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The role of *Trichoderma* spp. and silica gel in plant defence mechanisms and insect response in vineyard

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Abstract

Several elicitors, stimulating induced resistance mechanisms, have potential in preventing or mitigating pathogen infections. Some of these compounds, triggering the production of jasmonic acid (JA), a precursor of herbivore-induced plant volatiles, could also play a central role in indirect resistance to pest species, by improving beneficial arthropod performance, and necrotrophic pathogens. In the current work, *Trichoderma gamsii*/*T. asperellum* and silica gel treatments – alone and in combination – were studied to evaluate the plant defence mechanism on grapevines (*Vitis vinifera* L.) by laboratory and field trials. JA production level was measured before and after *Plasmopara viticola* infection on potted vines. JA production induced by silica gel was higher than that caused by *Trichoderma* before infection. In *Trichoderma*-treated plants, JA production increased after *P. viticola* inoculation. In vineyard field trials, Mymaridae (Hymenoptera: Chalcidoidea) showed higher captures in transparent sticky traps on silica gel-treated plants, in comparison with control. On the other hand, no significant attraction was detected for Ichneumonoidea and other Chalcidoidea in silica gel and *T. gamsii*/*T. asperellum*-treated plants. The potential effects of elicitors are discussed, in the frame of attract and reward strategy.

Keywords: *Trichoderma gamsii*, *Trichoderma asperellum*, silica gel, vines, induced resistance, JA production, natural enemies, herbivores

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Introduction

Plants have a wide range of pests and diseases, and they have protected themselves by evolving defence mechanisms (Dicke, 2009). Plants can use several constitutive defences, such as physical barriers that prevent pathogen penetration or arthropod access to tissues (Walling, 2000), and induced mechanisms (Simpson *et al*., 2011*a*). The latter are defined as improvements of the plant's defensive capacity against diseases and pests, which are acquired after appropriate stimulation (Ramamoorthy *et al*., 2001). The stimulation of these defences is induced by some molecules called elicitors (Hahn, 1996). Induced resistance can activate two main signalling pathways: systemic-acquired resistance and induced systemic resistance. The first is mediated by salicylic acid dependent processes, whereas the second by the jasmonic acid (JA) and ethylene sensitive pathway (Walters *et al*., 2013).

Elicitors have been widely studied in plant pathology. A list of elicitors, employed in different plants against several pathogens, is available in Walters *et al*. (2013). The induced

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systemic resistance pathway can be triggered as a result of the interaction with beneficial microorganisms, which act as elicitors (Carvalhais *et al.*, 2013). Among these elicitors, *Trichoderma* spp. is one of the most studied. *Trichoderma* strains generally elicit induced systemic resistance and activate priming responses in the plant (Hermosa *et al.*, 2012). A number of authors recognized that *Trichoderma* strains are able to induce JA synthesis involved in induced systemic resistance development (Nawrocka & Malolepsza, 2013). Djonović *et al.* (2006), using specific inhibitors of JA/ethylene synthesis, showed that signalling pathways transduced by these molecules may be involved in the defence effect of *Trichoderma virens* (Mill., Giddens & Foster) against *Colletotrichum graminicola* (Ces.) G. W. Wilson in maize (Nawrocka & Malolepsza, 2013). Moreover, Shoresh *et al.* (2005) reported the involvement of JA and ethylene in the effect of *Trichoderma* spp. against *Pseudomonas syringae* pv. *lachrymans*. Finally, Perazzolli *et al.* (2011) highlighted that *Trichoderma harzianum* T39 is an important elicitor of grapevine resistance by means of the involvement of JA and ethylene signals in the defence responses against downy mildew.

For the mitigation and control of fungal and bacteria disease, also silicon has been reported as an effective tool (Bakhat *et al.*, 2018). Vivancos *et al.* (2015) showed the tolerance increase of silicon-treated *Arabidopsis thaliana* (L.) Heynh against powdery mildew, whereas Conceicao *et al.* (2014) found that the disease caused by *Xanthomonas translucens* pv. *undulosa* (Smith, Jones & Reddy) in wheat was reduced by the application of calcium silicate (Bakhat *et al.*, 2018).

Herbivore-induced plant volatiles represent one of the main defence strategies that plants implement to control herbivores (Dicke, 2009). Plants respond to herbivore feeding damage by producing mixtures of volatiles that are characterized by a considerable level of specificity in blend composition. They can not only differ in the quantity of volatiles released (per unit of biomass) but also in the composition of the volatile blend (Dicke & van Loon, 2000). The induced emission of plant volatiles attracts natural enemies and it occurs in response to herbivore attacks; beyond parasitoids and predators, volatiles can display an effect also on herbivores (Heil, 2008). Nevertheless, it is difficult to predict whether herbivores are repelled or attracted to herbivore-induced plant volatiles, because the cues may represent weakened plants, but also plants that are less attractive from a nutritional point of view (Dicke & van Loon, 2000). Under natural conditions, herbivore oral secretions induce the activation of signal transduction pathways. Indeed, the production of herbivore-induced plant volatiles is mediated by phytohormones such as JA, salicylic acid and ethylene (Dicke, 2009). It has also been demonstrated that an herbivore species uses jasmonate and salicylate to activate cytochrome P450 genes that are associated with detoxification either before or concomitantly with the biosynthesis of allelochemicals; the ability to 'eavesdrop' on plant defence signals protects the phytophagous against toxins produced by host plants (Li *et al.*, 2002).

Elicitors as a tool in integrated pest management have not fully explored. There is a growing interest in the potential field use of elicitors in applied entomology, including silicon (Si). The primal role of Si as beneficial element for plants under a range of abiotic and biotic stresses is beyond doubt (Reynolds *et al.*, 2009; Bakhat *et al.*, 2018). A number of studies have shown increased resistance of plants treated (soil and/or foliar application) with silicon to insect herbivores and other arthropods, such as folivores, borers, phloem and xylem feeders, mites and nematodes. The majority of studies were carried out following a two trophic level design, whereas few studies considered species belonging to the third trophic level (Reynolds *et al.*, 2016). Besides silicon, also biotic elicitors, such as *T. harzianum* T22, showed to enhance tomato indirect defences against aphids (Coppola *et al.*, 2017).

The aim of this study was to evaluate the role of *Trichoderma* spp. and silica gel (a specific silicon compound), including their combination, to induce defence mechanisms in vine plants (*Vitis vinifera* L.). In particular, a laboratory experiment was performed to detect the production and dynamic of JA, before and after *Plasmopara viticola* (Berk & Curtis) inoculation. A field experiment was carried out in order to assess if field treatments of silica gel and *Trichoderma* in vineyard, alone or in combination, are able to affect the attraction of natural enemies or herbivores. Our hypothesis is that treatments of *Trichoderma* or silica gel, modifying the JA dynamics, could change the attractiveness of the plants towards beneficial insects, boosting the indirect resistance mechanisms of the plants. We considered the vineyard as case study, for its economic importance and for evaluating the potential use of elicitors to induce a multi-task resistance, including attraction to beneficial insects for pest suppression (this study) and in the perspective of a future use for mitigation of fungal diseases, which are very damaging in grapevines. In fact, elicitors may represent a valid alternative to fungicides, including copper, which is the only allowed effective fungicide against downy mildew in Italian organic viticulture. The use of copper has been recently dropped by the European community at a maximum of 4 kg per ha per year in Italy and alternative tools to reduce its use represent a recent challenge in viticulture scenario.

Materials and methods

Laboratory experiment

Plant material and product application

We performed trials using 20 vines, 2 years old, cv. Sangiovese grafted onto K5BB grown in pots $14 \times 14 \times 16$ cm^3 containing about 2.8 litres of peat mixed with expanded clay. We grew vines in open frame and each potted vine was watered daily by a drip irrigation system. We set up four treatments: (i) *Trichoderma* product; (ii) silica gel; (iii) combination of *Trichoderma* product + silica gel and (iv) untreated control. *Trichoderma* product consists of Remedier®, a commercial formulation of *Trichoderma asperellum* ICC012 and *Trichoderma gamsii* ICC080 (Gowan Italia, Faenza, Italy). We incorporated Remedier into the soil at 89 mg l^{-1} soil (250 mg per vine) per plant, immediately before transplantation of plants in the pots. Silica gel is a specific silicon compound containing treated amorphous silicates, quartz sand and diatomaceous earths (Siqur Salute, Vigonza, Italy). We applied silica gel by means of foliar spray at 0.12 g l^{-1} aqueous solution of micronized powder at the stage of ten leaves per shoot, and one shoot per vine.

Artificial inoculation

We performed the experiment in a growth chamber and used five vines per treatment. We performed artificial inoculation of *P. viticola* on each vine of the four treatments, 21 days after silica gel application. We made the inoculation by spraying the abaxial surface of leaves with conidial suspension at 10^6 sporangia of *P. viticola* per ml, assessed in a Thoma cell

counting chamber (a laboratory tool for counting suspended cells in a given volume) (Hajji-Hedfi *et al.*, 2018). We grew vines under controlled conditions with a light cycle of 8/16 night/day at 25°C and about 220 μmol m^{-2} s^{-1} of photosynthetically active radiation. We constantly maintained inoculated plants in a wet chamber (plastic bag) until the evasion of sporangia of the pathogen on the leaf generally noticed at 6 days after inoculation.

Biochemical analysis

We evaluated the production of methyl jasmonate (MeJa) in treated and control leaves, collected before and after the artificial inoculation and stored at −80°C until use. We performed each sampling by taking one leaf per plant, for a total of five leaves per treatment, each leaf representing a repetition. For each treatment and time of sampling, we collected leaves of the same type and position in the plant. We used the samples before the artificial inoculation to evaluate the MeJa production at time 0 and considered the basal production of JA by plants, only due to the application of the products. For each treatment, we evaluated the production of MeJa by collecting leaves for the first 4 days after inoculation.

We performed the analysis of MeJa by means of a solid phase microextraction (SPME) system (Zadra *et al.*, 2006) purchased from Supelco (Bellafonte, PA, USA) in the headspace followed by gas chromatography.

For each sample, we ground 0.5 g of tissue of a single leaf in liquid nitrogen. Then, we suspended the sample in 1 ml of 30% NaCl and immediately subjected it to the headspace extraction by stirring for 30 minutes at 67°C. We extracted the MeJa delivered in the head space with a fibre coated with PDMS 100 μm film thickness mounted on a manual fibre SPME holder.

We performed GC analyses using a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a 1177 split/splitless injector, a 30 m × 0.25 mm i.d., 0.25 μm, CP-Sil8CB capillary column (Varian), a FID detector and Star Chromatography Workstation software 5.51 (Varian Inc.). We directly made desorption of the fibre into the injector port for 5 min at 250°C in splitless mode. The injector split/splitless programme mode was: 0–5 min splitless; 5.01–5.75 min at 1:50 split ratio. We programmed the column oven at 60°C (1 min) to 280°C (2 min) at 25°C min^{-1}. The temperatures of the injector port and detector were 250 and 280°C, respectively. We used helium as a carrier gas and maintained its pressure constant at 10.0 psi (1 ml min^{-1}). After desorption, we cleaned the fibre with an additional step of desorption at 250°C for 15 min.

We performed standard curve adding a known amount of a solution of 1.03 g ml^{-1} MeJa (Sigma-Aldrich, St. Louis, MO, USA) to a 1 ml of 30% NaCl in distilled water. For each run, we calculated the area of a well recognizable peak at 5.8 min and converted peaks of the samples to milligram by comparing with the standard curve. The values are presented as mg of MeJa per g of leaf tissue.

Before the use, we activated the new fibre by placing it at 250°C in the oven of a gas chromatograph, according to the instructions of the manufacturer.

Field experiment

Vineyard characteristics

We carried out the trial in an organic commercial vineyard located in Monteveglio, Province of Bologna (Northern Italy). We considered a representative cultivar of this grape area as 'Pignoletto', 20 years old, spurred cordon trained. The experimental site was characterized by simple habitat complexity; scarce woody vegetation and hedgerows were present in the perimeter and around the vineyard.

Experimental planning

The experiment included four treatments: *Trichoderma* (T), silica gel (S), *Trichoderma* plus silica gel (T × S) and control (C).

In the field trial, we used the same *Trichoderma* and silica gel commercial formulations of lab experiment. For *Trichoderma*, we used a concentration of 2.5 kg hl^{-1}, applying 2 litre per vine plant on soil with an injector pole. We sprayed silica gel on vines foliage at inflorescences swelling, using 12 g of silica gel per hl. We performed the treatment with *Trichoderma* on 4th of May (BBCH 53), while we sprayed silica gel on 20th of May (BBCH 61). In T × S treatment, we carried out both soil and foliar application with *Trichoderma* and silica gel respectively. We made no applications in the control. We carried out each treatment in plots of 90 m^2. The minimum distance among each plot was 20 m, using a complete randomization. We carried out five replicates per treatment; each replicate was represented by six plants.

Arthropod sampling

We used two transparent sticky traps (12 × 15 cm^2) for each treatment replicate to sample insects, for a total of 40 traps per each sampling date. We made traps in laboratory using glue (Tanglefoot) applied only on one side in order to simplify their management. We selected transparent sticky traps in order to avoid distortion in captures due to a potential colour attraction towards flying insect (Irvin *et al.*, 2016). Moreover, non-attractive sticky traps used in this study can be considered an effective trapping method to sample parasitoid taxa, as demonstrated in the experiments carried out in Australia (Simpson *et al.*, 2011*a*, *b*).

We placed sticky traps at 1.50 m high on vines foliage after 11 days from elicitor treatment. We collected traps after 7 days, and this procedure was repeated in three consecutive weeks (7-14-21 June), for a total of 120 sticky traps in the whole experiment. For each sampling date, we collected traps, moved to laboratory and checked under a stereomicroscope. We identified all the arthropods captured to family, superfamily, sub-order and orders.

Data analysis

Laboratory experiment

For the post-infection phase, we studied the effects of silica gel (S) and *Trichoderma* (T) on JA production by means of repeated measures analysis of variance (ANOVA). We considered silica gel and *Trichoderma* as 'between-groups' factors (with two levels each) and included days after *P. viticola* infection as repeated measures ('within-group' factors). We also tested all the possible interactions. Given that the interaction silica gel × *Trichoderma* × days post infection was statistically significant (i.e. the trend in time of JA production was different among treatments), we ran a factorial ANOVA for each one of the days post-infection. In this model, we considered silica gel and *Trichoderma* as factors and we also included the interaction

silica gel × *Trichoderma*. We performed factorial ANOVA also for the pre-infection phase.

Field experiment

We considered various data structures for beneficial insects collected by sticky traps and final selection of error distribution was based on lowest Akaike's information criteria values. The data were analysed by generalized estimating equations with negative binomial error distribution and log link function. We considered the number of individuals for each taxon as the dependent variable; we used silica gel and *Trichoderma* as fixed factors, whereas we considered sampling dates as repeated measures. We tested the interactions silica gel × *Trichoderma* × dates, silica gel × dates, *Trichoderma* × dates and silica gel × *Trichoderma* as well.

We carried out statistical analysis with IBM SPSS statistical packages and Statistica version 10 software (StatSoft™).

Results

Laboratory experiment

In the pre-infection phase, silica gel showed a significant effect on JA production (df = 1; $F = 22.823$; $P < 0.001$), while *Trichoderma* did not affect the production of JA (df = 1; $F = 2.114$; $P > 0.05$); a significant *Trichoderma* × silica gel interaction was detected (df = 1; $F = 4.990$; $P < 0.05$), showing that JA synthesis caused by silica gel was affected by the presence of *Trichoderma*. In particular, in *Trichoderma* × silica gel treatment the JA production was higher than control and *Trichoderma* alone, but lower than recorded with silica gel alone (fig. 1).

Regarding the post-infection phase, repeated measures ANOVA showed a significant effect of days, silica gel, *Trichoderma* and also the interactions between them (table 1).

In the first day after inoculation, silica gel induced the highest level of JA production in the plants. Silica gel had a significant effect on the level of JA (df = 1; $F = 36.211$; $P < 0.001$), as well as *Trichoderma* × silica gel (df = 1; $F = 6.095$; $P < 0.05$), which presented an intermediate JA level production between silica gel and *Trichoderma*. On the other hand, *Trichoderma* did not show any significant effect (df = 1; $F = 4.078$; $P > 0.05$) (fig. 2a).

Trichoderma caused a significant increase of JA production 2 days after inoculation (df = 1; $F = 271.25$; $P < 0.001$), inducing the highest JA level production among treatments. Also *Trichoderma* × silica gel induced a significant increase of the phytohormone (df = 1; $F = 14.84$; $P < 0.01$), whereas silica gel had no significant effect in this day (df = 1; $F = 0.02$; $P > 0.05$) (fig. 2b).

In the third day after inoculation, JA production changed again. Silica gel significantly increased the JA level (df = 1; $F = 94.652$; $P < 0.001$), more than the other two treatments, which had still significant effect on JA production (T: df = 1; $F = 27.774$; $P < 0.001$; T × S: df = 1; $F = 34.794$; $P < 0.001$) (fig. 2c).

Finally, control had a higher level of JA production compared with other treatments in the fourth day, even though silica gel and *Trichoderma* × silica gel had a significant effect on the phytohormone production (S: df = 1; $F = 15.864$; $P < 0.01$; T × S: df = 1; $F = 16.075$; $P < 0.01$) (fig. 2d).

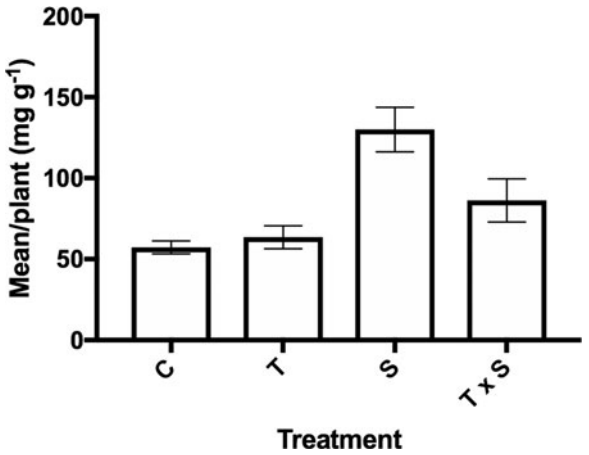

Fig. 1. Mean JA production per plant (each plant represented by one leaf) in each treatment and their combination, before *P. viticola* infection. C, untreated control; T, *Trichoderma* treatment; S, silica gel treatment; T × S, combination of *Trichoderma* and silica gel.

Table 1. Repeated measures ANOVA results concerning JA production after *P. viticola* infection.

Effect	F	df	*P*
S	86.057	1,16	<0.01
T	216.139	1,16	<0.01
D	732.399	1,16	<0.01
T × S	50.366	1,16	<0.01
S × D	118.565	1,16	<0.01
T × D	203.855	1,16	<0.01
S × T × D	23.055	1,16	<0.01

S, silica gel treatment; T, *Trichoderma* treatment; D, days.
P value shows a significant effect of days, silica gel, *Trichoderma* and all the interactions between them.

In conclusion, *Trichoderma* showed a peak of JA production 2 days after *P. viticola* infection and, after this increase, the level of JA decreased in the following days. On the other hand, the trend of silica gel seems to be more constant over time, with a peak on the third day after infection. Finally, *Trichoderma* × silica gel seems to present an intermediate trend between T and S, which clearly highlights the interaction between *Trichoderma* and silica gel.

Field experiment results

Faunistic analysis

A total of 41,456 arthropods were collected using sticky traps in three sampling dates. Diptera represented 96% of the collected individuals, while Hymenoptera accounted for 1.74% of the total. Nematocera were the most numerous taxon of Diptera sampled (98.23%) followed by Phoridae (1.39% of Diptera); other Diptera (Stratiomydae, Empididae, Agromizidae, Opomyzidae, Drosophilidae, Tachinidae, Sphaeroceridae) accounted for relative abundances lower than 1%. Within Hymenoptera, Mymaridae family was the most represented (48.19%), followed by other Chalcidoidea (28.19%), Braconidae (8.61%) and Ichneumonidae (1.53%).

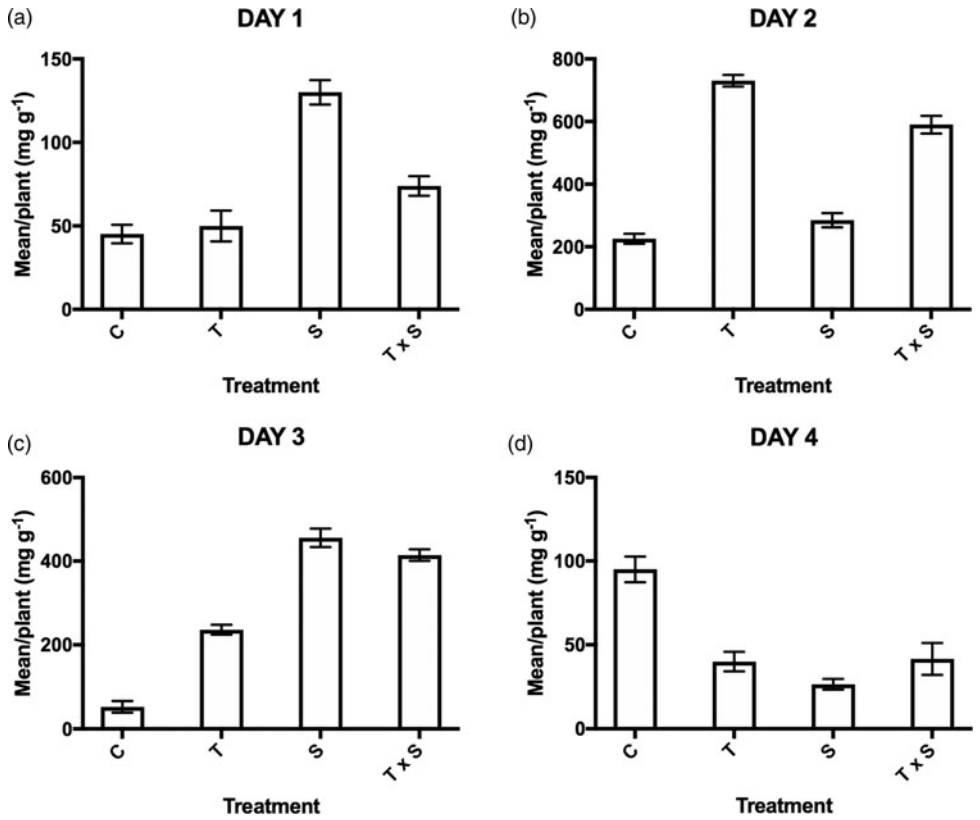

Fig. 2. Mean JA production per plant (each plant represented by one leaf) in each treatment and their combination in 4 days after *P. viticola* infection. C, untreated control; T, *Trichoderma* treatment; S, silica gel treatment; T × S, combination of *Trichoderma* and silica gel.

Finally, Thysanoptera represented the third abundant order (1.03% of the total). Other taxa such as Coleoptera (Coccinellidae and Staphylinidae) were poorly represented, with relative abundances lower than 1% of the total.

Data analysis was performed only for Mymaridae, other Chalcidoidea and Ichneumonoidea (Braconidae and Ichneumonidae), being the most abundant Hymenoptera taxa and for their important role in sustaining ecosystem services in vineyard. For statistical analysis, Braconidae and Ichneumonidae were pooled into the superfamily of Ichneumonoidea, due to the low number of specimens collected for these families.

Mymaridae is an important family of leafhoppers parasitoids, such as *Empoasca vitis* Goëthe. Other Chalcidoidea and Ichneumonoidea include several parasitoids of many vineyard pests, including *Planococcus ficus* Signoret and *Lobesia botrana* (Denis & Schiffermüller).

Functional analysis of the effect of elicitors and their combination on insect taxa

Tables 2–4 show the results of the generalized estimated equations with negative binomial error distribution analysis.

Silica gel treatment demonstrated to significantly boost ($P < 0.05$) the captures of Mymaridae (Hymenoptera). The other two taxa, including other Chalcidoidea and Ichneumonoidea, did not show any significant attraction towards plants treated with *Trichoderma* and silica gel. Only time (dates) had a significant effect on all three taxa (tables 2–4).

Table 2. Generalized estimating equations results for Mymaridae.

Effect	Wald χ^2	df	*P*
S	4.009	1	**0.045**
T	2.070	1	0.150
D	13.098	2	**0.001**
T × S	0.201	1	0.654
S × D	0.234	2	0.890
T × D	2.115	2	0.347
S × T × D	3.355	2	0.187

S, silica gel treatment; T, *Trichoderma* treatment; D, dates.
P value shows that silica gel significantly attracts Mymaridae parasitoids.

Mymaridae

The significant attraction of silica gel towards Mymaridae (table 2) can be evinced by the main effect, which compares the mean captures between treatments with and without this elicitor. Indeed, there were 6.77±0.7 (SE) Mymaridae in treatments containing silica gel, but only 4.8±0.7 (SE) in treatments without the elicitor. The increase of captures seems to be noticeable mainly in the second date (14th of June) (fig. 3), though the interaction between silica gel and date was not significant.

Plants treated with *Trichoderma* captured less Mymaridae than control, but this variation was not statistically significant (fig. 3 and table 2). Also the interaction between *Trichoderma* and silica gel was not significant (fig. 3).

Table 3. Generalized estimating equations results for other Chalcidoidea.

Effect	Wald χ^2	df	*P*
S	0.182	1	0.669
T	0.159	1	0.690
D	16.670	2	**0.000**
T × S	1.432	1	0.231
S × D	2.295	2	0.317
T × D	5.948	2	0.051
S × T × D	0.185	2	0.911

S, silica gel treatment; T, *Trichoderma* treatment; D, dates.
All treatments do not show any significant effect in attracting this taxon.

Table 4. Generalized estimating equations results for Ichneumonoidea.

Effect	Wald χ^2	df	*P*
S	0.476	1	0.490
T	0.259	1	0.611
D	8.071	2	**0.018**
T × S	0.635	1	0.425
S × D	1.261	2	0.532
T × D	1.582	2	0.453
S × T × D	0.014	2	0.993

S, silica gel treatment; T, *Trichoderma* treatment; D, dates.
All treatments do not show any significant effect in attracting this taxon.

Other Chalcidoidea

For the other Chalcidoidea taxon, silica gel and *Trichoderma* did not show any significant increase of captures (table 3). The mean captures during the three dates were lower in *Trichoderma* treatment than the control, with the exception of the first one (fig. 4). Finally, there was a higher number of captures in *Trichoderma* × silica gel treatment than those of the control, though interaction between the elicitors was not significant (table 3; Fig. 4).

Ichneumonoidea

Both treatments did not display any significant effect on Ichneumonoidea (table 4). Also *Trichoderma* × silica gel and control did not present any significant differences, but *Trichoderma* × silica gel tended to have lower means than the control in all three dates (fig. 5).

Discussion and conclusions

Laboratory experiment showed that silica gel and *Trichoderma* triggered defence mechanisms, confirming previous studies (Fauteux *et al.*, 2005; Perazzolli *et al.*, 2011; Nawrocka & Malolepsza, 2013; Wang *et al.*, 2017; Bakhat *et al.*, 2018). The dynamic of JA production in silica gel-treated plants was different in comparison with those treated with *Trichoderma*. In particular, silica gel produced a higher level of JA in comparison with control before *P. viticola* inoculation. *Trichoderma* did not show a significant increase of JA compared with the control before *P. viticola* infection, triggering the production of JA only after inoculation. Therefore, only a priming effect was showed for *Trichoderma*, with the highest peak of JA production 2 days after infection, which in turn activate the plant defence as faster and/or more intense responses to the pathogen attack, with the production of JA (Tucci *et al.*, 2012). The production of JA by *Trichoderma* only lasted for a limited period, associated with the infection.

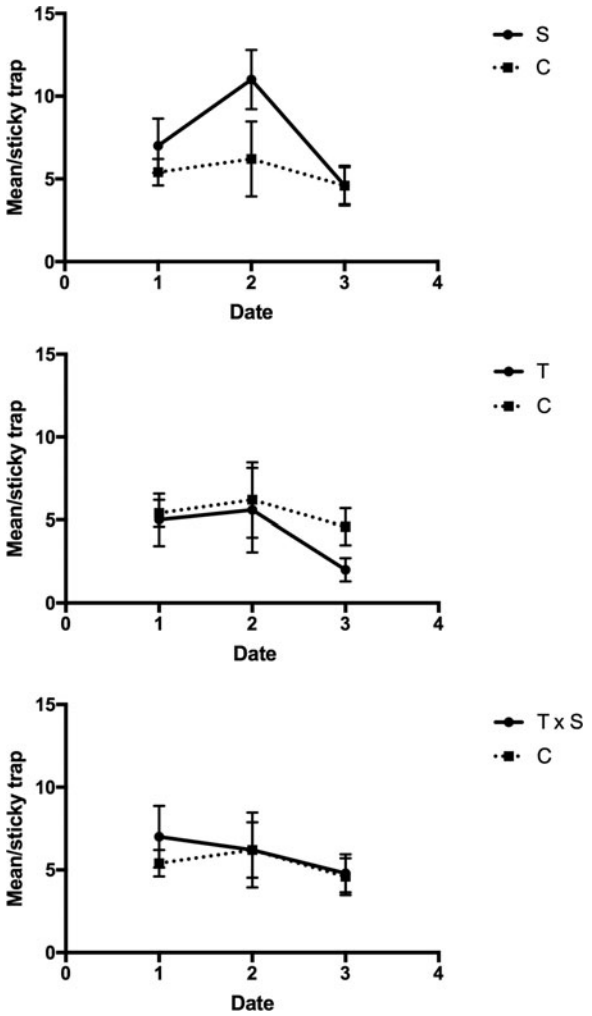

Fig. 3. Mean captures of Mymaridae per sticky trap in each treatment and date. Bars represent the standard errors of means (for full dataset, see Supplementary Table 1). C, untreated control; T, *Trichoderma* treatment; S, silica gel treatment; T × S, combination of *Trichoderma* and silica gel.

On the contrary, silica gel seems to be effective at enhancing plant defence pathways, both before and after *P. viticola* inoculation.

The field study demonstrated that captures of Mymaridae were higher in the plants treated with silica gel elicitor in comparison with those of control. In particular, the increase of Mymaridae captures in silica gel treatment was evident in the second sampling date. This peak can be explained by the JA dynamic evinced in the laboratory study, both before and after infection. Indeed, the enhance of Mymaridae capture in silica gel treatment seems to coincide with JA production peak of silica gel after 2–3 days from pathogen inoculation. Actually, a slight infection appeared around 9th of June, exactly in the week of the second sampling, which occurred

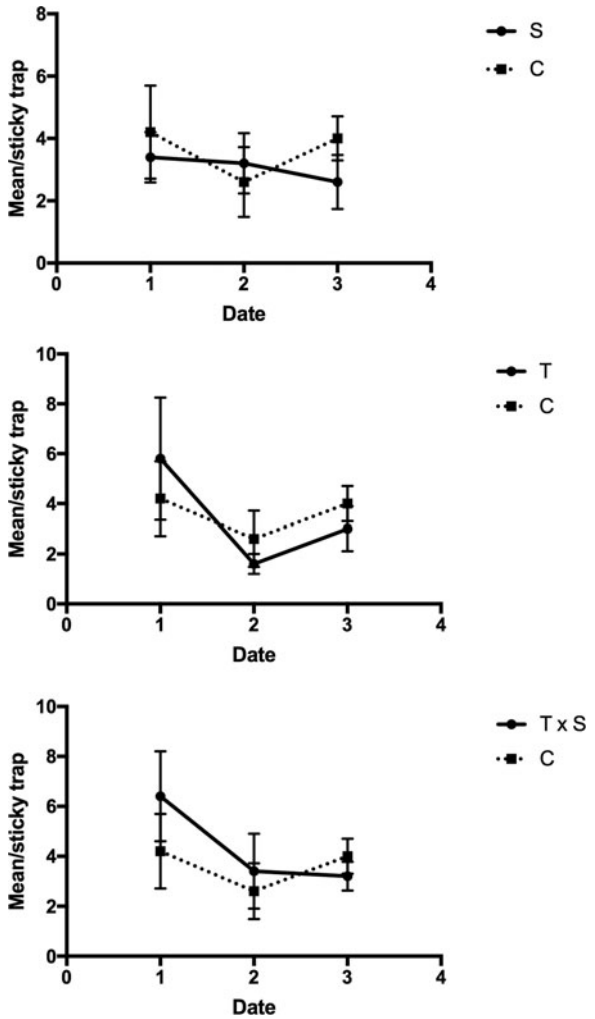

Fig. 4. Mean captures of other Chalcidoidea per sticky trap in each treatment and date. Bars represent the standard errors of means (for full dataset, see Supplementary Table 1). C, untreated control; T, *Trichoderma* treatment; S, silica gel treatment; T × S, combination of *Trichoderma* and silica gel.

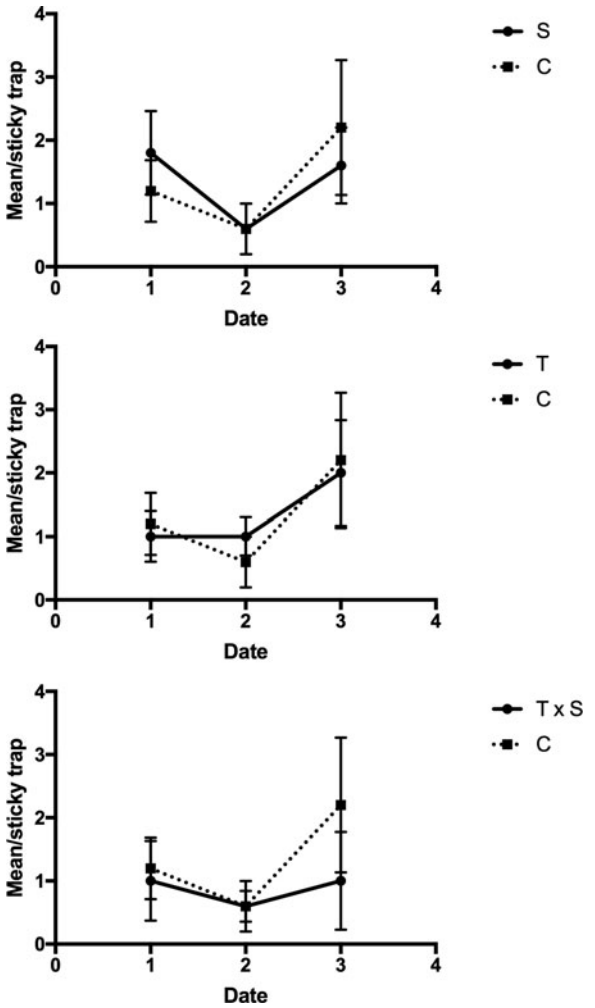

Fig. 5. Mean captures of Ichneumonoidea per sticky trap in each treatment and date. Bars represent the standard errors of means (for full dataset, see Supplementary Table 1). C, untreated control; T, *Trichoderma* treatment; S, silica gel treatment; T × S, combination of *Trichoderma* and silica gel.

between 7th and 14th of June. However, *Trichoderma* did not show any attraction or repulsion towards the studied taxa. Actually, according to Mymaridae captures, it seems that the triggering of JA due to infection can attract Mymaridae only if preceded by a production of JA before the infection, as it is recorded for silica gel especially, while the combination *Trichoderma* × silica gel does not seem sufficient to achieve the same effect.

Only few earlier studies have been conducted about the effects of elicitors on insects. Rostás and Turlings (2008) showed that maize plants treated with benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH), after caterpillar infestation, boosted more females of the braconid parasitoid *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) than untreated plants (Sobhy *et al*., 2014). Moreover, Sobhy *et al*. (2014) demonstrated that foliar application of BTH enhanced the attractiveness of *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) infested plants to three different parasitoid species (*M. rufiventris*, *Cotesia marginiventris* (Cresson; Hymenoptera: Braconidae) and *Campoletis sonorensis* (Cameron; Hymenoptera: Ichneumonidae)). Similar to BTH, also laminarin attracted two parasitoid species to herbivore-induced plants.

Also, about silicon only few experimental studies are known. Moraes *et al*. (2004) tested the Si effect on natural enemies, demonstrating detrimental results of Si on the pest *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), but no effect on natural enemies. Another study showed that silicon applied to plants with a consequent pest infestation enhances the attractiveness of plants to natural enemies (Kvedaras *et al*., 2010). The adult *Dicranolaius bellulus* (Guérin-Méneville) (Coleoptera: Melyridae) were significantly more attracted to cucumber plants infested by *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) larvae and supplied with potassium silicate, than pest-infested and untreated plants (Kvedaras *et al*., 2010). Lastly, in a field experiment, predation was higher for infested and potassium silicate-treated plants (Kvedaras *et al*., 2010). Moreover, Liu

et al. (2017) demonstrated that both adult female of *Trathala flavo-orbitalis* (Cameron) (Hymenoptera: Ichneumonidae) and *Microplitis mediator* Haliday (Hymenoptera: Braconidae) manifested greater attraction to the herbivore-induced plant volatile blend of rice plants infested with their own insect hosts and treated with a sodium metasilicate (Na_2SiO_3) hydroponic solution compared with Na_2SiO_3-untreated and infested plants.

Under the condition of our field study, silica gel proves to be an effective elicitor and to have a great capacity of attractiveness to Mymaridae. Therefore, our hypothesis is that silica gel, by means of JA production, induces the release of herbivore-induced plant volatiles.

Trichoderma, on the other hand, did not show any significant effect on the studied taxa. The priming effect of this elicitor that occurred after *P. viticola* infection could explain this result. This study confirmed the elicitor effect of *Trichoderma* on JA production as strongly affected by the presence of the infection. Moreover, this effect lasts a limited time compared with silica gel, probably too short to influence the attractiveness or repulsion of the investigated insects. However, *T. harzianum* T22 boosted aphid parasitoid attraction towards 'elicitor treated and infested plants' compared with 'untreated and not infested ones' under controlled conditions (Coppola *et al*., 2017).

Silica gel did not show any effect on other Hymenoptera parasitoids, including Ichneumonoidea. In the experimental vineyard, Ichneumonoidea were characterized by a relative low level of captures depending on the scarce presence of hosts or by the lack of biodiversity near the vineyard. Indeed, in the perimeter area there were not hedgerows or woody vegetation. Several studies highlighted the importance of non-crop habitats in enhancing parasitoid populations (Thomson & Hoffmann, 2009; Simpson *et al*., 2011*b*; Loni & Lucchi, 2014; Hassan *et al*., 2016). Otherwise, Mymaridae, which is an important family of leafhoppers parasitoids, were captured in higher numbers on sticky traps, in comparison with other parasitoid taxa. Thomson & Hoffmann (2010) demonstrated that the spatial scale at which non-crop vegetation influences beneficial abundance may differ. For parasitoids, the spatial scale is variable and associated with their size which influences their dispersal activity. Therefore, we hypothesize that Mymaridae, being a group of small natural enemies, are more closely tied to habitat resources than larger parasitoids (i.e. Ichneumonidae and Braconidae) that need to be preserved by larger undisturbed areas (Thomson & Hoffmann, 2010). The abundance of small parasitoids may be more influenced by local features, such as ground cover and floral resources (Smith *et al*., 2015).

Our preliminary field experiment should be replicated under other field conditions, in order to study potential effect of elicitor attraction on natural enemies in different receiving environments. Considering that the capture efficiency can be affected by the abundance of beneficial populations, including Hymenoptera parasitoids, other field trials could evoke different results. Also, the integration of agroecology and chemical ecology, including the testing of other elicitors, represent a potential strategy to implement conservation biological control (Simpson *et al*., 2011*b*). Recently, applications of synthetic plant volatiles as attractants for beneficial insects were tested by field studies (James & Price, 2004; Simpson *et al*., 2011*a*; Lucchi *et al*., 2017). Moreover, the use of cover/plants management is considered an agroecological technique to enhance the vineyard functional biodiversity (Duso *et al*., 1993, 2004, 2012; Gurr *et al*., 2007; Thomson & Hoffmann, 2007; Altieri *et al*., 2010; Burgio *et al*., 2016). For this reason, using elicitors as an attractant for natural enemies and flowering plants to provide them food and shelter, represents potential to reduce the lag between the build-up of natural enemies and taking control of pests (Simpson *et al*., 2011*b*). In this way, the 'attract and reward' approach could be an efficient tool for enhancing conservation biological control.

Moreover, further research should be addressed to quantify the influence of elicitors also on intensity of ecosystem services, including parasitization or predation. Besides the quantitative analysis of arthropod fauna dynamics, further studies should be addressed to assess the precise role of 'attract and reward' approach in parasitism rate of the most damaging pests of vineyard, including *L. botrana*, *P. ficus* and leafhoppers. Overall, this field experiment, whether confirmed in other contexts, could represent a potential strategy in the frame of the modern approach of integrated pest management.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S0007485319000075

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