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Survival Time, Mortality Rate, and Feeding Damage of Adult *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae) Exposed to Biopesticides in Laboratory Bioassays

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Abstract: Survival time, percentage mortality, and feeding behavior were evaluated for adults of the weevil *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) exposed to biopesticides by using a Petri dish plant-based bioassay system. Treatments consisted of three registered entomopathogenic fungal formulations (BotaniGard ES, PFR-97 20% WDG, and Met52 EC) and three biochemical formulations (AzaMax, Entrust, and PyGanic EC); a synthetic chemical insecticide (Sevin SL) and distilled water served as control treatments. Thirty adult *M. undecimpustulatus undatus* (ten per bioassay cage replicate, three replicates per treatment) were given cocoplum leaves sprayed to the point of runoff with a treatment. Survivorship of weevils was checked daily for 15 days. Five trials (15 total replicates per treatment) were performed. Survival times of weevils in all product treatments were shorter than those of weevils in the water control treatment. Percentages of beetle mortality in the Entrust and BotaniGard treatments were about two-fold higher than in the other treatments. Mean percentages of mycosis for beetles in the BotaniGard, PFR-97, and Met 52 treatments were 90%, 8%, and 5%, respectively. Weevils that consumed BotaniGard-treated leaves caused significantly more leaf damage than weevils that consumed leaves with PFR-97 or Met52. Weevils on leaves treated with Entrust, PyGanic, AzaMax, and Sevin consumed significantly less than those in the fungal and water only treatments. The high mortality rates caused by Entrust and BotaniGard suggest that populations of adult *M. undecimpustulatus undatus* might be well-managed using these two commercially available biopesticides. Additional testing is needed in the field to corroborate our results obtained under controlled laboratory conditions.

Keywords: weevil; entomopathogenic fungi; biopesticides; cocoplum; mycosis; plant damage rating index

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1. Introduction

Management of the polyphagous pest weevil *Myllocerus undecimpustulatus undatus* Marshall (Coleoptera: Curculionidae) by using synthetic insecticides has proven to be unsustainable [1,2]. Concern for environmental impacts, pesticide resistance, and the desire for effective and sustainable pest control point to the necessity of an integrated pest management (IPM) approach [3]. Implementing IPM through the combination of various tactics to overcome the limitations of specific practices should include the use of biopesticides [4].

Biopesticides are compounds containing living organisms or substances derived from animals, plants, fungi, bacteria, or certain minerals [5]. According to their active ingredient, biopesticides are categorized into one of three types: microorganisms; biochemicals; or semiochemicals [4]. The microorganisms in microbial pesticides, e.g., entomopathogenic fungi, naturally occur in soils worldwide and may infect a wide range

of insect hosts [6–8]. The biochemical pesticides that discourage herbivores from feeding are made from plants that produce secondary metabolites such as pyrethrins produced by *Chrysanthemum cinerariaefolium* Vis. (pyrethrum) and phenolic acids in the seeds of *Azadirachta indica* A. Juss (neem) [4,8]. The biochemical pesticide spinosad is a mixture of natural metabolites (spinosads A and D) produced by aerial fermentation of a soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao [4,9].

To date, studies on control strategies with biopesticides for *Myllocerus* species are limited. Shanthipriya and Misra [10] evaluated nine pesticide treatments against adult *Myllocerus maculosus* Desbrochers des Loges on field grown okra. Padan (a neonicotinoid), spinosad, Daman (*Beauveria bassiana* (Balsamo) Vuillemin), and Ozoneem (*A. indica*) reduced adult weevil numbers by 91%, 48%, 30%, and 48%, respectively, 5 days after treatment compared with the control. By day 10, *B. bassiana* produced a further reduction in the number of weevils per plant to 62%.

The purpose of our study was to comparatively evaluate survival times, mortality rates, and feeding damage of adult *M. undecimpustulatus undatus* after exposure to six biopesticides on foliage in laboratory assays. These selected fungal and biochemical biopesticides have been developed and registered for use in the United States. Additionally, the efficacy of each biopesticide was compared with a standard synthetic insecticide used by growers.

2. Materials and Methods

2.1. Plants

Cocoplum, *Chrysobalanus icaco* L. (Chrysobalanaceae), leaves on stems were used as a food source for weevils in the experiments. The leaves fit inside a Petri dish without needing to trim them. Twenty-four cocoplum branches cut to 25 cm in length each and with 4–6 leaves were washed and kept hydrated until use.

2.2. Insects

A laboratory colony of *M. undecimpustulatus undatus* was established with adults collected from mature Australian pine, *Casuarina equisetifolia* L. (Casuarinaceae), in Fort Pierce, FL, USA (27°29′15.43″ N, 80°24′33.79″ W). The weevils were maintained in mesh-screened Bug Dorms (60 × 60 × 60 cm, BioQuip Products, Rancho Dominguez, CA, USA) with the temperature at 24 ± 2 °C, 60% relative humidity, and 14 h photoperiod. Three 40-dram vials with a snap-on lid with a 1 cm hole cut into the lid were filled with water, and 2–3 branches of cocoplum, each 25–30 cm long, were inserted through the hole in the lid. In each Bug Dorm, three plastic containers measuring 13 cm high × 12 cm wide (at top) and narrowing to 10 cm (at bottom) were lined with a brown paper towel for an oviposition substrate, and one vial with cocoplum was placed into each container. Two small Petri dishes (60 × 15 mm) were filled with a moistened, crumpled brown paper towel to provide water for the weevils. Plants were changed weekly, and water was added to the dishes 2–3 times per week. Three days prior to use, 240 field-collected *M. undecimpustulatus undatus* adults were removed from the laboratory colony and placed into a Bug Dorm cage with a water source, but no food.

2.3. Preparation of Commercial Products

Three commercially available entomopathogenic fungal formulations were tested: BotaniGard® ES that contained conidia of *B. bassiana* (Hypocreales: Cordycipitaceae) strain GHA (a.i. 11.3%) in an oil emulsifiable suspension (Laverlam International Corporation, Butte, MT, USA); PFR-97™ 20% WDG that contained desiccation-tolerant blastospores of *Cordyceps javanica* (Frieder. and Bally) Kepler, B. Shrestha and Spatafora (Hypocreales: Cordycipitaceae) strain Apopka 97 (a.i. 20%) in the form of wettable dispersible granules (Certis, Columbia, MD, USA); and Met52® EC that contained conidia of *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) strain F52 (a.i. 11%) in an oil emulsifiable concentrate (Novozymes Biologicals Inc., Salem, VA, USA). Fungal

suspensions were prepared by mixing each fungal product in distilled water in a beaker with a magnetic stirring bar. For PFR-97, 0.66 g were mixed in 300 mL of distilled water. The beaker was placed on a stir plate for 30 min, and the suspension was then allowed to settle for 30 min. This allowed the inert ingredient to precipitate, thereby leaving the supernatant containing the blastospores. For BotaniGard and Met52, 750 µL and 960 µL, respectively, of the product were mixed in 300 mL of distilled water for 10 min. The number of spores per ml was counted by using a disposable plastic C-Chip™ DHC Neubauer hemocytometer (Incyto Co., Ltd., Chungnam-do, Republic of Korea). Each suspension was then adjusted to a rate of 10^7 spores/mL by adding either distilled water or product according to the formula in Avery et al. [11]. Viability of the spores was assessed by spreading 100 µL of 10^3 spores/mL of each fungal suspension onto potato dextrose agar plates containing bactericides [12]. The plates were sealed with Parafilm™ (Beemis Co., Inc., Neenah, WI, USA), transferred to an environmentally controlled chamber set at 25 °C with a 14 h photophase for 7 d. Colony-forming units (CFUs) were counted to check the quality of the fungal suspensions by comparing the number of CFUs obtained with the number of CFUs specified on the label of each product. The viability of all fungal products used in the trials was >90%.

Three biochemical products approved for organic production by the Organic Materials Review Institute were tested: AzaMax™ (a.i. 1.2% azadirachtin) (Parry America, Inc. Sacramento, CA, USA); Entrust® (a.i. 80% spinosad: mixture of A and D) (Dow AgroSciences LLC, Indianapolis, IN, USA); and PyGanic® EC 1.4 II (a.i. 1.4% pyrethrins) (McLaughlin Gormley King Co., Minneapolis, MN, USA). Each solution was mixed in 300 mL of distilled water with the following amounts: 90 µL (equivalent rate of 5 mL/L) of AzaMax; 0.26 g (equivalent rate of 240 g/L) of Entrust; 480 µL (equivalent rate of 19.8 mL/L) of PyGanic. Each solution was mixed in a beaker with a magnetic stirring bar on a stirring plate for 10 min.

A synthetic chemical standard insecticide, Sevin® SL (a.i. 43% carbaryl) (Bayer Environmental Science, Research Triangle Park, NC, USA), was chosen for its broad-spectrum activity and because it is a popular choice for pest control by growers and homeowners. The pesticide was prepared by adding 90 µL of the product to 300 mL of distilled water (equivalent rate of 2.5 mL/L) in a beaker containing a magnetic stirring bar that was placed on a stirring plate for 10 min. Distilled water only was used as the negative control treatment.

2.4. Design of Bioassay Cages and Application of Treatments

A bioassay cage was designed to evaluate the seven commercial products (product treatments) applied on cocoplum leaves for toxicity to adult *M. undecimpustulatus undatus*. The bioassay system was modified from one developed by McKenzie et al. [13]. Each bioassay cage consisted of a conical tube inserted into a vented polystyrene Petri dish (150 × 20 mm), into which plant material and weevils were added; the dish was sealed with Parafilm® (Figure 1). Polystyrene conical centrifuge tubes (15 mL, 17 × 120 mm) held the stem of the plant material. A hole was drilled near the top of the tube to accommodate adding water as needed throughout the duration of the bioassay. A small piece of 1 cm diameter wooden dowel was inserted into the conical tube to prevent breaking or cracking the tube during drilling. A small drill bit was used to make a starter hole into the conical tube, and a larger drill bit was used to bore a hole to accommodate a squirt bottle tip. A hole was drilled through the side of a closed Petri dish using a step drill bit (the exact size of the conical tube). Another hole, approximately 120 mm in diameter, was made with a hole-saw bit through the center of the small plate of the Petri dish (for ventilation). A piece of thrips-proof screen slightly larger than the hole was attached to the Petri dish by a clear, fast-curing cement for joining acrylic (Weld-On Acrylic Cement-IPS Corporation, Compton, CA, USA). This screened window provided ventilation for the plant and insects inside the cage during the bioassay.

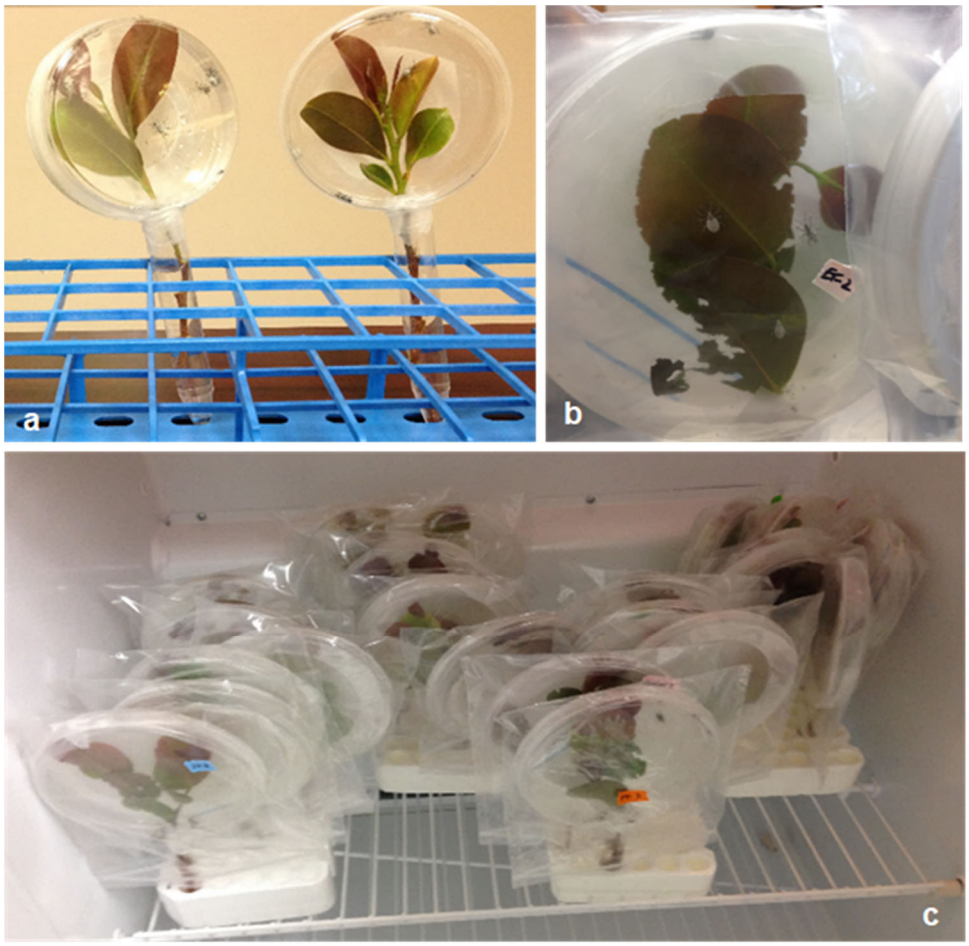

Figure 1. (**a**) Petri dish bioassay cages modified from McKenzie et al. [13]. (**b**) Ten adult weevils on cocoplum leaves in a Petri dish bioassay cage inside a plastic bag. (**c**) Randomized block design of treatments and replicates in an environmentally controlled chamber.

The centrifuge tubes were filled with approximately 35 mL of water up to 5 mL below the watering hole, and the mouth was covered with 2.5 × 2.5 cm^2 of Parafilm that was secured to the sides of each tube to prevent insects from crawling in and drowning, as well as to provide support to the plant stem. Plant stems with leaves were inserted through the Parafilm and into the tube as far as possible, keeping only the foliage exposed. Another square of Parafilm was added around the stem and secured to the tube.

All treatment solutions were applied with separate pressurized hand-pump 180-mL Nalgene® aerosol spray bottles (Nalgene Nunc International, Rochester, NY, USA) with fine mist nozzles to deliver consistent spray coverage. Each bottle was labeled with one of eight treatments and filled with 80 mL of the corresponding suspension, solution or water only. The bottle was pressurized with 8–10 pumps prior to each application. The cocoplum leaves in each replicate were sprayed on both surfaces to the point of runoff. After treatment application, the tubes were set in a wire tube rack to air dry. The tube was then placed into the side hole in the bottom half of the Petri dish, ten weevils were added to the dish, and the dish was immediately closed with the top half and secured with a 10 × 2.5 cm strip of Parafilm around the rim (Figure 1a). Bioassay cages were individually covered with a liter-size (17.7 × 18.8 cm) plastic bag (Ziploc® S. C. Johnson & Son, Inc., Racine, WI, USA) to increase humidity around the cage and prevent cross-contamination. Each bag was labeled with treatment and replicate number (three replicates per treatment) (Figure 1b). Six cages were randomized in one of four Styrofoam racks. Racks were placed in an environmentally controlled chamber (Percival Scientific, Inc., Perry, IA, USA) at 25 °C ± 1° with 20% relative humidity and a 14 h photophase for the duration of the

experiment (Figure 1c). The experiment was repeated four times for a total of five trials (each treatment with 15 total replicates).

2.5. Survival Time and Percentage Mortality

Weevils in all treatments were checked daily for mortality over a 15 d observation period. The survival time was determined by utilizing the time of death for each weevil per bioassay cage. Data from all trials combined were used to calculate the median survival values for 50% of the population inside the bioassay cages (ST_{50}) in days per treatment. If the ST_{50} values were significantly different among the treatments, then the mean survival time during the 15 d observation period was determined.

Percentage mortality for each treatment per trial at the end of the 15 d observation period was calculated using the formula: % mortality = [Td/10] × 100, where Td = total number of weevils dead per cage on day 15.

2.6. Mycosis

All dead insects per treatment at 15 d were removed from the bioassay cages, surfaced sterilized according to Lacey and Brooks [14], grouped by treatment in separate Petri dishes (100 × 15 mm) lined with filter paper moistened with 800 µL of distilled water, and observed daily until mycosis of the fungal pathogen phenotype could be identified (7–14 d).

Percentage mycosis for each cage was calculated using the formula: % mycosis = [Cm/Ct] × 100, where Cm = total number of cadavers mycosed; Ct = total number of cadavers.

2.7. Feeding Damage

Leaves were removed from the bioassay cages on day 15, and the feeding damage was assessed. Percentage leaf damage was estimated by using the Plant Damage Rating Index (PDRI) modified from Maletta et al. [15]. The PDRI rating was 1 = 0–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, 5 = 76–100% (Figure 2).

2.8. Statistical Analysis

The ST_{50} per treatment was determined and treatment values were compared using Kaplan–Meier survival analysis (α = 0.05) followed by a log rank test (JMP® PRO 13.1 Modeling and multivariate methods) (SAS Institute Inc., Cary, NC, USA, 2017). Mean ST_{50} values for all treatments were subjected to ANOVA, and treatment means were separated and compared using Tukey–Kramer HSD test (α = 0.05). Mortality percentages were modified with an arcsine square root transformation prior to the ANOVA, and treatment means were separated and compared using Tukey–Kramer HSD test (α = 0.05). Mean plant damage rating indices were subjected to ANOVA, and treatment means were separated and compared using Tukey–Kramer HSD test (α = 0.05). All ANOVA tests were performed using PROC GLM in SAS v. 9.2 (SAS Institute, Cary, NC, USA, 2009).

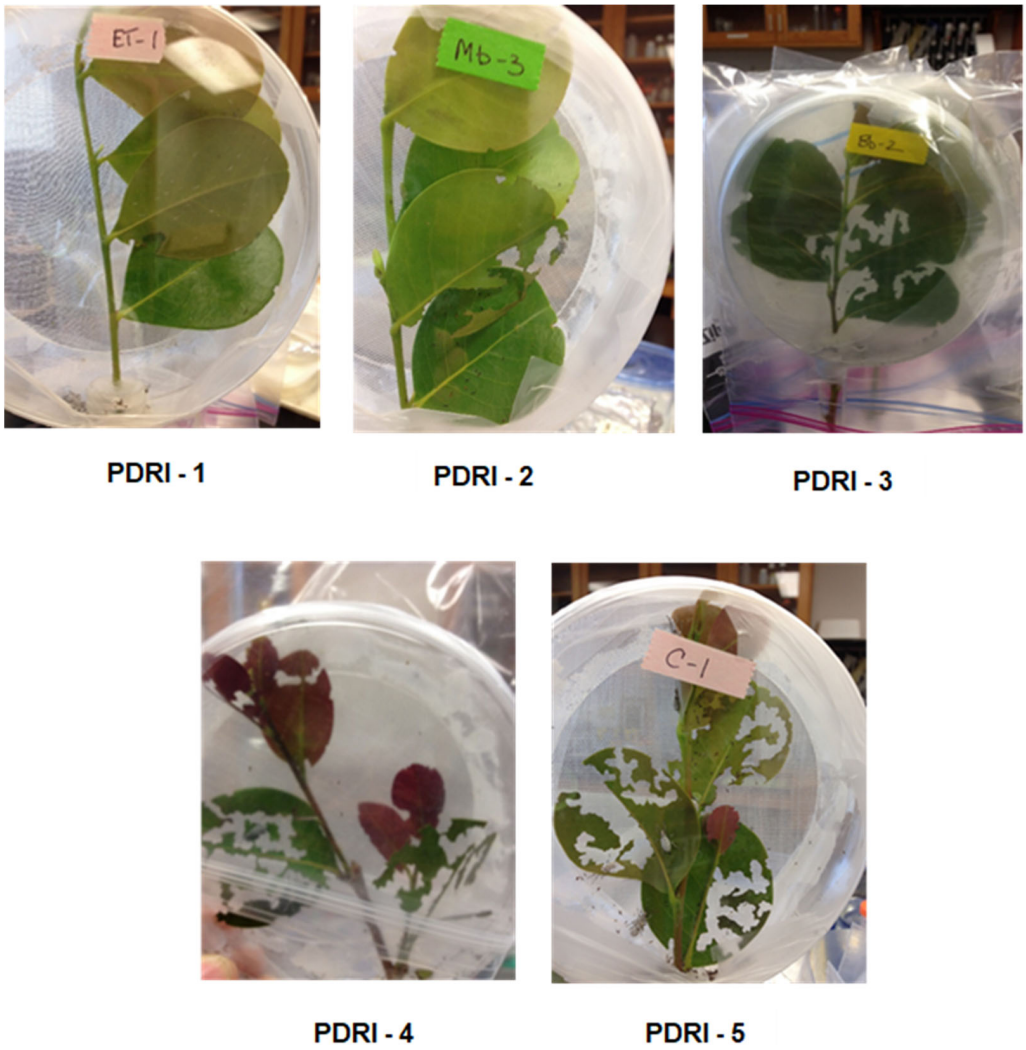

Figure 2. Plant Damage Rating Index (PDRI) examples of adult *Myllocerus undecimpustulatus undatus* feeding on treated cocoplum leaves. Percent leaf damage was estimated at day 15 using a rating scale as follows: 1 = 0–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, 5 = >76%.

3. Results

3.1. Survival Time and Percentage Mortality

The ST_{50} of weevils varied significantly among treatments in each trial when censored at 15 d (log rank X^2 = 399.75, df = 8, $p < 0.0001$). Trials were compared and not found to be significantly different ($F_{4, 1015}$ = 1.64; p = 0.169); therefore, the data from all trials were pooled and re-analyzed. The mean survival times during the 15-d observation period revealed that the weevils exposed to the control (water only), PFR-97, Met52, and PyGanic treatments survived significantly ($F_{7, 1043}$ = 134.21; $p < 0.0001$) longer (each at 14 d) than in the AzaMax and Sevin (13 d), BotaniGard (12 d), and Entrust (9 d) treatments (Figure 3). The mean survival times for weevils exposed to BotaniGard and Entrust were significantly shorter than the mean survival times for weevils exposed to AzaMax and Sevin. Beetles exposed to Entrust lived significantly shorter time than weevils exposed to BotaniGard.

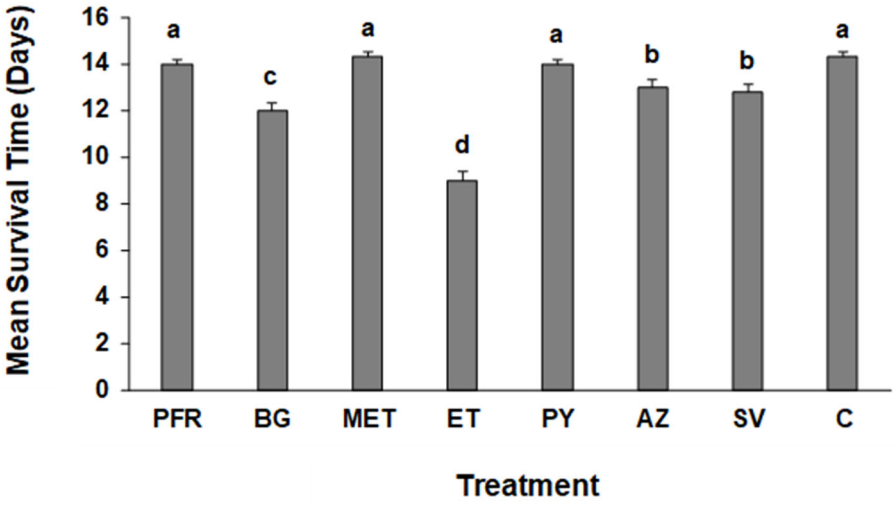

Figure 3. Comparison of mean survival times (days) of adult *Myllocerus undecimpustulatus undatus* on cocoplum leaves treated with PFR-97™ 20% WDG (PFR), BotaniGard® ES (BG), Met52®EC (MET), Entrust® SC (ET), PyGanic® EC 1.4II (PY), Aza-Max™ (AZ), Sevin® SL (SV), and water only (C). Bars are means ± SEM of five trials combined (three replicates per treatment per trial). Treatments not followed by the same letter above the bars are significantly different (Tukey–Kramer HSD test, $p < 0.0001$).

Weevils had significantly greater mortality rates at 15 d after exposure to leaves treated with Entrust and BotaniGard compared with the other treatments ($F_{7, 98} = 28.39$; $p < 0.0001$) (Figure 4). Percentage mortalities with Entrust and BotaniGard were about two–four-fold higher than with the other treatments.

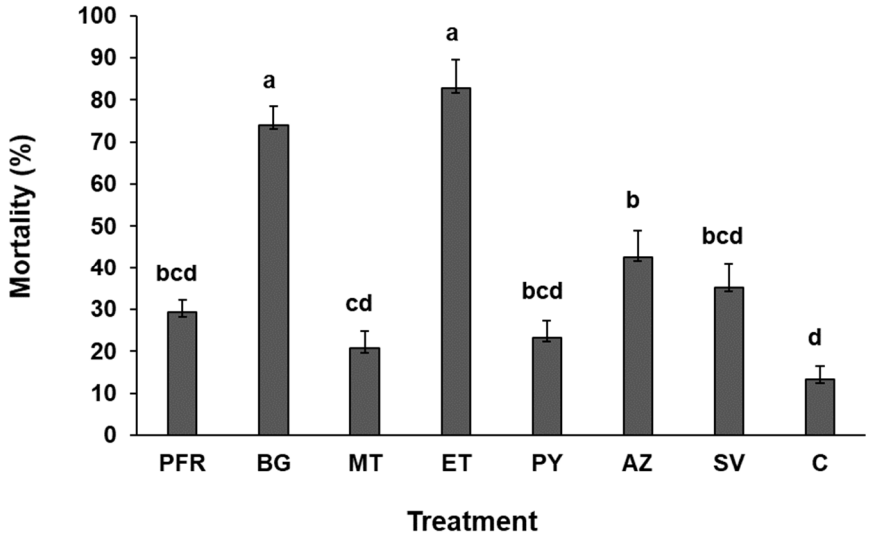

Figure 4. Adult *Myllocerus undecimpustulatus undatus* mortality rates 15 d after treatment applications. Treatments included PFR-97™ 20% WDG (PFR), BotaniGard® ES (BG), Met52®EC (MT), Entrust® SC (ET), PyGanic® EC 1.4II (PY), Aza-Max™ (AZ), Sevin® SL (SV), and water only (C). Bars are means ± SEM of five trials combined (three replicates per treatment per trial). Treatments not followed by the same letter above the bars are significantly different (Tukey–Kramer HSD test, $p < 0.0001$).

3.2. Mycosis

Before the end of the trials and prior to surface sterilization, dead weevils inside the bioassay cages of the BotaniGard treatment exhibited mycosis (Figure 5). In contrast, dead weevils from the other fungal treatments failed to show any external mycelial growth

prior to surface sterilization. The mean percentages ± SE of mycosis across the five trials for weevils in the BotaniGard, PFR-97, and Met 52 treatments were 90% ± 0.2, 8% ± 0.0, and 5% ± 0.0, respectively. No mycosis was observed in dead weevils in the non-fungal treatments.

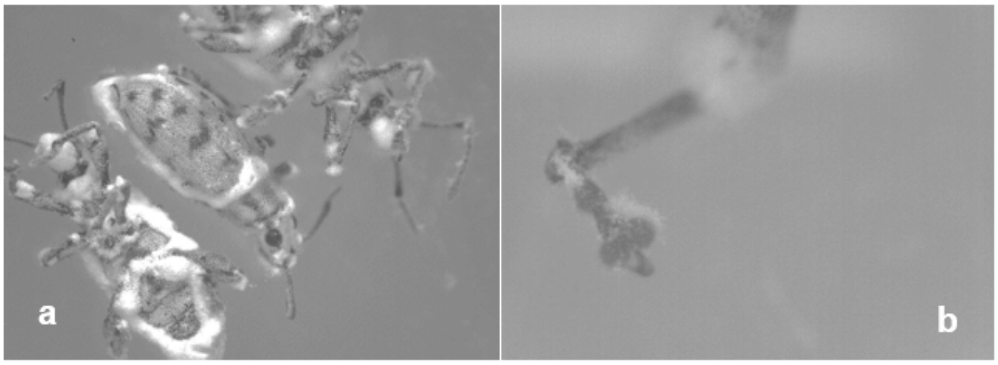

Figure 5. (**a**) *Myllocerus undecimpustulatus undatus* adults displaying mycosis from infection with *Beauveria bassiana*. (**b**) Close-up of *Beauveria bassiana* conidiogenesis on the weevil's tibia and tarsus.

3.3. Feeding Damage Assessment (PDRI)

There were significant differences ($F_{7,9}$ = 124.71; $p < 0.0001$) in leaf consumption ratings (PDRI) among treatments (Figure 6). The highest mean PDRI occurred in the water control (4.9), followed in decreasing order by the BotaniGard (3.3), PFR-97 (2.7), Met52 (2.5), Sevin (1.6), AzaMax (1.4), Entrust (1.0), and PyGanic (1.0) treatments. Of the fungal biopesticides, weevils exposed to BotaniGard-treated leaves consumed significantly more than those with PFR-97 or Met52. Weevils on leaves treated with Entrust, PyGanic, AzaMax, and Sevin consumed significantly less than weevils in other treatments.

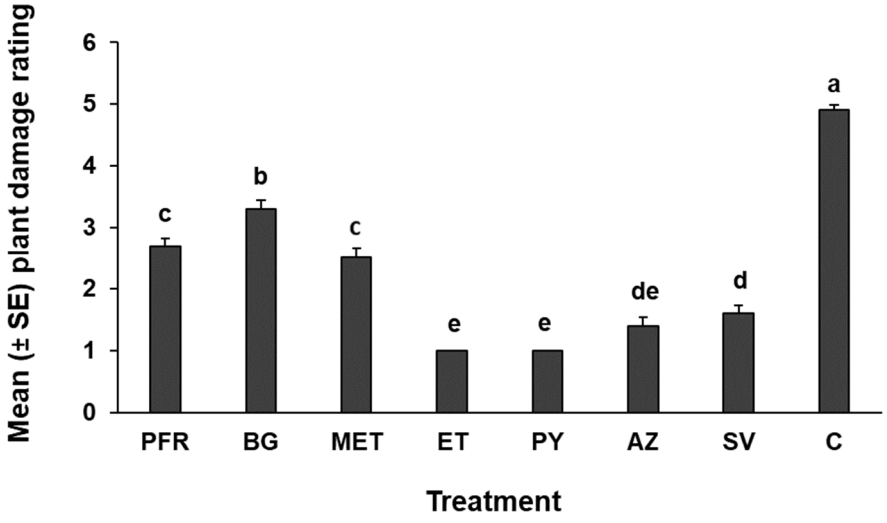

Figure 6. Plant damage rating index of treated cocoplum leaves fed on by adult *Myllocerus undecimpustulatus undatus* at 15 days after treatment application. Treatments included PFR-97™ 20% WDG (PFR), BotaniGard® ES (BG), Met52®EC (MET), Entrust® SC (ET), PyGanic® EC 1.4II (PY), Aza-Max™ (AZ), Sevin® SL (SV), and water only (C). Data are for five combined trials. Treatments not followed by the same letter above the bars are significantly different (Tukey–Kramer HSD test, $p < 0.0001$). SE = 0 for ET and PY.

4. Discussion

Of the seven pesticides tested, Entrust consistently performed better in killing *M. undecimpustulatus undatus* adults (83% mortality) and reducing plant damage (1.0 PDRI).

The mode of action of spinosad, the active ingredient in Entrust, is by contact and ingestion [16]. Balusu and Fadamiro [17] obtained similar results with Entrust applied against the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae); Entrust consistently achieved high efficacy among botanical and microbial insecticides tested compared with the control. This pesticide also reduced plant damage (1 = <10% PDRI) by the yellowmargined leaf beetle.

Our study is the first investigation of a spinosad product being tested against *M. undecimpustulatus undatus.* Entrust is labeled for leaf beetles (Chrysomelidae), but not for weevils (Curculionidae). The rate used in our bioassays, 27 g a.i./ha, was one-fifth the highest recommended rate of 140 g a.i./ha. The rate we used was chosen to determine the efficacy of a lower recommended rate. McLeod and Rashid [18] reported 100% mortality of eggplant flea beetles, *Epitrix fuscula* Crotch (Coleoptera: Chrysomelidae), at the highest recommended rate of Entrust; reduced feeding started the first day. Bažok et al. [19], using 72 g a.i./ha, compared contact and ingestion activity of spinosad against adult sugar beet weevils, *Bothynoderes punctiventris* Germar (Coleoptera: Curculionidae). Five days after treatment, the mortality rate was almost 2.5 times higher via ingestion (95%) than via contact (39%).

Our experiments are also the first investigation of entomopathogenic fungi infecting adult *M. undecimpustulatus undatus.* Of the three entomopathogenic fungi tested, BotaniGard consistently resulted in infection and mycosis. BotaniGard was over two times more effective than PFR-97 and Met52 in killing the weevils. Observation of fungal outgrowth on insect cadavers has been used to verify death by fungal infection [20]. In our study, mycosis by *B. bassiana* was observed prior to and after surface sterilization. The effectiveness of *B. bassiana* against other pest beetles has also been studied. For example, the red palm weevil, *Rhychophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), showed signs of infection 3–5 days after inoculation [21]. A cohort of the pine weevil, *Hylobius abietis* (Linnaeus) (Coleoptera: Curculionidae), had 100% mortality in the laboratory 6–7 days after exposure to *B. bassiana* [22]. For the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), 79% mortality was observed 9 days after laboratory exposure to *B. bassiana* [23]. Lastly, a laboratory population of the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Brentidae), had 93% mortality 7–8 days after exposure to *B. bassiana* [24].

The poor efficacy of PFR-97 and Met52 could be related to various factors. A beetle's first line of defense against entomopathogens is the strongly sclerotized, hydrophobic exoskeleton that is difficult to penetrate [25]. BotaniGard and Met52 formulations contain hydrophobic conidia suspended in oil with emulsifiers, whereas PFR-97 contains dry, desiccation-tolerant hydrophilic blastospores with no emulsifiers but suspends well when added to water [11,26]. Dunlap et al. [26] observed that *C. javanica* (reported as *Paecilomyces fumosoroseus*) hydrophilic blastospores will not bind strongly with hydrophobic surfaces, such as insect exoskeleton, and thereby may be removed more easily in comparison with conidia. Additionally, some studies have indicated that conidia suspended in oil have superior infectivity in comparison to a pure aqueous application [27,28].

Another possibility for the low efficacy of PFR-97 and Met52 is the plant cuticle. Cocoplum is a drought-, salt-, and wind-tolerant plant species [29] usually identified as having a thick waxy cuticular layer [30]. The plant cuticle is a hydrophobic layer composed of cutin and waxes serving as a primary barrier to water loss and adverse interactions with the environment [31]. In general, plant surface chemistry, e.g., plant exudates affecting conidia directly, herbivore-induced plant volatiles affecting sporulation or germination, and plant cuticle altering spore persistence, are possibilities for the reduction in infectivity of both entomopathogens [32]. Silva et al. [33] noted that the leaf extract of cocoplum has antifungal activity against oral clinical isolates of *Candida* species. Therefore, further research is warranted to determine if the cocoplum leaf has a similar antifungal effect when exposed to entomopathogenic fungi.

Genomic data revealed that even closely related fungal pathogens, e.g., *B. bassiana* and *M. anisopliae*, have different molecular mechanisms that mediate virulence [34]. *Beauveria bassiana* has many more bacteria-like toxins and Cry-like delta endotoxins than *M. anisopliae* and other fungi, suggesting the possibility of greater oral toxicity than other entomopathogenic fungi [34]. There is evidence that *B. bassiana* may infect insects *per os*, especially in insects with chewing mouthparts such as beetles [35]. Recent studies investigated *B. bassiana* as a fungal endophyte in plant defense against the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) [36], and the banana weevil, *Cosmopolities sordidus* (Germar) (Coleoptera: Curculionidae) [37]. Further investigation is needed to determine if the higher incidence of infection by *B. bassiana* in *M. undecimpustulatus undatus* is due to entry *per os*.

PyGanic and AzaMax, both producing less than 50% mortality, were less effective than Entrust and BotaniGard. Although both former products are contact insecticides, AzaMax also gains entry *per os* [38]. The repellency effect by PyGanic and AzaMax might explain the lower feeding damage ratings of 1.0 and 1.4 in these treatments, respectively. Other reports recognized the reduced effectiveness of azadirachtin-based insecticides with adult insects, e.g., the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) [39]; the rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae); and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) [40]. PyGanic was recognized as ineffective against adult apple flea weevils, *Orchestes pallicornis* (Say) (Coleoptera: Curculionidae) [41], and adult alfalfa weevils, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) [42].

5. Conclusions

High mortality was caused by Entrust and BotaniGard, suggesting that *M. undecimpustulatus undatus* adults might potentially be well-managed in plant protection strategies by using these two commercially available biopesticides in combination. However, it is important to point out that the effectiveness of the products was tested under controlled conditions in the laboratory. Therefore, additional testing should be carried out under field conditions, where environmental conditions will vary and the biopesticide products may differ in their efficacies compared with those obtained under optimum laboratory conditions. Future studies will focus on the use of these two biopesticides for management of *M. undecimpustulatus undatus* adults on fruit crops such as peaches grown in Florida.

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