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The Impact of Microbial and Botanical Insecticides on Grape Berry Moths and Their Effects on Secondary Pests and Beneficials

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Abstract: According to the European Directive 2009/128/EC and the subsequent provisions activated in member states, conventional pesticides should be progressively replaced by "non-chemical tools and/or measures". The identification of reliable alternatives to pesticides is crucial to achieve this objective. A European project (PURE) was funded to investigate this topic with reference to annual and perennial crops. In this framework, a number of natural insecticides, in particular microbial and botanical ones (*Bacillus thuringiensis* ssp. *kurstaki*, *Beauveria bassiana*, azadirachtin, pyrethrins and spinosad) were selected to test their effectiveness against grape berry moths, the key pests in most European vineyards. Trials were conducted in 2011 and 2012 in two experimental vineyards located in Italy (Tuscany and Veneto regions), following a randomized block design. Additional investigations were carried out in the Veneto region during 2013. Trial results stressed the high performance of spinosad and *B. thuringiensis* in controlling berry moth densities and the related damage. The use of *B. bassiana* mixed with *B. thuringiensis* did not significantly improve the impact of *B. thuringiensis* alone. Azadirachtin, and especially pyrethrins, proved to be less effective on berry moths than previous insecticides. The use of selected insecticides caused side-effects on a number of secondary pests, in particular leafhoppers. In 2011, densities of *Empoasca vitis* were higher in spinosad-treated plots probably because of a reduced egg parasitization rate. One year later, the population density of *Zygina rhamni* was higher in the plots treated with spinosad or pyrethrins. This trend was confirmed on spinosad-treated plots in the last experimental year. At the same time, spinosad and pyrethrins significantly reduced the predatory mite populations compared to other treatments. The use of these insecticides in viticulture is discussed in the framework of organic viticulture and Integrated Pest Management (IPM).

Keywords: *Lobesia botrana*; *Eupoecilia ambiguella*; leafhoppers; predatory mites; natural insecticides; damage; side-effects

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1. Introduction

The European grapevine moth *Lobesia botrana* (Denis & Schiffermüller) is a traditional pest of grapes in Europe and the Middle East [1,2] and a new threat in the Americas [3,4]. *Lobesia botrana* coexists with the grape berry moth *Eupoecilia ambiguella* (Hübner) in several European regions, such as northern Italy, where they develop three generations per year in most areas and seasons [5]. The impact of *L. botrana* is increasing in Europe, probably because of climate change [2,6]. Clusters damaged by berry moths can be invaded by fungal diseases and contaminated by ochratoxins [7,8]. Therefore, control measures against berry moths are considered crucial in European vineyards and elsewhere [9]. The active ingredients used most in European viticulture against berry moths are methoxyfenozide, emamectin-benzoate and chlorantraniliprole. Another major issue for grapevine is Flavescence dorée, a disease associated with phytoplasmas transmitted mainly by the leafhopper *Scaphoideus titanus* Ball [10]. Some leafhoppers, such as *Empoasca vitis* (Goethe), coccids, and mealybugs, such as *Parthenolecanium corni* Bouché and *Planococcus ficus* (Signoret), are locally important in European vineyards. Problems with spider mites, mainly *Panonychus ulmi* (Koch) and *Eotetranychus carpini* (Oudemans), have also been reported [11].

The use of insecticides in European viticulture is usually lower than that of fungicides, e.g., 3.1 insecticide applications vs. 10.6 fungicide applications per year in Italy in the 2010 growing season [12]. However, this figure can increase dramatically in table grapes due to risks posed by *L. botrana*, mealybugs and thrips. The occurrence of new pests, such as *Drosophila suzukii* Matsumura and *Erasmoneura vulnerata* (Fitch), poses new risks to grapevine production and is increasing insecticide use in some areas [13,14]. Pesticide use has been associated with toxicological and environmental problems; a number of frequently used toxic compounds have been banned in the European Union according to Regulation (EC) 1107/2009, and the availability of other active ingredients/formulations in expected to decrease in coming years. Resistance to insecticides and acaricides is a major concern, even if in viticulture this problem is more crucial for pathogens than for arthropod pests. On the other hand, pest resurgence and pesticide-induced pests represent typical implications of insecticide use in viticulture. The reduction in pesticide use by adopting alternative control measures is the objective of Directive 2009/128/EEC. Mating disruption is a reliable control technique against grape berry moths [15], but its performance can be reduced in fragmented habitats and/or hilly and windy areas [16,17]. In some areas, *S. titanus* is more important than berry moths and control measures against this pest are largely based on insecticides. Natural insecticides (e.g., microbial and botanical based insecticides) can be considered alternatives to conventional insecticides to reduce risks to human health and the environment. However, their efficacy is considered low to moderate and, thus, their use is often limited to organic viticulture. Little is known on the effects of natural insecticides against berry moths and other pests of grapes, with the exception of formulations based on *Bacillus thuringiensis* ssp. *kurstaki* [18]; at the same time, most studies refer to laboratory trials [7,19]. In this study, we evaluate the effectiveness of a number of microbial and botanical insecticides against grape berry moths in two vineyards located in northern and central Italy, in three experimental growing seasons. The potential of natural insecticides in reducing berry moth damage on grapevine yield was evaluated in the last growing season. Their effects on other grapevine pests (e.g., leafhoppers and scales) and some beneficials (e.g., predatory mites) are also considered.

2. Materials and Methods

Trials were conducted in two experimental vineyards located in Tuscany (Montepaldi, 2011–2012, vineyard 1) and Veneto (Meolo, 2011–2013, vineyard 2) regions. Average maximum temperatures of the last decades were 20.1 and 18.2 °C for Montepaldi and Meolo, respectively, while average minimum temperatures were 9.2 and 8.3 °C, respectively. Average rainfall was similar for the two sites (914.8 and 893.8 mm for Montepaldi and Meolo, respectively). Both vineyards were trained with the espalier system. The vineyard located in Tuscany comprised the Sangiovese cultivar, while that in Veneto the Cabernet Franc cultivar. A number of microbial (i.e., *Bacillus thuringiensis* ssp. *kurstaki*, *Beauveria bassiana*, spinosad) and botanical (i.e., pyrethrins and azadirachtin) insecticides were applied against the second generation of *L. botrana*, following a randomized block design (4–5 replicates per treatment, 8–30 vines per replicate). Regarding *B. thuringiensis* and *B. bassiana*, the strains EG 2348 and ATCC 74040 (Rapax® and Naturalis®, respectively, Biogard) were used. An untreated control was included for comparison. An additional treatment (i.e., indoxacarb as a toxic reference) was comprised in trials of 2013. Details of insecticide applications are reported in Tables 1 and 2. Vineyards were treated with fungicides to control downy and powdery mildews.

Table 1. Experimental design of trials conducted in Tuscany (vineyard 1) in 2011 and 2012.

Treatment	Active Ingredient/Microorganism	Dose	2011	2012
1	*Bacillus thuringiensis* (Bt)	100 g/hL	20 and 27 June	25 June and 3 July
2	Azadirachtin	300 g/hL	27 June	3 July
3	Bt + *Beauveria bassiana*	100 g/hL + 120 g/hL	20 and 27 June	25 June and 3 July
4	Spinosad	20 mL/hL	20 and 27 June	25 June and 3 July
5	Pyrethrins	250 g/hL	27 June and 3 July	3 and 11 July
6	Control	-	-	-

Table 2. Experimental design of trials conducted in Veneto (vineyard 2) from 2011 to 2013.

Treatment	Active Ingredient/Microorganism	Dose	2011	2012	2013
1	*Bacillus thuringiensis* (Bt)	100 g/hL	16 and 24 June	30 June and 7 July	2 and 9 July
2	Azadirachtin	300 g/hL	24 and 29 June	7 and 14 July	9 and 16 July
3	Bt + *Beauveria bassiana*	100 g/hL + 120 g/hL	16 and 24 June	30 June and 7 July	2 and 9 July
4	Spinosad	20 mL/hL	16 and 24 June	30 June and 7 July	2 and 9 July
5	Pyrethrins	250 g/hL	24 and 29 June	7 and 14 July	9 and 16 July
6	Indoxacarb	15 g/hL	-	-	2 July
7	Control	-	-	-	-

A total of 100 clusters per treatment (25 per replicate) were sampled approximately one month after the last insecticide applications to assess berry moth damage (% of infested clusters, number of larval nests per cluster). In 2011, leafhopper populations were sampled in the vineyards by analyzing 100 leaves per treatment (25 leaves per replicate). Additional leaf samples were analyzed in the laboratory to assess densities of leafhoppers, coccids, mealybugs and mites. Regarding the impact of insecticides on beneficial arthropods, observations focused on predatory mites belonging to the Phytoseiidae family. The latter are widely considered as key taxa in the studies on the side-effects of pesticides on non-target organisms [20–22]. Additional observations were conducted on egg parasitism of leafhoppers (vineyard 2). The parasitization rate of *E. vitis* and *Z. rhamni* eggs by *Anagrus* spp. (Hymenoptera: Chalcidoidea Mymaridae) was calculated by dividing the number of eggs with parasitoid emergence holes by the sum of normally hatched leafhopper eggs and eggs with parasitoid emergence holes.

Data on berry moth incidence (% of infested clusters) were analyzed using a logistic regression with the GENMOD procedure of SAS® (ver. 9.4) and considering the ratio between infested clusters over the total number of clusters as dependent variable with binomial distribution. The treatment effect was evaluated with an χ^2 test (α = 0.05). Using the LSMEAN statement, we performed a Wald χ^2 test on the pairwise comparison of different treatments (α = 0.05). Data on larval nests per cluster were analyzed using general linear model with the GLM procedure of SAS®. An F-test (α = 0.05) was used to assess effect of treatment, followed by a Tukey post hoc test (α = 0.05). Data on larval nests per cluster were checked for normality and homoscedasticity prior to the analysis, and a log (x + 1) transformation was applied to meet model assumptions.

Data obtained by the analysis of leaf samples were analyzed with a linear mixed repeated measures model with MIXED procedure of SAS® (ver. 9.4). Data on leafhoppers, scales and mites were analyzed separately and considered as response variables with repeated measures determined at different times corresponding to the different sampling dates. Using an F-test (α = 0.05), we evaluated the effect of treatment, time and their interactions. Slice option of the LSMEANS statement was used for the F-test partition of interactions between insecticide application and time. Contrasts were designed to assess differences among treatments and tested using a *t*-test (α = 0.05). Moreover, differences among treatments at each sampling date were evaluated using a t-test to the least-square means ($\alpha = 0.05$). The Kenward–Roger method was used for degrees of freedom estimation, which can yield degrees of freedom with non-integer values. Data were checked for analysis assumptions prior to the analysis, and log (x + 1) transformation was used to meet model assumptions.

3. Results

3.1. Effects on Grape Berry Moths

In both vineyards, captures of berry moth adults on pheromone traps revealed the dominance of *L. botrana* over *E. ambiguella.* Most larvae of berry moths found on clusters belonged to *L. botrana.* Therefore, the effects of insecticides were considered with reference to this species. In vineyard 1, insecticide use affected the berry moth incidence in the first experimental season (2011) ($\chi^2 = 28.78$; df = 5; $p < 0.0001$); however, only spinosad obtained a significant control of *L. botrana* (Figure 1). The effect of azadirachtin did not differ from the control, and the remaining treatments were associated with intermediate values. No differences among treatments were noticed in terms of larval densities (F = 0.78; df = 5, 18; $p = 0.574$; Figure 1). In 2012, the effect of insecticides was significant ($\chi^2 = 18.63$; df = 5; $p = 0.002$; F = 1.88; df = 5, 20; $p = 0.143$, respectively, for berry moth damage and larval densities), but only spinosad was effective in reducing berry moth damage and larval densities (Figure 1).

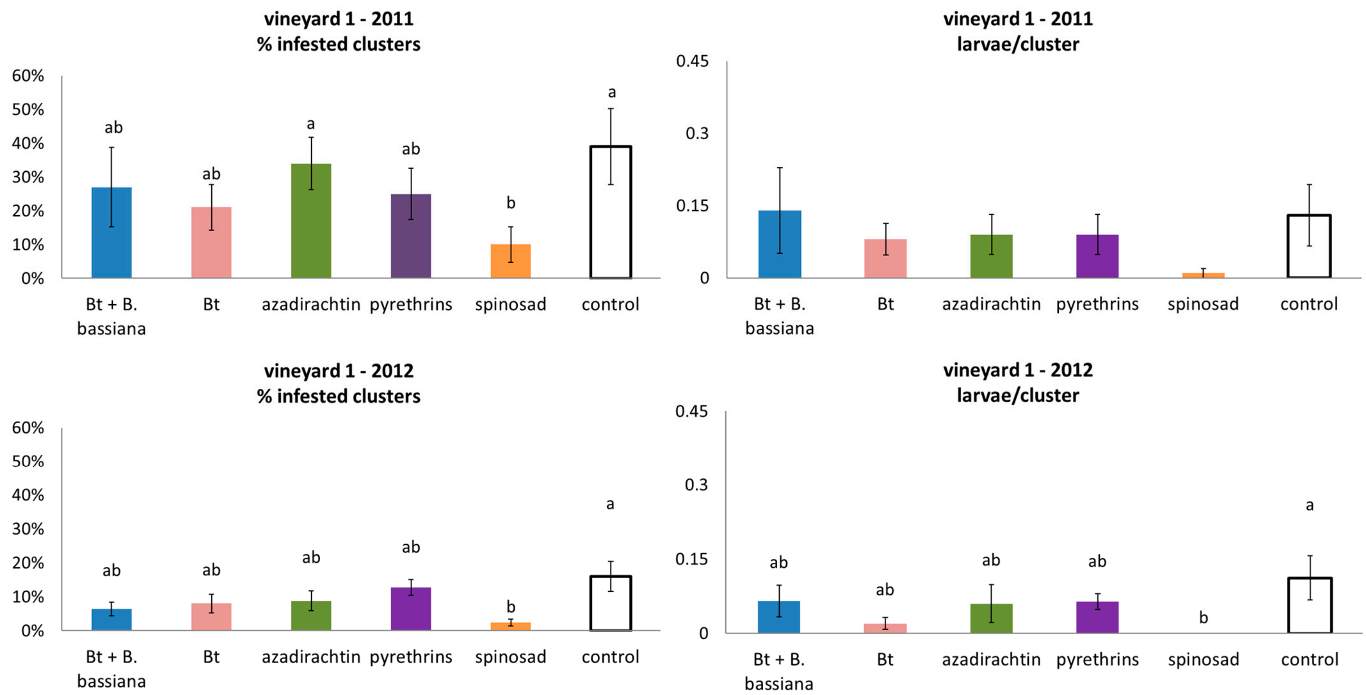

Figure 1. Effects of insecticide treatments on damage caused by berry moth larvae and their densities in two subsequent seasons (vineyard 1, Montepaldi, Tuscany, Italy) (Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test ($p = 0.05$).

In vineyard 2, insecticides applied in 2011 caused significant effects on both berry moth incidence and larval densities ($\chi^2 = 61.81$; df = 5; $p < 0.0001$; F = 12.27; df = 5, 15; $p < 0.0001$, respectively). The best infestation reduction was noticed when spinosad and *B. thuringiensis* (with or without *B. bassiana*) were applied (Figure 2). The impact of azadirachtin was also significant in contrast with that of pyrethrins. The effects of these insecticides on larval densities followed the previous trend (Figure 2). In 2012, the effects of insecticides were confirmed to be significant and berry moth control in terms of incidence was higher when spinosad, azadirachtin and *B. thuringiensis* (especially with *B. bassiana*) were applied ($\chi^2 = 42.38$; df = 5; $p < 0.0001$; Figure 2). Larval densities were limited following a similar tendency (F = 11.54; df = 5, 15; $p < 0.0001$; Figure 2). The infestation level of 2013 was higher than that observed in previous growing seasons. The effect of insecticides on the berry moth incidence was significant ($\chi^2 = 145.07$; df = 5; $p < 0.0001$) and spinosad obtained the best results in terms of berry moth control (Figure 2). The effectiveness of *B. thuringiensis* was comparable to that of indoxacarb; azadirachtin and pyrethrins showed a lower impact. The control level of larval populations obtained using spinosad did not differ from that of indoxacarb, and results obtained using the latter insecticide were not significantly different from those reported for *B. thuringiensis* or azadirachtin (F = 26.21; df = 5, 19; $p < 0.0001$).

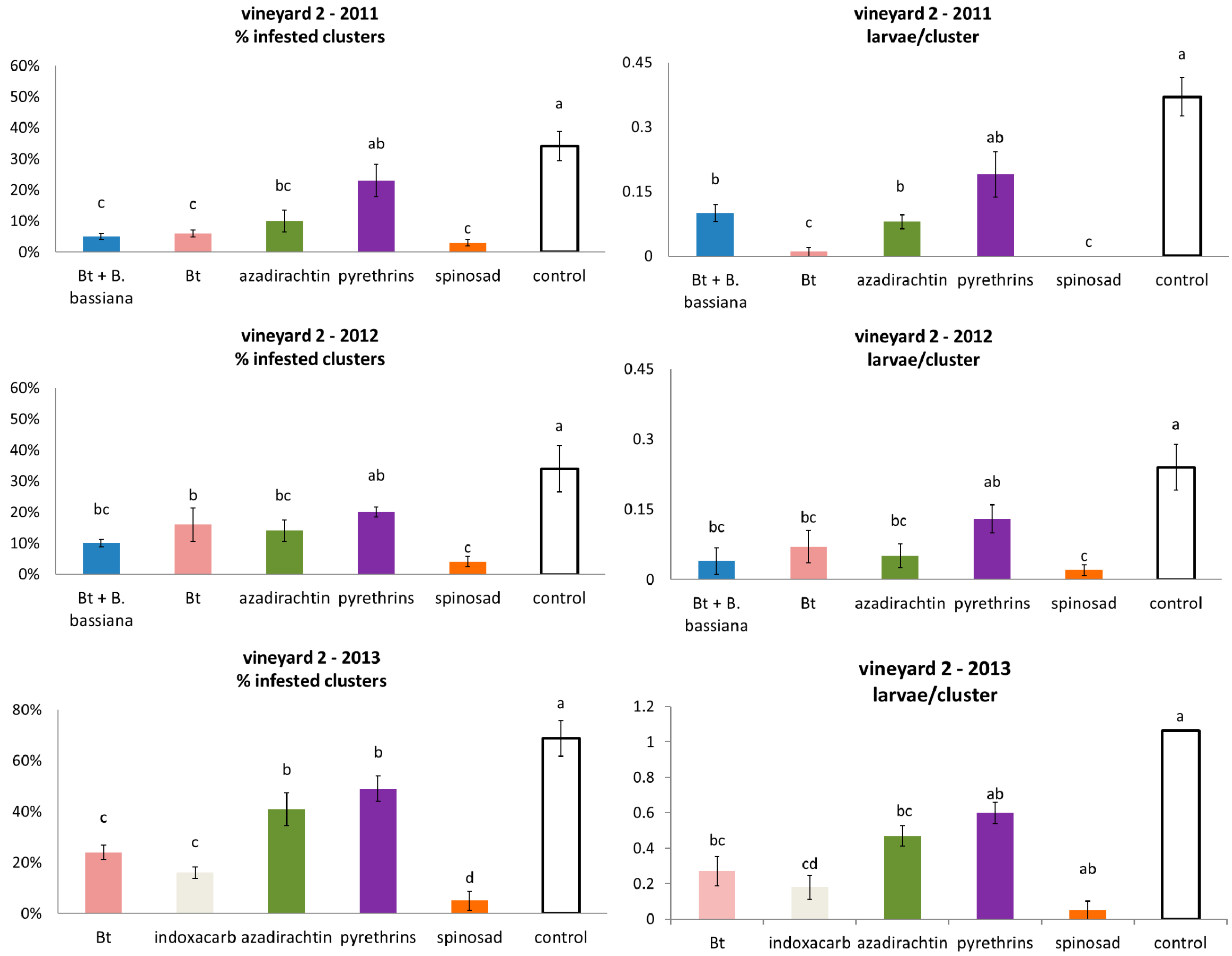

Figure 2. Effects of insecticide treatments on damage caused by berry moth larvae and their densities in three subsequent seasons (vineyard 2, Meolo, Veneto, Italy) (Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test ($p = 0.05$).

3.2. Effects on Damage Caused by Berry Moths

The impact of berry moths on the grapevine yield was assessed in 2013. The effect of the treatment was significant (F = 4.52; df = 5, 114 $p = 0.0009$) and the cluster weight was significantly reduced in the control plots compared to those treated with spinosad, *B. thuringiensis* and indoxacarb (Figure 3). The remaining treatments showed intermediate results.

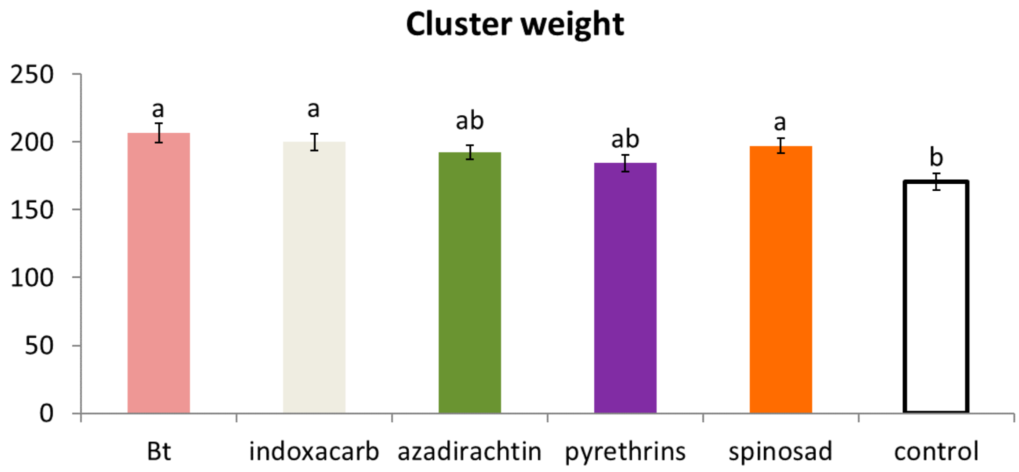

Figure 3. Effect on grape yield (mean cluster weight in g) of natural and conventional insecticides applied to control berry moths in vineyard 2 in 2013 (Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test (p = 0.05).

3.3. Effects on Leafhoppers

During 2011, the occurrence of leafhoppers in vineyard 1 was negligible. In the same year, the most interesting results were observed in vineyard 2, where *E. vitis* and *Z. rhamni* were common. A field evaluation was performed in late July when differences among treatments were significant for *E. vitis* (F = 7.93; df = 5, 15; $p < 0.001$). The highest leafhopper densities were found on spinosad plots compared to the control and other treatment plots (Figure 4). Regarding *Z. rhamni*, there were no differences among treatments (F = 1.13; df = 5, 15; $p = 0.385$; Figure 4). When the second generation of *E. vitis* was completed (early August), the parasitization rate of leafhopper eggs caused by *Anagrus* spp. was evaluated. The parasitization rate was significantly lower on spinosad plots compared to the other treatments ($\chi^2 = 16.55$; df = 5; $p < 0.001$; Figure 4).

A picture of the leafhopper seasonal abundance in 2011 was obtained by analyzing leaf samples in the laboratory. Regarding *E. vitis*, results showed significant differences among treatments (Table 3; Figure 5), with higher *E. vitis* densities occurring on spinosad-treated plots as compared to other treatments, except for *B. bassiana*, which showed intermediate infestation levels (Figure 5). The occurrence of *Z. rhamni* was lower and data were not analyzed (Table 3; Figure 5).

Table 3. Results of linear mixed repeated measures model analysis of data on leafhoppers, scales and mites observed in 2011, 2012 and 2013.

		Treatment	Time	Treatment × Time
Empoasca vitis	2011	F = 2.99; df = 5, 32.5; p = 0.025	F = 15.53; df = 4, 69.7; p < 0.0001	F = 1.44; df =20, 70.9; p = 0.132
	2012	F = 1.55; df = 5, 46.9; p = 0.692	F = 12.53; df = 6, 101; p < 0.0001	F = 1.55; df = 30, 102; p = 0.055
	2013	F = 5.47; df = 5, 43.7; p < 0.001	F = 57.81; df = 7, 120; p < 0.0001	F = 2.21, df = 35, 122; p < 0.001
Zygina rhamni	2011	n.a.	n.a.	n.a.
	2012	F = 1.23; df = 5, 23.8; p = 0.324	F = 112.49; df = 4, 67.1; p < 0.0001	F = 3.55; df = 20, 67.6; p < 0.0001
	2013	F = 2.65; df = 5, 40.9; p = 0.037	F = 93.34; df = 7, 117.0; p < 0.0001	F = 1.11; df = 35, 120; p = 0.331
Parthenolecanium corni	2012	F = 2.14, df = 5, 53.8; p = 0.074	F = 56.04, df = 6, 139; p < 0.0001	F = 1.55, df = 30, 142; p = 0.048
Predatory mites—vineyard 1	2011	F = 3.12; df = 5, 80; p = 0.013	F = 12.42; df = 4, 80; p < 0.0001	F = 0.87; df = 20, 80; p = 0.618
	2012	F = 5.71; df = 5, 168; p < 0.0001	F = 27.80; df = 6, 168; p < 0.0001	F = 1.18; df = 30, 168; p = 0.258
Predatory mites—vineyard 2	2011	F = 10.04; df = 5, 80; p < 0.0001	F = 70.30; df = 4, 80; p < 0.0001	F = 2.00; df = 20, 80; p = 0.016
	2012	F = 40.96; df = 5, 108; p < 0.0001	F = 16.50; df = 5, 108; p < 0.0001	F = 6.33; df = 25, 108; p < 0.0001
	2013	F = 18.24; df = 5, 43.7; p < 0.0001	F = 31.24; df = 7, 115; p < 0.0001	F = 2.82; df =35, 119; p < 0.0001

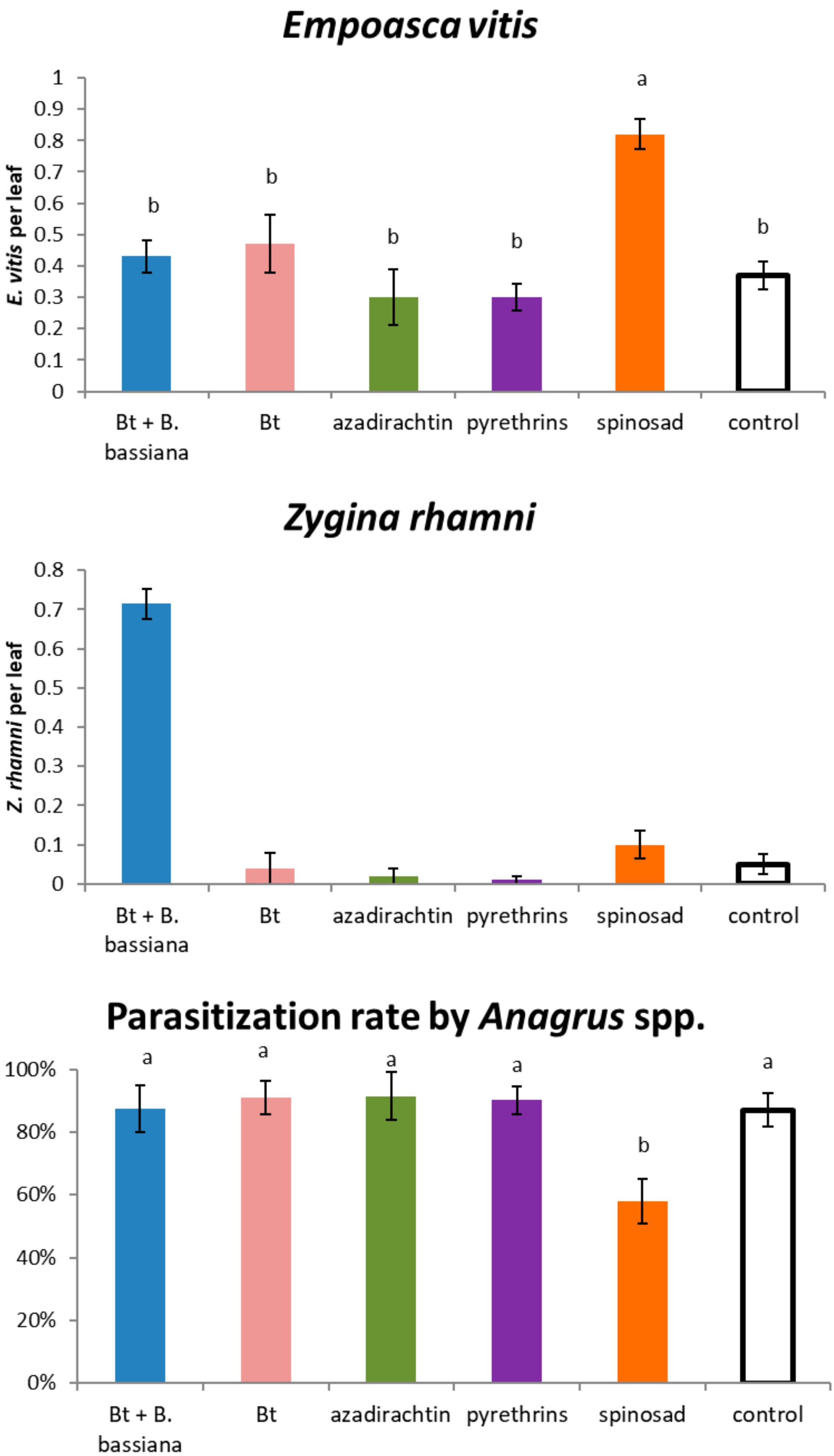

Figure 4. Effects of insecticide treatments on leafhopper density and parasitization rate of leafhopper eggs in vineyard 1 in the 2011 growing season (Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test (p = 0.05).

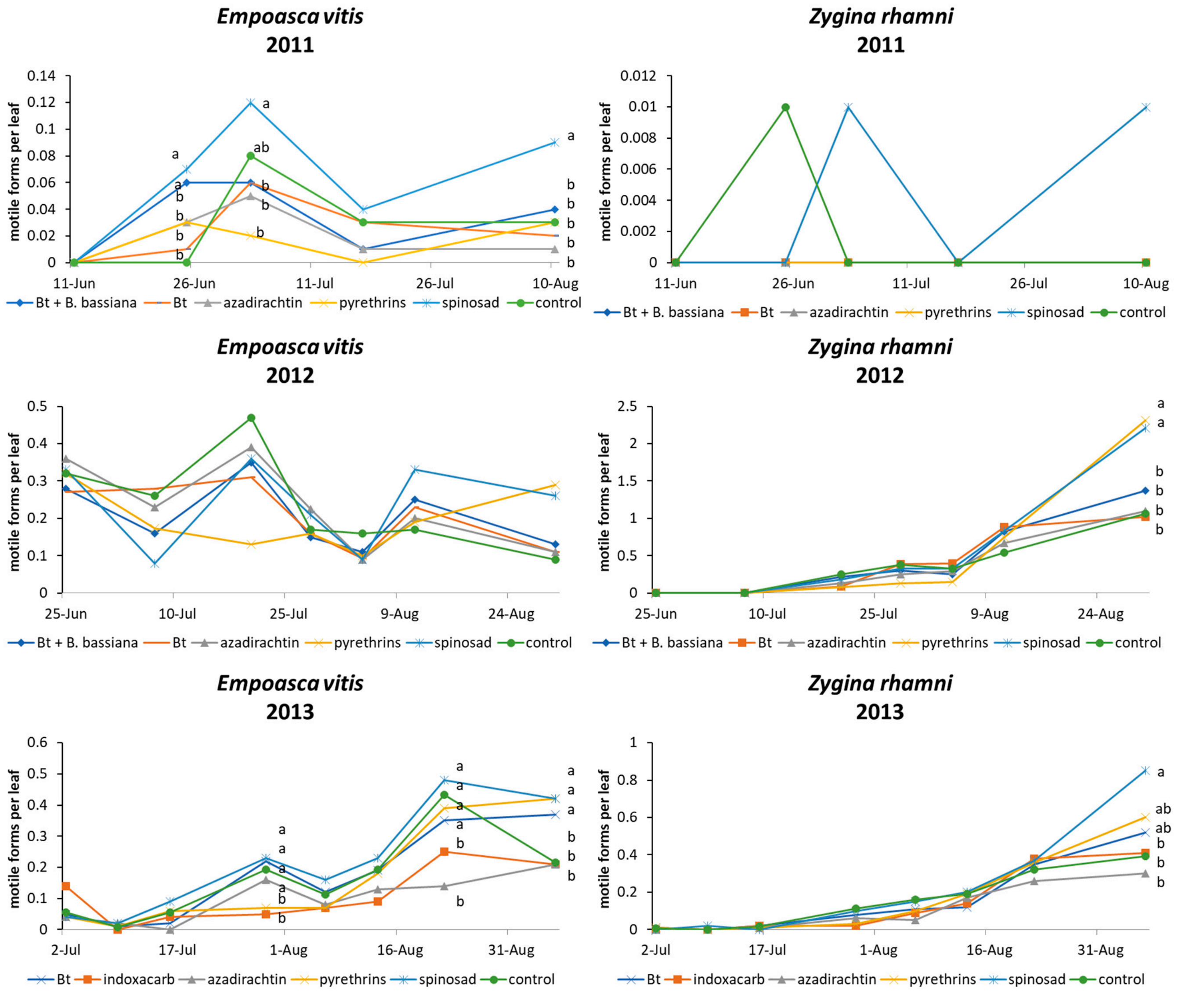

Figure 5. Effects of insecticide treatments on leafhopper population in vineyard 2 during three subsequent growing seasons (Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test (p = 0.05) among treatments on the same sampling date.

In 2012, leafhopper population densities were estimated analyzing leaf samples in the laboratory. Regarding the *E. vitis* abundance, there were no significant differences among treatments (Table 2). The effect of time was significant (Table 3) in contrast with the interaction "time × treatment" (Table 3). Regarding *Z. rhamni*, differences among treatments were not significant (Table 3), while the effect of time and interaction "treatment × time" were (Table 3). From the analysis performed over the sampling period, differences emerged in late season (Table 3), when higher densities of *Z. rhamni* were found on spinosad and pyrethrins than on the other treatments (Figure 5). The parasitization rate by *Anagrus* spp. attained the highest values (34%) in the control and the lowest (13%) in spinosad plots. However, differences among treatments were not significant (χ^2 = 0.87; df = 5; p = 0.522).

Moderate *E. vitis* densities occurred in leaf samples in 2013, but differences among treatments were significant (Table 3); in particular, higher densities were detected in the control, *B. thuringiensis* and spinosad compared to indoxacarb and azadirachtin treatments (Figure 5). The effect of time and the interaction "time × treatment" were also significant (Table 3). *Z. rhamni* populations increased in late season and differences among treatments were significant (Table 3; Figure 5); leafhopper densities were higher on spinosad than on indoxacarb and azadirachtin-treated plots (Figure 5). The effect of time was significant (Table 3) in contrast with interaction "treatment × time" (Table 3). In 2013, the parasitism rate by the Mymaridae reached low levels (<20%) with no differences among treatments.

3.4. Effects on Scales

The occurrence of coccids and mealybugs in both vineyards was generally low. However, insecticides caused significant effects on *P. corni* populations in the 2012 growing season. In vineyard 1, the effect of treatments was not significant (Table 3) in contrast with that of time and interaction "treatment × time" (Table 3). Differences among treatments were recorded in July, when higher coccid densities were found in the control, azadirachtin and spinosad than in the *B. thuringiensis* + *B. bassiana* treatment (Figure 6).

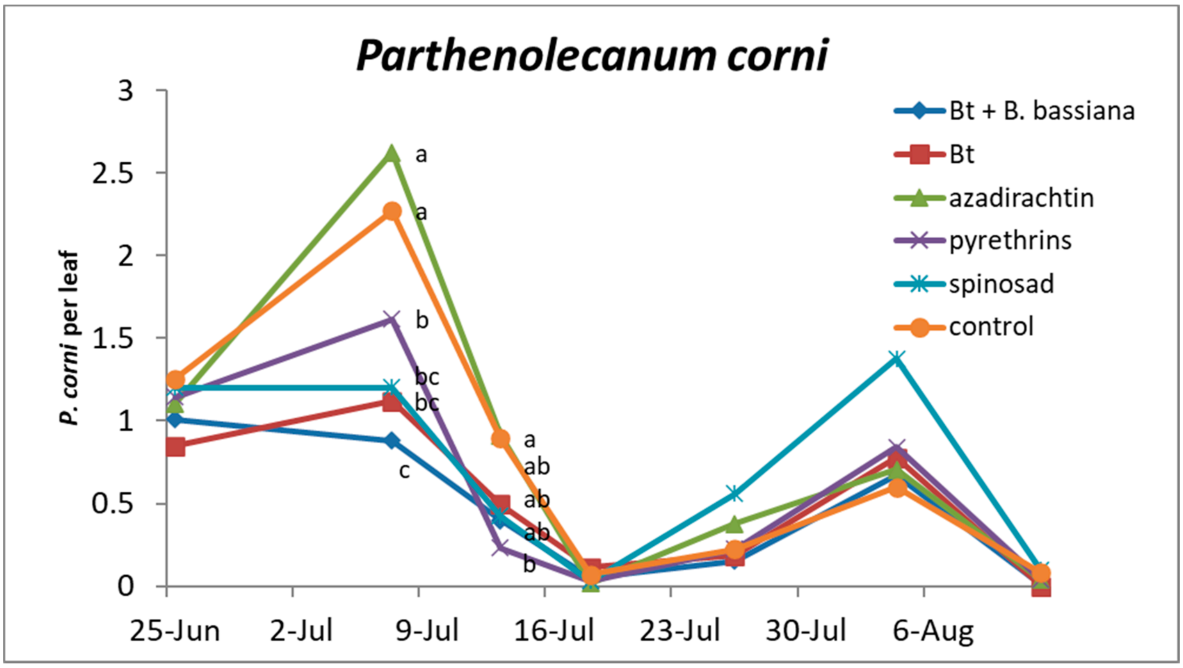

Figure 6. Effects of insecticide treatments on *Parthenolecanium corni* in vineyard 1 in the 2011 growing season (Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test ($p = 0.05$) among treatments on the same sampling date.

In 2013, mealybugs reached moderate levels in vineyard 2, but differences among treatments were not significant ($F = 1.11$; df = 5, 20.3; $p = 0.387$; data not shown).

3.5. Effects on Predatory Mites

Phytophagous mites (Acari: Tetranychoidea, Eriophyoidea) were recorded at very low densities in both vineyards. Predatory mites belonging to the Phytoseiidae family were commonly detected, in particular *Typhlodromus pyri* Scheuten, which dominated in vineyard 1, *Amblyseius andersoni* (Chant) and *Phytoseius finitimus* Ribaga in vineyard 2.

In vineyard 1, a number of insecticides applied in 2011 caused significant effects on predatory mite populations (Table 3). The effect of time was significant (Table 3) in contrast with that of the interaction "time × treatment" (Table 3). Prior to insecticides applications, there were no differences among treatments (Table 3). Later, predatory mite densities were significantly reduced in spinosad-treated plots compared to the control, *B. thuringiensis* and azadirachtin plots (Figure 7). There were no differences between spinosad, pyrethrins and *B. thuringiensis* + *B. bassiana*.

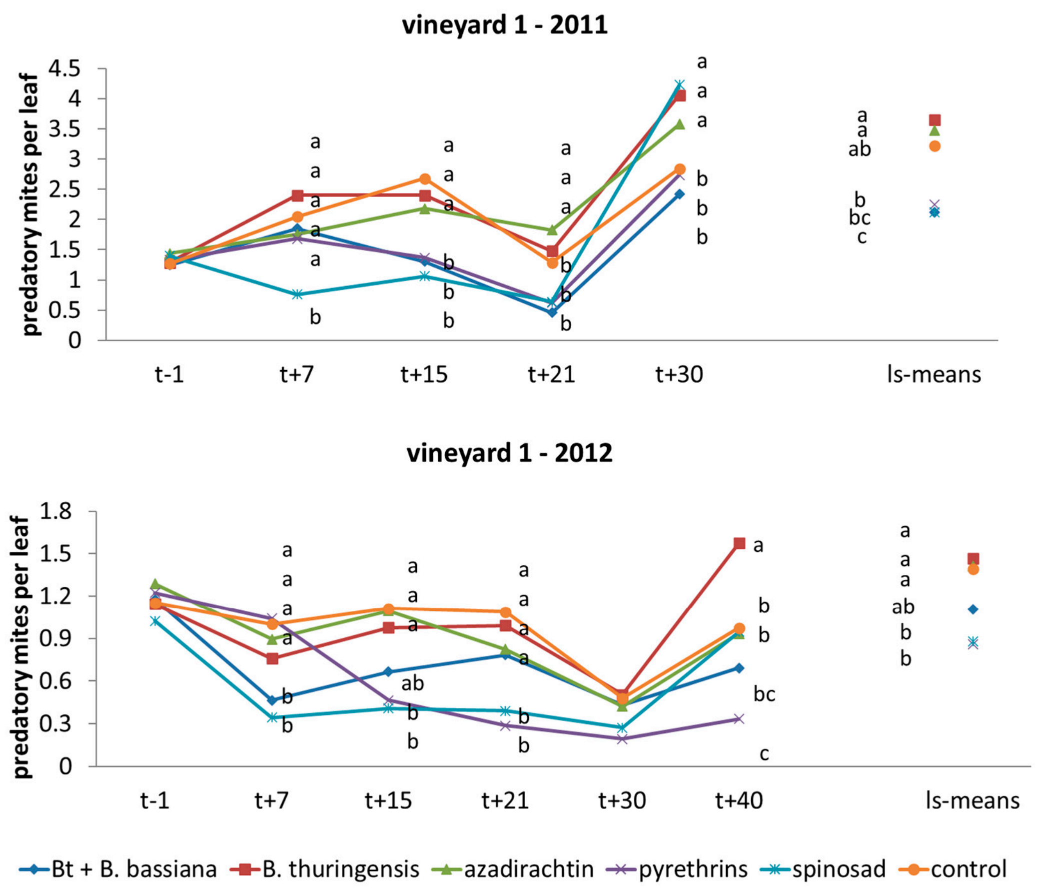

Figure 7. Effects of insecticide treatments on predatory mites in two subsequent growing seasons (2011, 2012) in vineyard 1 (Montepaldi, Tuscany, Italy; Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test ($p = 0.05$).

One year later, the effect of insecticides on *T. pyri* populations was confirmed to be significant (Table 3). The effect of time was also significant (Table 3), in contrast with the interaction "time × treatment" (Table 3). There were no differences among treatments before insecticide applications (F = 0.11; df = 5, 168; $p = 0.989$). Then, the use of spinosad and pyrethrins was associated with a decrease in predatory mite numbers compared to the control, *B. thuringiensis* and azadirachtin (Figure 7). There were no differences between *B. thuringiensis* + *B. bassiana* and the other treatments.

The effect of insecticides on predatory mites was also significant in vineyard 2 (Table 3). The effects of time were significant in the three growing seasons (Table 3). The interaction "time × treatment" was also significant (Table 3).

Prior to insecticide applications, differences among treatments were not significant (F = 0.29; df = 5, 80; $p = 0.917$; F = 0.21; df = 5, 108; $p = 0.958$; F = 1.05; df = 5, 151; $p = 0.384$; respectively in 2011, 2012 and 2013), while significant effects were observed later. In the first season, the use of spinosad and of pyrethrins reduced predatory mite densities compared to the control, *B. thuringiensis* and azadirachtin (Figure 8). The mixture between *B. thuringiensis* and *B. bassiana* was also associated with lower densities compared to the control (Figure 8). One year later, the impact of spinosad and pyrethrins was the most severe, followed by that of *B. thuringiensis* + *B. bassiana* and azadirachtin (Figure 8). In the third season, the applications of spinosad and pyrethrins caused significant reductions in the number of predatory mites as compared to other treatments (Figure 8).

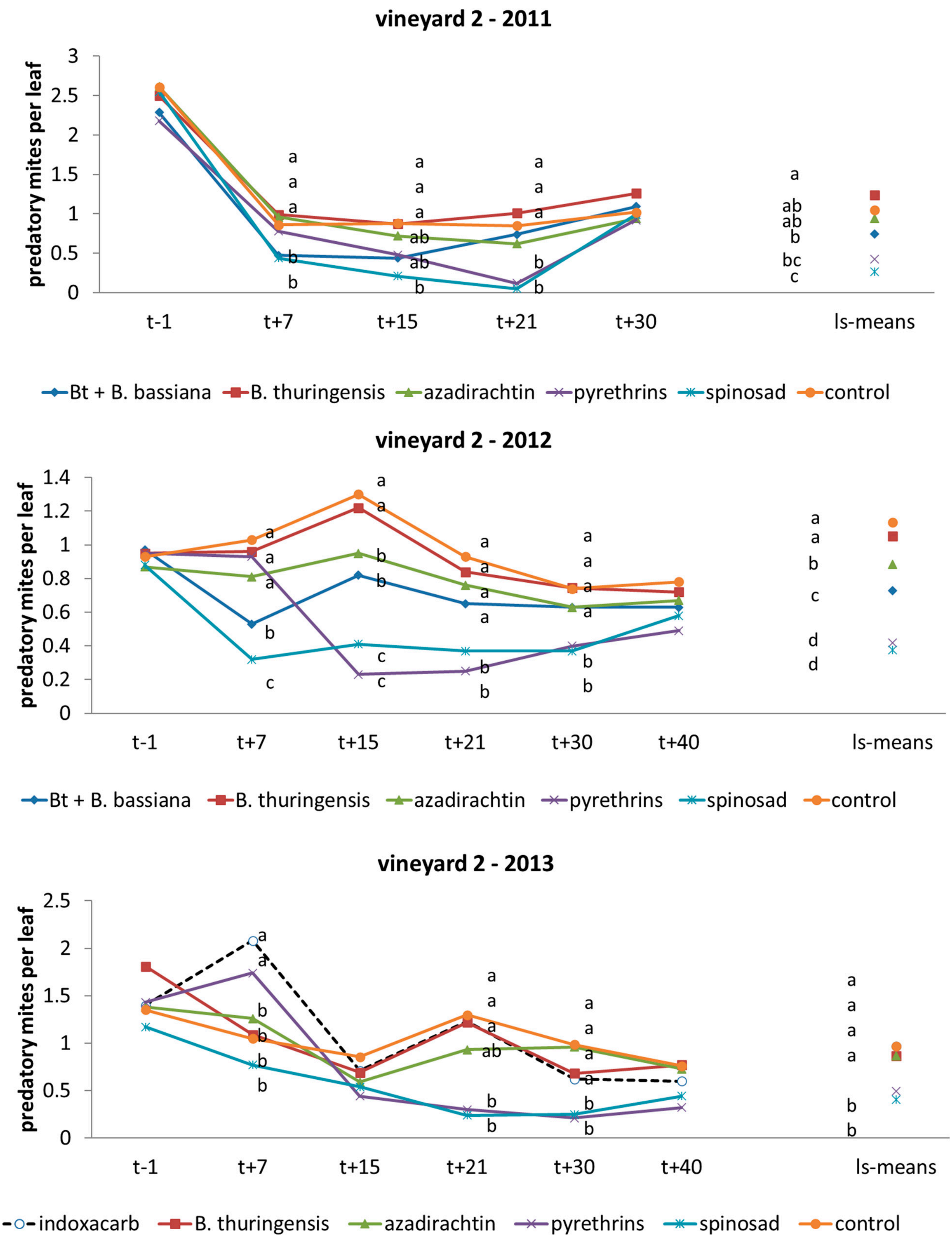

Figure 8. Effects of insecticide treatments on predatory mites in three subsequent growing seasons (2011, 2012, 2013) in vineyard 2 (Meolo, Veneto, Italy; Bt = *Bacillus thuringiensis*). Different letters indicate significant differences at *t*-test (p = 0.05).

4. Discussion

The main scope of these trials was to identify reliable alternatives to conventional insecticides in controlling grape berry moths. The evaluation of these tools was extended to their side-effects on some secondary pests and natural enemies to delineate a more comprehensive picture of their impact on arthropod communities associated with a definite crop system. Results highlighted the efficacy of spinosad and *B. thuringiensis* against grape berry moths compared to the other insecticides, including indoxacarb. Therefore, biopesticides can compete with conventional insecticides in controlling key pests. On the other hand, side-effects of tested insecticides were very different providing interesting data in different scenarios.

Spinosad was the most effective insecticide in controlling berry moths in the five trials, and in the last experimental year it was more effective than indoxacarb, a widely used insecticide in conventional viticulture. It is remarkable that the effects of spinosad on grapevine pests were poorly explored in the literature. It proved to be an effective tool in controlling *L. botrana* and thrips in southern Europe [23]. More recently, the efficacy of spinosad in controlling *D. suzukii* before oviposition was suggested in laboratory trials [24]. On the other hand, spinosad was tested against *S. titanus*, obtaining unsatisfactory results compared with other natural pesticides [25]. Our results showed that the use of spinosad can be associated with an increase in leafhopper and scale densities. Moreover, we found a negative effect of spinosad on the natural antagonists of pests. In the first experimental season, spinosad affected the leafhopper parasitization rate by mymarids, but this effect was not confirmed in the subsequent trials. Spinosad dramatically reduced predatory mite densities, confirming the results of trials conducted on other predatory mite species [26,27]. Caution about spinosad use has been expressed in a review devoted to the effects of spynosins on beneficial arthropods [28], and recent literature provides several examples of such effects [29,30].

Bacillus thuringiensis obtained a satisfactory control of berry moths in vineyard 2, while it was less effective in vineyard 1. Factors affecting these differences were not clear; perhaps the higher population densities of berry moths in vineyard 2 could have been involved. *B. thuringiensis* was considered an alternative to conventional pesticides in the 1970s, but the contrasting results in terms of efficacy against berry moths limited its practical use. New formulations characterized by a higher efficacy and stability were recently proposed and an improved knowledge about *B. thuringiensis* (strains, formulation, timing and frequency of application, spray volume) allowed Bt-based formulations (ssp. *kurstaki* or *aizawai*) to be evaluated again [18,31].

The mixture between *B. thuringiensis* and *B. bassiana* did not improve the results obtained by *B. thuringiensis* alone. Various entomopathogen fungi, including *B. bassiana*, have been proposed as biocontrol agents against different pests, but results obtained in viticulture are limited to a few species. An experimental strain of *B. bassiana* 432 (ITEM-1559) was tested against *L. botrana* in southern Italy, obtaining a reduction in berry moth damage and OTA contamination [7]. *Beauveria bassiana* has been tested against spider mites and thrips with good results [32] (Simoni S., pers. comm). Therefore, a formulation based on *B. bassiana* (ATCC 74040 strain) was authorized for the control of thrips and spider mites in Italian vineyards, and this formulation was used in our trials. While *B. thuringiensis* was confirmed to be a fully selective bioinsecticide, the present work showed that the mixture between *B. thuringiensis* and *B. bassiana* reduced predatory mite densities in some trials.

The effect of botanical pesticides on grapevine pests, including berry moths, has not been widely investigated. In some of our trials, azadirachtin showed results comparable with those of *B. thuringiensis,* while in others, its performance was lower. The application of azadirachtin to *L. botrana* larvae resulted in an inability to molt properly as well as deformities, a reduction in fecundity and fertility and a reduction in egg hatching [19]. In our trials, azadirachtin proved to be relatively harmless towards predatory mites.

Finally, the use of pyrethrins was associated with the worst results in terms of berry moth control and a decline in predatory mite densities. This insecticide proved to be the most effective in controlling *S. titanus* as compared to other natural pesticides [25], but is not recommended for the control of berry moths.

5. Conclusions

Natural insecticides can replace conventional ones in controlling grape berry moths, the key grapevine pests in Europe and the Middle East and reduce yield losses caused by them. However, the most promising, spinosad and *B. thuringiensis*, showed substantial differences in terms of their side-effects. Spinosad caused detrimental effects on predatory mites and was associated with a decline in the parasitization rate of leafhoppers. It was also associated with an increase in leafhopper population densities as a possible consequence of detrimental effects on beneficials. In contrast, *B. thuringiensis* proved to be selective, confirming data coming from the literature, and did not promote outbreaks of secondary pests. Differences between the two insecticides probably depend on their mode of action towards the target and non-target arthropods. *Bacillus thuringiensis* acts through ingestion, while spinosad through contact and ingestion. For a long time, the use of *B. thuringiensis* has been relegated to organic viticulture, but our results showed its potential in farms managed with Integrated Pest Management (IPM).

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