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Case report of experimental management of white grub (*Melolontha* sp.) in a greenhouse strawberry cultivation using biological agents and soil solarization

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ABSTRACT

The present case report communicates a pilot approach to managing a white grub infestation in a productive, 1-acre (4 stremma), strawberry var. Fortuna greenhouse, where a severe collapse of young seedlings was reported. In this work, the damage caused by *Melolontha* sp. larvae was monitored for the whole area of the greenhouse (~20,000 plants). Monitoring included detection and recording of the number of affected plants, i.e plants in which a partial or complete absence of the root system upon where the specific larvae were feeding was observed. Other symptoms recorded included smaller plant size, reduced production and complete wilting. A series of non-chemical insecticide formulations (mainly entomopathogenic fungi and entomopathogenic nematodes) were applied alone or in different combinations, in order to test and assess their effectivity against this significant problem. At completion of the cultivation/experimentation period, a 12 ton/acre final production was estimated by the producer, which is considered satisfactory.

KEY WORDS: White grub, greenhouse strawberry, biological agents.

Introduction

The common name "white grub" refers to the larvae of *Melolontha* spp., a coleopteran genus of the family Scarabaeidae, subfamily Melolonthinae (Freude et al, 1969). To date, some 62 *Melolontha* species have been recorded worldwide, six of which are found in Europe, with four having been recorded from Greece, i.e. *M. albida*, *M. melolontha*, *M. pectoralis* and *M. taygetana* (Fauna Europea, 2022; Catalogue of Life, 2022).

During the recent years, there is a notable increase in the presence of white grub in nurseries and horticultural crops, reported for many European countries, and this has led to significant economic losses, especially when the larvae of the pest are in the last instar stage (Dolci et al. 2006; Łabanowska and Bednarek 2005; Malusá et al, 2020; Nageleisen et al. 2015).

Symptoms – Current control approaches

White grub infestation symptoms include leaf damage induced by direct feeding of adults, and wilting, chlorosis and necrosis due to the feeding activity of larvae on the roots. Occasionally, fruit lesions may be recorded. The attack of the root system by the soil-dwelling larvae may be decisive at high or widespread infestations that cause total necrosis of the affected plants.

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Unfortunately, the treatment of white grubs is an intractable problem due to the biology of the species, extended life span, soil inhabiting behavior and low possibility of controlling the adults by leaf insecticide applications, while for many crops, such as strawberry, a very important culture for the Prefecture of Achaia, Greece, there are no licensed insecticides for soil applications.

Historically, during the pre-industrial era, *Melolontha* adults were collected by hand to be subsequently used as animal feed or even for human consumption in some areas. Later, as economic damage and need for food availability escalated, the application of synthetic insecticides provided sufficient control. However today, the European regime for the sustainable use of pesticides (Directive 2009/128/EC), and the implementation of European Union legal provisions and the Green Deal plan, the reduction of pesticide applications is imperative so the use of insecticides in agriculture, forces us to curtail such interventions.

Entomopathogenic nematodes

Entomopathogenic nematodes have repeatedly been reported for their ability to efficiently kill several species of insect larvae and actively disperse in the soil, although the effectiveness of most species against white grub larvae is reported as quite low (Gerritsen et al., 1998). Also, although the species *Heterorhabditis bacteriophora* has delivered positive results against *Melolontha*, Peters (2000) postulated that this method is not economically advantageous. On the other hand, *H. bacteriophora* application could reduce the population of white grubs by up to 65%, at the rate of 1,000.000 infective juveniles (IJs) per m^2 (Berner & Schnetter, 2001).

So far, *Steinernema glaseri* has given the most promising results against white grub larvae, while Peters (2004) stated that laboratory tests with *S. scarabaei* produced very promising results, however, the problem with the latter is that mass breeding has been proved unsuccessful (Huiting et al, 2006).

Entomopathogenic fungi

Beauveria brongniartii is considered the main natural enemy of white grubs (Keller, 2000). This fungus has the ability to infect all life stages of the insect. Two methods of application of these fungal species have been developed to control white grub larvae: 1) spray application against adults with fungal blastospores, that are transferred by the insects to their breeding areas, thus infecting other members of the population and 2) soil application of barley seeds colonized by the fungus.

Survival and spread of *B. brongniartii* requires certain conditions to be met, such as the prevalence of wet conditions, which is not always possible, while the application must be performed at a depth of about five centimeters, to avoid the inactivation of the pathogen by UV radiation (Meinert et al., 2001). Strasser (2004) also states that an immediate effect on the insect is not expected, but unlike chemicals whose action weakens over time, the activity of the fungus increases, even more as the insect population increases. The same researcher claims that this specific fungus affects all life stages of the insect and for an interval of three to four generations.

Other non-chemical methods

Meinert et al. (2001) tested the application of an insecticide with the active substance plant-derived azadirachtin, with spray applications at the dose of 3l/ha, during which an infestation reduction was observed, possibly due to feeding cessation, while Hummel & Kleeberg (2004) found 100% mortality after 9 days, under laboratory conditions. They also report that this particular insecticide caused a reduction in egg production from 14.9 to 3.9 per female and a reduction in the percentage of hatchable eggs from 53 to 15%.

Furthermore, Huiting et al (2006) reported that *Bacillus thuringiensis* and *B. popiliae* could be effective against *Melolontha* larvae, but so far, these bacterial species have not been extensively tested against this insect and, thus, no successful efficacy has been reported.

Chemical application against *Melolontha* larvae

One crucial characteristic for the control of white grub larvae is that they complete the larval stage of their biological cycle underground and move up and down in the soil during their development. Due to this, their treatment by chemical means becomes almost impossible. Nevertheless, there are several reports regarding the successful treatment with chlorpyrifos, albeit controversial. Additionally, several chemical methods have also been applied in combination with non-chemical applications, such as thiamethoxam or acetamiprid and subsequent soil cultivation before strawberry planting, that controlled white grub almost by 80% (Łabanowska et al., 2003).

The Case Report

A few years ago, in the forest of Strofylia, Prefecture of Achaia, Greece, adults of white grub were being observed, though there was no report on the specific species. Their presence gradually began to spread and now the problem is dispersed throughout the area of Manolada, an area traditionally dedicated to the cultivation of strawberries, and worldwide known as most produce is exported, thus local producers make their living dependent on this culture.

In November 2019, in a productive one-acre greenhouse, where the strawberry variety Fortuna was planted, a scattered plant collapse was recorded, for which a cultivation autopsy confirmed the presence of white grub larvae (Figs 1-4).

As aforementioned, there are no approved insecticides for soil applications for the control of this particular crop-pest combination (Greek Ministry of Rural Development and Foods, http://www.minagric.gr/syspest/).

Therefore, in an effort to "rescue" the annual yield of the producer, we started to monitor the crop (initiation date 19/11/2019) by recording the number of infested plants. More specifically, collapsing plants were being uprooted and when the root system appeared cut off, the soil around the plant rhizosphere was thoroughly scrutinized for the presence of larvae. When larvae were retrieved, the plant was reported as infested.

FIG 1.: Cut off root system of white grub infested strawberry plants.

The Pilot Implementation

As the situation appeared alarming, with the number of infested plants being considered as crucial by the producer, a pilot biological control schedule was designed, using various biological agents in the form of approved plant protection products. Application of the agents was performed on 29/11/2019. The applied products were:

Nemabac and Nemafelt (Bio-insecta GR), containing *Heterorhabditis bacteriophora* and *Steinernema feltiae* respectively)

Botaniguard 10,7SC (KNE Certis GR), containing *Beauveria bassiana* strain GHA 10,735%)

Metarhizium anisopliae var anisopliae strain F52 (Met52), containing *M. anisopliae* var *anisopliae* strain F52)

Biorend R1 (Bio-insecta GR), containing chitosan 1,88%.

Application rates were according to manufacturer's instructions: 500mi/acre, 500mi/acre, 150ml/100lt, 125ml/acre και 400ml/acre respectively). The products were applied singly or in combinations, as follows:

1. Botaniguard
2. Botaniguard + Metarhizium anisopliae
3. Botaniguard + Metarhizium anisopliae + Chitosan
4. Botaniguard + Metarhizium anisopliae + Nemabac + Nemafelt + Chitosan
5. Nemabac + Nemafelt + Chitosan
6. Botaniguard + Chitosan
7. Metarhizium anisopliae + Chitosan
8. Metarhizium anisopliae

Recording of infested plants was carried out at regular intervals until February 2020, when high levels of rainfall ceased to allow it, and finally stopped completely due to the measures imposed by the COVID-19 pandemic regulations in March 2020.

FIG. 2: Cut off root system of white grub infested plant, and isolated white grub larva from the rhizosphere soil.

Results

In all above applications, an impressive reduction in the number of infested plants was observed, which was maintained until the last recording. The timing of this reduction (ca 1 month after application) is in accordance with scientific data, since entomopathogenic agents do not cause a knock down effect of insect populations but require some time for their establishment and action. The reduction of white grub population is caused due to the action of the applied products but also to the decrease of ambient temperature during the specific time period. Unfortunately, the lack of recordings from February 2020 onwards, does not serve to draw absolute conclusions. However, it is interesting that the final yield of 3 tons/ha that was estimated by the producer, was completely satisfactory based on the history of the crop. In addition, coming May 2020, no adult flights were recorded.

After the end of the cultivation period, the greenhouse was treated with soil solarization for two months (July and August 2020), using black polyethylene sheets. The following year, a new strawberry plantation was established in the same greenhouse, during which period no presence of white grubs was reported, even after thorough soil and plant monitoring. Subsequently, soil solarization is applied annually and no white grub recurrence has been observed.

The results of the present study show a tendency to regulate the problem of white grub infestation and crop losses, although there is a need for further investigation regarding the doses, frequency of application and form of the aforementioned biological factors.

FIG.3: White grub larvae of various instar stages retrieved for the soil rhizosphere of symptomatic strawberry plants.

FIG. 4: Part of the strawberry (var. Fortuna) greenhouse where the pilot study was performed. The rightmost plant row shows severity of infestation.

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Αναφορά περίπτωσης πειραματικής διαχείρισης μηλολόνθης (*Melolontha* sp.) σε θερμοκηπιακή καλλιέργεια φράουλας με χρήση βιολογικών παραγόντων και ηλιοαπολύμανση

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ΠΕΡΙΛΗΨΗ

Στην παρούσα εργασία παρακολουθήθηκε η ζημιά παραγωγικού θερμοκηπίου φράουλας, ποικιλίας Fortuna, τεσσάρων στρεμμάτων, για το οποίο τον Νοέμβριο 2019 είχε διαπιστωθεί παρουσία προνυμφών *Melolontha* sp. Η παρακολούθηση ξεκίνησε στις 19/11/19 και καταγράφονταν το πλήθος προσβεβλημένων φυτών (παρατηρούνταν αποκοπή του ριζικού συστήματος στο ύψος της κορώνας και η προσβολή επιβεβαιωνόταν με την ανίχνευση προνυμφών του είδους στην περιοχή της ριζόσφαιρας). Για το συγκεκριμένο συνδυασμό καλλιέργειας-εχθρού, δεν υπάρχουν εγκεκριμένα εντομοκτόνα για εφαρμογές εδάφους. Συνεπώς, σε μια προσπάθεια «διάσωσης» της παραγωγής, εφαρμόστηκαν βιολογικοί παράγοντες σε μορφή εγκεκριμένων σκευασμάτων που εκτιμήθηκαν ως προς τη δυνατότητα αποτελεσματικής χρήσης τους για το δεδομένο πρόβλημα. Χρησιμοποιήθηκαν τα σκευάσματα Nemabac3, Nemafelt3, Biorend R3, Botaniguard 10,7SC4 και Metarhizium anisopliae var anisopliae strain F525 σε δόσεις σύμφωνα με τις οδηγίες των παρασκευαστών οίκων 500mi/στρ., 500mi/στρ., 400ml/στρ., 150ml/100lt και 125ml/στρ. αντίστοιχα. Οι εφαρμογές έγιναν σε διάφορους συνδυασμούς, στις 29/11/19, οπότε παρατηρήθηκε ραγδαία και επικίνδυνη αύξηση του αριθμού των προσβεβλημένων φυτών. Οι καταγραφές πραγματοποιούνταν ανά τακτά χρονικά διαστήματα μέχρι τον Φεβρουάριο 2020 που τα υψηλές βροχοπτώσεις έπαψαν να το επιτρέπουν, ενώ τελικά σταμάτησαν πλήρως, λόγω επιβολής των έκτακτων μέτρων για την πανδημία COVID-19, τον Μάρτιο. Σε όλες τις εφαρμογές παρατηρήθηκε μεγάλη μείωση του πλήθους προσβεβλημένων φυτών, η οποία διατηρήθηκε μέχρι και την τελευταία καταγραφή. Η χρονική τοποθέτηση αυτής της μείωσης είναι σύμφωνη με τα σχετικά επιστημονικά δεδομένα, αφού τα βιολογικά σκευάσματα δεν παρουσιάζουν φαινόμενα άμεσης κατάρρευσης των εντομολογικών πληθυσμών, αλλά απαιτείται χρόνος για την εγκατάσταση και δράση των εντομοπαθογόνων παραγόντων. Η μείωση των πληθυσμών μηλολόνθης οφείλεται τόσο στην πτώση της θερμοκρασίας περιβάλλοντος τη συγκεκριμένη χρονική περίοδο, όσο και στη δράση των σκευασμάτων, ενώ τυχόν αρνητικές συσχετίσεις μπορούν να αποδοθούν στα ακανόνιστα ποτίσματα, την εφαρμογή διαφυλλικών μυκητοκτόνων και την έλλειψη επαρκούς οργανικής ουσίας. Δυστυχώς, η αδυναμία επιπλέον καταγραφών το ακόλουθο διάστημα δεν εξυπηρετεί την εξαγωγή απόλυτων συμπερασμάτων. Πάντως ενδιαφέρον είναι το ότι η παραγωγή ήταν 3 τόνοι/στρ., δηλαδή απολύτως ικανοποιητική με βάση το καλλιεργητικό ιστορικό. Επιπροσθέτως, μετά την έλευση του μηνός Μαΐου, δεν παρατηρήθηκαν πτήσεις ακμαίων. Εν συνεχεία, το καλοκαίρι του 2020 έγινε εφαρμογή ηλιοπολύμανσης και έκτοτε δεν έχει παρατηρηθεί επανεμφάνιση της προσβολής. Τα αποτελέσματα δείχνουν μια τάση ρύθμισης του προβλήματος, που σε μερικές περιοχές τείνει να είναι δυσεπίλυτο, λόγω της βιολογίας του είδους, της διαβίωσής του για μεγάλο χρονικό διάστημα βαθιά στο έδαφος και της μικρής πιθανότητας ελέγχου των ακμαίων με ψεκασμό, αν και υπάρχει ανάγκη περαιτέρω διερεύνησης σχετικά με τις δόσεις, την συχνότητα εφαρμογής και την μορφή των προαναφερόμενων σκευασμάτων.

