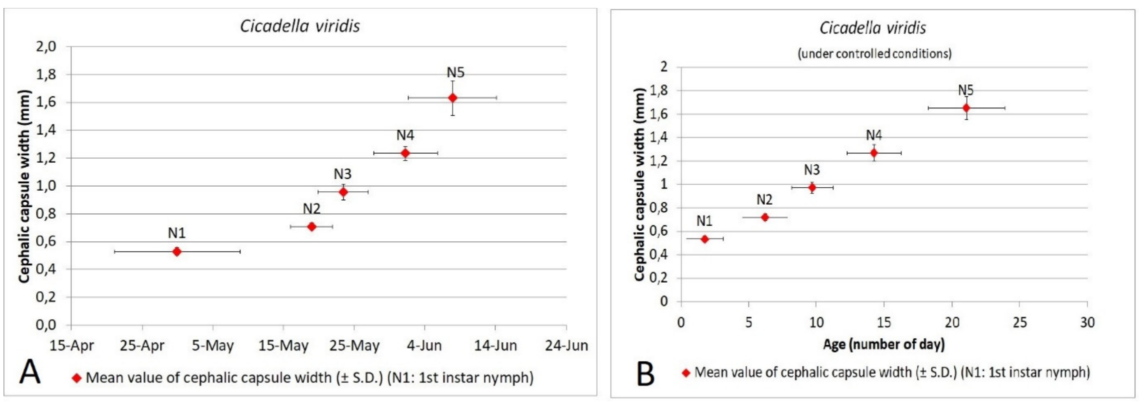

Fig. 6A. Immature development of *Cicadella viridis* under outdoor conditions (Ixelles, Belgium, 2016.

Fig. 6B. Immature development of *Cicadella viridis* under controlled conditions (21.5°C; D:L= 9:15).

Cercopis vulnerata

Distribution in Belgium

C. vulnerata was found in all the ecological regions of the country (Fig. 7), in 21 sites out of 36, in very diverse environments (meadows, vineyard, urban parks, forest edges, orchards, roadsides) and on various plants, mainly grasses, but also tree species such as oaks, fruit trees, elderberries, dogwoods (Table 4).

Adult phenology

The adult stage is relatively short, occurring mostly in May, with a few individuals to be found in April and in June-August (Fig. 8).

Aphrophora alni

Distribution in Belgium

A. alni was found in all the ecological regions of the country (Fig. 9). It was collected in 28 sites out of 36, in various environments (tree rows, meadows, urban parks, forest edges, orchards) and on various plants (Table 4).

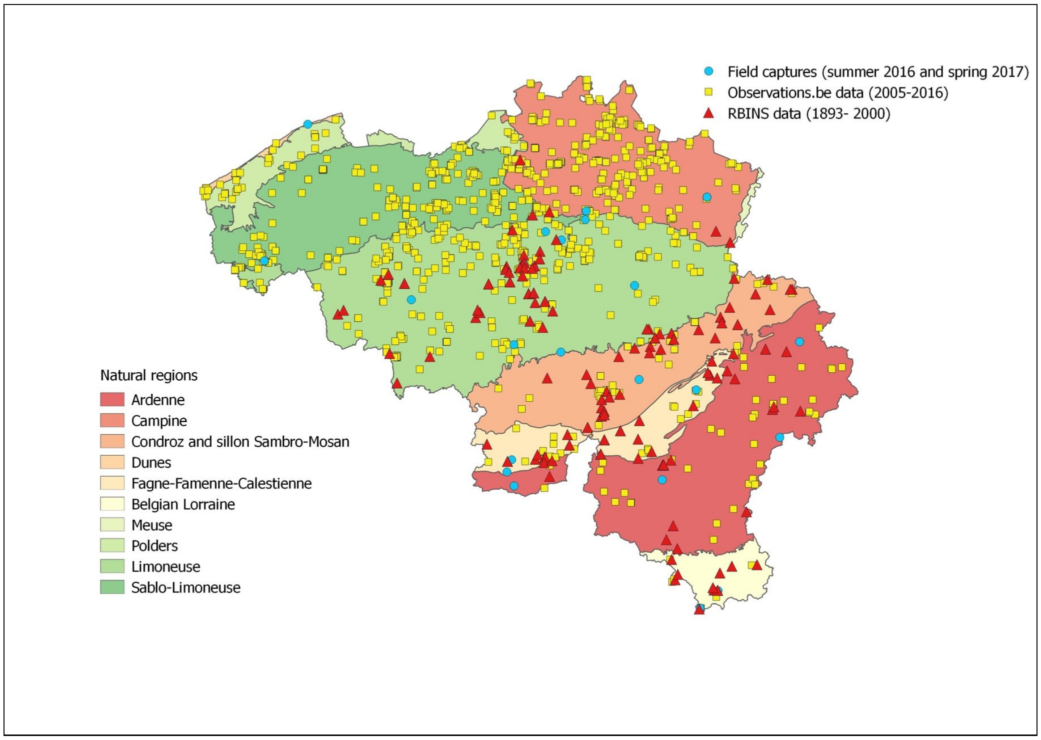

Fig. 7. Distribution map for *Cercopis vulnerata* in Belgium: Data: RBINS; *Observations.be* (2010-2016); our own samplings (2016-2017).

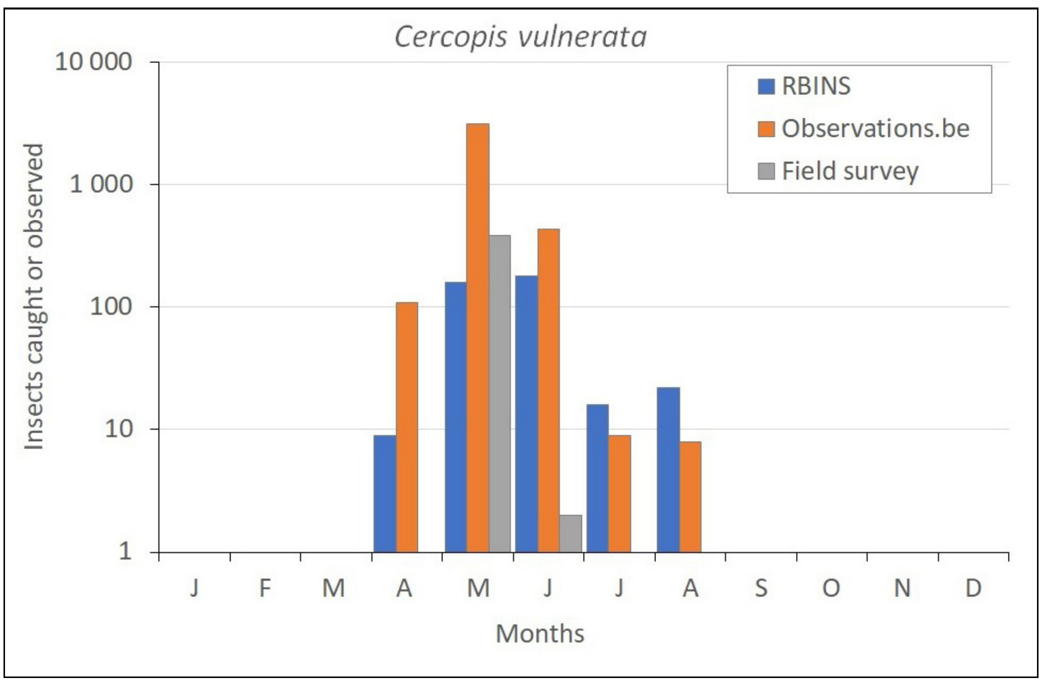

Fig. 8. Phenology of adult *Cercopis vulnerata* in Belgium. Data: RBINS; *Observations.be* (2005-2017); our own samplings (2016-2017).

Adult phenology

Adults were caught from May to November (Fig. 10), with a peak in July (our samplings) or two peaks, in July and September respectively (*Observations.be*).

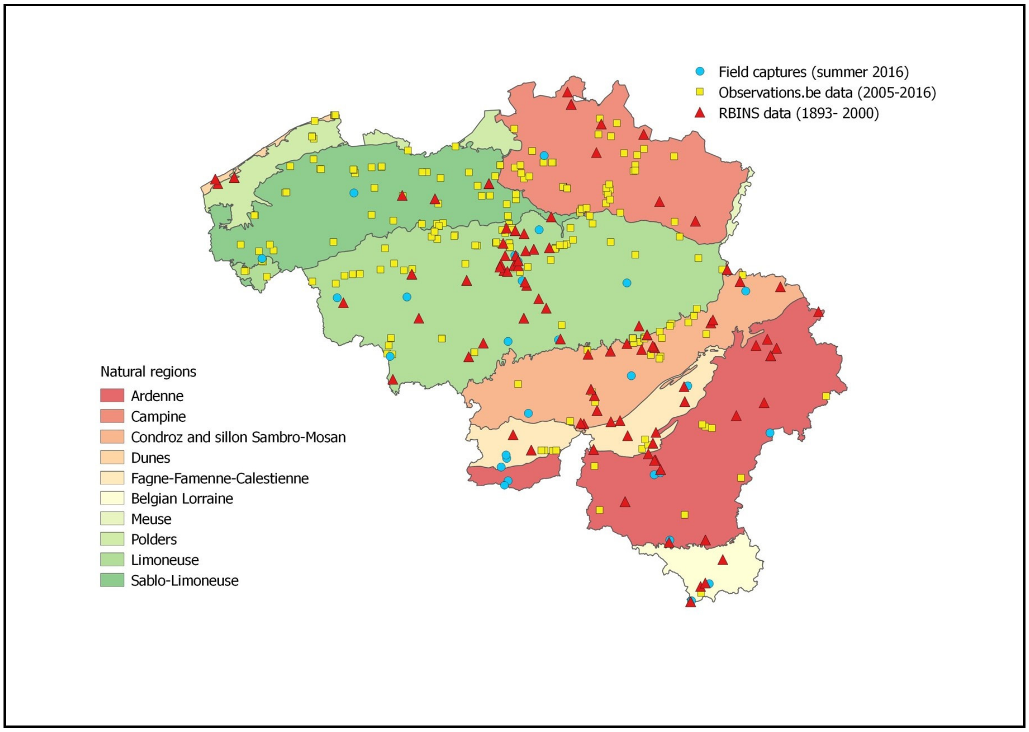

Fig. 9. Distribution map for *Aphrophora alni* in Belgium. Data: RBINS; *Observations.be* (2010-2016); our own samplings (2016-2017).

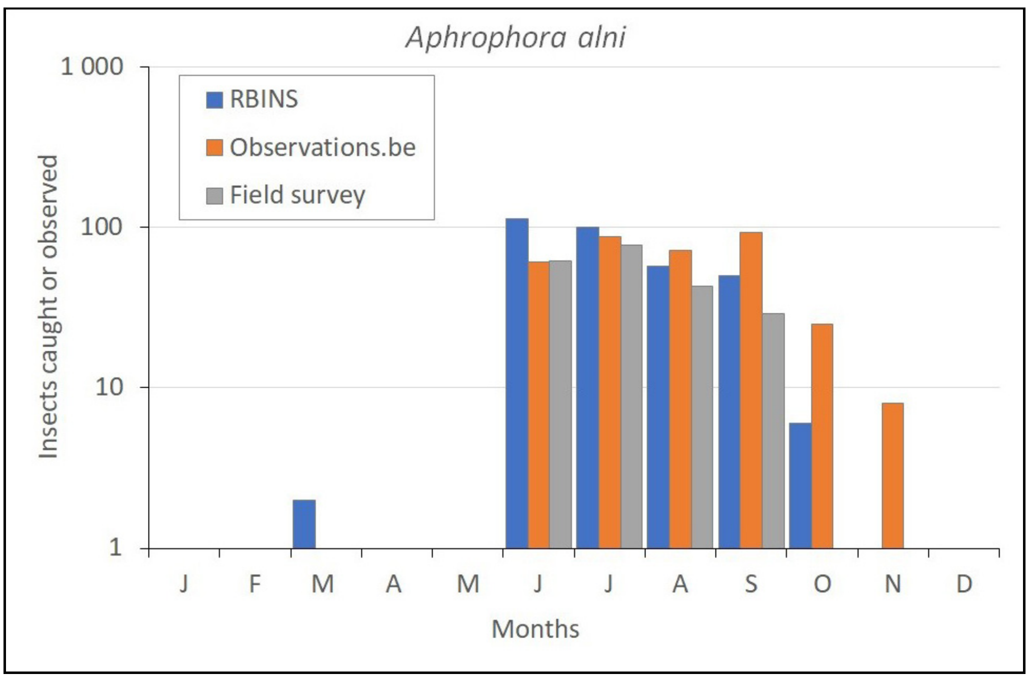

Fig. 10. Phenology of adult *Aphrophora alni* in Belgium. Data: RBINS; *Observations.be* (2005-2017); our own samplings (2016-2017).

Aphrophora salicina

Distribution in Belgium

A. salicina was not found to be very widespread in the country (Fig. 11). We caught it only in 6 sites out of 36, always on *Salix* spp. (table 4).

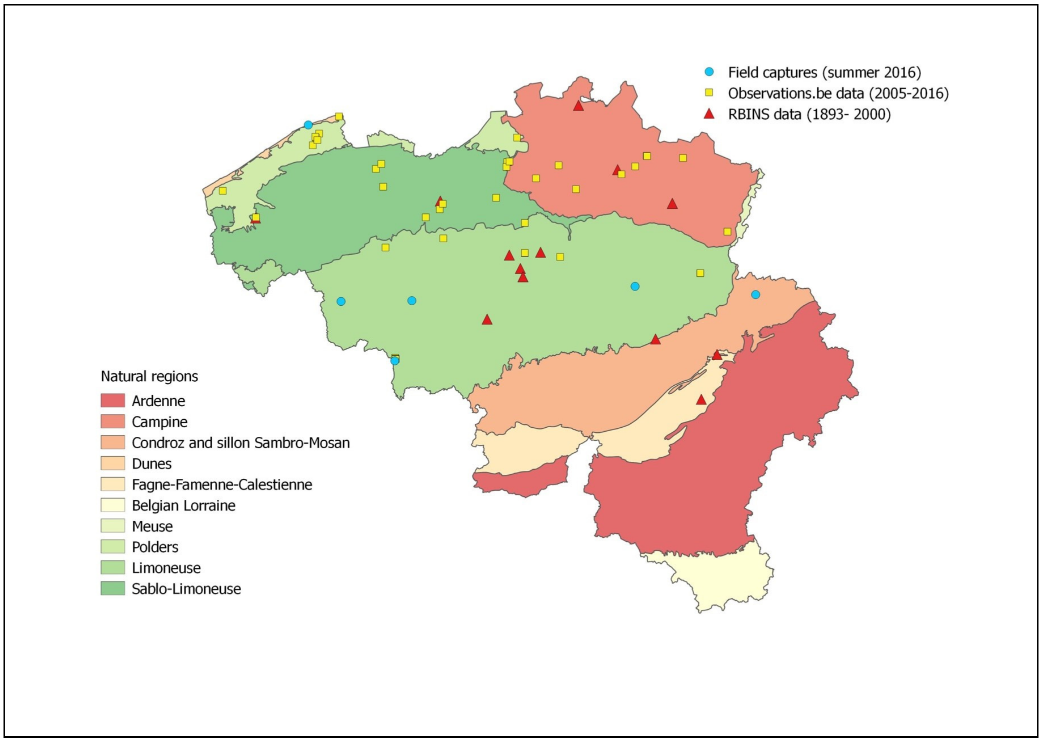

Fig. 11. Distribution map for *Aphrophora salicina* in Belgium. Data: RBINS; *Observations.be* (2010-2016); our own samplings (2016-2017).

Adult phenology

Adults were found from June to October (Fig. 12). We caught *A. salicina* in higher numbers in July and August.

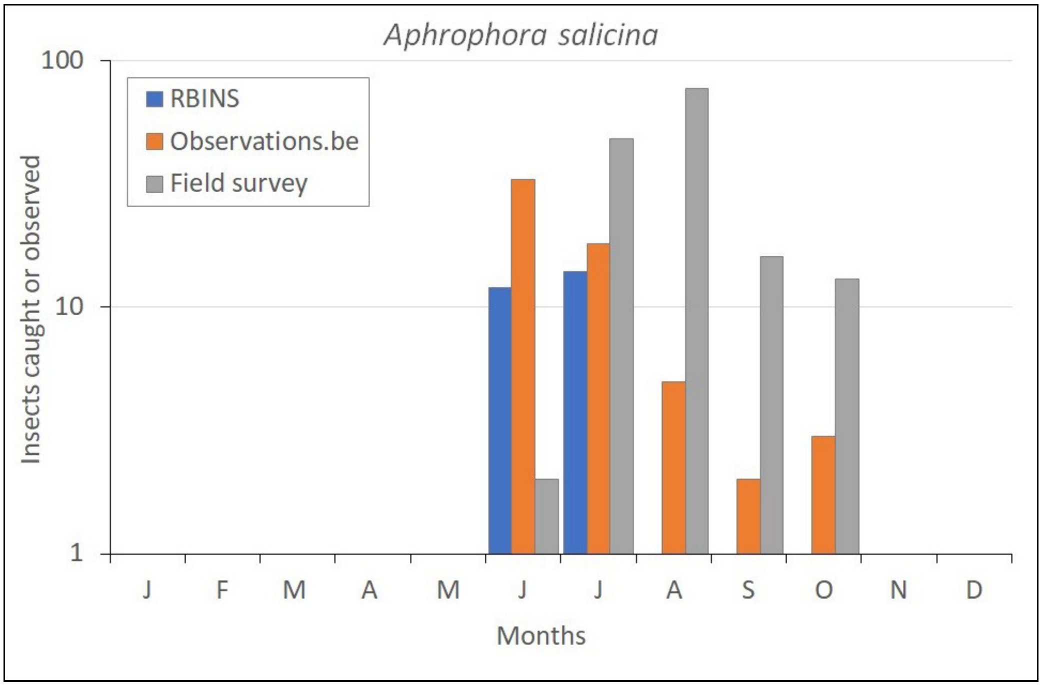

Fig. 12. Phenology of adult *Aphrophora salicina* in Belgium. Data: RBINS; *Observations.be* (2005-2017); our own samplings (2016-2017).

Oviposition, egg winter diapause and voltinism

Oviposition was observed in slits at the tip of *Salix* shoots. In this univoltine species, the eggs overwinter and need a winter period to lift diapause.

Immature development under outdoor conditions

It took place between early April and early June (Fig. 13). There are five instars, with spittle protection. The cephalic capsule width was 0.52,0 mm according to nymphal instar. Immature development lasted 63–76 days. The adults appeared between June 6 and 19.

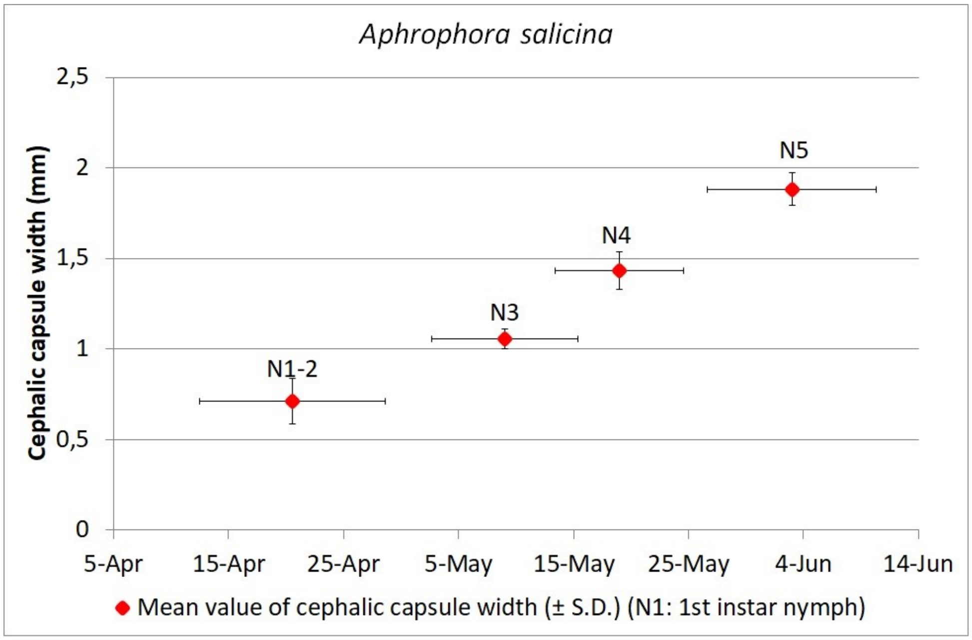

Fig. 13. Immature development of *Aphrophora salicina* under outdoor conditions (Ixelles, Belgium, 2016.

Discussion

The data relative to a total of 21,350 insects were used for this survey: 3,958 (18.5%) from the RBINS collections, 15,073 (70.6%) from *Observations.be* and 2,319 (10.9%) from our own surveys (Table 2). In order of abundance in the collections and surveys (which does not necessarily translate into abundance in the field as these records are clearly not intended to provide relative abundance of species), the species can be ranked as follows according to the RBINS collections and our own surveys: *P. spumarius* > *C. viridis* > *C. vulnerata* > *A. alni* > *A. salicina.* The data from *Observations.be* show another ranking: *C. viridis* > *C. vulnerata* > *P. spumarius* > *A. alni* > *A. salicina*. The reasons for this discrepancy are unclear, but it is possible that, being easier to identify, *C. viridis* and *C. vulnerata* have been notified more often than the less recognisable (and highly polymorphic) *P. spumarius*.

P. spumarius was not only found in higher numbers in our survey, it was also found in all sites. This highly polyphagous insect (WEAVER & KING, 1954; CORNARA *et al.,* 2018) is the most abundant species in olive groves of Italy, where it acts as a vector of Xf (BEN MOUSSA *et al*., 2016; DI SERIO *et al*., 2019; DONGIOVANNI *et al*., 2019). *P. spumarius,* with *Neophilaenus campestris,* is also the main spittlebug species in olive plantations in the Iberian peninsula (MORENTE *et al*., 2018b). *C. viridis*, *C. vulnerata* and *A. alni* were also found on many plant species although, according to ARZONE (1972), *C. viridis* prefers *Juncus* spp. and *Ranunculus* spp. for summer oviposition, *Juncus* spp., *Alnus glutinosa* and *Fraxinus excelsior* for autumn oviposition, and Poaceae for feeding. According to EFSA (2015) and NICKEL & REMANE

(2002), *A. alni* has a preference for *Alnus* sp. The multiple hosts on which we found these species also explain why they were found in most sites. At the opposite, *A. salicina,* which is unanimously considered as genus-specific on *Salix* (EFSA, 2018; ENDRESTOL, 2013; GERMAIN, 2016; KMIEĆ *et al.,* 2018; KULA, 2002; NICKEL, 2008; NICKEL & REMANE, 2002; SELJAK, 2004; SÖDERMAN *et al.*, 2009), was found only in six sites, all containing willows. It was absent, however, from two other sites also containing willows.

Fig. 14. *Aphrophora salicina* eggs in a willow twig.

Polyphagy, ubiquity and abundance would favour the vectoring of Xf by *P. spumarius*, *C. viridis*, *C. vulnerata* and *A. alni*. The very narrow adult phenological window of *C. vulnerata*, however, would probably reduce its role as a vector. *A. salicina* would be able to transmit the bacteria only between neighbouring *Salix* sp. trees, but transmission at longer range would require either passive transportation of the insects in vehicles or alternatively, other, more polyphagous vector species able to use other plants as "stepping stones" between *Salix* sp. trees.

Our rearing experiments showed that it is possible to produce several generations of *C. viridis* within one year. This could be used for experimental acquisition experiments. MORENTE *et al.* (2018a) recently succeeded to shorten the life cycle of *P. spumarius* to three months, also opening new experimental prospects with this species.

Table 6. Egg parasitism of *Cicadella viridis* by *Anagrus incarnatus* in three samples from La Hulpe.

Sample	Nº of eggs	Nº of parasitised eggs	Proportion of parasitised eggs (%)	Nº of parasitoids
1	302	16	6.79	32
2	244	10	3.48	20
3	168	19	11.77	38
Total	714	45	6.67	90

Parasitism by *Anagrus incarnatus* Haliday, 1833 was widespread in the sampled site, affecting on the average nearly 7% of the eggs (Table 6). *A. incarnatus* is a facultative gregarious parasitoid, producing one to eight adults from each single parasitised egg, depending on the size of the host egg (TRIAPITSYN *et al*., 2018). In our samples, two adults emerged from each parasitized *C. viridis* egg (Table 6). In a study in Italy, ARZONE (1972) found that one to seven adults per parasitized egg were produced. She identified four egg parasitoids associated to *C. viridis*: three mymarids (*Polynema woodi* Hincks, 1950, *Gonatocerus longicornis* Nees, 1834 [as its current synonym *Gonatocerus cicadellae*], *Anagrus incarnatus*) and one trichogrammatid (*Oligosita krygeri* Girault, 1929). These four species together parasitised 30-35% of the *C. viridis* eggs investigated, and *A. incarnatus* was responsible for 5-8% parasitism, a figure similar to ours.

Fig. 15. *Cicadella viridis* eggs in common rush.

A. incarnatus is a widespread species in the Holarctic and Oriental regions attacking a large number of leafhoppers and planthoppers (TRIAPITSYN *et al*., 2018), among which several species such as the delphacid *Nilaparvata lugens* Stâl, 1854 on rice, are important agricultural pests. *A. atomus* Linnaeus, 1767 is known as a natural enemy of the cicadellid *Empoasca vitis* (Göthe, 1875) in Europe (DAANE *et al.,* 2018). If *C. viridis* proved to be effectively a vector for Xf, *A. incarnatus* might be considered as a promising component of conservation biological control. We did not investigate the natural enemies of the other potential vector species, which constitute potentially promising areas of research as well.

Much more additional knowledge is still needed on potential vectors for *Xylella fastidiosa*. Of particular interest is the actual lifetime efficacy of these species as vectors, i.e. their real dispersal capacity over a lifetime, their ability to use alternative host plants as "stepping stones" in a landscape to vector bacteria over large distances and bacterial retention time in these insects. Laboratory acquisition and flight mill experiments, field release-recaptures and modelling might yield new and useful information in this respect.

Some other species would also deserve closer scrutiny. According to *Observations.be* (2019), the xylem-sap sucking species *Neophilaenus campestris* (Fallén, 1805) and *N. lineatus* (Linnaeus, 1758) appear to be less frequent in Belgium than the species we investigated, but *N. campestris* has been identified as a vector of Xf (CAVALIERI et al., 2019). The North-American *Graphocephala fennahi* Young, 1977 (Cicadellidae), presently well established in Europe (DE JONG et al., 2014), including Belgium (OBSERVATIONS.BE, 2019), is also a potential vector worth to be followed (DI SERIO *et al*., 2019)

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