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ACLEES CF. SP. *FOVEATUS* (COLEOPTERA CURCULIONIDAE), AN EXOTIC PEST OF *FICUS CARICA* IN ITALY: PRELIMINARY STUDY ON A SUSTAINABLE APPROACH TO DEFENCE.

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Gargani E., Simoni S., Benvenuti C., Frosinini R., Barzanti G.P., Roversi P.F., Caselli A., Guidotti M. - *Aclees* cf. sp. *foveatus* (Coleoptera Curculionidae) an exotic pest of *Ficus carica* in Italy: preliminary study on a sustainable approach to defense.

The entrance of alien pest in new countries has become more and more a problem, due to the loss of biodiversity, alteration of ecosystems, and damages to agricultural economy and human health. Insects are one of the taxonomic groups with the highest frequency of new introduction. The exceptionally frequent entries are a major source of concern for the farmers who have to protect their crops from unknown insects, often without natural enemies in the new areas. A new pest belonging to the Molytinae family (Coleoptera: Curculionidae), tribe Hylobiini, reported as *Aclees* sp. cf. *foveatus* Voss, was recently introduced into Italy. The species is responsible for severe damages in many Italian fig nurseries and orchards, particularly in the Italian Central Northern regions, *i.e.* Tuscany, Liguria and Latium. Currently, no active ingredients are registered against this insect on fig crops. An innovative and eco-friendly approach for controlling this exotic weevil infestation was investigated, by using montmorillonite-based clays, either in their native state or containing copper(II) species, and clinoptilolite zeolites, in order to check the perception of the adults' weevil towards the different solid materials and, subsequently, to evaluate the capability of these innovative products to act as repellents. The formulations containing copper(II)-exchanged clay and clinoptilolite zeolite showed preliminary promising results in terms of insect repellency and environmental sustainability.

KEY WORDS: Asian fig weevil, alien pest, control, copper-containing clay, clinoptilolite zeolite, montmorillonite.

INTRODUCTION

The exponential rise in the movement of goods, food products and people are having, as a side consequence, an ever-growing number of exotic species (also referred as alien) introduced into new areas. Over the centuries, introductions of alien species have sometimes been intentional, especially in the field of organisms useful to humans, such as animals of zootechnical interest, beneficial insects, such as honey bees or different entomophagous insects, various crop plants (*e.g.* corn,

potato, tomato, etc.) as well as ornamental species. However, often, in the case of arthropods, the entrances in new countries might have been unintentional, in particular for species which resulted to be harmful to plants or, even, to humans. In particular, some exotic insects are able to adapt themselves in order to fit into a new environment. However, the species which become acclimated in the new countries (also thanks to climate changes) can increase numerically in such a way as to be harmful to crop plants, favoured, above all, by the absence of specific natural enemies. Despite complex international and national legislations aiming at hindering the diffusion of alien species from one country to another (see, for instance, EU Dir. 2000/29/CE; or Italian Law Decree 214/2005), the phenomenon is becoming increasingly topical. With regard to arthropods, it is estimated that the current rate of introduction of new insects in Italy is, at least, 6-7 new species a year (Inghilesi *et al.*, 2013).

In 2005, a new pest belonging to the genus *Aclees* (Coleoptera: Curculionidae), was reported as the responsible of severe damages in fig nurseries in Tuscany (Central Italy). To date, the infestations have been spread across many fig orchards, particularly in the Italian Central Northern regions, *i.e.* Tuscany, Liguria and Latium, with considerable harvest yield losses and plant deaths (Gargani *et al.*, 2016). The Curculionids, belonging to Molytinae family, tribe Hylobiini, are reported as *Aclees* sp. cf. *foveatus* Voss (Benelli *et al.*, 2014). The species, probably of Asian origin, is strictly related to *Ficus carica* L. Adults, indeed, feed on the epigeal part of the plants, while larvae are xylophagous during their whole development and can cause severe damages, destroying the wood tissues of the root and the trunk. Fig plants are infested in any season, adults showing two different peaks of activity in spring and in summer. However, when climatic conditions are suitable, they can feed on the plants all year round. Up to now, no fig cultivars resistant to the attacks of this pest and very few control strategies capable of containing the adult infestations of this beetle, have been reported: treatments with entomopathogenic fungi, *Beauveria bassiana*, gave interesting results (Gargani *et al.*, 2016). Therefore, as the presence of the insect spreads from nurseries to scattered figs, if the problem will not be addressed systematically, the fig cultivation on the Italian territory might be likely decimated in a short time.

The study of eco-friendly control strategies against exotic insects is more and more important, not only in terms of sustainability, but also in view of the results. In fact, an efficient eradication of a newly introduced alien species, endangering plants, has never been achieved with traditional agrochemicals only. Furthermore, when a new pest is introduced in Italy, farmers have no authorized means to control the new alien species, since the current legislation requires that each

agrochemical product is registered on a specific crop and also against a specific list of noxious insects.

For this reason, a round of experimental trials using innovative solids to study innovative control strategies against this new pest of Italian fig, was performed.

In this aim, three types of solids expected to have a detrimental effect on the ethology of *Aclees cf sp foveatus* were selected and prepared, *i.e.* 1) a montmorillonite-based clay from mineral origin, also known as bentonite, 2) a montmorillonite clay containing copper(II) species, obtained through cationic exchange and 3) a clinoptilolite-type zeolite.

Clays and zeolite were here chosen as preferential materials for fig crop protection, thanks to their toxicological safety, environmental compatibility and particular physico-chemical characteristics. Finely ground clays are indeed able to form a homogeneous particle film on plant leaves and tissues and this proved to be a viable strategy for controlling pests and diseases (Glenn *et al.*, 2005; Chitu *et al.*, 2009; Silva *et al.*, 2013; Sharma *et al.*, 2016). In addition, since clays have a layered porous structure and can accommodate and immobilise active ingredients within the layers of their phyllosilicate structure, clays can be efficiently used as a device for the controlled release of biocides, herbicides and/or fertilisers onto host plants (Choy *et al.*, 2007; Singh *et al.*, 2009). Micronized zeolites as well are attractive candidates as crop protection products, since they create uniform films, which do not interfere with the metabolic lifecycle of the plant and, on the contrary, enhance its resistance towards high temperatures and strong solar irradiation (De Smedt *et al.*, 2015). Moreover, clay- or zeolite-based formulations typically contain chemically inert minerals and the thin particle films obtained from them can be easily removed from harvested fruits by a simple washing with water. In the present case, the materials were used either without any further modification in their micronized powder form or, for the montmorillonite clay only, after a treatment of cationic exchange, in order to insert copper(II) into the interlayer spaces of the solid structure. Since the current trend is to avoid, or at least minimize, the use of copper-containing formulations (Dagostin *et al.*, 2011; Kuehne *et al.*, 2017), immobilization of Cu(II) ions within the montmorillonite framework might help in reducing the overall amount of active metal and in having a controlled, smooth release of Cu(II) species on the host plant and eventually into the environment (Hu *et al.*, 2006).

Then, laboratory-scale and preliminary field trials were carried out to verify their effectiveness in terms of repellence or attraction towards adults of *A. cf sp foveatus*. From ethological studies conducted so far, *Aclees* seems to be closely related to its host plant, *F. carica*. For this reason, we

have studied the responses of weevil adults to plants treated with different formulations of solids covering the fig canopy.

MATERIALS AND METHODS

Laboratory tests: experimental apparatus

An all-plastic five-arm device was developed at CREA DC facilities. The system consists of a central plastic chamber covered with a screen tissue and having a surface suitable for the insects' walking, connected with five plastic tube to five little entomological cages, containing small fig plants in pot. During the test, a group of ten individuals or single insect, during the different trials, were put inside the central chamber, leaving them free to move and walk towards the cages. In the five lateral cages, three fig plants treated with the solid materials to be tested, one fig treated with Naturalis™, a well-known bioinsecticide, and a fig plant treated with tap water only, as control plant, were placed.

Chemicals and preparation of the solid materials

Bentonite Globalfeed AR, hereafter Ben, is a montmorillonite-containing natural clay of mineral origin and was kindly obtained from Laviosa Chimica Mineraria SpA (Livorno, Italy).

ROTA Mining Zeolite, hereafter Zeo, is a clinoptilolite-containing natural clay of mineral origin and was kindly obtained from Biohelp Your Planet s.r.l. (Tarquinia, Italy).

Copper(II) nitrate hemipentahydrate (Sigma-Aldrich, 95%) was used as received.

Each batch of Ben and Zeo to be tested in laboratory and field trials was prepared as follows. A 240 g batch of Ben or Zeo was washed carefully with *ca.* 1 L of deionised water (10 MΩ.cm, Elix-70, Millipore-Merck purifying apparatus), in order to remove any water-soluble species. The solid was then dispersed in 7.5 L of tap water just prior to use, by vigorous shaking and a 3.1 wt.% dispersion of the solid in water was hence obtained.

The elemental composition of Ben and Zeo and their appearance are reported in Table 1 and Figure 1, respectively. The C,H,N elemental analysis was performed on a Perkin Elmer apparatus.

The initial Ben clay contained more than 90% of montmorillonite, the rest being a mixture of minor amounts of amorphous silicate and/or aluminate oxides. The initial Zeo contained at least 85% of crystalline clinoptilolite zeolite, the rest being a mixture of minor amounts of amorphous inorganic oxides.

The average particle size of Ben and Zeo is 20 µm and 200 µm, respectively.

The copper-containing batch, Cu-Ben, on the contrary, was prepared from the pristine Ben material by cationic exchange with aqueous solution of copper(II) salt precursor. In detail, 72.5 g of $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$ were dissolved in 250 mL of deionised water. Then, 240 g of Ben-AR was slowly added to the solution in small aliquots, under mechanical stirring at 250 rpm. After the addition, the total volume of the suspension was brought to 1 L by a further addition of deionised water and homogeneously stirred for 4 h. The suspension was then decanted and thoroughly rinsed with 1 L of fresh deionised water, in order to remove Cu(II) species in excess from the liquid phase. The rinsing step was repeated 8 times, so to obtain an almost colourless upper layer above the decanted solid. The concentrated suspension was finally diluted to 7.5 L, by addition of tap water just prior to use, by vigorous shaking and an overall 3.1 wt.% dispersion of the solid in water was hence obtained.

A small fraction of the Cu-Ben was withdrawn and dried. The content of Cu(II) in the solid was 3.43 wt.%, as obtained by indirect iodometric titration of Cu(II) species in the residual aqueous phase. The content of C and N, as from elemental analysis, was 0.17 wt.% and 0.18 wt.%, respectively.

Naturalis™, the fourth treatment/thesis (Nat), is a commercial registered bio insecticide, based on living spores of naturally occurring strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin (Strain ATCC74040, 7.16 g, equal to 2.3×10^7 viable spores/mL).

Test insects

The Asian fig weevils, *A. cf sp. foveatus*, were obtained collecting adults in fig crops in Carmignano area (Prato province, Tuscany), a very well-known zone for fig production in Central Italy. The gathering step and, consequently, the laboratory trials were carried out in summer (July) and autumn (September). Before the experimental treatments, the adults were provided with fig fruits and leaves as food in entomological cages (100×60×60 cm) and were maintained under standard conditions. One pool of 10 adults, chosen randomly amongst the ones collected from the field, were used for each single trial. Twenty-four hours before the trial, the chosen adults were starved. The weevils were inserted into the central chamber and then their movements were monitored by an observer. Controls were carried out after 30 min, 1 h, 2 h, 3 h and 24 h, counting the number of adults in the different treated cages/plants (T1 – T5).

Field test

At the end of July, the experimental solids were tested in preliminary field trials. In the fig production area of Carmignano (Prato province, Tuscany), three young fig plants/thesis were treated with the 3.1 wt.% aqueous suspensions, as described above. The solid-in-water fine suspension of the products was spread onto the crop trees by means of a backpack sprayer at normal volume. Controls, performed with visual observation on the damage on the fig plants, were carried out at the end of September.

Statistical analysis

The effect of collection period of the insects to be tested, of the check time and of the treatment were analysed by GLM (General Linear Model) and significance evaluated by Duncan test ($P < 0.05$).

RESULTS AND DISCUSSION

It is worth highlighting that the insects' choice is more stable after 24 hours from the start of the test: at this check time, we registered a consolidate selection for the plant/cage. During the laboratory trials, we observed that the insects' movement was affected by the treatment/thesis: they, in fact, differently colonized the five cages ($F_{1.5} = 3.01$; $P = 0.02$). As shown in the graph, the formulations that the fig weevils chose less, were the Cu-Ben and Zeo samples (Fig. I). It is thus a first indication that these two solids could be active as repellent substances against the weevils, likely confusing the insects in the search for the host plant. The presence of a coating film of zeolite or metal-exchanged clay indeed proved to be able to modify the physico-chemical characteristics of the crop surface (in particular, the pH and the emission of volatile organics from fruits and leaves) and this may disorient the insect during the egg deposition activity (Larentzaki *et al.*, 2008; De Smedt *et al.*, 2015; Sharma *et al.*, 2015). The slow release of Cu(II) soluble species, via leaching out of the clay structure, can additionally have a detrimental effect on the surface microorganisms present on the target plant (Walters, 2006), thus causing a supplementary disorienting factor for the weevil.

The two different pools of insects, collected during summer or autumn, gave different results in terms of performance of their movements. The adults collected in summer were more mobile and made their choice amongst the cages more quickly (Fig. II). On the other hand, the adults collected in September showed the typical traits in approaching winter period.

With regard to the preliminary field trials, from a visual control and counting on the healthy and damaged fig sprouts, it was confirmed that the formulations containing the Cu(II)-modified

montmorillonite clay, Cu-Ben, and the natural clinoptilolite zeolite, Zeo, showed good results, as they were less preferred. In fact, while the plants treated with the unmodified clay, Ben, and the check figs presented a high number of shoots with erosions, the plants of the two theses treated with Cu-Ben and Zeo were healthy.

As far as the preparation of the formulation is concerned, since the Zeo sample was used in its natural form, after a simple rinsing and suspension in water, without any further time-consuming ion-exchange procedures, it proved to be a viable and economically sustainable alternative repellent against the fig weevil. Moreover, the absence of added copper species in the Zeo formulation makes it more promising and environmentally sustainable than other currently available commercial products containing copper salts as active ingredients, whose use is being gradually reduced or, better, phased out, especially in organic and/or sustainable farming (Cabus *et al.*, 2017; Kuehne *et al.*, 2017).

CONCLUSIONS

Although the exotic weevil *Aclees cf sp foveatus* is not included in the list of quarantine species, the problems resulting from its infestations on the Italian production of the fig tree could be relevant. To date, its infestations are present in Tuscany, Latium and Liguria, but its population can spread across the Country, reaching regions where fig crops are an important economical resource. No products for the control of the insect populations have been registered so far. Furthermore, the ethology of the weevil, which spends the entire period of its preimaginal development inside the fig tree tissues, makes it very difficult to think of a possible use of the most common synthetic agrochemicals. Now, the new strategic approach presented in this study, studying innovative substances that could have an impact on the selection of the target plant by the insect, by masking the chemical signals of the fig tree to the weevil, could be a successful strategy to solve the problem.

In this case, a Cu(II)-containing montmorillonite clay, Cu-Ben, and a clinoptilolite zeolite from mineral origin, Zeo, displayed the most promising results, both in laboratory-scale tests and in open-field trials. The application of the aqueous formulations containing one of these two solids onto the fig plants resulted in no evident attacks by *A. cf sp foveatus*. Furthermore, Zeo-based formulation was obtained via a simple and cheap preparation sequence, without any addition of copper(II) salt precursors. For these reasons, clinoptilolite zeolite Zeo, together with copper-exchanged clay Cu-Ben, may be considered economically and environmentally sustainable innovative repellents for *Aclees cf sp foveatus*, as an alternative to commercial insecticide products.

Even in the case of alien pests, an integrated and multidisciplinary approach to defence turns out to be the winning strategy and so, also the use of inorganic oxide zeolite- or phyllosilicates-based products could deserve further investigation, as an initial control strategy against alien insect species.

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RIASSUNTO

A seguito di sempre più numerose e più frequenti introduzioni di insetti esotici nel nostro Paese, la problematica dei cosiddetti “alien pest” rappresenta motivo di sempre maggiore preoccupazione per gran parte degli agricoltori. Infatti questi ultimi si trovano a dover fronteggiare nuove infestazioni di insetti fitofagi esotici che recano danno alle colture e non hanno a disposizione conoscenze e mezzi per contrastarle. *Aclees* sp. cf. *foveatus* (Coleoptera: Curculionidae) si sta rapidamente diffondendo nelle zone dell'Italia centro settentrionale, causando gravi infestazioni su alberi di fico. Questo fitofago svolge tutto il proprio ciclo vitale su piante di *Ficus carica*, che pare essere il suo unico ospite, portando le piante a progressivo deperimento fino alla morte. Finora non sono segnalati nemici naturali, né strategie di controllo efficaci. In questo articolo, si riportano prove di laboratorio e preliminari prove di campo, che prevedono l'impiego di innovative sostanze quali argille montmorillonitiche, allo stato nativo o contenenti specie rameiche Cu(II) e zeoliti naturali contenenti clinoptilolite, per verificare la percezione degli adulti del punteruolo del fico nei confronti dei diversi materiali solidi e, successivamente, valutare la capacità di questi prodotti innovativi ad agire come repellenti. Le formulazioni a base di montmorillonite scambiata con rame(II) e di clinoptilolite hanno mostrato proprietà promettenti, in termini di capacità insettorepellente e di sostenibilità ambientale. Tali preliminari risultati permettono di guardare a nuove strategie di controllo del curculionide con prodotti ad elevata sostenibilità ambientale.

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