

Compatibility of Zineb, Dimethoate and *Beauveria bassiana* (Balsamo) Vuillemin Against Tarnished Plant Bug (Hemiptera: Miridae)¹

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Abstract Experiments were conducted under laboratory and field conditions to assess the effect of the fungicide Zineb (zinc dimethyl-dithiocarbamate) and the insecticide Cygon (dimethoate) on the pathogenicity of the isolate MK 2001 of *Beauveria bassiana* (Balsamo) Vuillemin, a potential microbial agent for biological control of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois). The isolate was highly pathogenic to adults yielding an LC_{50} of 7.2×10^3 conidia/mL at 6 d post treatment and an LT_{50} of 3.07 d at a concentration of 1×10^8 conidia/mL. Zineb inhibited the *in vitro* growth of the fungus in Sabouraud's dextrose broth and on Sabouraud's dextrose agar. It had no insecticidal activity against adult *L. lineolaris* in laboratory bioassays. A field test with caged *L. lineolaris* adults on lettuce *Lactuca sativa* L. var. *longifolia*, at a phenological stage of 50 leaves further demonstrated that Zineb and Dimethoate had no effect on *B. bassiana* against *L. lineolaris*. Adult mortality 10 d after application did not differ significantly in treatments with *B. bassiana* alone from treatments with *B. bassiana* + Zineb, dimethoate alone, *B. bassiana* + dimethoate, *B. bassiana* + Zineb, and *B. bassiana* + Zineb + dimethoate. No larva was observed in cages treated with *B. bassiana* alone or in combination with Zineb or dimethoate. On the contrary, 11 larvae/plant were recorded in the untreated cages and 5 larvae/plant in the dimethoate-treated cages.

Key Words Entomopathogenic fungus, *Beauveria bassiana*, *Lygus lineolaris*, Zineb, zinc dimethyldithiocarbamate, Cygon, dimethoate, biological control

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a polyphagous insect with over 300 recorded hosts, 130 of which are economically-important crops (Young 1986, Taksdal 1961, Scott 1987, Snodgrass et al. 1984). Adults and larvae damage plants and reduce crop yield by directly feeding on foliage and fruit causing fruit abscission, and by transmission of plant pathogens (Strong 1970). *Lygus lineolaris* populations have developed resistance to insecticides in several locations in North America (Steinkraus et al. 1997, Ronald et al. 1994).

Natural enemies such as hymenopteran and dipteran parasitoids (Day et al. 1990, Day 1995, Hedlund and Graham 1987), polyphagous predators (Bostanian and Mailoux 1994), and nematodes (Painter 1929) had been catalogued from *L. lineolaris*, but none appear to have potential as remedial biocontrol agents. The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin reportedly infects *L. lineolaris* (Snodgrass and Elzen 1994, Steinkraus and Tugwell 1997) and is an excellent can-

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didate for development as microbial insecticide (McCoy 1990, Faria and Wraight 2001).

Chemical pesticides used in crop protection may have deleterious effects on entomopathogenic fungi, including *B. bassiana* (Olmert and Kenneth 1974, Roberts and Campbell 1977, Gardner and Storey 1985, Vänninen and Hokkanen 1988, Majchrowicz and Poprawski 1993, Poprawski and Majchrowicz 1995). It appears that these impacts are by direct toxicity (Vänninen and Hokkanen 1988, Majchrowicz and Poprawski 1993) and by depletion of natural hosts (Humber 1991). Other studies have demonstrated the compatibility of some *B. bassiana* isolates with pesticides (Hall and Dunn 1959, Loria et al. 1983, Majchrowicz and Poprawski 1993, Todorova et al. 1998).

Zineb™ (zinc dimethyl-dithiocarbamate, United Agri Products, Dorchester, Ontario, Canada) and Cygon™ (dimethoate, Cheminova Canada Inc. Mississauga, Ontario, Canada) are among the most-used pesticides in Quebec, Canada; the former for controlling several phytopathogenous fungi diseases including powdery mildew, sclerotinia, alternaria, cercospora and anthracnose (Ronald et al. 1994) and the second for the control of several insect pests. The fungicide and insecticide are routinely used in cropping systems in which *L. lineolaris* is a persistent pest. Compatibility of Zineb and dimethoate with *B. bassiana* will be critical in successfully using *B. bassiana* as a microbial insecticide in those systems. Therefore, the objective of this study was to evaluate the effect of Zineb and dimethoate, commonly used pesticides, on the field efficacy of *B. bassiana* in controlling the tarnished plant bug.

Materials and Methods

Insects. Adult *L. lineolaris* used in these tests were collected with sweep nets from *Solidago* sp. and radish (*Raphanus sativa* var. *oleiformis*) in Île Perrot (45°23'N; 73°57'W) Quebec, Canada. These were reared on potato sprouts (*Solanum tuberosum* L.) (Slaymaker and Tugwell 1982) and celery (*Apium graveolens* L.) petiole as food sources and oviposition substrates in a 30 × 30 × 60 cm meshed cage. A Petri dish (150 × 10 mm) containing moistened cotton was placed on the bottom of the cage to supply water. The insects were reared at 26°C, 60 to 70% RH, with a photoperiod of 16:8 (L:D). Each week, potato sprouts and celery were removed and placed in a cage covered with organdy to allow eggs to hatch. This method synchronized cohorts of adults for bioassays. The adults used were from second-generation laboratory colony and were free of fungal disease.

Fungus. The MK 2001 isolate was initially isolated from *Lygus* sp. in Quebec. In preliminary tests, MK 2001 killed a greater percentage of *L. lineolaris* adults within a shorter period of time than 21 other isolates of *B. bassiana* (Kouassi et al., unpubl. data). The isolate was passed twice through *L. lineolaris* adults by the immersion method (Butt et al. 1994) at a concentration of 10⁷ conidia/mL to prevent virulence attenuation. A colony was established from a single conidium that was aseptically harvested from 2-d-old culture on a selective oatmeal dodine medium (ODM) (Chase et al. 1986). One colony-forming unit (CFU) was transferred onto Sabouraud's dextrose agar (SDA) and incubated in the dark at 24°C for 20 d. Conidia were harvested with a sterile cotton swab and mixed in deionized water with 0.1% Triton X-100 for 15 min. The viability of the conidia was assessed by determining % germination following 24 h of growth in Sabouraud's dextrose broth (SDB).

Bioassay. The concentration-mortality response of *L. lineolaris* adults to the MK 2001 isolate was determined by the immersion method (Butt et al. 1994). Thirty adults

were placed on filter paper (Whatman No. 4) in a 9-cm diam Buchner funnel and submerged in 50 mL of a conidial or Zineb suspension for 10 s. The suspension was then drawn off by suction using a filtration system described by Hall (1976). Treated insects were placed 5 per dish in Petri dishes (150 × 10 mm) lined with moistened filter paper and soaked cotton with 4-cm petiole of celery for food. There were 8 replicates for each treatment including *B. bassiana* alone, *B. bassiana* + Zineb, Zineb alone and control. The treatment with Zineb alone was included to assess the Zineb insecticidal effect on adult insects. Fresh celery leaves were provided every 2 d, and the filter paper and cotton were moistened daily. These were kept at 24°C, 90% RH and a photoperiod of 16:8 (L:D). Mortality was recorded daily for 15 d. All dishes from each treatment were incubated in isolation to avoid the influence on adult *L. lineolaris* mortality from volatile Zineb products. Cadavers were removed and placed in Petri dishes lined with moistened filter paper and kept at 24°C in growth chambers for mummification and sporulation to confirm cause of death.

In vitro assays. The effect of Zineb on *B. bassiana* growth was determined by colony and mycelial growth. Zineb was mixed with SDA cooled to 45°C at rate of 1.68 kg/1000 L (lettuce field recommended rate (1X)). The solution was drop plated into Petri dishes (10 × 1 cm) and allowed to solidify at room temperature under a biological containment hood (Microzone corporation, Northside Road, Nepean, Ontario, Canada). A 1 mm deep × 10 mm diam disk of unsporulated mycelium from a 4-d-old culture of MK 2001 was placed in the center of each Petri dish. The dishes were incubated at 24°C in the dark. The radial growth was recorded at 4, 8, 12, and 16 d after placement with vernier calipers to the nearest 0.05 mm on the four cardinal points previously drawn on the bottom of Petri dish. The treatments were replicated five times. Each dish was incubated in isolation to avoid the influence of any volatile product on fungal growth among treatments.

Mycelial growth also was measured by mycelial dry weight produced in broth containing Zineb. An appropriate concentration (1X) of Zineb was added to 100 mL of SDB in 250 mL Erlenmeyer flasks and inoculated with 1 mL of *B. bassiana* suspension at a concentration of 1×10^7 conidia/mL. The control consisted of inoculating *B. bassiana* in 100 mL of SDB free of fungicide. Eight replicates were done for each treatment. The flasks were incubated in an orbital shaker (160 rpm) at 24°C for 7 d in isolation. After incubation, the content of each flask was filtered by vacuum using previously weighed filter paper (Whatman No. 4). The resulting mycelial mats were oven-dried at 120°C for 10 min and weighed.

Field test. One field evaluation was conducted in lettuce, *Lactuca sativa* L. var. *longifolia* (Asteraceae), in Sherrington (45°11'00"N; 73°31'00"W), Montreal, Quebec, Canada. Row spacing was 30 cm, and plants within the same row were 36 cm apart. The lettuce was at a phenological stage of 50 leaves (15 externals, 35 internals), 36 cm height, and 38 cm apical foliar span diam with a 14-cm heart. Nine plants of lettuce on 2 rows were enclosed in a 120 × 80 × 60 cm meshed cage.

For application, *B. bassiana* conidia were gently harvested with a sterile cotton swab and suspended in deionized water containing 0.1% Triton X-100. The suspension was mixed and homogenized with a stir bar for 15 min and serially diluted in order to obtain the targeted concentration of 1×10^8 conidia/mL. This concentration was confirmed by counting the average number of conidia per mL with an improved Neubauer hemacytometer (Hausser Scientific, USA, Levy Hemacytometer, 0.100 mm deep) as described by Cantwell (1970). The fungicide Zineb and the insecticide

dimethoate were prepared at the manufacturer recommendation rate of 1.68 g per L of water and 7 mL per 10 L of water, respectively.

Treatments were *B. bassiana* alone, dimethoate alone, *B. bassiana* + Zineb, *B. bassiana* + dimethoate, *B. bassiana* + dimethoate + Zineb, and control (deionized water with 0.1% Triton X-100). They were arranged in a randomized complete block design with four replicates per treatment. Three-liter suspensions of each treatment were prepared 2 h prior to use. The whole experiment was repeated twice (early June and August) during the season. The same plot location was not used more than once.

Lygus lineolaris adults were collected with sweep nets from untreated *Solidago sp.* and radish *Raphanus sativa* var. *oleiformis* around the lettuce field prior to being introduced into cages. Seventy-two individuals were introduced the day before treatment into 120 × 80 × 60 cm cages covered with 4-mm² mesh net. In the preliminary field test, we evaluated 3 types of cages (1 mm², 2.25 mm² and 4 mm² mesh net) for abiotic conditions and uncaged conditions comparison. Daily weather recorded with a Campbell Scientific (Logan, UT) data logger demonstrated that abiotic conditions did not differ significantly from uncaged conditions for the 4-mm² mesh net. The top of the cage was equipped with a removable piece of meshed mosquito net fixed to the frame with Velcro® (Velcro Canada Inc., Brampton, Ontario, Canada) and allowed assessment of mortality. Caged plants were sampled for insects 4, 7, and 10 d after treatment. On the fourth and seventh day, plants were vigorously shaken. Insects dislodged from the foliage and insects on the cage were collected and counted. On the tenth day, the lettuce leaves were harvested one-by-one and checked for the presence of adults, larvae, and feeding damage.

Collected insects were transported to the laboratory and frozen. The cadavers were removed and placed in dishes lined with wet filter paper and kept at 24°C in growth chambers for evidence of conidiation. Conidia were harvested from cadavers with a sterile cotton swab and inoculated on ODM. One well-differentiated colony was then isolated and inoculated on SDA medium in order to assess the morphological characteristics, which was used to confirm that the fungus recovered from the insects was *B. bassiana*.

Treatments were applied at 0530 to 0745 h on 22 Aug 2000. Suspensions were applied at a rate of 100 mL per plot to runoff with a knapsack SP0 4-gallon knapsack sprayer (SP Systems, LLC, M. K. Ritthenhouse & Sons, Ltd.) equipped with a nozzle having a small spray shield of 22 cm (80°). The height of the boom was 90 cm. Cages were surrounded with a 1.5 m high polyethylene sheet to reduce drift during spraying.

Incoming solar radiation with a daily UV peak, daily temperature (min, max), daily relative humidity, wind velocity, and direction were all monitored by the Agriculture Canada Weather station located near the lettuce field. Rainfall was recorded directly in the field using a rainfall apparatus device.

Mortality was corrected as described by Abbott (1925). Comparison of mortality was analyzed with a one or two-way analysis of variance (ANOVA) followed by a Fisher's Protected LSD test. Statistical tests were performed with the software Super-ANOVA for Macintosh computers from Abacus Concept (1989). LC₅₀ was determined after 6 d post-treatment using a probit analysis program (Finney 1971) performed with SAS software (SAS 1999).

Data on mycelial linear growth and mycelial weight were recorded on day 4, 8, 12, and 16 post-treatment and expressed as the percentage of the maximum growth rate of the control of each treatment. Statistical tests were performed on the linear growth, and the mycelial weight on the eighth day post-treatment with the software Super-

ANOVA for Macintosh from Abacus Concept (1989). Comparisons between treatments were analyzed with one, two, or three-way analyses of variance (ANOVA) followed by Fisher's Protected LSD test.

Results and Discussion

Laboratory bioassay. *Lygus lineolaris* adults were highly susceptible to isolate MK 2001 of *B. bassiana*. The MK 2001 isolate yielded an LC_{50} at 6 d after exposure of 7.2×10^3 conidia/mL against *L. lineolaris* adults and an LT_{50} at the concentration of 1×10^8 conidia/mL of 3 days (Table 1). Bidochka et al. (1993) reported an LT_{50} of 4.9 d and 90% mortality after 7 d for *L. borealis* (Kelton), *L. lineolaris* and *L. desertinus* Knight under laboratory conditions using an unreported conidial concentration of *B. bassiana*. Bajan and Bilewicz-Pawinska (1971) obtained similar results with nymphs and adults of *L. rugilipennis* Poppius at concentrations of 5×10^5 and 2×10^6 conidia/mL. Steinkraus and Tugwell (1997) obtained a LC_{50} of 2.2×10^6 conidia/mL at 5 d post-treatment with isolate ARSEF 3769 on both larvae and adults of *L. lineolaris*. Noma and Strickler (1999) reported a LC_{50} of 1.92×10^6 conidia/mL for *L. hesperus* (Knight) adults. The low LC_{50} (7.2×10^3 conidia/mL) and LT_{50} (3 d with concentration of 10^8 conidia/mL) values obtained in our study with MK 2001 indicate a relatively high pathogenicity for *L. lineolaris*. Zineb had no insecticidal effects on *L. lineolaris*.

Laboratory compatibility. The mycelial mean weight of the MK 2001 isolate for the SDB + Zineb treatment did not differ significantly from the control treatment (LSD, $P = 0.89$). The mycelial dry weight recorded on the eighth day post treatment was 0.23 g (SE = ± 0.01) compared to 0.24 g (SE = ± 0.01) for the control.

The mycelial mean linear growth of the MK 2001 isolate on the SDA + Zineb treatment also did not differ significantly from the control treatment (LSD, $P = 0.68$). The mycelial linear growth recorded on the eighth day post-treatment was 3.20 cm (SE = ± 0.13) compared to 4.33 cm (SE = ± 0.13) for the control. Growth inhibition is a useful criterion for testing the compatibility of pathogens with pesticides. In our study, Zineb did not significantly affect *B. bassiana* mycelial growth on solid or in liquid media and was neither fungicidal nor fungistatic to isolate MK 2001. Olmert and Kenneth (1974), Majchrowicz and Poprawski (1993), and Todorova et al. (1998) reported contradictory results on the compatibility of Zineb with *B. bassiana*. Hall and Dunn (1959) demonstrated that Zineb, at one-third the recommended dose, exhibited various inhibitory effects (0 to 100%) to five entomophthoralean fungi, all pathogens of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). Zineb is a dithiocarbamate which is a class of broad-spectrum fungicides. They have multi-side and non-

Table 1. Lethal concentrations and lethal times of *L. lineolaris* adults to the MK 2001 isolate of *B. bassiana*

Response	Value	95% Fiducial limits
LC_{50}	7.2×10^3 conidia/mL	6.9×10^2 - 2.7×10^4
LC_{90}	6.5×10^5 conidia/mL	2.0×10^5 - 3.6×10^6
LT_{50}	3.07 d	2.41-3.42
LT_{90}	4.45 d	4.03-5.43

specific modes of action against fungi (Griffith et al. 1992). Lyr (1977) reported that certain differences in sensitivity of phytopathogenic fungi to nonspecifically-acting fungicides, like dithiocarbamates, could be attributed to variations in the receptor sites or other structures, the potency of the defense mechanism, or in penetration efficiency of the active fungicidal compound. The MK 2001 isolate of *B. bassiana* was compatible with Zineb.

Field test. The mean incoming solar radiation at this site during this test was relatively high (10901 kJ/m²). The daily hourly mean temperature was relatively cool 18.85°C (SE = ±2.78), and the daily air temperature maximums ranged from 8.10°C to 30°C (mean = 24.94°C, SE = ±3.61) and minimums from 6.3°C to 28.7°C (mean = 12.77°C, SE = ±3.47). A rainfall of 8 mL occurred a few hours after application. Hourly relative humidity ranged from 38.7% to 100% with a mean of 81.02%, SE = ±8.87.

In spite of solar radiation, relatively low mean temperature, fluctuating relative humidity and rain during the field trial, each treatment (*B. bassiana* alone, dimethoate alone, *B. bassiana* + Zineb, *B. bassiana* + dimethoate, *B. bassiana* + dimethoate + Zineb) caused high mortality of *L. lineolaris* adults (Table 2). Mortality differed significantly between treatments on the fourth day post treatment but not on the seventh or tenth (ANOVA; Day 4: $F = 21.698$; $df = 2,6$; $P = 0.0018$; Day 7: $F = 4.056$; $df = 2,6$; $P = 0.0768$; Day 10: $F = 0.651$; $df = 2,6$; $P = 0.5547$). On the fourth day post treatment, mortality in the *B. bassiana* treatment differed significantly from treatment with dimethoate (LSD; $P = 0.0009$). However, no significant difference was observed between treatments with *B. bassiana* and *B. bassiana* + Zineb (LSD, $P = 0.5070$).

Treatment with dimethoate yielded 98.49% mortality compared to 73.5% for *B. bassiana* alone and 66.5% for *B. bassiana* + Zineb. Mortality increased significantly when adding dimethoate to *B. bassiana* (LSD; $P = 0.0001$). However, no significant difference was observed between the mortality of the treatment with *B. bassiana* + dimethoate and dimethoate alone (LSD; $P = 0.4966$); whereas, a significant difference was observed between the treatment *B. bassiana* and *B. bassiana* + dimethoate (LSD; $P = 0.0018$). A synergistic effect was observed when we compared the mortality of the treatment with *B. bassiana* alone to the combination of *B. bassiana* + dimethoate.

Table 2. Field mortality in percentage of *L. lineolaris* adults at 4, 7, and 10 d after application*

Treatment	Day after application		
	4	7	10
MK 2001	73.5 ^a	91.5 ^{ab}	98 ^a
Dimethoate	98.49 ^b	100 ^c	100 ^a
MK 2001 + Zineb	66.5 ^a	86.49 ^b	93.13 ^a
MK 2001 + Dimethoate	95.49 ^b	96.99 ^a	96.99 ^a
MK 2001 + Dimethoate + Zineb	99 ^b	99 ^c	100 ^a
Control	4.86	6.94	7.42

* Corrected from the control (Abbott 1925). Means within the same columns followed by the same letters are not significantly different (Fisher's Protected LSD tests, $P < 0.05$).

On the seventh day, no significant difference was observed between the mortality of the treatment with *B. bassiana* and dimethoate + *B. bassiana* (LSD; $P = 0.1190$) and also between *B. bassiana* and *B. bassiana* + Zineb (LSD; $P = 0.5070$). However, a significant difference was observed between the mortality of the treatment with *B. bassiana* alone and dimethoate (LSD; $P = 0.0308$). Dimethoate induced 100% mortality compared to 91.5% for *B. bassiana*.

On the tenth day, the treatments exhibited 100%, 100%, 98%, 96.99%, and 95.13% mortality for dimethoate, MK 2001 + dimethoate + Zineb, *B. bassiana* alone, *B. bassiana* + dimethoate and *B. bassiana* + Zineb, respectively (Table 2). When comparing the mean mortality, there were no significant differences between the treatment with *B. bassiana* and *B. bassiana* + Zineb (LSD, $P = 0.5921$); between *B. bassiana* and dimethoate (LSD; $P = 0.4826$), between dimethoate and *B. bassiana* + dimethoate (LSD; $P = 0.3054$), or between *B. bassiana* and *B. bassiana* + dimethoate (LSD; $P = 0.7225$). There was neither a synergistic effect nor an antagonistic effect when combining *B. bassiana* and dimethoate. The combination *B. bassiana* + dimethoate did not substantially enhance the mortality on the tenth day post treatment and did not differ significantly from *B. bassiana* alone or dimethoate alone.

Our field test revealed that (1) the MK 2001 isolate was pathogenic to *L. lineolaris* adults at ambient environmental conditions, and (2) adult mortality did not differ between treatment with *B. bassiana* alone, the insecticide dimethoate alone, *B. bassiana* + Zineb, and *B. bassiana* + dimethoate. The fungal treatment was as efficient as the insecticide treatment. In fact, the 98% or 95.13% mortality observed on the tenth day after application of MK 2001 alone or MK 2001 + Zineb could be an acceptable delay for controlling a damaging population of *L. lineolaris* under field conditions, depending on the host plant tolerance.

Entomopathogenic fungi, unlike insecticides, usually take several days to kill their host (Bajan and Bilewicz-Pawinska 1971, Bidochka et al. 1993, Steinkraus and Tugwell 1997, Todorova et al. 1998, Noma and Strickler 2000), depending on their virulence and host physiology. In our study, after relatively short latency phase, isolate MK 2001 at the field economic feasible concentration of 1×10^8 conidia/mL induced mortality as high as the dimethoate. Live *L. lineolaris* in the fungal-treated cages were observed moribund, and stopped feeding. Feeding damage decreased substantially or stopped when *L. lineolaris* were contaminated by *B. bassiana* (Kouassi et al., unpubl. data). Our findings disagree with Noma and Strickler (2000) who reported the increase of *L. hesperus* (Knight) feeding rate when treated with *B. bassiana*. The decrease of feeding rate and feeding damage following contamination must be considered when evaluating field performance of *B. bassiana* against *L. lineolaris*.

The harvest and leaf check on the tenth day post application revealed an absence of larva in cages treated with *B. bassiana* alone or in combination with the fungicide Zineb or insecticide dimethoate; whereas, a mean of 5.6 larvae/plant ($SE = \pm 1$) and 11 larvae/plant ($SE = \pm 2$) were observed in the dimethoate treatment and the control, respectively. Applications of the MK 2001 isolate of *B. bassiana* both in combination or alone with Zineb or dimethoate provided substantial and long-term foliar protection. The absence of larvae in the fungal-treated cages in contrast to the treatment with dimethoate could be explained by the potential of the MK 2001 isolate to control either *L. lineolaris* adults, larvae and probably to prevent egg hatchability. The profuse sporulation on the insect cadavers observed in the cages treated with the MK 2001 isolate could favor horizontal transmission or initiate an epizootic in *L. lineolaris* field populations.

The MK 2001 isolate could be used as a potential alternative to many common pesticides applied as field controls for *L. lineolaris*. Its compatibility with Zineb and dimethoate increases its potential use in a biological control or IPM program. Anderson et al. (1988) reported that insecticide and pathogen combinations introduced multiple mortality factors against target pest.

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