

- nents, and grain yield in durum wheat cultivars. *Crop Sci.* 22:287–290.
- Gibson, L.R., and G.M. Paulsen. 1999. Yield components of wheat grown under high temperature stress during reproductive growth. *Crop Sci.* 39:1841–1846.
- Hanft, J.M., and R.D. Wych. 1982. Visual indicators of physiological maturity of hard red spring wheat. *Crop Sci.* 22:584–588.
- Hartung, R.C., C.G. Poneleit, and P.L. Cornelius. 1989. Direct and correlated responses to selection for rate and duration of grain fill in maize. *Crop Sci.* 29:740–745.
- Housley, T.L., A.W. Kirleis, H.W. Ohm, and F.L. Patterson. 1982. Dry matter accumulation in soft red winter wheat seeds. *Crop Sci.* 22:290–294.
- Knott, D.R., and G. Gebeyehou. 1987. Relationships between the lengths of the vegetative and grain filling periods and agronomic characters in three durum wheat crosses. *Crop Sci.* 27:857–860.
- McNeal, F.H., C.F. McGuire, and M.A. Berg. 1978. Recurrent selection for grain protein content in hard red spring wheat. *Crop Sci.* 18:779–782.
- Metzger, D.D., S.J. Czaplewski, and D.C. Rasmusson. 1984. Grain filling duration and yield in spring barley. *Crop Sci.* 24:1101–1105.
- Mou, B., and W.E. Kronstad. 1994. Duration and rate of grain filling in selected winter wheat populations: I. Inheritance. *Crop Sci.* 34:833–837.
- Mou, B., W.E. Kronstad, and N.N. Saulescu. 1994. Grain filling parameters and protein content in selected winter wheat populations: II. Associations. *Crop Sci.* 34:838–841.
- Nass, H.G., and B. Reiser. 1975. Grain filling period and grain yield relationships in spring wheat. *Can. J. Plant. Sci.* 55:673–678.
- Ottaviano, E., and A. Camusi. 1981. Phenotypic and genetic relationships between yield components in maize. *Euphytica* 30:601–609.
- Rencher, A.C. 1995. *Methods of multivariate analysis.* John Wiley & Sons, New York.
- SAS Institute Inc. 1988. *SAS/STAT Users guide, Release 6.03 ed.,* Cary, NC.
- SAS Institute Inc. 1997. *SAS/STAT Software: Changes and enhancements through release 6.12.* SAS Institute Inc., Cary, NC.

Assessment of Plant Pathogenicity of Endophytic *Beauveria bassiana* in Bt Transgenic and Non-Transgenic Corn

Leslie C. Lewis,* Denny J. Bruck, Robert D. Gunnarson, and Keith G. Bidne

ABSTRACT

Field and greenhouse studies were conducted to determine the proclivity of *Bacillus thuringiensis* (Bt) (Berliner)-transgenic corn (*Zea mays* L.) to form an endophytic relationship with *Beauveria bassiana*, and to evaluate the corn for possible plant pathological effects associated with this relationship. *Beauveria bassiana* (Balsamo) Vuillemin was applied as a granular formulation to two separate lines of corn, expressing Bt events MON802 and MON810, and their corresponding isolines. There were no significant differences in levels of endophytism between transgenic events or their near-isolines. In greenhouse studies, *B. bassiana* was applied as a liquid seed treatment to Bt transgenic corn hybrids Pioneer 34R06 (event MON810) and Ciba Max 454 (event 176) and their near isolines at a rate of 2×10^{10} conidia per ml. There were no significant differences in seed germination or presence of root pathogens in transgenic or isolate seeds soaked in a *B. bassiana* suspension. The same lines of corn were used in field experiments with treatments of seeds soaked in a suspension of *B. bassiana*, a foliar application of a granular formulation of *B. bassiana*, and corresponding untreated checks. Plants were sampled throughout the growing season and evaluated for growth of individual plant components, including sheaths, leaves, stem, husk, ear, plant leaf-to-stem ratio and overall plant growth. There were no significant differences in overall plant growth between the *B. bassiana* treatments or in the growth of each plant component. The results of this study indicate that *B. bassiana* readily forms an endophytic relationship with transgenic and non-transgenic corn and causes no plant pathology.

1991). Only recently have investigations focused on relationships between *B. bassiana* and the corn plant. *Beauveria bassiana* forms an endophytic relationship with the corn plant and provides season-long suppression of *O. nubilalis* (Lewis and Cossentine, 1986; Lewis and Bing, 1991; Wagner and Lewis, 2000). Two areas of research must be investigated before acceptance by producers of *B. bassiana* as a management tool: (i) will *B. bassiana* colonize *Bacillus thuringiensis* (Bt) transformed corn plants as well as non-transformed plants, and (ii) is the endophytic relationship void of plant pathological symptoms in transformed plants and/or their isolines?

This paper reports the results of research to determine the proclivity of *B. bassiana* to form an endophytic relationship with Bt transgenic corn and whether endophytism causes a plant pathology. Field studies were conducted in 1994 and 1995 to determine the ability of Bt-transgenic corn to form an endophytic relationship with *B. bassiana*. Greenhouse studies were performed in 1997 to determine the effect of *B. bassiana* on seed germination and plant growth on two lines of Bt corn and their genetic isolines. Field studies in 1997 were conducted to determine any possible pathogenic effects of *B. bassiana* on overall plant growth and dry matter accumulation.

MATERIALS AND METHODS

1994 and 1995 Field Studies

A randomized complete block design with four replications and treatments arranged as split plots was used. The whole plots were a foliar application of *B. bassiana* and an untreated control. The split plots were corn hybrid, Bt transgenic corn (Jeremy event 802) and its near-isoline (an experimental hybrid with B73 \times Mo17 background). Corn was planted on an

THE ENTOMOPATHOGENIC FUNGUS *Beauveria bassiana* (Balsamo) Vuillemin has been used as a plant protectant to suppress populations of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) on corn, *Zea mays* L. (Bartlett and Lefebvre, 1934; Stirrett et al., 1937; Beall et al., 1939; York, 1958; Riba, 1984; Marcandier and Riba, 1986; Lewis and Bing,

USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, Iowa 50011. Received 30 Oct. 2000. *Corresponding author (leslewis@iastate.edu).

Abbreviations: GLM, General linear models; CFU, colony forming units; ARSEF, ARS collection of entomopathogenic fungi.

Iowa State University Research Farm, approximately 2 km away from the nearest corn field. Experimental plantings were in rows on 0.75-m centers separated by two guard rows of a commercial hybrid (Garst 8543). Accepted agronomic practices of fertilizer and herbicide were used. *Beauveria bassiana* (ARSEF 3113, USDA-ARS, Entomopathogenic Fungi Collection, Ithaca, NY), a soil isolate passed through *O. nubilalis*, was formulated on corn kernel granules and applied at 1×10^6 conidia per plant with a hand-held inoculator (Davis and Oswalt, 1979). In 1994, granules containing *B. bassiana* were applied to plants only at V6, or at V6 and at R1 growth stage (Ritchie et al., 1997). In 1995, *B. bassiana* granules were applied to plants at either the V6 or R1. Corresponding controls which received no fungal applications were used in 1994 and 1995.

The techniques of Lewis and Bing (1991) were used to evaluate the occurrence of endophytic *B. bassiana*. Samples of corn pith were taken with aseptic techniques and plated on agar that favors the growth of *B. bassiana* (Doberski and Tribe, 1980). Five plants per treatment were sampled. If fungal applications were at V6, samples were taken at V12, R1, and R6; 30, 45, and 60 d following fungal application, respectively. If application was at V6 and again at R1, samples were taken at R1, R6, and senescence. If fungal application was at R1, samples were taken at R6 and senescence. Agar plates with pith samples were incubated in total darkness at 28°C for 10 d at which time samples were examined for the growth of *B. bassiana*.

Statistical Analysis

Data were analyzed with analysis of variance using the General Linear Model procedure (GLM) with the *B. bassiana* treatment as the whole plot and genetic makeup of the corn as the split plot. The effects of each treatment on endophytism levels were analyzed and means separated using Student's *t*-test at $P \leq 0.05$ (SAS Institute, 1995). Endophytism data were analyzed separately for the two granular applications for each plating date and pooled over all plating dates.

1997 Studies

Greenhouse

The experiment was a randomized complete block design with four replications and a factorial arrangement of the four lines of corn (Pioneer 34R06 event MON810, and Ciba Max 454 event 176, and their genetic isolines Pioneer 3489 and Ciba 4494, respectively) and three seed treatments: seeds soaked in a 0.1% tween and distilled water suspension of *B. bassiana* (2×10^{10} conidia per ml), seeds soaked in sterile 0.1% tween and distilled water, and unsoaked seeds. Seeds were soaked for 10 min, placed on sterile filter paper, and air-dried under a bioflow hood. The dry seeds were placed into sterile packets and taken to the greenhouse for planting. The unsoaked seed was taken directly from the commercial bag and also placed in sterile packets before planting. A replication consisted of ten seeds of each hybrid planted into individual 10 cm plastic pots containing sterile vermiculite. Pots were watered at planting and thereafter when the surface of the vermiculite was dry to touch. After the first week of plant growth, pots were fertilized weekly with liquid soluble fertilizer (N-P-K, 20-20-20). Conditions in the greenhouse were maintained at photoperiod 14:10 (L:D), 80% RH, and 27°C during the day and 21°C at night.

Emergence was recorded 1 wk after planting and weekly for five weeks. Plant heights were measured beginning the

second week after planting for three consecutive weeks. Measurements were taken from the base of the plant (vermiculite surface) to the tip of the longest leaf. After five weeks, five plants from each treatment were randomly selected for examination of endophytic *B. bassiana*. Plants were cut at their base, placed in a plastic bag, and returned to the laboratory for fungal isolation. Each plant surface was disinfected by wiping its exterior with 95% EtOH. With sterile laboratory techniques, the stem of each plant was split longitudinally with the portion from the base to the growing tip excised and placed on an agar medium favoring the growth of *B. bassiana* (Doberski and Tribe, 1980). Plates containing the plant material were allowed to incubate in darkness at 28°C for 10 d at which time the plant tissue was examined for growth of *B. bassiana*.

The remaining plants from each treatment were examined for presence of root disease. Plants were pulled from their pots and placed into a 19-L pail containing cool water. Plants were dipped in the water repeatedly until the roots were free of vermiculite. All plants then were inspected for signs of disease in the root system by Dr. Gary Munkvold (Plant Pathologist, Department of Botany, Iowa State University, Ames, IA).

Field Studies

The experiment was a randomized complete block design with four replications. Plots were planted with a four row planter on 12 May 1997. Hybrids used were the two transgenic corn lines and their near isolines used in the 1997 greenhouse studies. Whole plot treatments (10 m in length) consisted of corn seeds soaked for 3 min in an emulsifiable formulation of *B. bassiana* containing 2.1×10^8 conidia per ml or a 0.4 g foliar application of a granular formulation of Mycotech 726 containing 8.8×10^7 conidia per plant and an untreated control. Plants were treated on 16 June when they reached V6 stage of development. Harvests over time served as the split plot. The two different application techniques for *B. bassiana* were performed to confirm results found in the greenhouse and to test for any adverse effects on plant growth due to granular *B. bassiana* application to V-stage corn. Sampling of plants began 42 d after planting at which time the corn had reached the V7 growth stage. Sampling of plants was conducted every 2 wk for 16 wk. Plants were selected from the center two rows of each four row plot on each of eight sample dates. Plants within 0.1 m of the beginning and end of each plot were not sampled to eliminate any effect that the alleyways may have had on plant growth. The remaining 9.8 m of row in each of the center two rows of the plot were sampled approximately every 1 m in succession along the length of the plot. One plant was selected from each of the center two rows that was evenly spaced with the adjacent plants. The distance from the selected plant to the two adjacent plants was measured and divided by two to determine the amount of row space that the selected plant occupied. The selected plant was cut at the soil surface and its height measured from the base to the longest outstretched leaf or tassel. Plants were folded and placed into a plastic bag and returned to the laboratory for processing. Plants were held in a cold room at 4°C until processed.

Leaf blades were removed at the junction of the sheath and blade. Sheaths were circumcised at their base around the stalk. As the plants matured and developed ears, the ears and ear shoots were removed. The husk, shank, and silks were separated from the cob and kernels. The leaves, sheaths, stalks, ears, and husk were placed in a brown paper bag. Bags containing the fresh material were weighed. Samples were placed in a drying oven for a minimum of 4 d at 57.2°C or until they

reached a constant dry weight. After drying, the samples were reweighed to obtain the dry weight and calculate dry matter percentage for each plant part.

To determine the amount of endophytic *B. bassiana*, the stalk surface was disinfected and split longitudinally, from the base to the sixth node (Lewis and Bing, 1991). Nodes were excised using sterile techniques and placed on an agar media favoring the growth of *B. bassiana* (Doberski and Tribe, 1980). Plates containing the plant material were allowed to incubate in total darkness at 28°C for 10 d at which point the plant tissue was examined for *B. bassiana* growth.

Statistical Analysis

Greenhouse data were analyzed as repeated measures using GLM with the line of corn as the whole plot and seed treatment as the split plot. The effects of each seed treatment on germination, plant growth, and endophyte formation were analyzed separately for each line of corn so as not to attribute differences between seed treatments to differences between hybrids. Means were separated using Student's *t*-test at $P \leq 0.05$ (SAS Institute, 1995). Field data were analyzed using GLM with the line of corn and *B. bassiana* treatment as the whole plot and the harvests over time the split plot. Differences in overall whole-plant dry weight, sheath dry weight, leaf dry weight, stem dry weight, husk dry weight, ear dry weight, and the dry leaf-to-stem ratio were determined between *B. bassiana* application methods along with the hybrid \times *B. bassiana* treatment interactions. Means were separated using Student's *t*-test at $P \leq 0.05$ (SAS Institute, 1995).

RESULTS

1994 and 1995 Field Studies

Whorl-stage (V6) application alone of *B. bassiana* in 1994 resulted in significant differences in whole plot effects when the plants were sampled at V12 ($F = 60.51$; $df = 1, 3$; $P = 0.004$), with treated plants having significantly more endophytism ($\bar{X} = 36.9 \pm 10.6$, $N = 8$) than the untreated check ($\bar{X} = 0.0 \pm 0.0$, $N = 8$). This was not the case for plants sampled for endophytism at R1 (Treated- $\bar{X} = 25.0 \pm 10.5$, $N = 8$; Check- $\bar{X} = 17.5 \pm 6.1$, $N = 8$) or R6 (Treated- $\bar{X} = 82.5 \pm 7.0$, $N = 8$; Check- $\bar{X} = 47.5 \pm 13.1$, $N = 8$). Genetic makeup of the plant played no significant role in the levels of endophytism observed when sampled at V12 (Transgenic- $\bar{X} = 28.1 \pm 12.8$, $N = 8$; Isoline- $\bar{X} = 10.0 \pm$

4.4, $N = 8$), R1 (Transgenic- $\bar{X} = 21.9 \pm 9.9$, $N = 8$; Isoline- $\bar{X} = 20.6 \pm 7.3$, $N = 8$), or R6 (Transgenic- $\bar{X} = 72.5 \pm 9.2$, $N = 8$; Isoline- $\bar{X} = 57.5 \pm 14.4$, $N = 8$). There were no significant interactions in endophytism levels between *B. bassiana* application and the genetic makeup of the corn for samples taken at V12, R1, or R6 (Table 1). Results of pooling all sample dates resulted in the same trends; i.e., an overall significant difference between *B. bassiana* applications ($F = 10.70$; $df = 1, 3$; $P = 0.05$) and no significant difference between plant types or a plant type \times *B. bassiana* interaction.

Whorl-stage and pollen shed (R1) application of *B. bassiana* in 1994 did not result in significant differences in whole plot effects when the plants were sampled at R1 (Treated- $\bar{X} = 26.3 \pm 7.4$, $N = 8$; Check- $\bar{X} = 27.5 \pm 7.4$, $N = 8$), R6 (Treated- $\bar{X} = 69.4 \pm 11.3$, $N = 8$; Check- $\bar{X} = 63.8 \pm 6.5$, $N = 8$), or senescence (Treated- $\bar{X} = 74.4 \pm 7.0$, $N = 8$; Check- $\bar{X} = 58.2 \pm 12.5$, $N = 8$). Genetic makeup of the plant played no significant role in the levels of endophytism observed when samples were taken at R1 (Transgenic- $\bar{X} = 25.0 \pm 6.7$, $N = 8$; Isoline- $\bar{X} = 28.8 \pm 8.1$, $N = 8$), R6 (Transgenic- $\bar{X} = 62.5 \pm 11.6$, $N = 8$; Isoline- $\bar{X} = 70.6 \pm 5.7$, $N = 8$), or senescence (Transgenic- $\bar{X} = 62.5 \pm 12.3$, $N = 8$; Isoline- $\bar{X} = 70.0 \pm 8.2$, $N = 8$). There were no significant interactions in endophytism levels between the *B. bassiana* application and the genetic makeup of the corn for samples taken at R1, R6, or senescence (Table 1). Results of pooling all sample dates resulted in the same overall trends with no significant difference between *B. bassiana* applications, plant types, or a plant type \times *B. bassiana* interaction.

Application of *B. bassiana* at V6 alone in 1995 did not result in significant differences in when the plants were sampled at V12 (Treated- $\bar{X} = 14.3 \pm 3.4$, $N = 8$; Check- $\bar{X} = 17.5 \pm 19.8$, $N = 8$), R1 (Treated- $\bar{X} = 25.0 \pm 10.5$, $N = 8$; Check- $\bar{X} = 10.0 \pm 5.3$, $N = 8$), or R6 (Treated- $\bar{X} = 7.5 \pm 5.3$, $N = 8$; Check- $\bar{X} = 7.5 \pm 5.3$, $N = 8$). Genetic makeup of the plant played no significant role in the levels of endophytism observed when sampled at V12 (Transgenic- $\bar{X} = 17.1 \pm 4.9$, $N = 8$; Isoline- $\bar{X} = 15.0 \pm 6.3$, $N = 8$), R1 (Transgenic- $\bar{X} = 15.0 \pm 9.8$, $N = 8$; Isoline- $\bar{X} = 20.0 \pm 7.6$, $N = 8$), or R6 (Transgenic- $\bar{X} = 5.0 \pm 4.9$, $N = 8$;

Table 1. Mean (\pm SEM) percentage of plants with endophytic *B. bassiana* from the 1994 field studies.

Hybrid†	<i>B. bassiana</i> Treatment‡	Time of Application	Percentage of plants with an endophyte (\pm SEM)		
			(V12)§	(R1)§	(R6)§
Transgenic	Treated	V6	17.5 \pm 6.0a	25.0 \pm 17.7a	85.0 \pm 9.6a
Isoline	Treated	V6	56.3 \pm 15.7a	25.0 \pm 14.4a	80.0 \pm 11.5a
Transgenic	Control	V6	0.0 \pm 0.0a	18.8 \pm 11.9a	60.0 \pm 14.1a
Isoline	Control	V6	0.0 \pm 0.0a	16.3 \pm 5.5a	35.0 \pm 22.2a
			Percentage of plants with an endophyte (\pm SEM)		
			(R1)§	(R6)§	(Senescence)§
Transgenic	Treated	V6 and R1	25.0 \pm 10.2a	55.0 \pm 5.0a	46.3 \pm 21.7a
Isoline	Treated	V6 and R1	30.0 \pm 12.2a	72.5 \pm 11.1a	70.0 \pm 12.9a
Transgenic	Control	V6 and R1	25.0 \pm 10.2a	70.0 \pm 23.8a	78.8 \pm 8.3a
Isoline	Control	V6 and R1	27.5 \pm 12.3a	68.8 \pm 5.2a	70.0 \pm 12.2a

† Hybrid: Transgenic - Jeremy event 802; Isoline - B73 \times Mo17.

‡ *B. bassiana* Treatment: Treated - ARSEF 3113 at 1×10^6 conidia per plant; control - untreated.

§ Means from the same growth stage and time of fungal application with the same letter are not significantly different $P \leq 0.05$ (SAS Institute, 1995).

Table 2. Mean (\pm SEM) percentage of plants with endophytic *B. bassiana* from the 1995 field studies.

Hybrid†	<i>B. bassiana</i> Treatment‡	Time of Application	Percentage of plants with an endophyte (\pm SEM)		
			(V12)§	(R1)§	(R6)§
Transgenic	Treated	V6	15.0 \pm 9.6a	5.0 \pm 5.0a	10.0 \pm 10.0a
Isoline	Treated	V6	20.0 \pm 11.5a	15.0 \pm 9.6a	5.0 \pm 5.0a
Transgenic	Control	V6	20.0 \pm 10.0a	25.0 \pm 18.9a	0.0 \pm 0.0a
Isoline	Control	V6	10.0 \pm 5.8a	25.0 \pm 12.3a	15.0 \pm 9.6a
			Percentage of plants with an endophyte (\pm SEM)		
			(R6)§	(Senescence)§	
Transgenic	Treated	R1	0.0 \pm 0.0a	40.0 \pm 18.3a	
Isoline	Treated	R1	50.0 \pm 10.0b	45.0 \pm 18.9a	
Transgenic	Control	R1	60.0 \pm 14.1b	45.0 \pm 20.6a	
Isoline	Control	R1	5.0 \pm 5.0a	30.0 \pm 10.0a	

† Hybrid: Transgenic - Jeremy event 802; Isoline - B73 \times Mo17.

‡ *B. bassiana* Treatment: Treated - ARSEF 3113 at 1×10^6 conidia per plant; control - untreated.

§ Means from the same growth stage and time of fungal application with the same letter are not significantly different $P \leq 0.05$ (SAS Institute, 1995).

Isoline- $\bar{X} = 10.0 \pm 5.3$, $N = 8$). There were no significant interactions in endophytism levels between the *B. bassiana* application and the genetic makeup of the corn for samples taken at V12, R1, or R6 (Table 2). Results of pooling all sample dates for the V6 application resulted in the same trends with no overall significant difference between *B. bassiana* applications, plant types, or a plant type \times *B. bassiana* interaction.

Application of *B. bassiana* at R1 alone in 1995 did not result in a significant difference in endophytism levels when plants were sampled at R6 (Treated- $\bar{X} = 17.5 \pm 9.6$, $N = 8$; Check- $\bar{X} = 50.0 \pm 9.3$, $N = 8$), early senescence (Treated- $\bar{X} = 25.0 \pm 10.5$, $N = 8$; Check- $\bar{X} = 32.5 \pm 12.5$, $N = 8$), or dry down (Treated- $\bar{X} = 42.5 \pm 12.2$, $N = 8$; Check- $\bar{X} = 37.5 \pm 10.9$, $N = 8$). Genetic makeup of the plant also played no significant role in the levels of endophytism observed when samples were taken at R6 (Transgenic- $\bar{X} = 35.0 \pm 11.2$, $N = 8$; Isoline- $\bar{X} = 32.5 \pm 11.2$, $N = 8$), early senescence (Transgenic- $\bar{X} = 30.0 \pm 13.1$, $N = 8$; Isoline- $\bar{X} = 27.5 \pm 9.9$, $N = 8$), or dry down (Transgenic- $\bar{X} = 42.5 \pm 12.8$, $N = 8$; Isoline- $\bar{X} = 37.5 \pm 10.3$, $N = 8$). There were no significant interactions in endophytism levels between the *B. bassiana* application and the genetic makeup of the corn for samples taken at early senescence or dry down (Table 2). There was, however, a significant interaction between endophytism levels and genetic makeup of the plants for samples taken at early senescence ($F = 33.92$; $df = 1, 6$; $P = 0.001$), with the transgenic corn with no *B. bassiana* applied (6%) and the *B. bassiana* granules applied to the isoline (50%) having significantly higher endophytism levels than the plants subjected to the other two treatments (Table 2). Results of pooling all sample dates showed no significant difference between *B. bassiana* applications, or between plant types but a significant plant type \times *B. bassiana* interaction ($F = 6.68$; $df = 1, 6$; $P = 0.04$).

1997 Greenhouse Studies

There were no significant differences in the total number of germinated seeds between treatments in Pioneer 3489, Pioneer 34R06, Ciba 4494, or Ciba Max 454 after

five weeks (Table 3). There were significant differences in plant growth due to seed treatment within three of the hybrids (Table 3). Endophytism levels within each hybrid did not vary significantly between treatments. No disease causing organisms were observed on roots in any of the treatments.

1997 Field Studies

There were no differences in whole plant dry weight, dry sheath weight, dry leaf weight, dry stem weight, dry husk weight, dry ear weight, or in the dry leaf-to-stem ratio between *B. bassiana* treatments. There were also no hybrid \times *B. bassiana* treatment interactions in whole plant dry weight, dry sheath weight, dry leaf weight, dry stem weight, dry husk weight, or in the dry leaf-to-stem ratio. There was a significant hybrid \times *B. bassiana* treatment interaction in dry ear weight when analyzed over all harvest dates ($F = 4.54$; $df = 6, 18$; $P = 0.006$). However, when the plants reached physiological maturity at harvest dates seven and eight, there was no longer a significant interaction between *B. bassiana* treatment and hybrid when harvest dates seven and eight are combined.

Table 3. Mean (\pm SEM) plant height (cm) at week three and the mean (\pm SEM) number of 10 seeds germinated from four corn hybrids with the following treatments in the 1997 greenhouse study.

Treatment†	Pioneer		Ciba	
	3489	34R06	4494	Max 454
B.b.				
Plant height	39.8 \pm 2.3a‡	40.7 \pm 0.9a	38.3 \pm 2.1b	36.2 \pm 2.3b
Seeds germinated	9.0 \pm 0.41a‡	10.0 \pm 0.0a	9.3 \pm 0.48a	8.8 \pm 1.3a
Unsoaked				
Plant height	40.9 \pm 1.5a	35.1 \pm 2.6a	40.8 \pm 1.8b	43.7 \pm 1.6a
Seeds germinated	9.8 \pm 0.3a	9.5 \pm 0.3a	9.5 \pm 0.5a	9.8 \pm 0.3a
Water				
Plant height	35.3 \pm 2.1a	36.2 \pm 2.0a	45.8 \pm 1.1a	36.3 \pm 2.3b
Seeds germinated	9.0 \pm 0.71a	9.0 \pm 0.6a	10.0 \pm 0.0a	9.3 \pm 0.8a

† Treatments: B.b. - seeds soaked in 0.1% Tween 80 suspension of *B. bassiana* (2×10^{10} conidia per ml) for 10 minutes; unsoaked - seeds not soaked and planted directly out of seed bag; water - seeds soaked in a sterile 0.1% tween suspension for 10 minutes.

‡ Treatment means of plant height and seed germination from a hybrid with the same letter are not significantly different $P \leq 0.05$.

Table 4. Mean (\pm SEM) percentage of plants with endophytic *B. bassiana* from the 1997 field studies summed over hybrids.

Treatment†	% of plants with an endophyte
Seed	13.7 \pm 2.4a‡
Leaf	11.7 \pm 2.0ab
Control	8.2 \pm 1.9b

† Treatments: Seed - seeds soaked for three minutes in a 0.1% tween suspension of *B. bassiana* (2.1×10^8 conidia per ml); Leaf - 8.8×10^7 conidia applied to whorl of V7-stage corn; control - untreated control.

‡ Means in the same column with the same letter are not significantly different $P \leq 0.05$.

The percentage of nodes sampled with endophytic *B. bassiana* was not significantly different between hybrids, but was significantly different between *B. bassiana* application methods ($F = 6.58$; $df = 2, 6$; $P = 0.03$). Seed treatment with *B. bassiana* resulted in a significantly larger percentage of the plants forming an endophytic relationship compared with untreated plants (Table 4). The overall percentage of plants with endophytic *B. bassiana* among all hybrids increased steadily from no endophyte present on the first two harvest dates to >35% of all of the plants collected endophytic at harvest eight. The percentage of endophytism increased in a similar fashion for each hybrid individually, although there was a drop during the time of seed formation (Fig. 1). Levels of endophytism did not drop at the same harvest date, but a decline occurred during one reproductive stage or another in all hybrids. The decline occurred during harvest 6 for Ciba 4494 (R5, dent stage), harvest 7 for Max 454 (R5, dent stage), harvest 5 for Pioneer 3489 (R4, dough stage), and harvest 4 for Pioneer 34R06 (R3, milk stage).

DISCUSSION

Results of studies with transgenic plants in 1994–1997 consistently showed that the incorporation of a gene for the production of the toxin from *B. thuringiensis* had no effect on the plants ability to form an endophyte with *B. bassiana*. The significant differences in endophytism observed in these studies occurred as a result of plots receiving an application of *B. bassiana* at V6 stage in 1994 having significantly higher endophytism levels regardless of plant type. There were unusual interactions in 1995 with the untreated transgenic plants ($\bar{X} = 60.0 \pm 14.14$, $N = 4$) and the treated isoline ($\bar{X} = 50.0 \pm 10.0$, $N = 4$) having significantly higher percentage endophytism levels than the treated transgenics and the untreated isoline. Although the differences were significant, they show that transgenic plants are as well suited to form an endophytic relationship with *B. bassiana* as nontransgenic corn. While *B. bassiana* application did not generally increase the percentage of plants with an endophyte, the intensity of the endophytic relationship may have been greater in the treated than the untreated plants (Bing, 1990).

Greenhouse studies demonstrated that seed treatment with *B. bassiana* had no detrimental impact on seed germination or seedling growth, and did not result in the formation of root disease. These results were confirmed when plants were allowed to grow to maturity under field conditions with no significant differences in plant growth between *B. bassiana* treatments for any of the hybrids. There were no ill effects of a *B. bassiana* seed treatment on transgenic corn or their isoline. Seed treatment did result in a significant increase in endo-

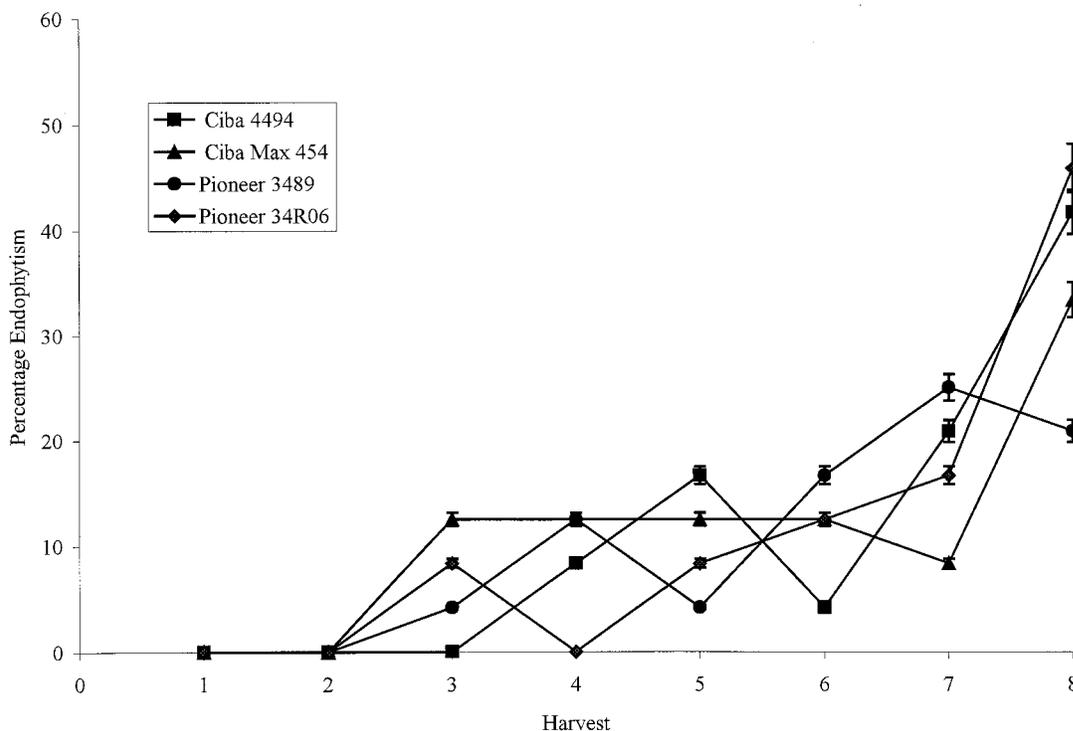


Fig. 1. Percentage endophytism for each hybrid over the course of the growing season in 1997 field studies. Hybrids: ■ = Ciba 4494; ▲ = Ciba Max 454; ● = Pioneer 3489; ◆ = Pioneer 34R06.

phytism. The reason for the lack of endophytism in the seeds treated with *B. bassiana* in the greenhouse study are unclear.

No significant differences were observed between hybrids in their ability to form an endophytic relationship with *B. bassiana* in 1997. Differences in plant growth on a dry-weight basis between hybrids were found but will not be discussed because they represent differences between hybrids only and not treatment effects. The proportion of plants exhibiting an endophytic relationship over all hybrids increased steadily from the third harvest date. The same pattern was true for each hybrid individually although a drop in the proportion of plants exhibiting an endophytic relationship occurred during ear set for each hybrid before endophytism levels rebounded (Fig. 1). A similar trend in a nontransgenic hybrid was reported by Bing and Lewis (1992a,b). When plants reached the point of physiological maturity the number of plants containing an endophyte increased dramatically. The reason for the dip in endophytism levels during the reproductive stages of the plant is unknown. Most likely the endophyte is still present, but not located in the nodal tissue where the samples are taken. *Beauveria bassiana* maybe mobile in the xylem or phloem tissue of the plant and is shunted to the ear along with many of the nutrients that the plant is using to produce grain.

Over the entire growing season, application of *B. bassiana* either as a seed or foliar application did not result in significant differences in dry matter accumulation. The corn plant grows normally and shows no signs that *B. bassiana* is acting as a plant pathogen. The corn plant benefits by having an insect defense system, and *B. bassiana* is able to survive in the moist, humid, nutrient-rich environment within the corn plant.

Results of this research are significant in terms of resistance management and concerns of transgenic plants forming a "biological control vacuum," i.e., incorporation of the gene for production of the toxin from *B. thuringiensis* into the corn plant had no adverse effect on the ability of *B. bassiana* to form an endophytic relationship. As a result *B. bassiana* inoculum loads will most likely remain stable in the environment even if large acreages of transgenic corn are planted. If *O. nubilalis* resistance to Bt occurs, naturally occurring or applied *B. bassiana* could be a significant tool in managing resistant *O. nubilalis* populations.

ACKNOWLEDGMENTS

This article is a joint contribution: USDA–ARS and Journal Paper No. J-18799 of the Iowa Agriculture and Home Eco-

nomics Experiment Station, Ames, Iowa. Project No. 3543. Names are necessary to report factually on available data; however, neither the USDA nor Iowa State University guarantees or warrants the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may be suitable.

REFERENCES

- Bartlett, K., and C. Lefebvre. 1934. Field experiments with *Beauveria bassiana* (Bals.) Vuill., a fungus attacking the European corn borer. *J. Econ. Entomol.* 27:1147–1157.
- Beall, G., G.M. Stirrett, and I.L. Connors. 1939. A field experiment on the control of the European corn borer, *Pyrausta nubilalis* Hübn., by *Beauveria bassiana* Vuill. *Sci. Agric.* 19:531–534.
- Bing, L.A. 1990. The entomopathogen, *Beauveria bassiana* (Balsamo) Vuillemin: Colonization and movement in *Zea mays* L. and potential for *Ostrinia nubilalis* (Hübner) suppression. M.S. thesis, Iowa State University, Ames.
- Bing, L.A., and L.C. Lewis. 1992a. Endophytic *Beauveria bassiana* (Balsamo) Vuillemin in corn: the influence of the plant growth stage and *Ostrinia nubilalis* (Hübner). *Biocontrol Sci. Technol.* 2:39–47.
- Bing, L.A., and L.C. Lewis. 1992b. Temporal relationships between *Zea mays*, *Ostrinia nubilalis* (Lep.: Pyralidae) and endophytic *Beauveria bassiana*. *Entomophaga* 37:525–536.
- Davis, F.M., and T.G. Oswalt. 1979. Hand inoculator for dispensing lepidopterous larvae. USDA-Science and Education Administration. *Advances in Agricultural Technology AAT-S-9.*
- Doberski, J.W., and H.T. Tribe. 1980. Isolation of entomogenous fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae*. *Trans. Br. Mycol. Soc.* 74:95–100.
- Lewis, L.C., and L.A. Bing. 1991. *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Balsamo) Vuillemin for European corn borer control: Potential for immediate and season-long suppression. *Can. Entomol.* 123:387–393.
- Lewis, L.C., and J.E. Cossentine. 1986. Season long intraplant epizootics of entomopathogens, *Beauveria bassiana* and *Nosema pyrausta*, in a corn agroecosystem. *Entomophaga* 31:363–369.
- Marcandier, S., and G. Riba. 1986. Endemisme de la mycose a *Beauveria bassiana* (Bals.) Vuillemin dans les populations géographiques de la pyrale du maïs, *Ostrinia nubilalis* (Hübner). *Acta Ecol. Applic.* 7:39–46.
- Riba, G. 1984. Application en essais parcellaires de plein champ d'un mutant artificiel du champignon entomopathogène *Beauveria bassiana* [Hyphomycete] contre la pyrale du maïs, *Ostrinia nubilalis* [Lep.: Pyralidae]. *Entomophaga* 29:41–48.
- Ritchie, S.W., J.J. Hanway, and G.O. Benson. 1997. How a corn plant develops. Iowa State Univ. *Sci. Tech. Spec. Rep.* 48.
- SAS Institute, Inc. 1995. JMP statistics and graphic guide, version 3.1. SAS Institute, Inc., Cary, NC.
- Stirrett, G.M., G. Beall, and M. Timonin. 1937. A field experiment on the control of the European corn borer, *Pyrausta nubilalis* Hübn., by *Beauveria bassiana* Vuill. *Sci. Agric.* 17:587–591.
- Wagner, B.L., and L.C. Lewis. 2000. Colonization of corn, *Zea mays*, by the entomopathogenic fungus *Beauveria bassiana*. *Appl. Environ. Microbiol.* 66:3468–3473.
- York, G.T. 1958. Field tests with the fungus *Beauveria* sp. for control of the European corn borer. *Iowa State J. Sci.* 33:123–129.