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Antifeedant and insecticidal effects of alfalfa saponins in the management of the Japanese beetle *Popillia japonica*

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Abstract

Popillia japonica is a quarantine pest of priority interest for the EU, given its potentially important economic, social and environmental impacts. Alternative strategies to chemical methods are essential to limit its spread in newly infested areas with favourable climatic and environmental conditions. Saponins are biologically active molecules widely distributed in plants, displaying a well-known repellent activity combined with a mortality effect against insects. In this context, saponins were extracted from alfalfa *Medicago sativa*, where medicagenic and zanhic acid glycosides and Soyasaponin I were the most abundant compounds and used in the laboratory and semi-field experiments for treating leaves of susceptible host plants for *P. japonica*. Under laboratory conditions, a food deterrence effect and a significant mortality rate were observed using *Corylus avellana* leaves treated at increasing saponin concentrations, ranging from 1% to 5% w/v. Semi-field condition experiment supported the food deterrence effect, as a significant food preference was observed for untreated plants of *Vitis vinifera* compared to treated plants. The promising results obtained suggest that alfalfa saponins could represent a potential eco-friendly approach for Japanese beetle control.

KEYWORDS
alien species, bioactive molecules, biopesticides, insect pest, integrated pest management, *Medicago sativa*

1 | INTRODUCTION

The Japanese beetle *Popillia japonica* Newman, 1841 (*Coleoptera*: *Scarabaeidae*) is an invasive pest, native to Japan and established in North America, where it was first discovered in New Jersey in 1916 (Dickerson & Weiss, 1918). Favourable environmental conditions and the absence of natural enemies (Fleming, 1968) have allowed this pest to spread easily in the US (Althoff & Rice, 2022) and Canada (CFIA, 2020). In addition, *P. japonica* is also established in Europe. The beetle was accidentally introduced to the Azores (Terceira Island) in the early 1970s (EPPO, 2019); since 2014 *P. japonica* was also detected, in the Ticino Regional Natural Park between the Piedmont and Lombardy regions, Northern Italy (EPPO, 2014; Pavesi, 2014). Probably, due to the natural spread of the nearby Italian populations, it was also found in Switzerland in 2017 (EPPO, 2017).

Popillia japonica is usually a univoltine species, and generally, adults emerge in summer with a lifespan of about 40 days (Campbell et al., 1989; Fleming, 1972; Potter & Held, 2002). *P. japonica* is a polyphagous species that feeds on over 300 species with a wide variety of trees, shrubs, wild plants and crops (Campbell et al., 1989; Fleming, 1972; Ladd Jr, 1987, 1989; Potter & Held, 2002). Plants are damaged by both adults and larvae: adults feed on leaves and fruits, whereas larvae feed just below the soil surface damaging the roots of host plants (Potter & Held, 2002).

The high reproductive rate, the pathway of introduction and the dispersal ability, as well as the polyphagous diet, have contributed to the classification of *P. japonica* as a quarantine pest of priority interest for the EU, according to Commission Delegated Regulation (EU) 2019/1702, given the potential economic, environmental and social impact for the European territory.

In the US, chemical methods are mainly used for the *P. japonica* control but with adverse effects on ecosystems, with the possibility of non-target effects (Althoff & Rice, 2022). For this reason, an eco-friendly approach is thus appreciated and encouraged, especially by the European Commission, which is pursuing a pesticide-reducing policy ('Farm to Fork' European Commission strategy —https://ec.europa.eu/food/system/files/2020-05/f2f_action-plan_2020_strategy-info_en.pdf).

Given the current limited distribution of *P. japonica* in mainland Europe, sustainable and effective containment strategies are essential to limit its spread in newly infested areas (EFSA, 2018). So far, environmentally friendly approaches have been proposed using attract and kill strategies (Marianelli et al., 2018; Paoli et al., 2023), biological control agents such as entomopathogenic nematodes (Marianelli et al., 2018; Mazza et al., 2017; Simões et al., 1993; Torrini et al., 2020) and entomopathogenic fungi (Barzanti et al., 2023; Benvenuti et al., 2019; Giroux et al., 2015). Nevertheless, the integrated pest management approach emphasizes a reduction in the use of synthetic pesticides and is crucial to increase the effectiveness of *P. japonica* containment with environmentally acceptable alternatives.

Saponins are biologically active glycosides widely distributed in plants, consisting of a sugar moiety linked to a hydrophobic aglycone (sapogenin) with a triterpenoid or a steroid structure (Hostettmann & Marston, 1995). These specialized metabolites play a central role in plant defence strategy preventing and deterring phytopathogenic agents, like fungi, bacteria and insects (Abdelrahman et al., 2017; Zaynab et al., 2021). It has been also reported that saponins have a significant effect on insects' reproduction, development, activity and mortality rate. Indeed, their repellent activity, combined with their ability to impair the digestion process, leads to a drastic reduction in food intake, which in turn leads to starvation and related health issues (Singh & Kaur, 2018). Moreover, saponins bind with a complex of cholesterol, causing the insects' ecdysial failure (De Geyter et al., 2012; Qasim et al., 2020; Taylor et al., 2004).

Saponins are particularly abundant in Fabaceae and especially in the genus *Medicago*. *Medicago sativa* L. (commonly named alfalfa) is the most cultivated forage crop in the world and represents an important source for the extraction of these bioactive molecules (Tava et al., 2022). Saponins from this genus display fungicidal, molluscicidal, nematicidal, antibacterial, antiviral and antitumoral properties (Abbruscato et al., 2014; Avato et al., 2017; D'Addabbo et al., 2011, 2020; Paparella et al., 2015; Tava & Avato, 2006). Recently, a high anthelmintic activity against gastrointestinal nematodes from donkeys and goats (Maestrini et al., 2019, 2020) was demonstrated for alfalfa saponin mixtures. Moreover, saponins extracted from alfalfa showed insecticidal and antifeedant effects on several pest insects (Qasim et al., 2020; Tava & Avato, 2006). In the pea aphid *Acyrthosiphon pisum* (Harris, 1776), alfalfa saponins caused a reduction in phloem ingestion, growth, survival and reproduction rate (Pedersen et al., 1976). Similar effects have been reported in the potato aphid *Aulacorthum solani* (Kaltenbach, 1843), where alfalfa saponins were provided with a liquid artificial diet (Mazahery-Laghab, 1997). Increased mortality has been reported also in leafhoppers (Cicadellidae) fed with alfalfa crude saponin (5%) (Qasim et al., 2020). The Colorado potato beetle *Leptinotarsa decemlineata* (Say, 1824) larvae fed with leaves treated with 0.5% alfalfa saponins significantly reduce food intake resulting in inhibition of growth rate combined with higher mortality (Qasim et al., 2020; Szczepaniak et al., 2001; Szczepanik et al., 2004).

Despite the above-mentioned significant effects on insect survival and feeding strategies, the effect of saponins against the major pest *P. japonica* has been poorly investigated. Kreuger and Potter (1994) highlighted that unripe holly fruits had a higher saponin content than ripe fruits, resulting in less desirable, probably also due to the low sugar level. By contrast, in another paper, Keathley and Potter (2008) reported no relationship between saponins and resistance and found that some of the more susceptible host plants, such as the sassafras *Sassafras albidum* (Nutt.) Nees, have a higher leaf content of saponins, sugar and tannins, comparing to resistant plants. Apart from these suggestions and correlational observations, to date, no direct experimental tests have investigated the effects of saponins on *P. japonica*.

In this study, we aimed to investigate alfalfa saponins regarding their possible effects as a repellent, feeding deterrent and/or biocidal capability at different concentrations against *P. japonica* adults under laboratory conditions and semi-field assay when applied to the leaves of two of the most susceptible host plants, hazelnut (*Corylus avellana* L.) and grapevine (*Vitis vinifera* L.).

2 | MATERIALS AND METHODS

2.1 | Extraction and purification of *M. sativa* leaf saponins

Alfalfa (*Medicago sativa* cv. Equipe) plants were grown in an open field at CREA-ZA (Lodi, Italy) and collected in July 2019. Leaf samples were oven-dried at 50°C for 2 days, ground and used for saponin extraction, following general procedures (Abbruscato et al., 2014; D'Addabbo et al., 2020; Tava et al., 2020). Briefly, about 800 g of

powdered alfalfa leaves were first defatted with $CHCl_3$ in a Soxhlet apparatus and then saponins were extracted with 80% MeOH under reflux (48 h). After removing the solvent with a rotary evaporator, the residue was resuspended in 30% MeOH and loaded onto a 200×60 mm C18 column (Lichroprep RP-18, 40–63 μm; Merck, Darmstadt, Germany), preconditioned with 30% MeOH. Elution was first performed with 40% MeOH to remove some polar compounds, and then the saponins were eluted with 90% MeOH and dried under reduced pressure. The saponin mixture obtained (2.5% yield) was kept in airtight vials until used. The saponin mixture was dissolved in distilled water and used at the indicated concentration for different experiments conducted in laboratory and semi-field conditions.

2.2 | Characterization of the saponin mixture

The saponin mixture was analysed by HPLC (Perkin–Elmer) equipped with an LC250 binary pump and a DAD 235 detector, using a 250 mm×4.6 mm i.d., 5 μm, Discovery® HS C18 column (Supelco) (Tava et al., 2005, 2009, 2017). The mobile phase consisted of solvent A, CH_3CN/0.05% CF_3COOH and solvent B H_2O/1% MeOH/0.05% CF_3COOH. Chromatographic runs were carried out under gradient elution from 20% (5 min isocratic condition) to 90% of solvent A in 100 min, and 20 μL of MeOH/H_2O (9:1) solutions (1 mg/mL) were injected. Saponins were eluted at 1.0 mL/min and detected by UV monitoring at 215 nm. Identification of compounds in the saponin mixture was performed by using saponin standards previously purified and identified in *Medicago* spp. (Paparella et al., 2015; Tava et al., 2009, 2011, 2020; Tava & Avato, 2006).

Evaluation of the saponin composition was also performed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) experiments, following methods previously reported (Abbruscato et al., 2014; D'Addabbo et al., 2020; Paparella et al., 2015; Tava et al., 2020).

Purified saponin mixtures were also characterized for their quantitative aglycone composition as previously reported by Tava et al. (2017). In brief, 5 mg of saponin mixtures were treated with 15 mL of 2 N HCl in 50% aqueous MeOH under reflux for 8 h. After cooling, MeOH was eliminated with a stream of N_2 and aglycones extracted with AcOEt (3×10 mL) and dried under N_2. Finally, gas chromatography-flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC–MS) (Clarus 500; Perkin–Elmer) analyses were performed on sapogenins as their methyl-trimethylsilyl derivatives. Gas-chromatographic analyses were performed with a 30 m×0.32 mm, 0.25 μm i.d., DB-5 capillary column. Retention times and mass spectra data were compared with those of previously purified and identified sapogenins (Tava et al., 2017).

2.3 | Insect collection

During the summer of 2021, adult specimens of *P. japonica* were collected in the province of Novara (45°27′11.9″ N 8°47′01.7″ E, Piedmont region, northern Italy). Specimens were captured with pheromone traps for Japanese beetles placed in the field (baited with 2-phenyl-ethyl-propionate, eugenol and geraniol, 3:7:3; Trécé). Traps were emptied every 30 min and only active, apparently healthy and free of morphological defects adults were used. After collection, *P. japonica* adults were transferred to the quarantine room at CREA-DC in Florence (26°C, 51.6% HR, 8:16 L:D) for laboratory experiments. Males and females were separated by the observation of the tibial shape and tarsal length, according to EPPO (2006) and kept in plastic boxes with perforated lids. *P. japonica* adults were fed with fresh leaves of the host plant *Corylus avellana* and used for the experiments after 5 days. A water-soaked cotton swab was placed in each box to hydrate the adults during rearing. For the semi-field experiment, the adults were collected as above, transferred to plastic boxes to the nursery 'Vivai Scarlatta' (Biella, Piedmont region, Italy) and used within 1 h.

2.4 | Food preference in laboratory conditions

The food preference between treated and untreated leaves of *C. avellana* was evaluated under laboratory conditions through a choice test. Leaves were collected from untreated hazelnut plants and randomly selected for control and treatment. Two leaves with a comparable surface, one soaked in water and the other one in a 1% saponin-water solution (10 mg/mL of saponin mixture in water) for 3 s, were placed at the maximum distance from each other in a plastic box (27×18×10 cm) together with one single specimen ($n=10$ males and $n=9$ females). We were not able to use the same number of specimens for both sexes because one female did not look in good health. Before the experiment, each leaf was scanned to record its original surface. Water was supplied using a cotton swab soaked in water and placed on the leaf petiole to wet both the *P. japonica* adults and the leaf itself. After 24 h, leaves were collected and scanned to quantify leaf consumption. Images were analysed using ImageJ 1.52a software (Schneider et al., 2012), and for each replicate, the eaten surface was calculated for both leaves by estimating the ratio between the final surface and the original one. As the differences between the pairs were not normally distributed (Shapiro Wilk test: $W=0.519$, p value$=7.451\times10^{-7}$), the non-parametric paired-samples Wilcoxon signed-rank test (statistic: V) was used to compare the ratio between the final surface and the original one in the control and the treated leaves. Data were analysed using R statistical software (version 4.1.3) (R Core Team, 2020).

2.5 | Deterrent and biocidal effects of saponins

Different concentrations of the saponin mixture were tested against *P. japonica* adults under controlled laboratory conditions in order to evaluate (1) the deterrent effect rating the percentage of leaf consumption and (2) the biocidal effect recording the dead individuals daily. The experimental unit consisted of a fresh leaf of *C. avellana*,

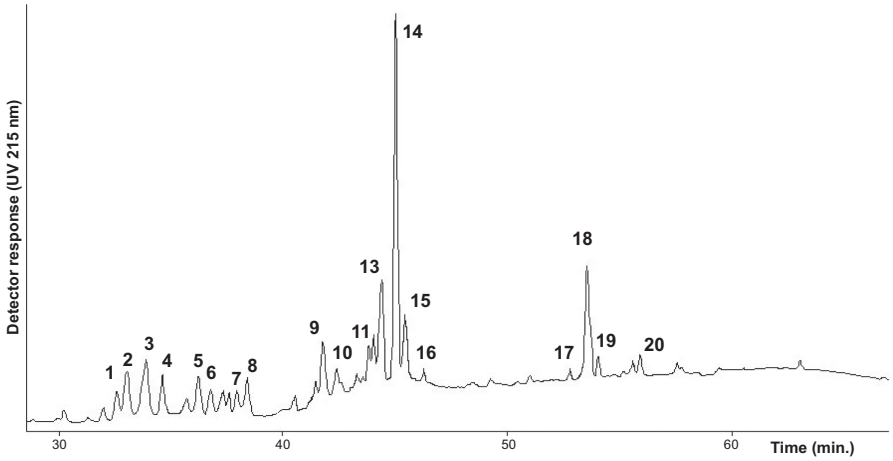


FIGURE 1 HPLC chromatogram of saponins from *Medicago sativa* leaves. The identified compounds are reported in Figure 2.

soaked in water for 3 s for the control or in the saponin-water solution (concentrations 1%, 3% and 5%, corresponding to 10 mg/mL, 30 mg/mL and 50 mg/mL, respectively) for the treatments and placed inside a plastic box (14 × 14 × 8 cm) containing 5 *P. japonica* adults. For each treatment, 10 replicates were carried out (a total of 50 adults for each of the four treatments). Before each treatment, each leaf was scanned to record its original surface. Water was supplied as described above. After 48 h, the leaves were collected and scanned to quantify leaf consumption and processed as previously described in paragraph 2.4, using ImageJ 1.52a software (Schneider et al., 2012). To assess potential long-term mortality, after the experiments, *P. japonica* adults were reared for 5 days and fed with untreated leaves moistened with cotton. Each box was checked every 24 h, and mortality was recorded daily.

As the assumption of normal distribution was not met by some of the data distributions (Shapiro Wilk test, $p < 0.05$), Kruskal–Wallis test (statistic: H) followed by post-hoc Dunn test (statistic: Z) with Benjamini–Hochberg correction for multiple comparisons were used to compare leaf consumption (i.e., the ratio between the eaten surface and the original one) and adult among mortality treatments (i.e., the control leaves and the treated leaves with 1%, 3% and 5% concentration of saponins). Data were analysed using R statistical software (version 4.1.3) (R Core Team, 2020).

2.6 | Food preference in semi-field conditions

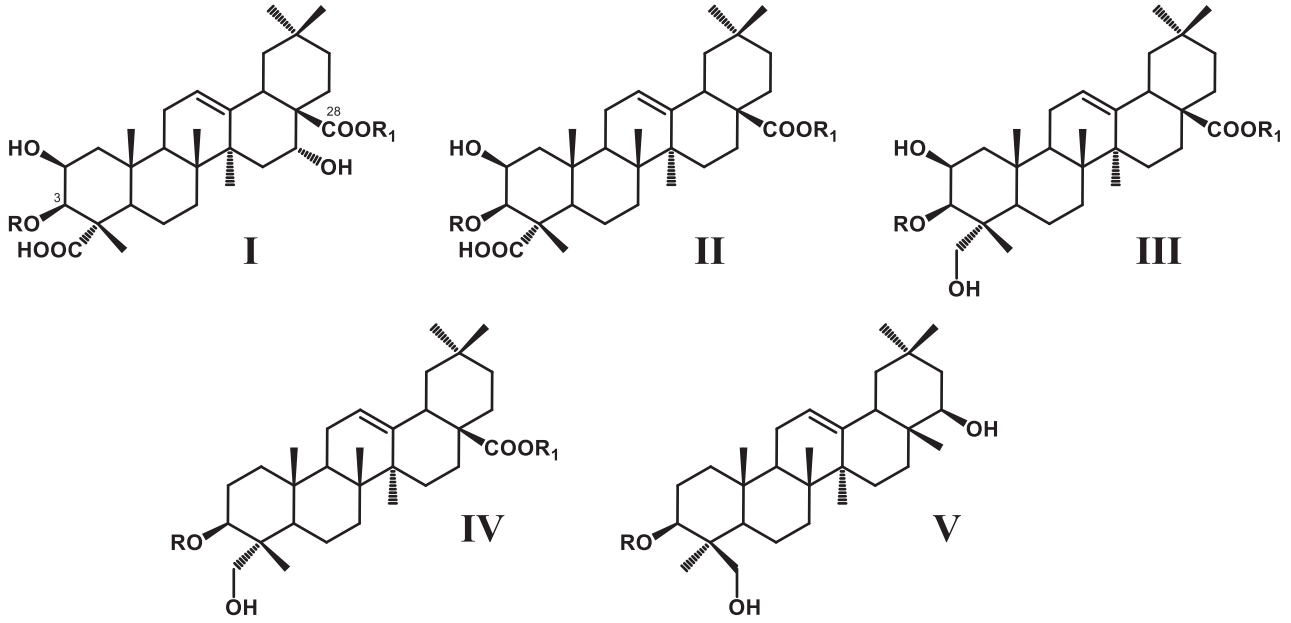
To evaluate the food preference between saponin-treated and untreated plants, a semi-field experiment was conducted in the Vivai Scarlatta nursery using nine bug-dorm cages (75 × 75 × 130 cm). Eighteen plants of *Vitis vinifera*, similar in size and number of leaves (10 leaves per plant), were used for the experiment. Nine plants were treated by spraying 1 mL of 3% saponin solution in water on each leaf, whereas the other nine plants of control were treated with 1 mL of water per leaf. One treated and one untreated plants were placed in each cage at the maximum distance (about 100 cm), and 50 *P. japonica* adults (25 females and 25 males) were released inside. After 48 h, the leaves of each plant were collected, sealed in a plastic bag and taken in a refrigerated container to the CREA-DC laboratories in Florence to be scanned. The captured images were analysed using ImageJ 1.52a software. Contrary to the previous experiments, the original leaf surface was calculated after the experiment through the remaining leaf margin, as the leaves that were eaten by *P. japonica* adults retain the vascular system, and it was thus possible to determine the original leaf perimeter and the initial surface. A generalized mixed model (tweedie distribution) was used to assess the effect of treatment on the leaf area eaten, using this parameter as a dependent variable, cage ID and plant ID as random factors, and treatment and total leaf area as predictors. The interaction term between treatment and total leaf area was not significant ($p = 0.996$) and it was thus removed from the analyses. This choice was confirmed by the lower AIC/BIC values of the model without the interaction. Data were analysed and visualized using R packages glmmTMB and Emmeans (Brooks et al., 2017; Russell, 2022; Team, 2013).

3 | RESULTS

3.1 | Purification and structural elucidation of *M. sativa* leaf saponins

Purified saponins were obtained as a whitish powder in a pure grade (85%–90%) and their purity was first inspected by HPLC (Figure 1). Medicagenic acid and zanhic acid were the most abundant detected sapogenins within the saponin mixture extracted from *M. sativa* leaves, representing 52.2% and 31.5% of the total, respectively, followed by soyasapogenol B (12.1%), bayogenin (2.0%) and hederagenin (1.5%) (Figure 2).

Alfalfa leaf saponin mixture was predominantly composed of bidesmosidic compounds, with two sugar chains attached to aglycons (Figure 2), accounting for more than 85% of the total saponins. The most abundant saponin was identified as a bidesmoside of medicagenic acid (compound **14**) quoted as 25.2% of the total saponins (Figures 1 and 2). High molecular weight saponins of medicagenic and zanhic acids containing up to seven sugar units in the molecule were detected in lower amounts (Figure 2). Low molecular weight mono



Compound	Aglycone	R	R_1	%
1	Zanhic acid (I)	β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara α-L-Ara(1→3)┘	2.3
2	Zanhic acid (I)	β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara α-L-Api(1→3)┘	5.6
3	Zanhic acid (I)	β-D-Glc(1→2)-β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara α-L-Ara(1→3)┘	8.1
4	Zanhic acid (I)	β-D-Glc(1→2)-β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara α-L-Api(1→3)┘	2.8
5	Zanhic acid (I)	β-D-Glc(1→2)-β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara	3.4
6	Medicagenic acid (II)	β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara	2.1
7	Zanhic acid (I)	β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara	2.1
8	Zanhic acid (I)	β-D-Glc	α-L-Ara (1→2)-α-L-Rha(1→2)-α-L-Ara	4.0
9	Medicagenic acid (II)	β-D-Glc(1→2)-β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara α-L-Ara(1→3)┘	5.2
10	Bayogenin (III)	α-L-Ara	β-D-Glc	3.0
11	Medicagenic acid (II)	β-D-Glc(1→2)-β-D-Glc(1→2)-β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara α-L-Api(1→3)┘	2.9
12	Medicagenic acid (II)	β-D-Glc(1→2)-β-D-Glc	α-L-Rha(1→2)-α-L-Ara	4.1
13	Medicagenic acid (II)	β-D-GluAc	α-L-Rha(1→2)-α-L-Ara	9.9
14	Medicagenic acid (II)	β-D-GluAc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara	25.2
15	Medicagenic acid (II)	β-D-Glc	β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara	4.4
16	Hederagenin (IV)	β-D-GluAc	β-D-Glc	0.6
17	Medicagenic acid (II)	β-D-Glc	H	0.5
18	Soyasapogenol B (V)	α-L-Rha(1→2)-β-D-Gal(1→2)-β-D-GluAc	-	9.9
19	Bayogenin (III)	α-L-Ara	H	1.1
20	Hederagenin (IV)	α-L-Ara	H	1.4

FIGURE 2 Chemical structure of identified saponins from *Medicago sativa* leaves used in this investigation. Number of compounds referred to as HPLC peaks is reported in Figure 1. Aglycones: bayo, bayogenin; hed, hederagenin; med ac, medicagenic acid; soya B, soyasapogenol B; zan ac, zanhic acid. Sugar moieties: β-D-Api, β-D-apiofuranosyl; α-L-Ara, α-L-arabinopyranosyl; β-D-Gal, β-D-galactopyranosyl; β-D-Glc, β-D-glucopyranosyl; β-D-GluAc, β-D-glucuronopyranosyl; α-L-Rha, α-L-rhamnopyranosyl; β-D-Xyl, β-D-xylopyranosyl.

and bidesmoside saponins of bayogenin (compounds **10** and **19**) and hederagenin (compounds **16** and **20**), together with a monodesmoside of medicagenic acid (compound **17**) were also identified in lower amounts. Soyasaponin I (compound **18**), a monodesmoside saponin of soyasapogenol B commonly found in the *Fabaceae* family, was also identified in the mixture and quoted as 9.9% of the total saponins.

3.2 | Food preference in laboratory conditions

The food preference for all the tested individuals ($n=19$) was evaluated by comparing the ratios of the final leaf surface and the original one between the control and treated leaves of *C. avellana* with 1% saponin-water solution. There was a significant difference in the relative amount of the leaf area eaten between control and treated leaves ($V=28$, $n=19$, $p=0.023$), showing that adults of *P. japonica* preferred to consume the control leaves with respect to the treated ones.

3.3 | Deterrent and biocidal effects of saponins

To evaluate the deterrent effect of the saponin mixture, the eaten surface of the treated leaves was compared with the untreated ones. As already observed in the choice test, the ratios between the eaten surface and the original one in the control and the treated leaves were significantly different ($H=24.4$, df$=3$, $p=2.11\times10^{-5}$; Figure 3a), showing that adults selected the control leaves over saponins-treated leaves at all concentrations (Figure 3b). According to the Dunn pairwise comparisons, significant differences were observed between 5% saponin-treated leaves compared both with control ($Z=4.47$; $p=4.67\times10^{-5}$) and 1% saponin-treated leaves ($Z=2.40$; $p=3.25\times10^{-2}$), whereas no statistical significance was remarked with the 3% saponins concentration ($Z=0.59$; $p=5.52\times10^{-1}$). At the same time, no statistical difference was found between 1% and 3% of saponins concentrations ($Z=1.81$; $p=8.45\times10^{-2}$) even though leaves treated with 3% were less consumed (Figure 3), while significant differences were observed between control and 3% saponin-treated leaves ($Z=3.88$; $p=3.17\times10^{-4}$). No difference was found between control and 1% ($Z=2.07$; $p=5.79\times10^{-2}$).

Concerning the survival, the number of alive adults was significantly higher in the control than in the treatments ($H=23.19$, df$=3$, $p=3.69\times10^{-5}$). Significant differences were observed between the control and the three treatments ($Z=-2.28$ and $p=4.5\times10^{-2}$ for 1%, $Z=-3.75$ and $p=5\times10^{-4}$ for 3%, $Z=-4.45$ and $p=5\times10^{-5}$ for 5% saponin-treated leaves). No significant differences were found between 1% and 3% saponin concentrations ($Z=-1.46$ and $p=1.7\times10^{-1}$) nor between 3% and 5% ($Z=-0.7$ and $p=4.8\times10^{-1}$), whereas there were significant differences between 1% and 5% saponin concentrations ($Z=-2.17$ and $p=4.4\times10^{-2}$) (Figure 4).

3.4 | Food preference in semi-field conditions

The laboratory choice test has shown that 3% saponin solution works as a deterrent as well as 5% one and we did not observe any difference between the leaves eaten surfaces treated with those concentrations, thus we decided to use the 3% solution in the semi-field experiments. We found an effect of the treatment on the amount of leaf area eaten, with greater leaf consumption on control plants than

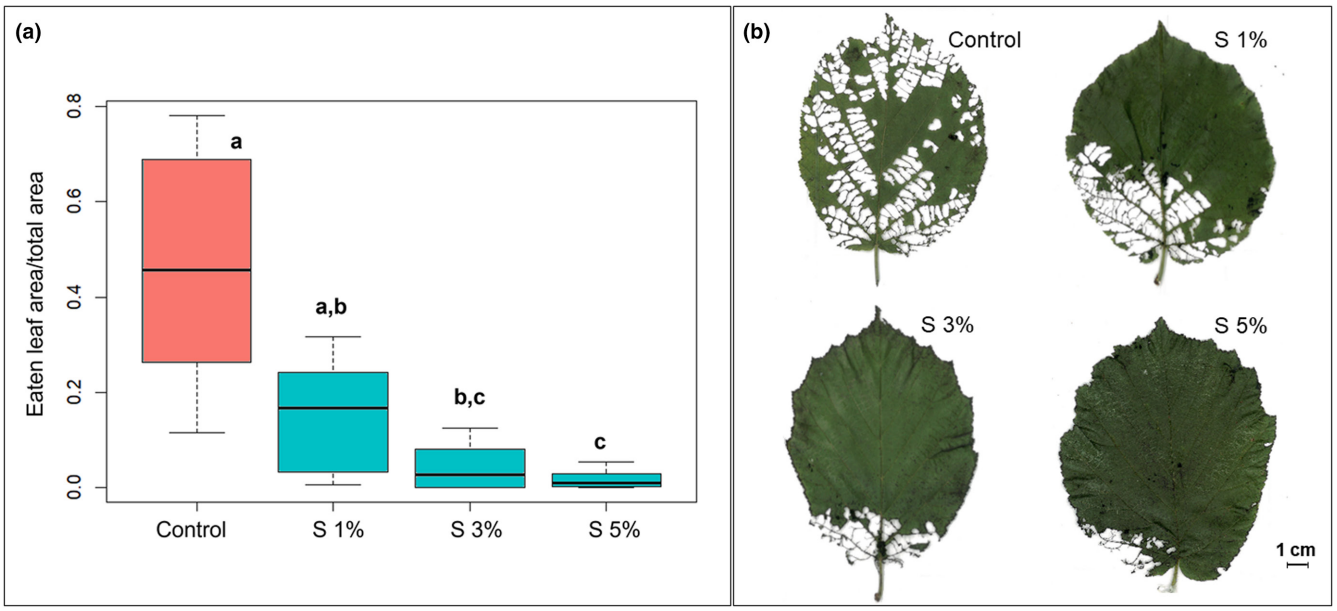


FIGURE 3 (a) Boxplots (red for control and blue for treatments) represent the median and the interquartile range for the ratio between the eaten leaf surface and the total one. Different letters among treatments indicate significant differences to Dunn's Multiple Comparison Test. (b) Representative leaves with a median level of eaten surface for control and each saponin concentration (1%, 3% and 5%) by *Popillia japonica*. [Colour figure can be viewed at wileyonlinelibrary.com]

on treated ones (Table 1, Figure 5a). We also found a significant effect on the total area, that is the larger the leaf, the greater the area eaten (Table 1, Figure 5b).

4 | DISCUSSION

In this work, we have investigated the possible effect of alfalfa saponins as a repellent, feeding deterrent, and insecticide, under laboratory and semi-field conditions, against *P. japonica* adults. Alfalfa leaf saponin mixture was characterized through LC–MS/MS analysis and single compounds quantitated by HPLC analysis. The most abundant compounds were identified as bidesmosides of medicagenic acid (compounds **13** and **14**) and Soyasaponin I (compound **18**, a monodesmoside saponin of soyasapogenol B), representing 9.9%, 25.2% and 9.9% of the total saponins, respectively (Figures 1 and 2). The activities of alfalfa saponins have been reported against some insect pests belonging to Hemiptera, Coleoptera and Lepidoptera but data are scanty and mainly concerning stored products (Singh & Kaur, 2018; Zaynab et al., 2021). The most observed effects are increased mortality, lowered food intake, weight reduction, delayed development and decreased reproduction (Qasim et al., 2020; Tava & Avato, 2006; Zaynab et al., 2021). Among various tested saponins, 3-GlcA-28-AraRhaXyl-medicagenic acid (compound **14**, Figure 2), the most abundant compound in our saponin mixture is reported to be potentially responsible for insecticidal activity (Singh & Kaur, 2018). However, as suggested by Li et al. (2022), Tian et al. (2021) and Ye et al. (2023), also some other minor constituents with particular functional groups could act as pesticides, but further research is needed to solve this topic. To the best of our knowledge, the employment of *M. sativa* saponins in the control of the *P. japonica* pest has not been investigated so far, while few works against other Coleoptera have been reported. Saponin extract from alfalfa roots has been used as a growth regulator in *Tropinota squalida*, disturbing the development and reproduction (Hussein et al., 2005). Moreover, saponins extracted from three *Medicago* species were included in the diet of Colorado potato beetle larvae showing a food deterrent and insecticidal effect (Szczepaniak et al., 2001; Szczepanik et al., 2004).

Several repellent and/or antifeedant deterrents have been tested in laboratory choice tests and field experiments against *P. japonica*. Among the plant-derived molecules, the cardenolide glycoside neriifolin showed a feeding deterrent effect for 5 days on soybeans in laboratory experiments (Reed et al., 1982) as well as the cucurbitacin B used in choice test with primrose leaves (Tallamy et al., 1997). Neem-based products have been also used both in laboratory tests and greenhouse experiments, proving to be effective against *P. japonica* using different plant hosts, such as soybean, linden, rose and sassafras (Held et al., 2001; Ladd Jr et al., 1978; Mmbaga & Oliver, 2007).

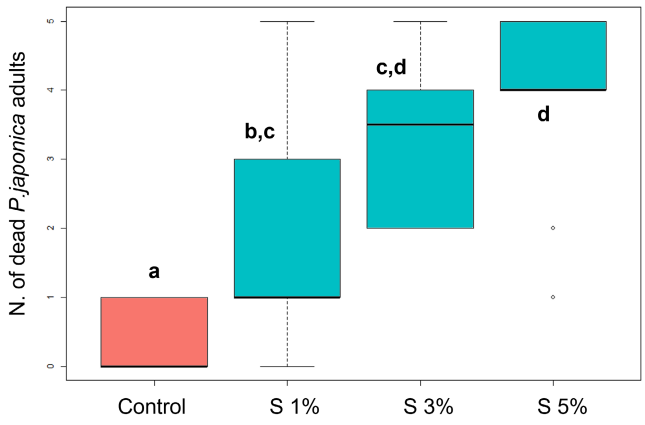


FIGURE 4 Effect of different saponin concentrations (1%, 3% and 5%) on the number of dead *Popillia japonica* adults. Different letters among treatments indicate significant differences. Boxplots (red for control and blue for treatments) represent the median and the interquartile range for the number of dead adults. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Summary of the GLMM testing for the effect of treatment on feeding preference in semi-field conditions. Leaf area eaten is modelled by plant treatment, total leaf area and their interaction.

	Estimate	SE	*z* Value	*p* Value
Treatment	0.26988	1.27706	0.211	0.8326
Total area	0.02848	0.01221	2.333	0.0196
Treatment × total area	−0.01314	0.01408	−0.934	0.3505

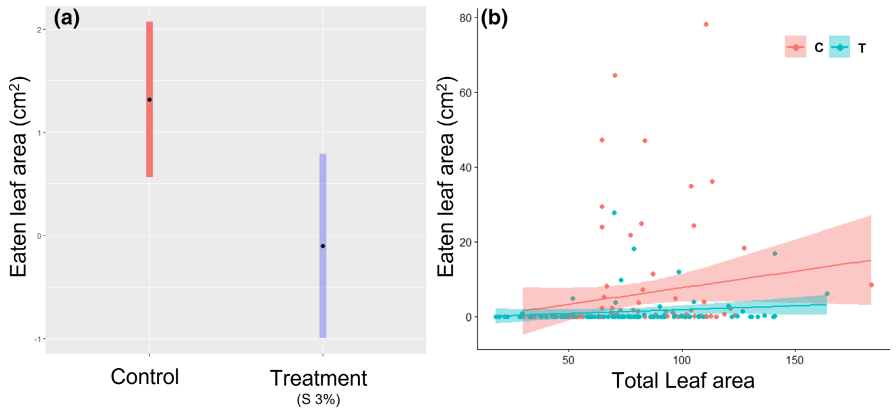


FIGURE 5 Results of the semi-field experiments: (a) the significant deterrence effect of treatment (saponins 3% vs. control) on the amount of leaf area eaten (dot represent estimated marginal means, bars represent confidence intervals); (b) effect of the total area of the leaf on the amount of leaf area eaten, for control (red) and treatment (blue) plants. [Colour figure can be viewed at wileyonlinelibrary.com]

In laboratory choice test experiments, we evaluated the food preference of *P. japonica* towards treated and untreated leaves of hazelnut, showing that adults preferred to eat on the control leaves with respect to the treated ones. Moreover, the deterrent effect of the saponin mixture was assessed using different concentrations (1%, 3% and 5%) based on previously reported data on alfalfa saponins tested against leafhoppers and Colorado potato beetle at concentrations of 5% and 0.5% respectively (Qasim et al., 2020; Szczepaniak et al., 2001; Szczepanik et al., 2004). Therefore, we evaluated the eaten leaf surface by observing that the consumed surface was lower as the concentration of the treatment increased, even if no statistical differences were highlighted between 1% and 3% of treated leaves, showing that the adults dislike leaves treated with 5% saponins over the other concentrations. The survival of adults of *P. japonica* was monitored for 5 days, observing that it was significantly higher in the control than in the treatments, without significant differences among saponin concentrations.

Finally, a semi-field experiment was conducted on *Vitis vinifera*, which is one of the most favourite host plants of *P. japonica*, to evaluate the food preference between treated and untreated leaves. We decided to use the saponin mixture at 3% concentration since in the laboratory choice tests no significant differences have been highlighted with respect to the highest concentration used. In addition, it may last longer on leaves in semi-field conditions and is still in the range of potential feasibility in terms of costs. With this experiment, we confirmed that saponins from *M. sativa* act as a feeding deterrent against *P. japonica* also in semi-field conditions.

Our study aimed to increase the knowledge about saponins as an ecologically sound and safe method for controlling the Japanese beetle. Our results, using saponins extracted from alfalfa, showed a food deterrence effect and a significant mortality rate. These promising results suggest that saponins could represent a potential eco-friendly approach for the control of this pest and require further research to deeply investigate this topic.

AUTHOR CONTRIBUTIONS

Immacolata Iovinella: Data curation; investigation; writing – original draft; writing – review and editing. **Francesco Barbieri:** Data curation; investigation; writing – original draft; writing – review and editing. **Elisa Biazzi:** Conceptualization; formal analysis; investigation; resources; writing – original draft; writing – review and editing. **Chiara Sciandra:** Data curation; investigation; writing – original draft; writing – review and editing. **Aldo Tava:** Conceptualization; formal analysis; investigation; resources; writing – review and editing. **Giuseppe Mazza:** Conceptualization; formal analysis; investigation; writing – review and editing. **Leonardo Marianelli:** Conceptualization; funding acquisition; investigation; resources; writing – review and editing. **Alessandro Cini:** Formal analysis; investigation; writing – review and editing. **Pio F. Roversi:** Writing – review and editing. **Giulia Torrini:** Conceptualization; investigation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in 'Mendeley Data Repository' at https://data.mendeley.com/datasets/j936prxxt7/2.

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REFERENCES

Abbruscato, P., Tosi, S., Crispino, L., Biazzi, E., Menin, B., Picco, A. M., Pecetti, L., Avato, P., & Tava, A. (2014). Triterpenoid glycosides from *Medicago sativa* as antifungal agents against *Pyricularia oryzae*. *Journal of Agricultural and Food Chemistry*, *62*, 11030–11036. https://doi.org/10.1021/jf5049063

Abdelrahman, M., El-Sayed, M., Sato, S., Hirakawa, H., Ito, S., Tanaka, K., Mine, Y., Sugiyama, N., Suzuki, Y., Yamauchi, N., & Shigyo, M. (2017). RNA-sequencing-based transcriptome and biochemical analyses of steroidal saponin pathway in a complete set of *Allium fistulosum*-a. cepa monosomic addition lines. *PLoS One*, *12*, e0181784. https://doi.org/10.1371/journal.pone.0181784

Althoff, E. R., & Rice, K. B. (2022). Japanese beetle (*Coleoptera: Scarabaeidae*) invasion of North America: History, ecology, and management. *Journal of Integrated Pest Management*, *13*, 1–11. https://doi.org/10.1093/jipm/pmab043

Avato, P., Migoni, D., Argentieri, M., Fanizzi, F. P., & Tava, A. (2017). Activity of saponins from *Medicago* species against HeLa and MCF-7 cell lines and their capacity to potentiate cisplatin effect. *Anti-Cancer Agents in Medicinal Chemistry*, *17*, 1508–1518. https://doi.org/10.2174/1871520617666170727152805

Barzanti, G. P., Enkerli, J., Benvenuti, C., Strangi, A., Mazza, G., Torrini, G., Simoncini, S., Paoli, F., & Marianelli, L. (2023). Genetic variability of

Metarhizium isolates from the Ticino Valley Natural Park (northern Italy) as a possible microbiological resource for the management of *Popillia japonica*. *Journal of Invertebrate Athology*, *197*, 107891. https://doi.org/10.1016/j.jip.2023.107891

Benvenuti, C., Barzanti, C., Marianelli, L., Sabbatini Peverieri, G., Paoli, F., Bosio, G., Venanzio, D., Giacometto, E., & Roversi, P. F. (2019). A new device for auto-disseminating entomopathologic fungi against *Popillia japonica*: A study case. *Bulletin of Insectology*, *72*, 219–225.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Mächler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, *9*, 378–400. https://doi.org/10.32614/RJ-2017-066

Campbell, J. M., Sarazin, M. J., & Lyons, B. (1989). Canadian beetles (*Coleoptera*) injurious to crops, ornamentals, stored products, and buildings. *Agriculture Canada Ottawa, Ontario Canada*, *1989*, 491.

CFIA. (2020). *Appendix 1: Regulatory status of areas in Canada and the United States for Japanese beetle (Popillia japonica)*. http://www.inspection.gc.ca/plants/plant-pests-invasive-species/directives/horticulture/d-96-15/appendix-1/eng/1346826626609/1346826990603

D'Addabbo, T., Argentieri, M. P., Żuchowski, J., Biazzi, E., Tava, A., Oleszek, W., & Avato, P. (2020). Activity of saponins from *Medicago* species against phytoparasitic nematodes. *Plants*, *9*, 443. https://doi.org/10.3390/plants9040443

D'Addabbo, T., Carbonara, T., Leonetti, P., Radicci, V., Tava, A., & Avato, P. (2011). Control of plant parasitic nematodes with active saponins and biomass from *Medicago sativa*. *Phytochemistry Reviews*, *10*, 503–519. https://doi.org/10.1007/s11101-010-9180-2

De Geyter, E., Smagghe, G., Rahbé, Y., & Geelen, D. (2012). Triterpene saponins of *Quillaja saponaria* show strong aphicidal and deterrent activity against the pea aphid *Acyrthosiphon pisum*. *Pest Managment Science*, *68*, 164–169. https://doi.org/10.1002/ps.2235

Dickerson, E. L., & Weiss, H. B. (1918). *Popilia japonica* Newm., a recently introduced Japanese pest. *The Canadian Entomologist*, *50*, 217–221. https://doi.org/10.4039/Ent50217-7

EFSA Plant Health Panel (EFSA PLH Panel), Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M. A., Jaques Miret, J. A., Justesen, A. F., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnell, S., Potting, R., Reignault, P. L., Thulke, H. H., Van der Werf, W., Vicent Civera, A., Yuen, J., Zappalà, L., … MacLeod, A. (2018). Scientific opinion on the pest categorisation of *Popillia japonica*. *EFSA Journal*, *16*, 5438. https://doi.org/10.2903/j.efsa.2018.5438

EPPO. (2006). *PM 7/74 (1) Popillia japonica*. EPPO Bulletin, 36, 447–450.

EPPO. (2014). *EPPO reporting service No. 10. Num. Article 2014/179 first report of Popillia japonica in Italy*. https://gd.eppo.int/reporting/article-3272

EPPO. (2017). *EPPO reporting service No. 09. Num. Article 2017/160 first report of Popillia japonica in Switzerland*. https://gd.eppo.int/reporting/article-6128

EPPO. (2019). *EPPO reporting service No. 08. Num. Article 2019/158 update of the situation of Popillia japonica in Portugal (Azores)*. https://gd.eppo.int/reporting/article-6588

Fleming, W. (1968). *Biological control of the Japanese beetle*. Technical bulletin No. 1383. Agricultural Research Service, US Department of Agriculture, Washington, DC https://doi.org/10.22004/AG.ECON.171592

Fleming, W. (1972). *Biology of the Japanese beetle*. Technical bulletin No. 1449, Agricultural Research Service, US Department of Agriculture, Washington, DC.

Giroux, F., Lavallée, R., Bauce, É., & Guertin, C. (2015). Susceptibility of the Japanese beetle, *Popillia japonica* (Newman) (*Coleoptera: Scarabaeidae*), to entomopathogenic *Hypocreales* fungi. *Phytoprotection*, *95*, 1–6. https://doi.org/10.7202/1028399ar

Held, D. W., Eaton, T., & Potter, D. A. (2001). Potential for habituation to a neem-based feeding deterrent in Japanese beetles, *Popillia japonica*. *Entomologia Experimentalis et Applicata*, *101*, 25–32. https://doi.org/10.1046/j.1570-7458.2001.00887.x

Hostettmann, K., & Marston, A. (1995). *Chemistry and pharmacology of natural products* (Vol. *59*, p. 96). Cambridge University Press. https://doi.org/10.1021/NP960011Z

Hussein, H. M., Dimetry, N., Zidan, Z., Iss-hak, R. R., & Sehnal, F. (2005). Effects of insect growth regulators on the hairy rose beetle, *Tropinota squalida* (Col., Scarabeidae). *Journal of Applied Entomology*, *129*, 142–148. https://doi.org/10.1111/j.1439-0418.2005.00891.x

Keathley, C. P., & Potter, D. A. (2008). Quantitative resistance traits and suitability of woody plant species for a polyphagous scarab, *Popillia japonica* Newman. *Environmental Entomology*, *37*, 1548–1557. https://doi.org/10.1603/0046-225X-37.6.1548

Kreuger, B., & Potter, D. A. (1994). Changes in saponins and tannins in ripening holly fruits and effects of fruit consumption on non-adapted insect herbivores. *The American Midland Naturalist Journal*, *132*, 183–191.

Ladd, T. L., Jr. (1987). Japanese beetle (Coleoptera: Scarabaeidae): Influence of favoured food plants on feeding response. *Journal of Economic Entomology*, *80*, 1014–1017. https://doi.org/10.1093/JEE/80.5.1014

Ladd, T. L., Jr. (1989). Japanese beetle (Coleoptera: Scarabaeidae): Feeding by adults on minor host and nonhost plants. *Journal of Economic Entomology*, *82*, 1616–1619. https://doi.org/10.1093/JEE/82.6.1616

Ladd, T. L., Jr., Jacobson, M., & Buriff, C. R. (1978). Japanese beetles: Extracts from neem tree seeds as feeding deterrents. *Journal of Economic Entomology*, *71*, 810–813. https://doi.org/10.1093/jee/71.5.810

Li, Y., Hao, N., Ye, S., Hu, Z., Zhao, L., Qi, Y., & Tian, X. (2022). New triterpenoid saponins from Clematis lasiandra and their mode of action against pea aphids Acyrthosiphon pisum. *Industrial Crops and Products*, *187*, 115517. https://doi.org/10.1016/j.indcrop.2022.115517

Maestrini, M., Tava, A., Mancini, S., Salari, F., & Perrucci, S. (2019). In vitro anthelmintic activity of saponins derived from *Medicago* spp. plants against donkey gastrointestinal nematodes. *Veterinary Sciences*, *6*, 35. https://doi.org/10.3390/vetsci6020035

Maestrini, M., Tava, A., Mancini, S., Tedesco, D., & Perrucci, S. (2020). In vitro anthelmintic activity of saponins from *Medicago* spp. against sheep gastrointestinal nematodes. *Molecules*, *25*, 242. https://doi.org/10.3390/molecules25020242

Marianelli, L., Paoli, F., Torrini, G., Mazza, G., Benvenuti, C., Binazzi, F., Sabbatini Peverieri, G., Bosio, G., Venanzio, D., Giacometto, E., Priori, S., Koppenhöfer, A. M., & Roversi, P. F. (2018). Entomopathogenic nematodes as potential biological control agents of *Popillia japonica* (*Coleoptera, Scarabaeidae*) in Piedmont region (Italy). *Journal of Applied Entomology*, *142*, 311–318.

Mazahery-Laghab, H. (1997). Endogenous resistance to insect pests in alfalfa: Engineering for enhanced resistance. Doctoral dissertation. Durham University.

Mazza, G., Paoli, F., Strangi, A., Torrini, G., Marianelli, L., Sabbatini Peverieri, G., Binazzi, F., Bosio, G., Sacchi, S., Benvenuti, C., Vananzio, D., Giacometto, E., Roversi, P. F., & Poinar, G. O., Jr. (2017). *Hexamermis Popilliae* N. Sp. (*Nematoda: Mermithidae*) parasitizing the Japanese beetle *Popillia japonica* Newman (*Coleoptera: Scarabaeidae*) in Italy. *Systematic Parasitology*, *94*, 915–926. https://doi.org/10.1007/s11230-017-9746-0

Mmbaga, M. T., & Oliver, J. B. (2007). Effect of biopesticides on foliar diseases and Japanese beetle (*Popillia japonica*) adults in roses (*Rosa* spp.), oakleaf hydrangea (*Hydrangea quercifolia*), and crapemyrtle (*Lagerstroemia indica*). *Journal of Arboriculture*, *33*, 210–219.

Paoli, F., Iovinella, I., Barbieri, F., Sciandra, C., Sabbatini Peverieri, G., Mazza, G., Torrini, G., Barzanti, G. P., Benvenuti, C., Strangi, A., Bosio, G., Mori, E., Roversi, P. F., & Marianelli, L. (2023). Effectiveness of field-exposed attract-and-kill devices against the

adults of *Popillia japonica* (Coleoptera: Scarabaeidae): A study on duration, form and storage. *Pest Management Science*. https://doi.org/10.1002/ps.7504

Paparella, S., Tava, A., Avato, P., Biazzi, E., Macovei, A., Biggiogera, M., Carbonera, D., & Balestrazzi, A. (2015). Cell wall integrity, genotoxic injury and PCD dynamics in alfalfa saponin-treated white poplar cells highlight a complex link between molecule structure and activity. *Phytochemistry*, *111*, 114–123. https://doi.org/10.1016/j.phytochem.2015.01.008

Pavesi, M. (2014). *Popillia japonica* specie aliena invasiva segnalata in Lombardia. *L'Informatore Agrario*, *32*, 53–55.

Pedersen, M. W., Barnes, D. K., Sorensen, E. L., Griffin, G. D., Nielson, M. W., Hill, R. R., Jr., Frosheiser, F. I., Sonoda, R. M., Hanson, C. H., Hunt, O. J., Peaden, R. N., Elgin, J. H., Jr., Devine, T. E., Anderson, M. J., Goplen, B. P., Elling, L. J., & Howarth, R. E. (1976). Effects of low and high saponin selection in alfalfa on agronomic and pest resistance traits and the interrelationship of these traits. *Crop Science*, *16*, 193–199. https://doi.org/10.2135/cropsci1976.0011183X001600020007x

Potter, D. A., & Held, D. W. (2002). Biology and management of the Japanese beetle. *Annual Review of Entomology*, *47*, 175–205. https://doi.org/10.1146/annurev.ento.47.091201.145153

Qasim, M., Islam, W., Ashraf, H. J., Ali, I., & Wang, L. (2020). Saponins in insect pest control. In *Co-evolution of secondary metabolites*, reference Series in Phytochemistry (pp. 897–924). Springer. https://doi.org/10.1007/978-3-319-96397-6_39

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing https://www.R-project.org/

Reed, D. K., Freedman, B., & Ladd, T. L., Jr. (1982). Insecticidal and Antifeedant activity of Neriifolin against codling moth, striped cucumber beetle, and Japanese beetle. *Journal of Economic Entomology*, *75*, 1093–1097. https://doi.org/10.1093/jee/75.6.1093

Russell, V. L. (2022). *Emmeans: Estimated marginal means, aka least-squares means*. R Package Version 1.7.2. https://CRAN.R-project.org/package=emmeans

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to image: 25 years of image analysis. *Nature Methods*, *9*, 671–675. https://doi.org/10.1038/nmeth.2089

Simões, N., Laumond, C., & Bonifassi, E. (1993). Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores. *Journal of Nematology*, *25*, 480–485.

Singh, B., & Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: A review. *LWT*, *87*, 93–101. https://doi.org/10.1016/j.lwt.2017.08.077

Szczepaniak, M., Krystkowiak, K., Jurzysta, M., & Biały, Z. (2001). Biological activity of saponins from alfalfa tops and roots against Colorado potato beetle larvae. *Acta Agrobotanica*, *54*, 35–45. https://doi.org/10.5586/aa.2001.021

Szczepanik, M., Biały, Z., & Jurzysta, M. (2004). The insecticidal activity of saponins from various *Medicago* spp. against Colorado potato beetle, *Leptinotarsa Decemlineata* say. *Allelopathy Journal*, *14*, 177–185.

Tallamy, D. W., Stull, J., Ehresman, N. P., Gorski, P. M., & Mason, C. E. (1997). Cucurbitacins as feeding and oviposition deterrents to insects. *Environmental Entomology*, *26*, 678–683. https://doi.org/10.1093/ee/26.3.678

Tava, A., & Avato, P. (2006). Chemical and biological activity of triterpene saponins from *Medicago* species. *Natural Product Communications*, *1*, 1159–1180. https://doi.org/10.1177/1934578X0600101217

Tava, A., Biazzi, E., Mella, M., Quadrelli, P., & Avato, P. (2017). Artefact formation during acid hydrolysis of saponins from *Medicago* spp. *Phytochemistry*, *138*, 116–127. https://doi.org/10.1016/j.phytochem.2017.02.018

Tava, A., Biazzi, E., Ronga, D., Mella, M., Doria, F., Accogli, R., Argentieri, M. P., & Avato, P. (2020). Triterpenic saponins from *Medicago marina* L. *Phytochemistry*, *174*, 112333. https://doi.org/10.1016/j.phytochem.2020.112333

Tava, A., Biazzi, E., Ronga, D., Pecetti, L., & Avato, P. (2022). Biologically active compounds from forage plants. *Phytochemistry Reviews*, *21*, 471–501. https://doi.org/10.1007/s11101-021-09779-9

Tava, A., Mella, M., Avato, P., Argentieri, M. P., Bialy, Z., & Yurzysta, M. (2005). Triterpene saponins from leaves of *Medicago arborea* L. *Journal of Agricultural and Food Chemistry*, *53*, 9954–9965. https://doi.org/10.1021/jf052468x

Tava, A., Mella, M., Avato, P., Biazzi, E., Pecetti, L., Bialy, Z., & Jurzysta, M. (2009). New triterpenic saponins from the aerial parts of *Medicago arabica* (L.) huds. *Journal of Agricultural and Food Chemistry*, *57*, 2826–2835. https://doi.org/10.1021/jf8036984

Tava, A., Pecetti, L., Romani, M., Mella, M., & Avato, P. (2011). Triterpenoid glycosides from the leaves of two cultivars of *Medicago polymorpha* L. *Journal of Agricultural and Food Chemistry*, *59*, 6142–6149. https://doi.org/10.1021/jf2005854

Taylor, W. G., Fields, P. G., & Sutherland, D. H. (2004). Insecticidal components from field pea extracts: Soyasaponins and lysolecithins. *Journal of Agricultural and Food Chemistry*, *52*, 7484–7490. https://doi.org/10.1021/jf0308051

Team, R.C. (2013). *R: A language and environment for statistical computing*. http://www.R-project.org/

Tian, X., Li, Y., Hao, N., Su, X., Du, J., Hu, J., & Tian, X. (2021). The antifeedant, insecticidal and insect growth inhibitory activities of triterpenoid saponins from *clematis aethusifolia* Turcz against *Plutella xylostella* (L.). *Pest Management Science*, *77*, 455–463. https://doi.org/10.1002/ps.6038

Torrini, G., Paoli, F., Mazza, G., Simoncini, S., Benvenuti, C., Strangi, A., Tarasco, E., Barzanti, G. P., Bosio, G., Cutino, I., Roversi, P. F., & Marianelli, L. (2020). Evaluation of indigenous entomopathogenic nematodes as potential biocontrol agents against *Popillia japonica* (*Coleoptera: Scarabaeidae*) in northern Italy. *Insects*, *11*, 804. https://doi.org/10.3390/insects11110804

Ye, S., Zhao, L., Qi, Y., Yang, H., Hu, Z., Hao, N., Li, Y., & Tian, X. (2023). Identification of azukisapogenol triterpenoid saponins from *Oxytropis hirta* Bunge and their aphicidal activities against pea aphid *Acyrthosiphon pisum* Harris. *Pest Management Science*, *79*, 55–67. https://doi.org/10.1002/ps.7172

Zaynab, M., Sharif, Y., Abbas, S., Afzal, M. Z., Qasim, M., Khalofah, A., Ansari, M. J., Khan, K. A., Tao, L., & Li, S. (2021). Saponin toxicity as key player in plant defence against pathogens. *Toxicon*, *193*, 21–27. https://doi.org/10.1016/j.toxicon.2021.01.009

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