Temperature Effects on the Susceptibility of the Colorado Potato Beetle (Coleoptera: Chrysomelidae) to *Beauveria bassiana* (Balsamo) Vuillemin in Poland, the Czech Republic and the United States[1,2]

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Abstract *Beauveria bassiana* (Balsamo) Vuillemin is a fungus with broad spectrum insecticidal activity. As a biological control agent used against Colorado potato beetles (*Leptinotarsa decemlineata* (Say)), this fungus has performed erratically in various field studies. This inconsistent performance has been attributed to formulation problems, UV sensitivity, and humidity. In a multi-site test, *B. bassiana* controlled Colorado potato beetle larvae in both Poland and the Czech Republic, but not in Maryland. Control was measured by reduction in populations of beetle larvae. One of the major differences among these sites was temperature. In Poland, the mean temperature ranged from 5°C to 23°C; in the Czech Republic the average temperature ranged from 6.7°C to 18.7°C; and in Maryland, temperatures at time of application exceeded 45°C at canopy level. This led us to examine *B. bassiana* growth *in vitro*. While *B. bassiana* grew in the laboratory from 16 to 30°C, the *B. bassiana* from a formulated product (Mycotrol™, Mycotech, Butte, MT) did not germinate at temperatures above 37°C. Germination and subsequent development of this entomopathogenic fungi are critical factors in the infection and control of the Colorado potato beetle. As a consequence of the inability to germinate at high temperatures, *B. bassiana* would not be expected to effectively control pest insects in climates with hot summers. This fungus, however, may be suitable for insect control in early spring or in cool temperature climates during the growing season.

Key Words Entomophagous fungi, *Beauveria bassiana, Leptinotarsa decemlineata*

Beauveria bassiana (Balsamo) Vuillemin has a wide insect host range (Feng et al. 1994) and, therefore, is considered a generalist pathogen. Various strains of *B. bassiana* have been found to be pathogenic to homopterans, coleopterans, lepidopterans, dipterans, and orthopterans (Tanada and Kaya 1993). This wide range of affected insects makes *B. bassiana* a good candidate for insect control. Commercial products incorporating *B. bassiana* are available for insect control in the U.S. including Mycotrol™ (Mycotech, Butte, MT).

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Pathogens, such as *B. bassiana,* in order to infect insects, must be able to adhere to the insect cuticle, emerge from a resting state (germinate), and multiply in the insect. The success of *B. bassiana* as a biological control agent against Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) has been erratic (Hajek et al. 1987). Initial formulations against Colorado potato beetle were designed for soil incorporation. Soils tend to heat slowly in the spring. Therefore, previous temperature studies have focused on lowest temperature needed for germination and growth (Hywell-Jones and Gillespie 1990). The optimal temperature for growth of many *B. bassiana* strains is 24 to 30°C (Teng 1962, Fargues et al. 1992, Mietkiewski et al. 1994). Foliar application formulations have overcome the problem of little to no growth of *B. bassiana* at low soil temperatures, but performance is still inconsistent. In Europe and in the northern United States, such as Rhode Island and Michigan, successful control of Colorado potato beetles has been achieved using *B. bassiana* (Dirlbekova 1986, Lipa et al. 1989, Groden and Lockwood 1991). This success has been inconsistent in warmer climates with success in Virginia in early-season applications (Poprawski et al. 1997) and failure in Maryland in late-season applications (Schroder, unpubl. data). Recently, it has been found that grasshoppers "cure" themselves of *B. bassiana* infection by using behavioral changes, including sitting in sunlight (Inglis et al. 1997).

This report on temperature effects on *B. bassiana* was part of a larger multinational project to control Colorado potato beetle using biological control agents. The focus of the multinational project was to reduce chemical pesticide usage in Central Europe. The objective of this study was to determine why *B. bassiana* successfully controlled Colorado potato beetles in European countries, but did not in Maryland.

Materials and Methods

Cultivars of potatoes (*Solanum tuberosum* L.) were planted as appropriate for the region: 'Mila' in Pozan, Poland; 'Désirée E' in Zabcice, Czech Republic; and 'Superior' in Salisbury, MD, United States, for research purposes. Temperatures were recorded as mean air temperatures, calculated as an average of the daily maximum and minimum temperatures. In Europe, the initial application date was determined on first hatch of the beetle eggs. In the United States, the application dates were determined by economic threshold levels of beetles. The economic threshold for potatoes is 4 small larvae (first and second instars) and 1 large larvae (third and fourth instars) per stem. In Poland, the economic threshold used was 15 small larvae per plant. For comparison purposes, the date of the first application is day 0. The chemical insecticides used as positive controls varied by region. Karate™ (Zeneca Agricultural Products, Wilmington, DE), a synthetic pyrethroid, in Poland was applied on days 0, 9, and 14; Marshal™ (FMC Corp., Philadelphia, PA), a synthetic pyrethroid, in the Czech Republic was applied on days 0 and 21; and Pravado™ (Bayer Agricultural Products, Kansas City, MO) an imidacloprid, in the United States was applied on days 0 and 11. *Beauveria bassiana* formulations were flowable concentrates. Mycotrol™ (Mycotech, Butte, MT) was applied at the rate of 2.5×10^{13} spores per ha. In the United States, a wettable powder formulation also was tested and applied at the same rate. In Poland, *B. bassiana* was applied at days 0, 6, 9, and 14; in the Czech Republic, at days 0, 2, 6, and 9; and in the U.S. at days 0 and 11. The application dates were in June and July for the European countries, and in late-July and early-August for the U.S. Treatments were applied in a complete randomized block design.

Counts were made of large larvae (third and fourth instar) on the dates shown in the data. For Europe the counts were on a per plant basis, for the U.S. the insect counts were on a 10 stem basis as the number of Colorado potato beetles was lower. Yield data are shown for the European countries. No yield data were available for the U.S.

In the U.S. only, leaf samples for recovery of *B. bassiana* were collected aseptically and individually stored in sterile Whirl Pak® bags (Nasco, Ft. Atkinson, WI) immediately after application, 6 h later, at 1 day, and at 2 days. Leaves were stored on ice until returned to the laboratory. Leaves were weighed. For removal of the fungi, 5 ml of water was added to each bag and each leaf was homogenized using a Stomacher™ blender (Techmar, Cincinnati, OH) set on low for 30 s. Dilutions were plated on potato dextrose agar (PDA, Oxoid, London) + 50 μg/ml chloramphenicol (Sigma, St. Louis, MO) to inhibit bacterial growth. Colonies were counted after 48 h of incubation at room temperature (approximately 25°C). Recovery was expressed as fungal colonies (colony forming units or CFU) per gram of leaf.

In vitro temperature studies were conducted using spread plates of PDA with the *B. bassiana* liquid formulation that was applied in the field. The same strain of *B. bassiana* was present in both formulations, so temperature studies were conducted using the liquid formulation. Dilutions of *B. bassiana* were plated in duplicate and incubated at 23, 25, 30, 33 and 36°C in order to determine optimal germination and growth temperature of this strain. The experiment was repeated 3 times. Mean CFU's and standard errors were calculated. In order to show that the first step in the formation of a colony was inhibited by temperature, plated conidia were shifted to 37°C for various times.

For *B. bassiana* recovery from leaves an analysis of variance was performed using the mixed procedure with treatment and time as fixed effects (SAS Institute Inc. 1997) with variance grouping.

Results

Field conditions. In Poland the temperatures were cool (averaging 10 to 24°C), but the relative humidity (RH) was 75 to 99%. Temperatures in the Czech Republic averaged 18 to 21°C but RH was not recorded (Fig. 1). In the U.S., the temperature the day of application was unusually hot. When the initial leaf samples of *B. bassiana* recovery were collected, the temperature was 37.9°C, with 38% RH, in the leaf canopy. For the next sample, taken 6 h later, the temperature was 45.7°C, with 22% RH. The mean temperatures were above 27°C and the relative humidity ranged from 50 to 67% during the remainder of the experiment. The mean temperatures were taken from weather stations at the sites.

Larval counts. In all three countries the larval pressure was high. In Poland, at this time there were no large larvae (Fig. 2a). The *B. bassiana* treatments were able to keep the mean number of large larvae to fewer than 10 per plant, whereas during the same time, the number of large larvae in the untreated controls rose to a mean of 50 larvae per plant. The synthetic pyrethroid controlled the beetle larvae to fewer than 5 per plant.

The Czech Republic potato plots had more large larvae per plant than the Polish plots at time of application (about 13/plant). Because the numbers were higher than optimal, the first application of *B. bassiana* was followed 2 d later by a second treatment. These two treatments reduced the number of large larvae to fewer than 10

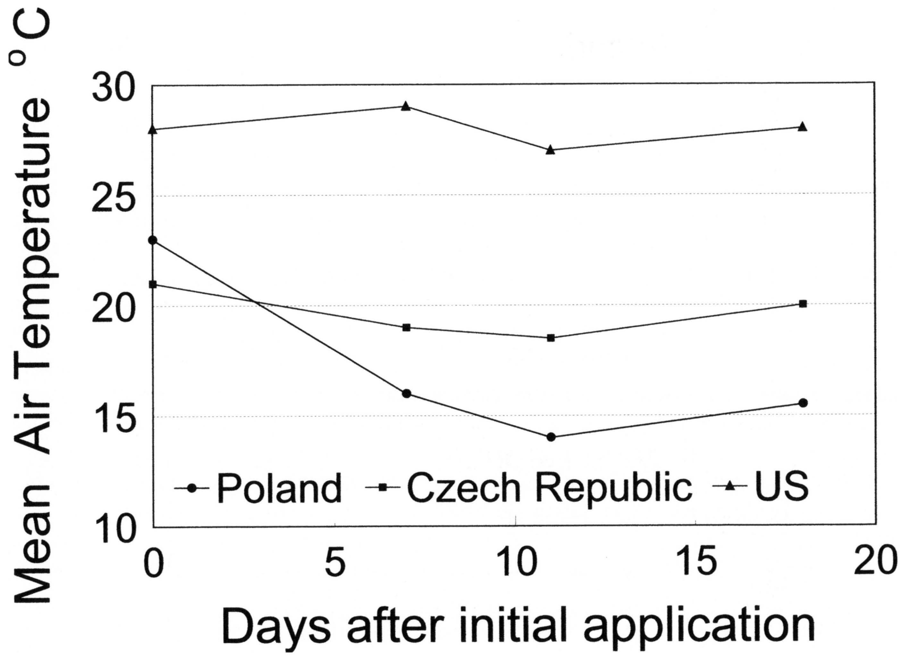

Fig. 1. Mean air temperature during field treatments in Poland, Czech Republic, and the U.S.

per plant (Fig. 2b). A third application of *B. bassiana* reduced the numbers to fewer than 5 larvae per plant. A single application of a synthetic pyrethroid controlled the number of large larvae to fewer than 8 per plant for 17 d. During this time the larval numbers in the untreated control rose to a high of 37 at 11 d (Fig. 2b).

Insects were counted in the United States 4 d after the first application of insecticide. The numbers of Colorado potato beetles were lower than in the European fields and, therefore, counted on a 10 stem basis as opposed to a per plant basis. Unexpectedly, the numbers of large larvae, in the *B. bassiana* treated rows, exceeded those in the untreated rows (Fig. 2c) and had the highest total number of larvae even following a second treatment. The Pravado insecticide kept the numbers of large larvae lower, but these were still higher than expected from chemical insecticide.

Yield. In Poland the yield for potatoes in the untreated control was 48.5 metric tons/ha. The yield for insecticide-treated potatoes was 55.5 metric tons/ha. The yield for *B. bassiana*-treated potatoes was significantly higher (66.6 metric tons/ha) than the untreated and insecticide-treated potatoes. In the Czech Republic the yield of potatoes was 38 metric tons/ha for the untreated control. The insecticide-treated potatoes yielded 45 metric tons/ha, and the *B. bassiana*-treated potatoes averaged 43 metric tons per ha. Both the insecticide-treated and *B. bassiana*-treated potatoes had significantly higher yields than the control, but were not significantly different from each other. Potatoes were not harvested in the United States because of adverse weather conditions.

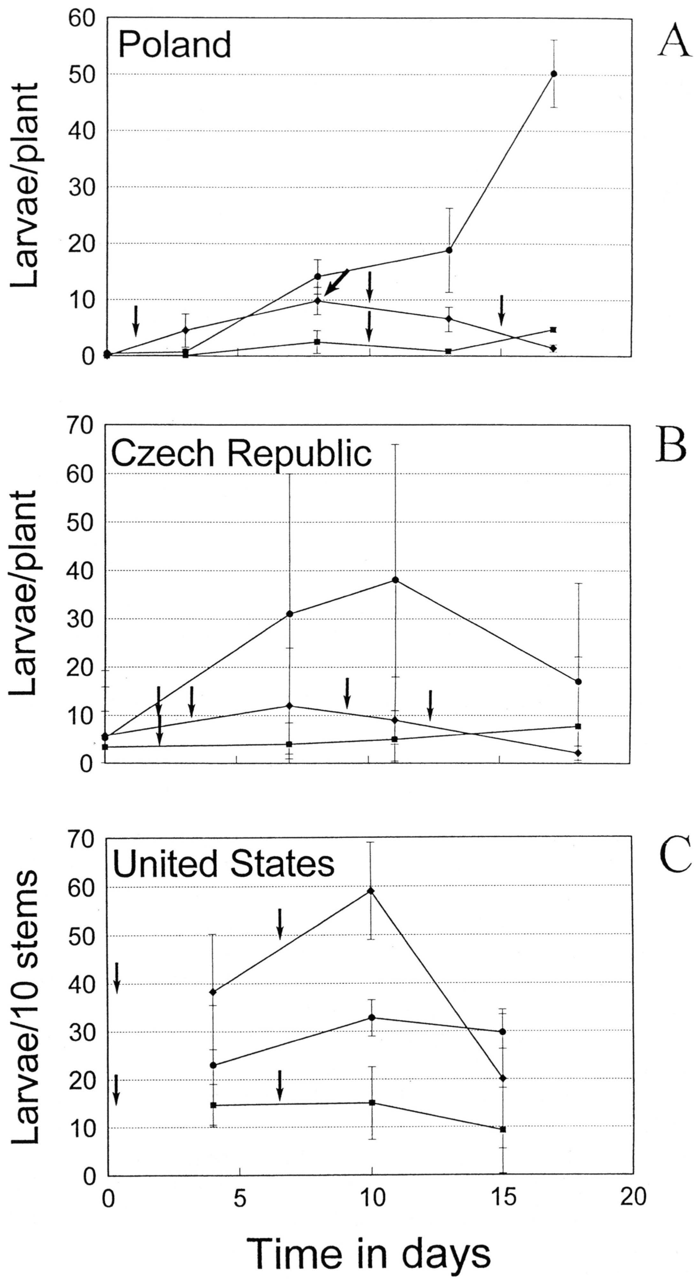

Fig. 2. Large Colorado potato beetle counts from Poland (A), Czech Republic (B), and the U.S. (C). Arrows indicate dates of application of *B. bassiana* or insecticide. ●-untreated control, ♦-*B. bassiana*, ■-insecticide.

Field recovery of *B. bassiana*. Recovery of *B. bassiana* from leaves to which either formulation of *B. bassiana* was applied was not significantly different (Table 1, $P = 0.0591$). In 6 h, the recovery of *B. bassiana* from leaves treated with the wettable powder formulation had dropped to 2% of the initial recovery, while recovery from leaves treated with the liquid concentrate formulation had dropped to 1%. By day 1, the recovery of *B. bassiana* (318 CFU/g) from leaves treated with the liquid concentrate formulation was not significantly different from zero ($P = 0.1152$) because of high variability in recovery. At this time, recovery of *B. bassiana* (646 CFU/g) from leaves treated with the wettable powder formulation also was not different from zero ($P = 0.062$).

Laboratory temperature studies. The inadvertent temperature study performed in the field was repeated in the laboratory with the liquid formulation. The most colonies were formed when propagated at 25°C (Table 2). At 5 degrees warmer, there was a reduction in colony formation from the same sample. At 33°C there was a 10-fold reduction in numbers of colonies formed. These colonies were much smaller and less dense. The number per plate was also more variable. At 36°C no colonies were formed. For the temperature shift studies to high temperatures, for short shift times as 1 h to 4 h there was no reduction in number of colonies formed, but formation of colonies was delayed. At 16 h at 37°C there was a 50% reduction in CFU's. After 3 days at 37°C, no colonies were formed even when 10 fold concentration was plated.

Discussion

Beauveria bassiana does not survive long on foliage in warmer climates, due to increased solar radiation, and as demonstrated in this study, elevated temperatures. This was confirmed by lab data showing reduced replication at 30°C. There was very little replication with poor growth at 33°C, and no replication at 36°C, temperatures below those observed in the Maryland field experiments. These data are slightly different than those reported by others based on radial growth (Fargues et al. 1992)

Table 1. Viable *Beauveria bassiana* colonies recovered from potato leaves

Formulation	Time after application (days)	CFUs recovered per g fresh weight of leaf	Standard error
Liquid	0	18798.91	7589.45
	0.25	227.10	122.57
	1	318.60	131.59
	2	86.10	63.89
Powder	0	43987.80	10846.05
	0.25	1088.20	357.40
	1	646.20	289.30
	2	402.50	238.24

Table 2. *Beauveria bassiana* response to various temperatures when cultured on potato dextrose agar

Temperature °C	CFU x 10^5	Standard error x 10^5
23	141.2	7.88
25	156.1	4.59
30	125.8	17.49
33	38.0	25.86
36	no growth	—

because they are based on CFUs which is also the way we determined recovery from leaves after spray. This takes into account both germination and growth, two conditions that are necessary for infection of Colorado potato beetle in the field. Preliminary data with temperature shifts also indicate that the varying temperatures in the field over the course of a day also affect the ability and the rate at which the fungi multiply and, thus, affect that aspect of biocontrol.

In all trials, the Colorado potato beetle populations were above the economic threshold. Survival of *B. bassiana* has been found to be reduced by UV light (Inglis et al. 1995). We have also found that survival declined with high temperatures. Unlike *Bacillus thuringiensis* Berliner, *B. bassiana* is a pathogen which needs to germinate and multiply in order to infect its host. The strain of *B. bassiana* used in these studies has an optimum growth temperature between 25°C and 27°C and does not germinate and multiply at mammalian body temperatures (37°C), which is considered a safety factor. However, because *B. bassiana* must multiply to infect insects whose body temperature is controlled by ambient conditions, its use is precluded in high temperature climates. Humidity is also a factor for fungal replication, but high relative humidity in these trials did not adversely affect *B. bassiana* conidial germination. Only in Europe did the mean air temperatures approach those for optimum growth of the fungi. In the U.S., mean air temperatures exceeded the optimum, and field temperatures at the level of the plant canopy exceeded 45°C.

Beauveria bassiana has been shown to be able to control Colorado potato beetle under cool weather conditions (Groden and Lockwood 1991, Poprawski, unpubl. data). In both Poland and the Czech Republic, numbers of large larvae were reduced compared to the untreated control. In both countries this reduction in beetle numbers resulted in yield increases over untreated controls, although the increase was less than the chemical control. Because of the extreme heat in the U.S., potato quality was poor and yields were not calculated.

Predictability of control of insects with microbes is complex. Many factors need to be taken into account in the application of microbes for control. This study demonstrates that temperature should be included as one of the factors, *B. bassiana* may be used in early spring, or perhaps applied at dusk when temperatures are more favorable to growth. Tests suggest that shifts to higher temperature reduces the number of conidia that germinate and that this reduction in germination is a function of time at the higher temperature.

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