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SUSCEPTIBILITY OF *MICROTHECA OCHROLOMA* (COLEOPTERA: CHRYSOMELIDAE) TO BOTANICAL AND MICROBIAL INSECTICIDE FORMULATIONS

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ABSTRACT

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), is a key pest of organic crucifer production in the southern United States. The susceptibility of larvae and adults of *M. ochroloma* to some botanical and microbial insecticide formulations was evaluated using laboratory leaf-dip bioassays. Insecticides evaluated included OMRI (Organic Material Review Institute) approved formulations of PyGanic®(pyrethrum), Entrust® (spinosad), Mycotrol O® (*Beauveria bassiana* strain GHA), and NOFLY® (*Isaria fumosoroseus* strain FE 9901). Others were MBI-203 (experimental organic formulation of *Chromobacterium subtsugae*) and BotaniGard® 22WP (conventional formulation of *Beauveria bassiana* strain GHA). The insecticides were first evaluated at the field recommended rate against *M. ochroloma* larvae and adults, followed by multiple-concentration assays to determine the LC_{50} and LT_{50} for promising formulations. At the field recommended rates, all tested formulations were toxic to the larvae compared to the untreated control, whereas only Entrust® and PyGanic® were effective against the adults. Entrust® and PyGanic® caused 100% mortality to the larvae and adults after just 24 h of exposure. The LC_{50} values of Entrust® and PyGanic® were 200 X and 15 X less than the field recommended rates, respectively. MBI-203 was effective against the larvae (100% mortality after 5 days) but not against the adults. The entomopathogenic fungal formulations, Mycotrol®, NOFLY®, and BotaniGard®, were much less toxic with LT_{50} values of 10, 12, and 9 days, respectively. Although all 3 fungal formulations caused significantly higher larval mortality than the untreated control after 5 days of exposure, none resulted in more than 50% larval or 14% adult mortalities over the 9-day exposure period.

Key Words: Yellowmargined leaf beetle, botanicals, biopesticides, LC_{50}, pyrethrum, spinosad

RESUMEN

El escarabajo de las hojas de margen amarillo, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), es una plaga clave de la producción de crucíferas orgánicas en el sur de los Estados Unidos. Se evaluó la susceptibilidad de las larvas y adultos de *M. ochroloma* a algunas formulaciones de insecticidas botánicos y microbianos mediante bioensayos de hojas sumergidas en el laboratorio. Los insecticidas evaluados incluyeron formulaciones aprobadas por el OMRI (Instituto de Revision de Materia Organica) como PyGanic ® (piretro), Entrust ® (spinosad), Mycotrol O® (*Beauveria bassiana* cepa GHA) y NOFLY ® (*Isaria fumosoroseus* cepa FE 9901). Los otras formulaciones fueron MBI-203 (formulación orgánica experimental de *Chromobacterium subtsugae*) y BotaniGard ® 22WP (formulación convencional de *Beauveria bassiana* cepa GHA). Los insecticidas fueron evaluados con dosis de campo recomendadas contra larvas y adultos de *M. ochroloma*, seguido por los ensayos de concentración múltiple para determinar la CL_{50} y LT_{50} de las formulaciones prometedoras. En las proporciones recomendadas de campo, todas las formulaciones probadas fueron tóxicas para las larvas en comparación con el control sin tratar, mientras que sólo Entrust® y PyGanic® fueron eficaces contra los adultos. Entrust® y PyGanic®causó una mortalidad del 100% de las larvas y los adultos después de sólo 24 horas de exposición. Los valores de LC_{50} de Entrust® y PyGanic® fueron 200x y 15x menores que las proporciones recomendadas de campo, respectivamente. La MBI-203 fue eficaz contra las larvas (100% de mortalidad después de 5 días), pero no contra los adultos. Las formulaciones de hongos entomopatógenos, Mycotrol®, NOFLY® y BotaniGard®, fueron menos tóxicos, con LT_{50} valores de 10, 12 y 9 días, respectivamente. Aunque todas las 3 formulaciones de hongos causaron una mortalidad de larvas significativamente más alta que el control sin tratar después de 5 días de exposición, ninguna resultó en una mortalidad de mas del 50% de larvas o 14% de adultos durante el período de exposición de 9 días.

Palabras clave: El escarabajo de las hojas del margen amarillo, botánicos, bioplaguicidas, ensayo de hojas sumergidas, LC_{50} , piretro, spinosad, *Isaria fumosoroseus*

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), is a major pest of cruciferous vegetables (Brassicales: Brassicaeae) in the southern United States (USA) (Chamberlin & Tippins 1948; Ameen & Story 1997a). This beetle, which was accidentally introduced into the USA from South America in the 1940s (Chamberlin & Tippins 1948), is now widely distributed in the southern USA with major field infestations reported in Alabama, Florida, Louisiana, Mississippi, South Carolina, North Carolina, and Texas (Ameen & Story 1997b). Both larvae and adults of *M. ochroloma* feed in groups on foliage and may cause complete defoliation of crucifers. Although *M. ochroloma* is often not a major problem in conventional crucifer production due to its susceptibility to synthetic foliar insecticides (Bowers 2003), the beetle poses a major threat to organic vegetable production since organic farmers cannot use synthetic insecticides. Organic production of crucifers is presently an emerging industry in Alabama and other parts of the southern USA, and *M. ochroloma* is often the predominant pest and a major factor limiting the growth and expansion of the industry (Balusu & Fadamiro 2012).

Several years of field studies by our group and others have identified only a few effective OMRI (Organic Material Review Institute) approved formulations, specifically Entrust® (spinosad), against *M. ochroloma* (Overall 2008; Balusu & Fadamiro 2012). PyGanic®(pyrethrum) was moderately effective, while other tested insecticides, including some entomopathogenic fungal formulations showed very little or no efficacy against larvae or adults (Balusu & Fadamiro 2012). Therefore, spinosad (Entrust®) and pyrethrum (PyGanic®) are currently recommended for management of this pest in organic crucifer vegetable production in Alabama. However, repeated application of these insecticides could lead to development of insecticide resistance. Identification of additional effective products could provide growers more control options and help to reduce the risk of insecticide resistance. Also, knowledge of baseline susceptibility response of *M. ochroloma* to spinosad (Entrust®) and pyrethrum (PyGanic®) is essential for keeping track of changes in toxicological response of the pest over time. Baseline susceptibility data can be used as reference values to diagnose shifts in susceptibility or to monitor insecticide resistance (Prabhaker et al. 2006).

Many of the microbial insecticides, including entomopathogenic fungal formulations, which showed poor field efficacy against *M. ochroloma* in our field study (Balusu & Fadamiro, 2012), have been reported as effective against other Chrysomelidae (Poprawski et al. 1997; Butt et al. 1992). Thus, an understanding of the factors responsible for the limited field efficacy of entomopathogenic fungal formulations against *M. ochroloma* is important to allow us to overcome constraints and thereby achieve efficacious control of *M. ochroloma*. Poor field performance of entomopathogenic fungal formulations is typically related to suboptimal ambient conditions such as relative humidity, temperature, and UV-radiation (Inglis et al. 1996). However, studying these factors under field conditions is extremely complex. Evaluation of the microbial formulations against *M. ochroloma* under controlled laboratory conditions will likely provide an insight into the factors responsible for their poor field efficacy.

Thus, the present study was conducted to evaluate the susceptibility of *M. ochroloma* larvae and adults to various botanical and microbial formulations under laboratory conditions. Many of the insecticides tested in the current paper are known to be effective against other Coleopteran insect pests (Andersen et al. 2006; Igrc Barcic et al. 2006; Isman 2006) and thus are expected to show activity against *M. ochroloma.*

Ultimately, it is hoped that the results of this laboratory study will help to understand the basis for the poor performance of the microbial insecticides tested in our field trials (Balusu & Fadamiro, 2012), and allow identification of additional effective organically acceptable insecticides which can be applied as stand-alone treatments or in rotation with Entrust® for effective management of *M. ochroloma* in organic crucifer production. Furthermore, knowledge of baseline susceptibility of *M. ochroloma* to various insecticides can be used to determine changes in susceptibility over time and the onset of resistance development.

Materials and Methods

Plants

Turnip (Brassica rapa L.var. rapa cv 'purple top white globe') seedlings were raised from seeds purchased from Johnny's Selected Seeds (Winslow, Maine) in 60- well seed trays at one seed per well under controlled greenhouse conditions (26 ± 2 °C and 55 ± 5% RH). Seedlings (3 wk old) were transplanted into 0.5 - L pots in Sunshine potting mixture #8 consisting of 70-80% Canadian sphagnum grower grade peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone for pH adjustment, gypsum, and wetting agent (SunGro Horticulture, Washington). Plants were irrigated daily and fertigated twice a wk with Scotts® peat lite special fertilizer (Scotts-Sierra Horticultural Product Company, Marysville, Ohio), a 20-10-20 water soluble NPK fertilizer mixture with micronutrients. Plants were grown using organic practices and no pesticides were applied. Plants used for the bioassays and insect rearing were about 4-5 wk post transplanting.

Insects

Adults of *M. ochroloma* collected from a commercial organic farm in central Alabama in October 2006 were used to start laboratory colonies, which were supplemented annually with field-collected adults. *Microtheca ochroloma* was reared on foliage of turnip plants (grown in the greenhouse as described above) in clear plastic Petri dishes (150 mm diam × 30 mm height) lined with paper towels (Bounty®, Procter & Gamble, Cincinnati, Ohio). The colony was maintained at 25 ± 2 °C, 50 ± 10% RH, and a photoperiod of 14: 10 h L: D.

Treatments

The materials evaluated (Table 1) included OMRI (Organic Material Review Institute) approved formulations such as PyGanic® (pyrethrum), Entrust® (spinosad), Mycotrol O® (*Beauveria bassiana* strain GHA), and NOFLY® (*Isaria fumosoroseus* strain FE 9901). Others were MBI-203 (experimental organic formulation of *Chromobacterium subtsugae*) and BotaniGard® 22WP (conventional formulation of *Beauveria bassiana* strain GHA). Entrust® WP (spinosad), the most effective treatment in our field trials, was evaluated as positive control, whereas BotaniGard® 22WP was evaluated as a positive control for organic standard.

Bioassay Method

Toxicity of the insecticides (Table 1) against *M. ochroloma* larvae and adults was determined in 2 experiments. Single (field recommended rate) concentration screening assays were first carried out, and the most promising treatments were further evaluated in multi-concentration assays. Solutions of each insecticide were made in distilled water (= control) and no adjuvants were used. All bioassays were performed using the leaf-dip method at ambient conditions, 26 °C, 14:10 h L: D, and 50% RH. Briefly, intact leaves on a turnip plant were

TABLE 1. INSECTICIDES TESTED AGAINST *MICROTHECA OCHROLOMA*.

Registered name	Active ingredient	Type	Company	Field recommended rate
PyGanic®1.4 EC	Pyrethrum	OMRI approved	McLaughlin Gormley King Company, Minneapolis, MN	2 qt/ac (4.68 L/ha)
Entrust® WP	Spinosad	OMRI approved	Dow AgroSciences LLC, , Indianapolis, IN	2 oz/ac (140 g/ha)
Mycotrol O® ES	*Beauveria bassiana* strain GHA	OMRI approved	Laverlam International Corporation, Butte, MT	1 qt/ac (2.34 l/ha)
NOFLY® WP	*Isaria fumosoroseus* strain FE 9901	OMRI approved	Natural Industries, Inc. Houston, Houston, TX	2 lb/ac (2.25 kg/ha)
MBI-203 SC	*Chromobacterium subtsugae*	Experimental	Marrone Bio Innovations, Davis, CA	2 qt/ac (4.68 L/ha)
BotaniGard® 22WP	*Beauveria bassiana* strain GHA	Conventional	Laverlam International Corporation, Butte, MT	2 lb/ac (2.25 kg/ha)

directly immersed in each insecticide solution for 5 s. The leaves were then removed and air dried for ~ 4 h to remove excess water on the leaf surface. A leaf disc (~90 mm) cut out of an excised treated leaf was placed in a 100 mm diam Petri dish lined with moist filter paper. To maintain higher humidity levels than the ambient RH, the Petri dishes were covered with damp paper towel and placed inside a large plastic container (25 × 45 cm). Mortality of insects was recorded daily for 14 days (or until pupation of larvae). Insects that failed to move when probed with a dissecting needle were recorded as dead and removed from the Petri dish. For the entomopathogenic fungal formulations, dead insects were removed and incubated separately in Petri dishes lined with damp filter paper. The Petri dishes were placed in a humidifier and inspected for the presence of mycelia on cadavers (mycosis) starting on day 7.

Toxicity at the Field Recommended Rates

In the first experiment, all insecticide formulations were evaluated at field recommended rates (i.e., single concentration assays) for efficacy against *M. ochroloma* larvae and adults. Test solutions of field recommended rates of the insecticides (Table 1) were prepared with distilled water. A group of 20 1st instar larvae (larvae that emerged within 24 h) from the same batch was placed in a Petri dish containing a treated leaf disc. Similarly, a group of 20 adults (4-5 days old) from the same batch was placed in a Petri dish containing a treated leaf disc by using a fine camel's hair brush (#00). The experiment was replicated 5 times per insecticide over time and insect mortality was determined as described above.

Multi-Concentration Assays

Promising treatments in the first experiments were selected for further evaluation in multi-concentration assays to determine the LC_{50} (lethal concentration that kills 50% of test insects or median lethal concentration) and LT_{50} (lethal time to kill 50% of test insects). Based on the results of the first experiment, all 6 insecticides were evaluated against the larvae, but only 2 (Entrust® and PyGanic®) were evaluated against the adults. Each insecticide was tested at 5 concentrations plus distilled water control for a total of 6 rates. The concentration range for each insecticide was determined based on the results of preliminary bioassays, which gave a mortality range of 10 to 90%. For each concentration, a group of 20 adults (4-5 days old) or 20 larvae (1 day old) from the same batch were placed in a Petri dish containing a treated leaf disc. The experiment was replicated 5 times per concentration over time and insect mortality was determined as described above.

Data Analysis

Mortality data did not meet the assumptions of independence. Thus, the data were analyzed using the Kruskal-Wallis non-parametric test ($P < 0.05$; JMP® 7.0.1, SAS Institute 2007). The data were further analyzed using the ordinary F-test after which the results were compared with the non-parametric test. When both procedures gave similar results, the ANOVA assumptions were assumed to be satisfied (http://faculty.evansville.edu/ch81/bio415f02/BIO415Topic7.pdf). Means were then separated using the Tukey Kramer honestly significant difference (HSD) test. The LC_{50} values expressed in ppm, LT_{50} values (in days), 95% fiducial limits (FL), and regression slopes were estimated by probit analysis (Finney 1971) using POLO PLUS software for Windows (LeOra software 2007). Tests of parallelism of probit regression lines for all treatments were conducted using chi-square goodness-of-fit tests (POLO PLUS, LeOra software 2007).

Results

Toxicity at Field Recommended Rates

There was a significant effect of insecticide treatment at the field recommended rate on mortality of *M. ochroloma* larvae ($F = 1251.2$, df = 6, 28, $P < 0.0001$) as early as 24 h of exposure to treated leaf discs (Fig. 1). Entrust® and PyGanic® resulted in 100% larval mortality after 24 h. MBI-203 also caused significantly greater mortality than the untreated control starting at 24 h; however 100% mortality was not attained until day 5. In contrast, no significant differences were recorded between the entomopathogenic fungal formulations (i.e. Botanigard®, Mycotrol O®, and

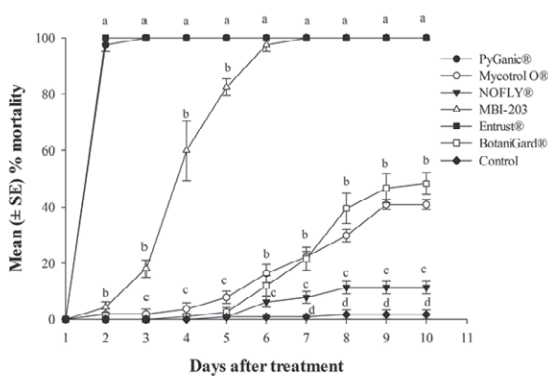

Fig. 1. Percent mortality of *Microtheca ochroloma* larvae exposed to field recommended rates of various insecticide formulations in leaf-dip bioassays. Means within a date having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, $P < 0.05$).

NOFLY®) and the untreated control on days 1-4. On day 5 the fungal formulations caused significantly higher mortality than the untreated control (F = 492.9, df = 6, 28, P < 0.0001). However no fungal formulation resulted in more than 50% larval mortality throughout the exposure period (Fig. 1). The average survival time for larvae treated with Entrust®, PyGanic®, MBI-203, Botanigard® Mycotrol O®, and NOFLY® at the field recommended rate was 1, 1, 5, 8, 8, and 7 days after treatment, respectively (Fig. 1).

For adults, Entrust® and PyGanic® were the most effective treatments with 100% mortality after 24 h, which was significantly greater than the other treatments or the untreated control (F = 1974, df = 6, 28, P < 0.0001) (Fig. 2). No significant differences were recorded between the other treatments and the untreated control on days 1-7. On day 8, Botanigard® (14% mortality) and Mycotrol O® (12% mortality) were significantly better than the untreated control (F = 979.1, df = 6, 28, P < 0.0001), whereas mortalities caused by MBI-203 (2%) and NOFLY® (2%) were not significantly different from the untreated control (Fig. 2). The average survival time for adults treated with Entrust®, PyGanic®, Botanigard®, and Mycotrol O® at the field recommended rate was 1, 1, 9, and 9 d, respectively (Fig. 2).

In general, the fungal formulations were slow-acting and relatively less efficacious against *M. ochroloma*. None resulted in more than 50% larval or 14% adult mortality over the 9-day exposure period. Comparing the 2 life stages, the larvae were more susceptible to the insecticides than adults.

Multi-Concentration Assays

The LC_{50} and LT_{50} values, 95% fiducial limits, slope, and chi-square values for the insecticides tested against larvae are presented in Table 2. All

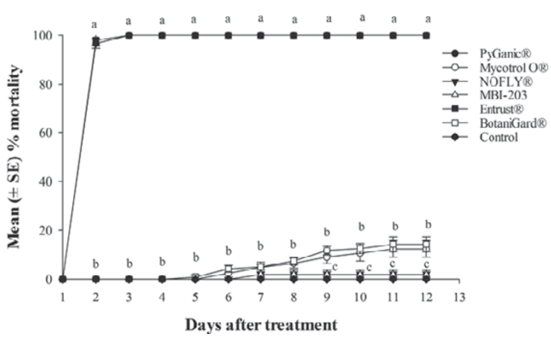

Fig. 2. Percent mortality of *Microtheca ochroloma* adults exposed to field recommended rates of various insecticide formulations in leaf-dip bioassays. Means within a date having no letter in common are significantly different (ANOVA, Tukey-Kramer HSD, P < 0.05).

chi-square values were not significant (α = 0.05) in Pearson's goodness-of-fit test on the probit model, indicating a good fit of regression line. Entrust® and PyGanic® had the lowest LC_{50} values against the larvae (Table 2), indicating higher toxicity. However, Entrust® (LC_{50} = 0.1 ppm) was ≈ 100 times more toxic to the larvae than PyGanic® (LC_{50} = 10.9 ppm). All other treatments (Mycotrol O®, NOFLY®, MBI-203, and BotaniGard®) were not significantly different after 24 h of exposure. Significant concentration-mortality responses of the larvae were observed for all insecticides tested, as indicated by the positive slope values (Table 2). MBI-203 had the highest slope (6.21 ± 0.66), followed by PyGanic® (2.86 ± 0.23), Mycotrol O® (1.99 ± 0.25), BotaniGard® (1.96 ± 0.27), NOFLY® (1.28 ± 0.29), and Entrust® (1.15 ± 0.08); higher slopes indicate greater concentration-mortality response. The second measure of efficacy was the LT_{50} values that were calculated for the field recommended rate (Table 3). LT_{50} values were not estimated for Entrust® and PyGanic® since both treatments caused complete mortality of larvae within 24 h at the field recommended rate. Among the remaining treatments, MBI-203 had significantly lower LT_{50} (2 days) than BotaniGard® (9 days), Mycotrol O® (10 days), or NOFLY® (12 days) (Table 3).

Since Entrust® and PyGanic® were the only effective treatments against the adults at the field recommended rate (experiment 1), LC_{50} values were estimated only for these two formulations against the adults (Table 4). Entrust® (LC_{50} = 2.4 ppm) was ≈ 10 times more toxic to the adults than PyGanic® (LC_{50} = 24.1 ppm). LT_{50} values were not estimated since both insecticides caused complete mortality within 24 h of exposure.

Discussion

The results of this laboratory study demonstrated varying levels of susceptibility of *M. ochroloma* to the tested insecticides. Among the formulations, Entrust® was the most effective causing 100% larval and adult mortality within 24 h, as well as having the lowest LC_{50} values and survival time. PyGanic®, the second best treatment, also caused complete mortality of both life stages within 24 h, but was ≈ 100 – fold and 10 – fold less toxic than Entrust® to the larvae and adults, respectively. The results further showed that the LC_{50} values of Entrust® and PyGanic® were only a fraction, 200 X and 15 X less than the actual field recommended rate, respectively. MBI-203 (experimental organic formulation of *Chromobacterium subtsugae*) was effective against the larvae but not against the adults. At the field recommended rate, the entomopathogenic fungal formulations (Mycotrol®, NOFLY®, and BotaniGard®) were comparatively less efficacious against the larvae and showed no efficacy against the adults.

TABLE 2. CONCENTRATION-MORTALITY RESPONSE OF *MICROTHECA OCHROLOMA* LARVAE EXPOSED TO VARIOUS INSECTICIDE FORMULATIONS IN LEAF-DIP ASSAYS.

Treatment	No. insects	Slope ± SE	LC_{50} (ppm of a.i)	95% Fiducial limits (ppm) Lower - Upper	χ^2
PyGanic®	121	2.86 ± 0.23	10.9 b	8.48 - 14.03	8.85
Mycotrol O®	118	1.99 ± 0.25	6.67×10^2 a	534.9 - 922.2	2.46
NOFLY®	114	1.28 ± 0.29	4.08×10^3 a	2018.5 - 25038	0.78
MBI-203	118	6.21 ± 0.66	5.21×10^3 a	4064.2 - 6417.2	8.55
Entrust®	118	1.15 ± 0.08	0.1 c	0.061 - 0.18	10.81
BotaniGard®	117	1.96 ± 0.27	1.3×10^3 a	829.4 - 5938.6	6.1

The efficacy of Entrust® or its active ingredient (spinosad) has also been documented against other beetles in the same family (Chrysomelidae) as *M. ochroloma*, including flea beetles, *Phyllotreta* spp., in crucifer crops (Andersen et al. 2006), *Epitrix tuberis* Gentner in potato (Chu et al. 2006), and Colorado potato beetle, *Leptinotarsa decemlineata* (Say) in potato (Igrc Barcic et al. 2006). Entrust® was also effective against lepidopteran pests of crucifer crops in Alabama (Maxwell & Fadamiro 2006). The efficacy of Entrust® recorded in the present study may be attributed to its broad spectrum activity (Cisneros et al. 2002), multiple modes of entry (Eger & Lindenberry 1998, Liu et al. 1999). The active ingredient in Entrust® is both a contact and stomach poison (Eger & Lindenberry 1998; Liu et al. 1999). The efficacy of PyGanic®, a botanical insecticide with pyrethrum as the active ingredient, against *M. ochroloma* was not surprising, since pyrethrum is known for its rapid knockdown effect on insects. PyGanic® has also been reported as effective against other insect pests including Colorado potato beetle (Igrc Barcic 2006), and harlequin bug, *Murgantia histrionica* (Hahn) (Overall 2008). In general, the results of this laboratory study are in agreement with our field data which identified Entrust® as the only effective treatment and PyGanic® as moderately effective (Balusu & Fadamiro 2012). The high activity of the experimental formulation MBI-203 against *M. ochroloma* larvae is very encouraging and suggests that (if registered) this insecticide may play a role in the management of *M. ochroloma*. *Chromobacterium subtsugae*, the active ingredient in MBI-203, has been reported to be toxic to several insects including larvae of Colorado potato beetle (Martin et al. 2004), as well as adults of southern corn rootworms, *Diabrotica undecimpunctata howardi* Barber and *Diabrotica virgifera virgifera* LeConte, and southern green stink bug, *Nezara viridula* (L.) (Martin et al. 2007).

The entomopathogenic fungal formulations were only slightly effective against *M. ochroloma* larvae and did not work against the adults. Although some studies have reported the efficacy of entomopathogenic fungal formulations against Colorado potato beetle (Poprawski et al. 1997) and other chrysomelid beetle species (Butt et al. 1992), our laboratory results, which also agree with our field data (Balusu & Fadamiro 2012), suggest that these fungal formulations are relatively non-toxic to *M. ochroloma*. The poor efficacy of the fungal formulations against *M. ochroloma* in this laboratory study is unlikely due to suboptimal environmental conditions, but may be attributed to some plant or insect-related factors as follows: First, low pathogenicity of fungal formulations may be due to potential negative interactions of surface chemicals of crucifer plants. Crucifer crops contain specialized secondary plant metabolites called glucosinolates that are transformed into isothiocyanates during insect damage. Isothiocyanates are known for their fungistatic properties. In fact, many studies have reported the inhibitory activity of isothiocyanates, specifically phenylethyl-, 2-chlorophenyl-,

TABLE 3. PROBIT ANALYSIS OF TIME-MORTALITY RESPONSE OF BIOASSAY WITH TEST FORMULATION AGAINST *MICROTHECA OCHROLOMA* LARVAE.

Treatment	No. insects	Slope ± Se	LT_{50} (days)	95% Fiducial limits (days) Lower - Upper	χ^2
Mycotrol O®	108	2.54 ± 0.25	10.44 a	7.82 - 21.52	34.88
NOFLY®	114	4.77 ± 0.68	12.26 a	10.77 - 15.27	3.09
MBI-203	112	5.34 ± 0.31	2.21 b	1.94 - 2.58	23.9
BotaniGard®	111	5.88 ± 0.58	9.03 a	8.52 - 9.76	0.67

TABLE 4. CONCENTRATION-MORTALITY RESPONSE OF *MICROTHECA OCHROLOMA* ADULTS EXPOSED TO VARIOUS INSECTICIDE FORMULATIONS IN LEAF-DIP ASSAYS

Treatment	No. insects	Slope ± SE	LC_{50} (ppm of a.i)	95% Fiducial limits (ppm) Lower - Upper	χ^2
PyGanic®	117	3.24 ± 0.27	24.14 a	19.71 - 31.22	8.85
Entrust®	118	1.15 ± 0.08	2.36 b	1.30 - 4.29	10.81

and allyl-isothiocyanates, on entomopathogenic fungal conidia germination and its ability to infect the insects (Inyang et al. 1999; Klingen et al. 2002). Second, the physio-chemical properties of host insect cuticle could also account for its lower susceptibility to fungal pathogens. *Microtheca ochroloma* may sequester plant-derived fungistatic compounds (isothiocyanates) from turnip plant as defense against potential microbial pathogens. The presence of fungistatic compounds in the insect cuticle is well demonstrated (Smith & Grula 1982; Butt et al. 1995). Butt (1990) showed that insect cuticle influences all stages of the infection process: adhesion, germination, and appressorium differentiation. Lastly, insect nutrition is another factor that plays an important role in the success of entomopathogens (Vago 1963). Inadequate nutrition often results in increased susceptibility to pathogens. Balusu & Fadamiro (2011) demonstrated that turnip is a preferred host of *M. ochroloma*. Therefore, it is possible that *M. ochroloma* raised on turnip leaves are more vigorous and less susceptible to fungal infection. Despite their poor efficacy in reducing adult mortality, we observed in this study some sub-lethal effects of some microbial formulations (i.e., MBI-203, Mycotrol O®, and BotaniGard® 22WP) on *M. ochroloma* in terms of reduced feeding and oviposition. If confirmed, these sub-lethal effects can be as valuable as direct insect mortality (Liu & Bauer 2008) in decreasing the pest status of *M. ochroloma*, and are worthy of further investigation.

The results showed that *M. ochroloma* larvae were more susceptible than the adults to the tested insecticides. For instance, the LC_{50} value of Entrust® against the larvae (0.1 ppm) was 20X lower than that of the adults (2.36 ppm). This difference in susceptibility may be attributed to the differential feeding rate of both life stages; larvae are more voracious feeders than adults (unpublished data). Another possible explanation for the differential susceptibility observed between the 2 life stages may be a difference in the ability of the insecticides to penetrate through the cuticle or a difference in the composition of the cuticle. Christie & Wright (1990) attributed marked differences in relative toxicity of the insecticide abamectin between larval instars of *Spodoptera littoralis* (Boisduval) to differences in the insecticide's penetration rate.

In summary, this study has identified some promising OMRI – acceptable biopesticides and effective rates against *M. ochroloma* larvae and adults. Entrust® and PyGanic® were the most effective insecticides followed by MBI-203. The data also showed that the actual lethal concentrations of Entrust® and PyGanic® were only fractions of the field recommended rates. Additional studies are necessary to further evaluate the field activity of the promising treatments identified in this study, in particular in rotation with Entrust® for effective management of *M. ochroloma* in organic crucifer production.

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