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Article

The control of the American leafhopper *Erasmoneura vulnerata* (Fitch) in European vineyards: impact of conventional and natural insecticides

Paola Tirello [1], Enrico Marchesini [2], Pamela Gherardo [1], Damiano Raniero [1], Filippo Rossetto [1], Alberto Pozzebon [1], Carlo Duso [1,*]

[1] Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, Agripolis, Legnaro, 35020 Padova, Italy; paola.tirello@unipd.it (P.T.); filippo.rossetto.1@studenti.unipd.it (F.R.); pamela.gherardo@studenti.unipd.it (P.G.); damiano.raniero@studenti.unipd.it (D.R.); alberto.pozzebon@unipd.it (A.P.)
[2] AGREA S.r.l. Centro Studi, Via Garibaldi 5/16, San Giovanni Lupatoto (VR), Italy; enrico.marchesini@agrea.it (E.M.)
* Correspondence: carlo.duso@unipd.it; Tel.: +39-049-8272805 (C.D.)

Simple Summary: *Erasmoneura vulnerata*, a grapevine leafhopper rarely harmful in North America has become a new pest in European vineyards. Winegrowers are worried because of severe leaf symptoms potentially associated to yield losses and the nuisance when large numbers of adults occur at harvest time. Outbreaks were detected in conventional vineyards despite the use of broad-spectrum insecticides as well as in organic vineyards treated with pyrethrins. Therefore, the identification of effective control tools is required. Studies on *E. vulnerata* phenology established that the second generation produces the largest population densities. We planned field trials to establish the most effective insecticides to be applied in conventional and organic vineyards. The most effective conventional insecticides were acetamiprid, flupyradifurone and lambda-cyhalothrin while the most effective natural product was kaolin.

Abstract: The American leafhopper *Erasmoneura vulnerata*, detected in Europe in early 2000s, has recently become a pest in North-Italian vineyards. Issues were recorded in organic and conventional vineyards despite the application of insecticides devoted to the control of other pests. *Erasmoneura vulnerata* completes three generations, and the second generation is frequently associated to large populations. The selection of appropriate active ingredients and the timing of their application is crucial for effective pest control. Field trials were carried out in North-eastern Italy, using a randomized design, to evaluate the impact of insecticides applied against other grapevine leafhoppers on *E. vulnerata* populations. The beginning of the second generation was selected as best timing for insecticide application. For a number of natural products, two applications were planned. Among the selected insecticides, the most effective were acetamiprid, flupyradifurone and lambda-cyhalothrin. Regarding natural products, the most effective was kaolin that could represent an alternative to pyrethrins in organic vineyards. The identification of pest threshold levels and the evaluation of side effects of the most effective insecticides on key natural enemies occurring in vineyards are required.

Keywords: *Erasmoneura vulnerata*; *Vitis vinifera*; insecticides; vineyard management

1. Introduction

The most important leafhoppers in European vineyards are *Empoasca vitis* (Göthe) and *Scaphoideus titanus* Ball. *Empoasca vitis* has been considered a pest in France, Italy, Switzerland, and other countries [1-7]. Traditionally, its control has been achieved using insecticides, sometimes specific (e.g., pyrethroids in France) or devoted to the control of

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berry moths and leafhoppers (e.g., organophosphates and chitin-inhibitors in Italy and Switzerland) [8-10]. In the 1990s, organophosphates' effectiveness in controlling *E. vitis* declined probably because of pesticide resistance [11]. At the same time research showed that *E. vitis* populations are limited by a number of natural enemies, namely the Hymenoptera Mymaridae [e.g., 12-16]. These findings, improved knowledge on cultivar susceptibility, and the adoption of action thresholds has reduced the attention posed on *E. vitis*. *Scaphoideus titanus* is the main vector of phytoplasma strains of the elm yellows group (16SrV) involved in the Flavescence dorée, a destructive disease for European vineyards [17-19]. In the 1990s Flavescence dorée phytoplasma was declared a quarantine pest by the EU and control measures were made mandatory in France and Italy. Although chemical control is considered crucial in this framework, issues with Flavescence dorée spread [20-22]. In contrast with *E. vitis*, the efficacy of organophosphates towards *S. titanus* has remained satisfactory [11] and thus, resistance is not a concern for this pest. Problems with Flavescence dorée are serious in organic vineyards where growers can use only natural products (e.g., pyrethrins) characterized by limited effectiveness and persistence [23,24]. Recently, new compounds replaced organophosphates in most viticultural areas and some of them (e.g., neonicotinoids) were very effective against leafhoppers [25-27]. However, in the last two years, several active ingredients have been banned in Europe and some of the remaining insecticides showed lower effectiveness against leafhoppers occurring in vineyards. The selection of active ingredients has become more important than in the past and chemical control must be integrated with agronomic and cultural measures to obtain adequate control of grapevine leafhoppers [20,22,28].

In this context, the American leafhopper *Erasmoneura vulnerata* (Fitch) (Hemiptera: Cicadellidae), first detected in Europe in 2004 [29], has become a pest in vineyards [30]. Although earlier records considered this species very harmful in North America, recent findings consider it a minor pest in leafhopper communities [31,32]. Initially *E. vulnerata* was localized in unsprayed vineyards in North-eastern Italy, then it spread to North-western Italy, Slovenia and Switzerland [33-35]. Currently, outbreaks involve both conventional and organic vineyards located in North-eastern Italy, particularly in the Veneto region [36]. Winegrowers are worried because of severe symptoms (leaf discoloration and leaf fall) potentially associated to yield losses and the nuisance to grape pickers when large numbers of adults are active at harvest time. Issues with *E. vulnerata* were detected, although insecticides were applied against *S. titanus*. Investigations on *E. vulnerata* biology, ecology and behavior were planned to implement effective control measures. This pest can complete three generations per year. Overwintered adults can damage shoots at sprouting, but the first nymph generation is usually not harmful. The second generation is associated with the highest population densities, while the third is sometimes a problem [36]. At the same time, we conducted field trials to evaluate the impact of insecticides used against other leafhoppers on *E. vulnerata* populations. The most interesting results obtained in chemical control trials are reported here.

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2. Materials and Methods

The effects of a number of insecticides on *E. vulnerata* populations were evaluated in three conventional vineyards located in Vicenza and Verona provinces (Veneto region, North-eastern Italy) during the 2017, 2018 and 2019 growing seasons. In 2017 trials were carried out in a vineyard located in the Vicenza plain (Lonigo, cv. Garganega, Sylvoz training system, planting space 2,70 m x 1,40 m). In 2018 a hilly vineyard (Monteforte d'Alpone, cv. Trebbiano, Guyot training system, planting space 2,30 m x 0,9 m) located in the Verona province was selected for trials. The vineyard selected in 2019 (Colognola ai Colli, cv. Garganega, pergola veronese training system, planting space 3,70 m x 0,90 m) was also located in the Verona province. In these vineyards, the occurrence of *E. vulnerata* had been reported in the season preceding the study. Insecticides commonly applied in vineyards (active ingredients authorized in the EU and products authorized in Italy) and other products (e.g., kaolin) potentially useful for leafhoppers control were selected for trials (Table 1). An untreated control was included in each trial.

Table 1. Insecticides selected for field trials.

Year	Active ingredients	Trade mark	Concentration	Dose	Group	Number of applications
2017	Untreated control	-	-	-	-	-
	Acetamiprid	Epik SL	50 g/L	150 mL/hL	Neonicotinoids	1
	Thiamethoxam	Actara 25 WG	25%	20 g/hL	Neonicotinoids	1
	Lambda-cyhalothrin	Karate Zeon	9.40%	25 mL/hL	Pyrethroids	1
	Buprofezin	Applaud Plus	25.00%	200 g/hL	Thiadiazines	1
	Chlorpyrifos-methyl	Reldan LO	21.40%	150 mL/hL	Organophosphates	1
	Pyrethrins	Biopiren Plus	18.6 g/L	160 mL/hL	Pyrethrins	2
	Pyrethrins+ mineral oil	Biopiren Plus Chemol Plus	18.6 g/L 80%	160 mL/hL 500 mL/hL	Pyrethrins Mineral oils	2
	Potassium salts	Ciopper	455 g/L	150 mL/hL	Salts	2
	Mineral oil	Chemol Plus	80%	500 mL/hL	Mineral oils	2
	Kaolin	Surround	95%	4 Kg/hL	Kaolin	2
2018	Untreated control	-	-	-	-	-
	Acetamiprid	Epik SL	50 g/L	150 mL/hL	Neonicotinoids	1
	Lambda-cyhalothrin	Karate Zeon	9.40%	25 mL/hL	Pyrethroids	1
	Chlorpyrifos-methyl	Reldan LO	21.40%	150 mL/hL	Organophosphates	1
	Pyrethrins	Biopiren Plus	18.6 g/L	160 mL/hL	Pyrethrins	2
	Potassium salts	Ciopper	455 g/L	150 mL/hL	Organic salts	2
	Mineral oil	Chemol Plus	80%	500 mL/hL	Mineral oils	2
	Kaolin	Surround	95%	4 Kg/hL	Kaolin	2
2019	Untreated	-	-	-	-	-
	Acetamiprid	Epik SL	50 g/L	150 mL/hL	Neonicotinoids	1
	Flupyradifurone	Sivanto Prime	200 g/L	60 mL/hL	Butenolides	1
	Pyrethrins	Biopiren Plus	18.6 g/L	160 mL/hL	Pyrethrins	2
	Pyrethrins + mineral oil	Biopiren Plus + Oliocin	18.6 g/L 80%	160 mL/hL 500 mL/hL	Pyrethrins + Mineral oils	2

Trials were carried out according to a completely randomized design where each treatment comprised four replicates of 8-10 vines. Insecticides were applied (1 or 2 applications depending on label instructions) against the second generation of *E. vulnerata* (Table 1). Sampling was conducted before and after (3, 7, 10, 14 and 21 days) insecticide applications. A total of 40 leaves per treatment (10 leaves per replicate) were removed and transferred to the laboratory where leafhoppers were identified at species and stage (for *E. vulnerata*: I-II instar nymphs, III-V instar nymphs, adults) levels under a dissecting microscope.

Data were analyzed using a repeated measures linear mixed model with the MIXED procedure of SAS® (ver. 9.3; SAS Institute Inc., Cary, NC, USA). Insecticide, time of sampling, and their interaction were considered as sources of variation in the model and tested using an F test ($\alpha = 0.05$). Pairwise comparisons of the abundance of *E. vulnerata* on

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different treatments were performed using t-test (α = 0.05) on the least-square means. The degrees of freedom were estimated with Kenward–Roger method. Prior to the analysis, data were checked for model assumptions. The model was run on data transformed to log (n + 1), while untransformed data are shown in figures. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods.

3. Results

3.1. 2017

Most of leafhopper specimens found in leaf samples belonged to *E. vulnerata* nymphs. Adults of this species were rarely detected. Therefore, statistical analyses were carried out on total nymphs, early (I-II instar) nymphs and older (III-V instar) nymphs. In 2017, the effects of treatment and time were significant (for treatment: F = 2.78; d.f. = 9, 33.4; P = 0.015; for time: F = 14.29; d.f. = 5, 140; P < 0.0001). Interaction "treatment*time" was not significant (F = 1.07; d.f. = 45, 136; P = 0.367). No differences among treatments were found prior to the first insecticide application (F = 0.14; d.f. = 9, 97.6; P = 0.999). Among insecticides, acetamiprid was more effective than potassium salts (Table 2, Figure 1).

Table 2. Results of t-test (α = 0.05) on the least-square means of *Erasmoneura vulnerata* nymphs observed on different treatments in the 2017 trial.

Active ingredient		Acetamiprid	Buprofezin	Chlorpyrifos-methyl	Lambda-cyhalothrin	Potassium salts	Pyrethrins	Pyrethrins + mineral oil	Thiamethoxam	Kaolin
Control	t	**4.20**	**3.18**	**3.57**	**3.28**	**2.15**	**3.30**	**3.39**	**3.76**	**2.71**
	P	**0.0002**	**0.0032**	**0.0011**	**0.0024**	**0.0393**	**0.0023**	**0.0018**	**0.0007**	**0.0105**
Acetamiprid	t		-1.03	-0.63	-0.92	**-2.06**	-0.90	-0.81	-0.44	-1.49
	P		0.3123	0.5309	0.3618	**0.0474**	0.3751	0.4214	0.6599	0.1447
Buprofezin	t			0.39	-0.10	-1.03	0.13	0.21	-0.58	-0.47
	P			0.6970	0.9199	0.3094	0.8998	0.8334	0.5645	0.6431
Chlorpyrifos-methyl	t				0.29	-1.42	-0.27	-0.18	-0.19	-0.86
	P				0.7726	0.1634	0.7920	0.8577	0.8510	0.3957
Lambda-cyhalothrin	t					-1.13	0.03	0.11	-0.48	-0.57
	P					0.2650	0.9798	0.9126	0.6339	0.5732
Potassium salts	t						-1.16	1.24	-1.61	0.56
	P						0.2546	0.2220	0.1159	0.5761
Pyrethrins	t							0.09	-0.46	-0.59
	P							0.9327	0.6520	0.5562
Pyrethrins + mineral oil	t								-0.37	-0.68
	P								0.7137	0.5014
Thiamethoxam	t									-1.05
	P									0.3014

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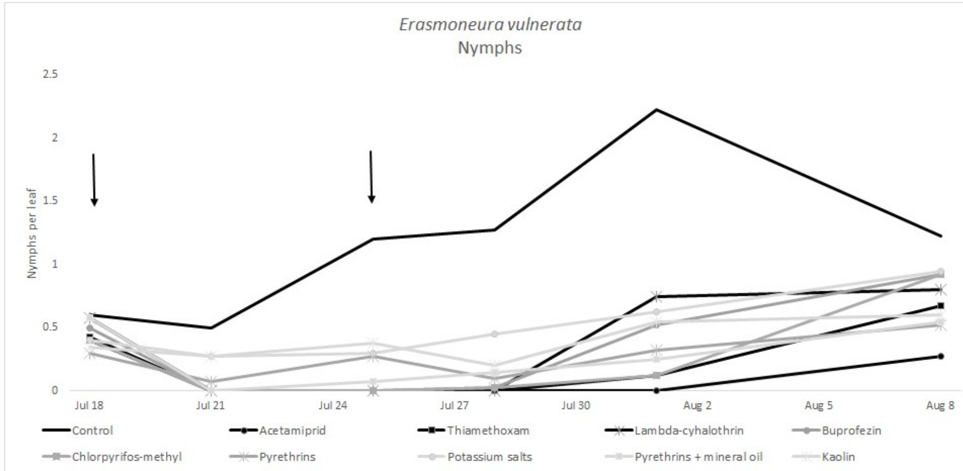

Figure 1. Dynamics of *Erasmoneura vulnerata* nymphs in the 2017 trial. Arrows indicate insecticide applications.

When early (I-II instar) and older (III-V instar) nymphs were considered separately, differences among treatments emerged only for the former (F = 3.03; d.f. = 9, 38.6; P = 0.008) (Tables 3, Figure 2). At the same time, there were no differences among insecticides (Table 3). Considering older nymphs, there were no differences among treatments (F = 2.16; d.f. = 9, 27.1; P = 0.058) (Figure 3). The effect of time emerged for early (F = 10.46; d.f. = 5, 143; P < 0.0001) as well as for older nymphs (F= 16.49; d.f. = 5, 127; P < 0.0001). Interaction "treatment*time" was not significant for early (F = 1.06; d.f. = 45, 140; P = 0.389) nor for older nymphs (F = 0.76; d.f. = 45, 126; P = 0.859)..

Table 3. Results of t-test on the least square means of *Erasmoneura vulnerata* early (I-II instar) nymphs observed on different treatments in the 2017 trial.

Active ingredient		Acetamiprid	Buprofezin	Chlorpyrifos-methyl	Lambda-cyhalothrin	Potassium salts	Pyrethrins	Pyrethrins + mineral oil	Thiamethoxam	Kaolin
Control	t	**4.46**	**3.16**	**3.81**	**3.29**	**2.70**	**3.48**	**3.61**	**4.02**	**2.63**
	P	**< 0.0001**	**0.0031**	**0.0005**	**0.0021**	**0.0101**	**0.0013**	**0.0009**	**0.0003**	**0.0122**
Acetamiprid	t		-1.31	-0.65	-1.17	-1.76	-0.98	-0.85	-0.44	-1.83
	P		0.1990	0.5182	0.2489	0.0866	0.3318	0.3990	0.6618	0.0747
Buprofezin	t			0.65	-0.14	-0.45	0.32	0.45	-0.87	-0.52
	P			0.5165	0.8923	0.6542	0.7476	0.6523	0.3919	0.6027
Chlorpyrifos-methyl	t				0.52	-1.11	-0.33	-0.20	-0.21	-1.18
	P				0.6071	0.2755	0.7427	0.8420	0.8338	0.2454
Lambda-cyhalothrin	t					-0.59	0.19	0.32	-0.73	-0.66
	P					0.5602	0.8520	0.7523	0.4700	0.5125
Potassium salts	t						-0.78	0.91	-1.32	-0.07
	P						0.4428	0.3709	0.1955	0.9418
Pyrethrins	t							0.13	-0.54	-0.85
	P							0.8973	0.5910	0.4012
Pyrethrins + mineral oil	t								-0.41	-0.98
	P								0.6827	0.3337
Thiamethoxam	t									-1.39
	P									0.1723

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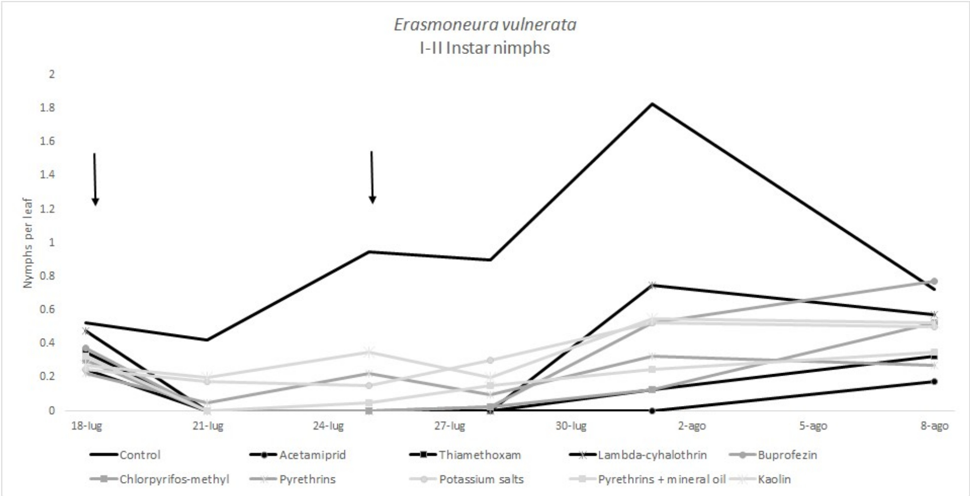

Figure 2. Dynamics of early (I-II instar) nymphs of *Erasmoneura vulnerata* in the 2017 trial. Arrows indicate insecticide applications.

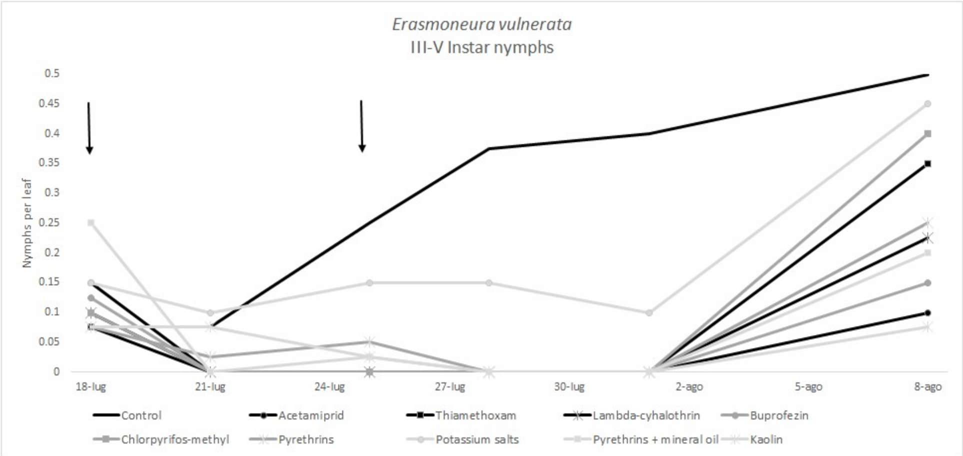

Figure 3. Dynamics of older (III-V instar) nymphs of *Erasmoneura vulnerata* in the 2017 trial. Arrows indicate insecticide applications

3.2. 2018

In the trial of 2018, insecticide applications showed significant effects on *E. vulnerata* nymphs (F = 8.87; d.f. = 7, 26.2; P < 0.0001). Nymph densities fluctuated over sampling dates (F = 27.43; d.f. = 5, 101; P < 0.0001) and interaction "treatment*time" was significant (F = 2.75; d.f. = 35, 103; P < 0.0001). There were no differences among treatments before insecticide application (F = 0.92; d.f. = 7, 106; P = 0.496). Higher *E. vulnerata* nymph densities were found in control compared to acetamiprid, lambda-cyhalothrin, mineral oil and kaolin treatments (Table 4, Figure 4). The application of chlorpyrifos-methyl, potassium salts and pyrethrins did not significantly decrease leafhopper densities than untreated control (Table 4). Acetamiprid was more effective than the remaining insecticides, followed by lambda-cyhalothrin (Table 4). The remaining insecticides were more effective than potassium salts (Table 4).

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Table 4. Results of t test (α = 0.05) on the least-square means of *Erasmoneura vulnerata* nymphs observed on different treatments during the 2018 trial.

Active ingredient		Acetamiprid	Lambda-cyhalothrin	Chlorpyrifos-methyl	Pyrethrins	Potassium salts	Mineral oil	Kaolin
Control	t	**5.86**	**4.20**	1.76	1.97	-0.36	**2.67**	**3.73**
	P	**< 0.0001**	**0.0003**	0.0906	0.0599	0.7183	**0.0128**	**0.0009**
Acetamiprid	t		-1.66	**-4.11**	**-3.90**	**-6.23**	**-3.19**	**-2.13**
	P		0.1088	**0.0004**	**0.0006**	**< 0.0001**	**0.0037**	**0.0427**
Lambda-cyhalothrin	t			-2.45	-2.24	**-4.57**	-1.53	-0.47
	P			0.0215	0.0341	**0.0001**	0.1380	0.6420
Chlorpyrifos-methyl	t				0.21	**-2.12**	0.91	1.97
	P				0.8358	**0.0435**	0.3686	0.0589
Pyrethrins	t					**-2.33**	0.71	1.77
	P					**0.0277**	0.4866	0.0891
Potassium salts	t						**3.04**	**4.10**
	P						**0.0054**	**0.0004**
Mineral oil	t							1.06
	P							0.2989

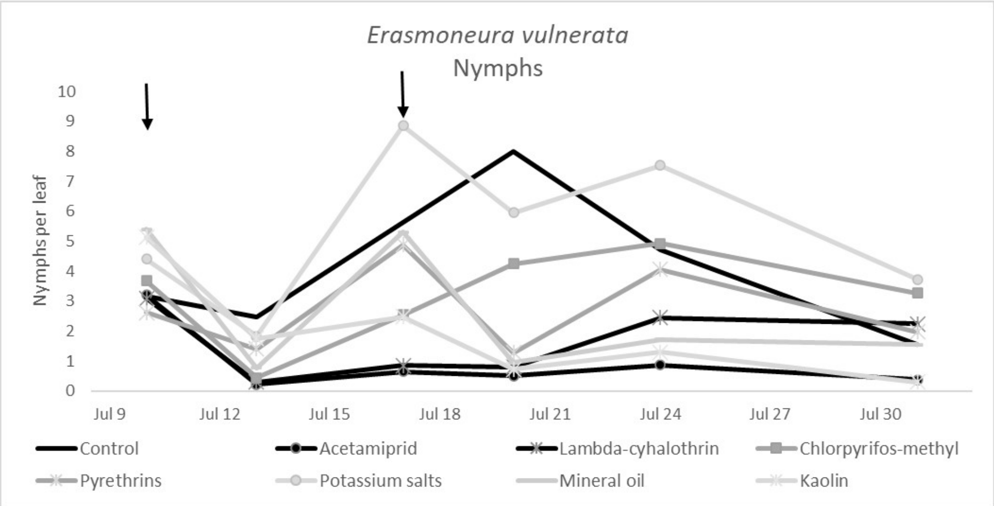

Figure 4. Dynamics of *Erasmoneura vulnerata* nymphs in the 2018 trial. Arrows indicate insecticide applications.

The effects of treatment, time and their interaction were significant also considering separately early (treatment: F = 9.61; d.f. = 7, 26.4; P < 0.0001; time: F = 35.72; d.f. = 5, 98.1; P < 0.0001; treatment*time: F = 2.6; d.f. = 35, 102; P = 0.0001) or older nymphs (treatment: F = 7.34; d.f. = 7, 26.3; P < 0.0001; time: F = 4.48; d.f. = 5, 101; P = 0.001; treatment*time: F = 2.26; d.f. = 35, 103; P = 0.0008) (Tables 5, 6, Figures 5, 6). No differences among treatments were found prior to the first insecticide application also considering separately early (F = 1.07; d.f. = 7, 115; P = 0.384) and older nymphs (F = 0.54; d.f. = 7, 108; P = 0.803) Acetamiprid and lambda-cyhalothrin confirmed to be the most effective insecticides on early nymphs but their impact was less significant on older nymphs. Considering early nymphs, kaolin was more effective than chlorpyrifos-methyl, pyrethrins and potassium salts (Tables 5, 6).

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Table 5. Results of t-test ($\alpha = 0.05$) on the least-square means of *Erasmoneura vulnerata* early (I-II instar) nymphs observed on different treatments in the 2018 trial.

Active ingredient		Acetamiprid	Lambda-cyhalothrin	Chlorpyrifos-methyl	Pyrethrins	Potassium salts	Mineral oil	Kaolin
Control	t	**5.93**	**4.45**	1.48	1.41	-0.39	**2.27**	**3.66**
	P	**< 0.0001**	**0.0001**	0.1508	0.1697	0.6984	**0.0315**	**0.0011**
Acetamiprid	t		-1.48	**-4.45**	**-4.52**	**-6.32**	**-3.66**	**-2.27**
	P		0.1505	**0.0001**	**0.0001**	**< 0.0001**	**0.0011**	**0.0314**
Lambda-cyhalothrin	t			**-2.97**	**-3.04**	**-4.84**	**-2.18**	-0.79
	P			**0.0063**	**0.0053**	**< 0.0001**	**0.0385**	0.4356
Chlorpyrifos-methyl	t				-0.07	-1.87	0.79	2.18
	P				0.9463	0.0724	0.4354	**0.0385**
Pyrethrins	t					-1.80	0.86	2.25
	P					0.0827	0.3975	**0.0332**
Potassium salts	t						**2.66**	**4.05**
	P						**0.0130**	**0.0004**
Mineral oil	t							1.39
	P							0.1772

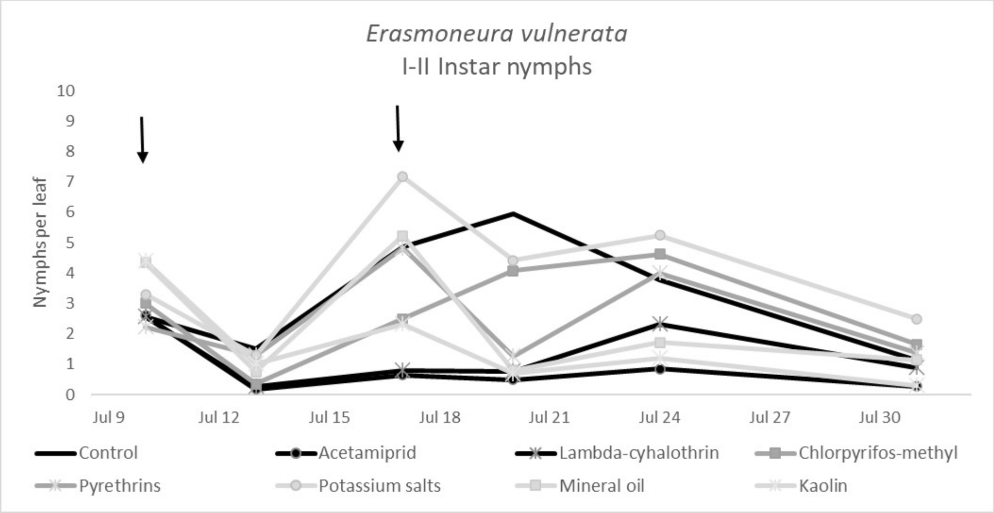

Figure 5. Dynamics of early (I-II instar) nymphs of *Erasmoneura vulnerata* in the 2018 trial. Arrows indicate insecticide applications.

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Table 6. Results of t-test ($\alpha = 0.05$) on the least-square means of *Erasmoneura vulnerata* older (III-V instar) nymphs observed on different treatments in the 2018 trial.

Active ingredient		Acetamiprid	Lambda-cyhalothrin	Chlorpyrifos-methyl	Pyrethrins	Potassium salts	Mineral oil	Kaolin
Control	t	**4.47**	**3.24**	**2.59**	**3.83**	-0.85	**3.64**	**3.38**
	P	**0.0001**	**0.0032**	**0.0154**	**0.0007**	0.4055	**0.0012**	**0.0023**
Acetamiprid	t		-1.23	-1.88	-0.64	**-5.32**	-0.83	-1.09
	P		0.2289	0.0714	0.5262	**< 0.0001**	0.4137	0.2849
Lambda-cyhalothrin	t			-0.65	0.59	**-4.08**	0.40	0.14
	P			0.5234	0.5606	**0.0004**	0.6914	0.8895
Chlorpyrifos-methyl	t				1.24	**-3.44**	1.05	0.79
	P				0.2273	**0.0020**	0.3042	0.4383
Pyrethrins	t					**-4.67**	-0.19	-0.45
	P					**< 0.0001**	0.8522	0.6570
Potassium salts	t						**4.49**	**4.22**
	P						**0.0001**	**0.0003**
Mineral oil	t							-0.26
	P							0.7961

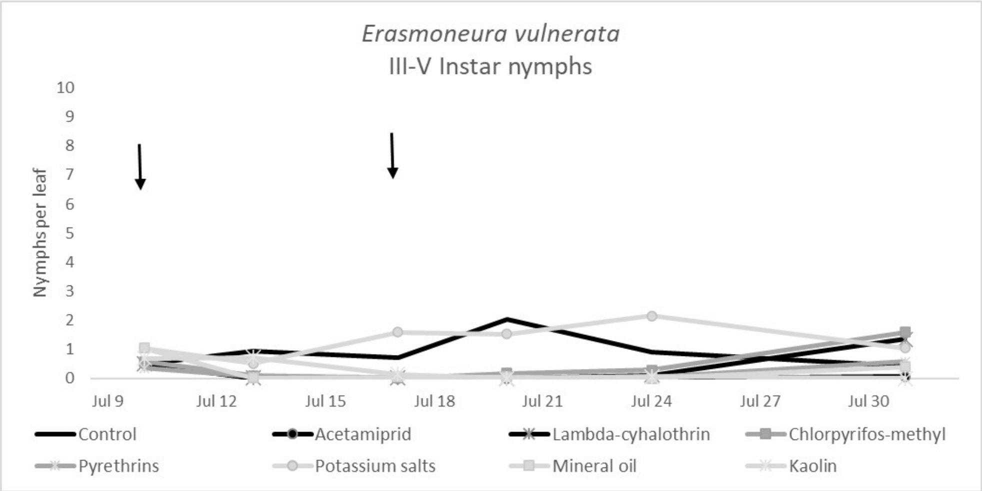

Figure 6. Dynamics of *Erasmoneura vulnerata* older (III-V instar) nymphs observed in the 2018 trial. Arrows indicate insecticide applications.

3.3. 2019

In 2019, the effect of insecticides on *E. vulnerata* nymphs confirmed to be significant (F = 5.17; d.f. = 4, 16.8; P = 0.007). Nymph densities changed over time (F = 13.7; d.f. = 5, 69.9; P < 0.0001) and a significant interaction "treatment*time" was found (F = 1.95; d.f. = 20, 69; P = 0.022). There were no differences among treatments before insecticide applications (F = 0.13; d.f. = 4, 47.8; P = 0.969). Acetamiprid and flupyradifurone reduced significantly *E. vulnerata* nymphs compared to the control, while pyrethrins and pyrethrins + mineral oil were not effective (Table 7, Figure 7). The last two treatments did not differ significantly.

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Table 7. Results of t-test (α = 0.05) on the least-square means of *Erasmoneura vulnerata* nymphs observed on different treatments during in the 2019 trial.

Active ingredient		Acetamiprid	Flupyradifurone	Pyrethrins	Pyrethrins + mineral oil
Control	t	**2.70**	**3.33**	-0.05	0.56
	P	**0.0153**	**0.004**	0.9619	0.5831
Acetamiprid	t		0.63	**-2.75**	**-2.14**
	P		0.5367	**0.0138**	**0.0471**
Flupyradifurone	t			**-3.38**	**-2.78**
	P			**0.0036**	**0.0132**
Pyrethrins	t				0.61
	P				0.5512

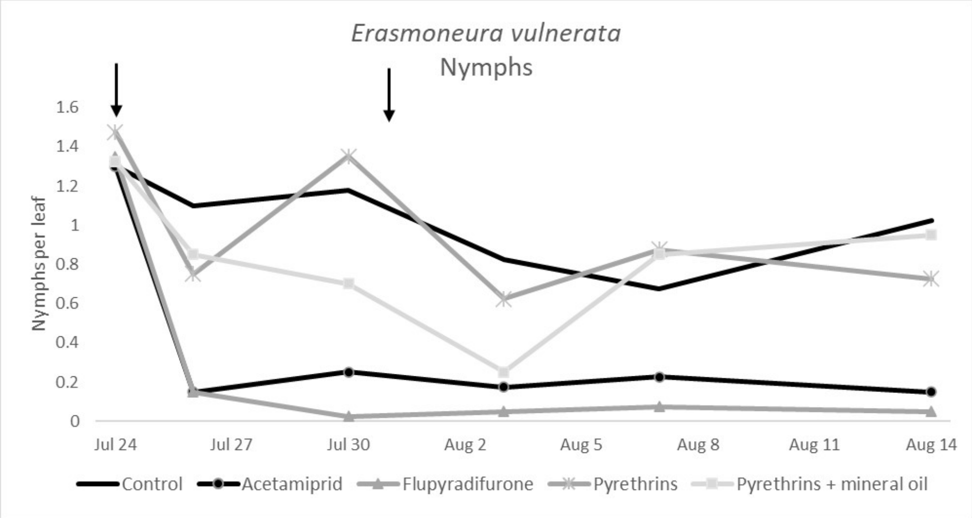

Figure 7. Dynamics of *Erasmoneura vulnerata* nymphs in the 2019 trial. Arrows indicate insecticide applications.

When the statistical analysis was conducted on early or older nymphs the effects of treatment, time and "treatment*time" were confirmed (for I-II instar nymphs - treatment effect: F = 4.59; d.f. = 4, 19.4; P = 0.009; time effect: F = 19.11; d.f. = 5, 71.6; P < 0.0001; treatment*time effect: F = 3.57; d.f. = 20, 70.7; P < 0.0001; for III-V instar nymphs - treatment effect: F = 3.18; d.f. = 4, 16.5; P = 0.041; time effect: F = 6.64; d.f. = 5, 67.8; P < 0.0001; treatment*time effect: F = 2.15; d.f. = 20, 67.4; P < 0.011) (Tables 8-9, Figures 8-9). No differences among treatments were found prior to the first insecticide application (early nymphs: F = 2.21; d.f. = 4, 55.4; P = 0.08; older nymphs: F = 1.33; d.f. = 4, 53.6; P = 0.271). Flupyradifurone and acetamiprid significantly reduced the abundance of early and older nymphs (Tables 8-9-, Figures 8-9).

Table 8. Results of t test on the least square means of *Erasmoneura vulnerata* early (I-II instar) nymphs observed on different treatments in the 2019 trial.

Active ingredient		Acetamiprid	Flupyradifurone	Pyrethrins	Pyrethrins + mineral oil
Control	t	1.29	**2.40**	-1.40	-0.60
	P	0.2114	**0.0267**	0.1768	0.5544
Acetamiprid	t		1.11	**-2.69**	-1.89
	P		0.2826	**0.0142**	0.0733
Flupyradifurone	t			**-3.8**	**-3**
	P			**0.0012**	**0.0073**
Pyrethrins	t				0.8
	P				0.4333

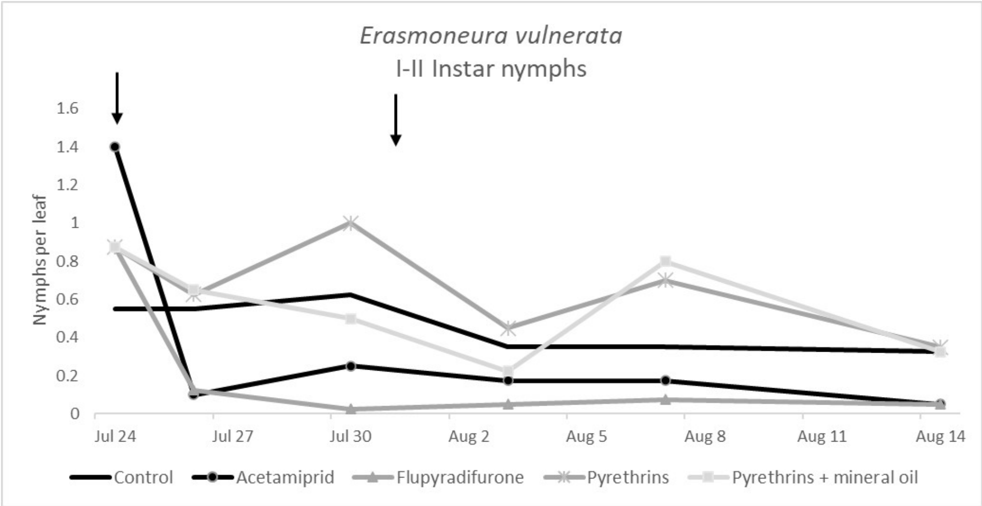

Figure 8. Dynamics of *Erasmoneura vulnerata* early (I-II instar) nymphs in the 2019 trial. Arrows indicate insecticide applications.

Table 9. Results of t-test ($\alpha = 0.05$) on the least square means of *Erasmoneura vulnerata* older (III-V instar) nymphs observed on different treatments in the 2019 trial.

Active ingredient		Acetamiprid	Flupyradifurone	Pyrethrins	Pyrethrins + mineral oil
Control	t	**2.73**	**3.07**	1.00	1.47
	P	**0.0144**	**0.0072**	0.3332	0.1613
Acetamiprid	t		0.33	-1.74	-1.27
	P		0.7448	0.1009	0.2223
Flupyradifurone	t			-2.07	-1.6
	P			0.0546	0.1287
Pyrethrins	t				0.47
	P				0.6449

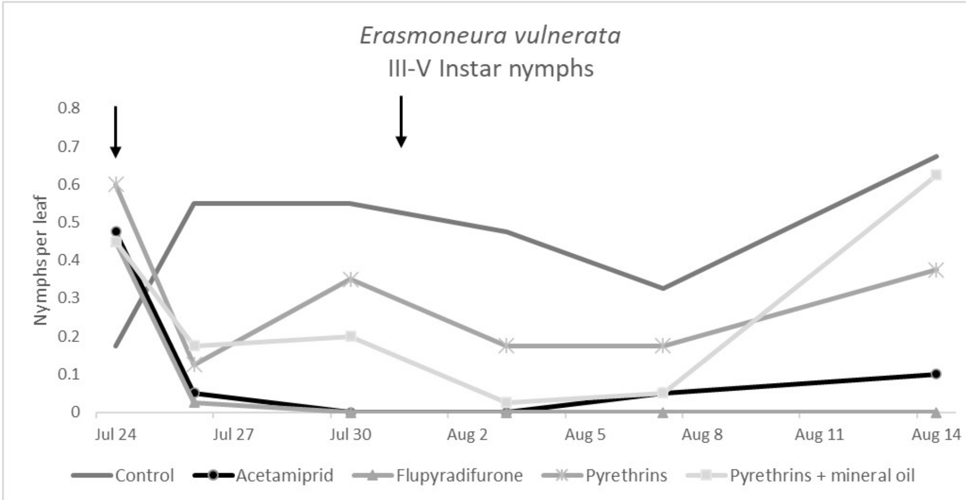

Figure 9. Dynamics of *Erasmoneura vulnerata* older (III-V instar) nymphs observed in the 2019 trial. Arrows indicate insecticide applications.

4. Discussion

Among the products tested in this study, those based on acetamiprid (IRAC Group 4A) resulted in the most effective in controlling *E. vulnerata* populations in two out of three trials. In 2019, its impact was slightly lower than that of flupyradifurone (IRAC Group 4D), a novel insecticide belonging to Butenolides recommended against sucking pests. Its effectiveness against *E. vulnerata* are consistent with those reported in the control of *Erythroneura elegantula* and *E. ziczac* in North America [37]. A single application of these two insecticides at the beginning of the second generation maintained *E. vulnerata* population densities at low levels for some weeks. Another neonicotinoid (IRAC Group 4A), i.e., thiamethoxam, proved to be very effective against *E. vulnerata* in 2017. It was largely employed against sucking insects, e.g., *E. vitis* and *S. titanus* [25-27,38]. No additional comparisons were planned as thiamethoxam was banned from the EU because of the adverse impact on pollinators [39,40]. Buprofezin (IRAC Group 16) was tested in 2017, showing good effectiveness, but it was also banned from the EU and thus excluded from further evaluations. Lambda-cyhalothrin (IRAC 3A Group) showed effectiveness slightly lower than acetamiprid, but leafhopper populations seemed to recover faster in the respective plots. Results obtained using the organophosphate chlorpyrifos-methyl (IRAC Group 1B) are of particular interest as this insecticide has been widely used against grapevine leafhoppers in Italy [41,38,26]. It was effective against *E. vulnerata* in 2017 but not in 2018. Different vineyards were selected for these trials, and thus a variation in susceptibility of leafhopper populations could explain the different results we obtained. It should be mentioned that the first outbreaks of *E. vulnerata* in Northern Italy were detected in vineyards frequently treated with chlorpyrifos-methyl. This observation led to suggest that resistance to insecticides could be a key factor explaining the unexpected outbreaks of this species [30]. It should be mentioned that the closely related chlorpyrifos has been widely used in European vineyards against *S. titanus*, berry moths, and scales [20] and resistance in *E. vitis* was strongly suspected [11]. Chlorpyrifos-methyl and chlorpyrifos have been banned from the UE in 2020 because of concerns for human health and thus research on leafhopper resistance to these insecticides was not planned.

As regards natural products, pyrethrin based insecticides (IRAC Group 3A) are widely used against *S. titanus* and other leafhoppers in organic vineyards in France, Italy and Switzerland [20]. In the current study, the application of pyrethrins gave contrasting results in controlling *E. vulnerata*. In 2017, pyrethrins significantly reduced nymph densities compared to the control, in 2018 they showed some effectiveness on older nymphs only, while in 2019 they reached unsatisfactory results in controlling leafhoppers. Min-

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eral oils were effective in 2018 and it was expected they could increase the impact of pyrethrins when mixed. This assumption was verified in 2017 but not in 2019. Potassium salts were slightly effective in 2017 but were associated to poor results in 2018. Finally, kaolin (an inert white clay not classified as an insecticide) significantly reduced *E. vulnerata* densities in 2017 and 2018. It was more effective than chlorpyrifos-methyl, pyrethrins and potassium salts against early nymphs in 2018 trial. Kaolin was harmful to some grapevine pests [42], particularly to *E. vitis* and *Z. rhamni* [43]. Inhibition of feeding was the main mode of action through which kaolin affected leafhopper nymph populations. Timing in applying kaolin against *E. vulnerata* and mechanisms underlined its mode of action are worthy of study.

5. Conclusions

Recent outbreaks of *E. vulnerata* in Europe warned winegrowers and suggested to test the impact of a number of conventional or natural insecticides on this species in vineyards. Among the insecticides tested, the most effective resulted in acetamiprid, flupyradifurone and lambda-cyhalothrin. A single application of these compounds reduced leafhopper population densities at low levels for some weeks. Regarding natural products, the most effective was kaolin that could represent an alternative to pyrethrins in organic vineyards and a complementary tool in conventional vineyards. The use of insecticides should be done at the correct timing and once threshold levels are exceeded. Our knowledge on the biology of *E. vulnerata* allowed to identify the best timing for insecticide application (i.e., at the beginning of the second generation) but threshold levels have not been defined yet [44]. Finally, insecticides' side effects on beneficials occurring in European vineyards should be known to optimize IPM strategies [45,46]. Information on the side effects of many of these insecticides on predatory mites belonging to the Phytoseiidae family is available [47,48] but is limited for other important beneficials.

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