Effect of Root Feeding by Striped Cucumber Beetle Larvae on the Incidence and Severity of Fusarium Wilt of Muskmelon

R. X. Latin and G. L. Reed

Assistant professor, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907 and research entomologist, Fruit and Vegetable Insects Research Laboratory, USDA, ARS, Vincennes, IN 47591 and Department of Entomology, Purdue University.

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ABSTRACT

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Incidence and severity of Fusarium wilt were evaluated on muskmelon seedlings grown in a soil-less substrate infested with microconidia of *Fusarium oxysporum* f. sp. *melonis* (FOM) and eggs of the striped cucumber beetle (*Acalymma vittatum*) (STCB). Inoculum levels of FOM were 0, 10^3, 10^5, and 10^7 microconidia per seedling and STCB infestation levels were 0, 5, 10, and 20 eggs per seedling. Incidence and severity of Fusarium wilt were significantly greater in treatments that included STCB infestations of 5, 10, and 20 eggs per plant. In another experiment, six levels of microconidia of FOM were applied to seedling substrate, either infested with six eggs of STCB per seedling or uninfested. Regression lines describing the relationship between log inoculum dose and log disease incidence for plants infested and uninfested with STCB eggs were unequal. Disease incidence was observed at a lower inoculum level where treatment included STCB infestation. The increased incidence and severity of Fusarium wilt due to root-feeding by STCB larvae provide the rationale for controlling root-feeding stages of insects on muskmelon.

Fusarium wilt, caused by *Fusarium oxysporum* Schlect. emend Snyder and Hansen f. sp. *melonis* Leach and Currence (FOM), is an economically important disease of muskmelon (*Cucumis melo* var. *reticulatus*) in southwestern Indiana. The most commonly grown commercial cultivars are not resistant to Fusarium wilt. Resistant cultivars are available but either are not suited to production in this area, or are not preferred at local or regional markets.

Successful inoculation of muskmelon seedlings with *F. oxysporum* f. sp. *melonis* (FOM) is facilitated by prior wounding of plant roots. For example, Bergeson (1) demonstrated that root injury from nematodes increased disease expression in melon cultivars inoculated with FOM. Wensley and McKeen (14) wounded roots by lifting individual seedlings from seed beds before inoculation while testing FOM isolates for pathogenicity. Uprooting individual seedlings prior to inoculation is a standard practice for determination of wilt resistance in melon genotypes (5,15).

An additional source of root injury is the feeding larvae of the striped cucumber beetle (*Acalymma vittatum* (F.)), the most common insect pest of cucurbits in the midwest (3). Striped cucumber beetles (STCB) overwinter as adults and lay eggs in the soil around young cucurbit plants in the spring (4). The rootworm larvae of STCB feed on roots and stems (6) and may cause serious damage (Fig. 1) There are two generations of STCB each year in Indiana with peak oviposition periods in June and August. Populations of STCB eggs in commercial muskmelon fields can approach 30 eggs per plant in spring and summer generations (10).

The STCB adults predispose muskmelons to infection by the gummy stem blight pathogen (*Mycosphaerella melonis*) and serve as vectors of the causal fungus (2). Adult STCB are also vectors of *Erwinia tracheophila*, the muskmelon bacterial wilt pathogen (9). The effect of this insect on Fusarium wilt has not been investigated.

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The objective of this research was to determine the effect of root feeding by STCB larvae on the incidence and severity of Fusarium wilt in muskmelon.

MATERIALS AND METHODS

Seedlings of the wilt-susceptible cultivar Gold Star (Jos. Harris Co. Inc., Rochester, NY) were grown in a soil-less, peat-Perlite growth substrate (Peat-lite; W. R. Grace & Co., Cambridge, MA) in plastic transplanting trays (Growing Systems, Inc., Milwaukee, WI) on a bench in the greenhouse where temperature was maintained at 24 ±2 C. Seedlings were raised at the USDA Fruit and Vegetable Insects Research Laboratory, Vincennes, IN, and then transported to West Lafayette, IN, after infestation with STCB eggs. Each seedling occupied a single cell (3.5-cm diameter and 3.8-cm deep) in the trays. The experimental design was a randomized complete block with a factorial arrangement of treatments. Treatments included four levels of inoculum of FOM (including no inoculum) and four infestation levels of STCB eggs (including no eggs). The 16 STCB-FOM treatment combinations were tested with 12 replicate seedlings per combination and the experiment was repeated four times.

Inoculum was prepared from a single spore isolate (828B) of FOM race 2 (11) originally obtained in 1982 from infected plants in southwestern Indiana. Stock cultures were transferred to acidified potato-dextrose agar in 9-cm-diameter petri dishes. After four days, 3-mm-diameter agar plugs from the margins of new colonies were transferred to 500-ml Erlenmeyer flasks (one plug per flask) containing 100 ml of potato-dextrose broth (10 g/L). The flasks were plugged with nonabsorbent cotton and placed on a shaker for 5 days at 23 C. The resulting spore suspension was strained through cheesecloth, bulked in a large flask, and diluted with distilled water to 10^7 microconidia per milliliter. This suspension was diluted with distilled water to obtain inoculum concentrations of 10^5 and 10^3 microconidia per milliliter. A hemacytometer was used to quantify spore suspensions. Inoculum levels of FOM were designated F0, F3, F5, and F7 for suspensions containing 0, 10^3, 10^5, and 10^7 microconidia per milliliter, respectively.

The eggs of STCB were from a colony collected in southwestern Indiana which was started during 1981 and reared for 11

generations on squash seedlings (10). Eggs were applied as an agar suspension (8) to the substrate around each seedling. STCB infestation levels were designated B0, B5, B10, and B20 for infestations of 0, 5, 10, and 20 eggs per seedling, respectively. Eggs were timed to hatch 12 hr after infestation.

Seedlings emerged 5 days after planting. At that time, the substrate around each seedling was infested with STCB eggs. Microconidia of FOM (5.2 ml of each suspension per seedling) were dispensed to the seedling substrate 24 hr after its infestation with STCB eggs.

Thirteen days after infestation with microconidia of FOM, each seedling was assessed for Fusarium wilt severity. Severity scores were 0 = no symptoms, 1 = chlorosis or wilting of cotyledons, 2 = chlorosis of true leaves, and 3 = dead seedling. Disease incidence was represented by the proportion of seedlings for each treatment combination of STCB-FOM that received a score greater than zero. Incidence and severity data were subjected to analysis of variance and means were separated by using an orthogonal comparison procedure (12, especially pages 177–181). Independent contrasts between means of FOM level included F7 versus F5, and F3 versus F5 and F7. Independent contrasts between STCB-level means included B20 versus B10, B5 versus B10 and B20 and, B0 versus others. Orthogonal comparisons were also used to identify significant interactions of STCB and FOM.

The disease response of seedlings to six concentrations of microconidia of FOM in the presence and absence of STCB larvae also was examined. Gold Star muskmelon seedlings were raised in plastic trays. Because transportation of seedlings in the first experiment was suspected to have caused a higher frequency of wilt than anticipated, seedlings for this experiment were raised at West Lafayette, IN, and STCB eggs were transported for the infestation. Six eggs of STCB were placed on small (25 mm^2) pieces of moistened filter paper with a camel's-hair brush. The pieces were inverted and placed on the substrate surface for each plant to be infested. The eggs were from a colony reared for nine generations on squash seedlings. Eggs were timed to hatch 12 hr after infestation; egg hatch averaged 85%. Inoculum of FOM was prepared as described above. Inoculum concentrations included 0, 10^3, 10^4, 10^5, 10^6, and 10^7 microconidia per milliliter. Two milliliters of each inoculum suspension were pipetted into the substrate around each seedling 24 hr after infestation with STCB eggs. Trays were placed in a greenhouse maintained at 24 ±2 C and seedlings were evaluated for Fusarium wilt incidence 13 days after infestation with inoculum of FOM.

Disease incidence proportions were estimated as $I = (x + 0.5) \cdot (N + 1)^{-1}$ in which x = the number of diseased individuals, and N = the number of observations per replication. This represents a slightly biased estimate of disease incidence but allows treatments which resulted in zero disease for one or two repetitions to be included in regression analyses. Data from treatments which resulted in zero disease incidence for three repetitions were not included in the analyses.

Log-transformed disease incidence data for STCB-infested and uninfested treatments were regressed against log-transformed inoculum concentrations of FOM. A general linear test approach and confidence interval estimation were used to compare regression lines and parameters, respectively (7, especially pages 160-167).

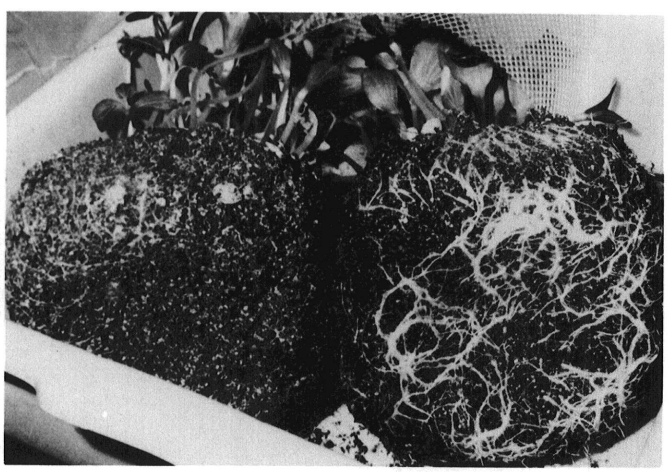

Fig. 1. Comparison of root growth of cucurbits grown in a peat-perlite substrate in pots infested (left) and uninfested (right) with larvae of the striped cucumber beetle .

RESULTS

Incidence and severity of Fusarium wilt increased with increasing concentrations of inoculum of FOM (Figs. 2 and 3). Analyses of variance showed that highly significant increases in disease resulted from increasing levels of inoculum of FOM over all levels of STCB infestation (Table 1). Disease incidence and severity data for treatments not inoculated with microconidia of FOM (F0) were not included in the analyses of variance. Such treatments resulted in zero wilt and would have zero variance. If included in the analyses, the error mean square would be artificially low and could result in false identification of significant effects. Therefore, only three inoculum levels of FOM (F3, F5, and F7) were included in the statistical analyses with four levels of STCB infestation. Single-degree-of-freedom contrasts among levels of STCB infestation identified highly significant differences in wilt incidence and severity between uninfested and infested treatments (B0 versus

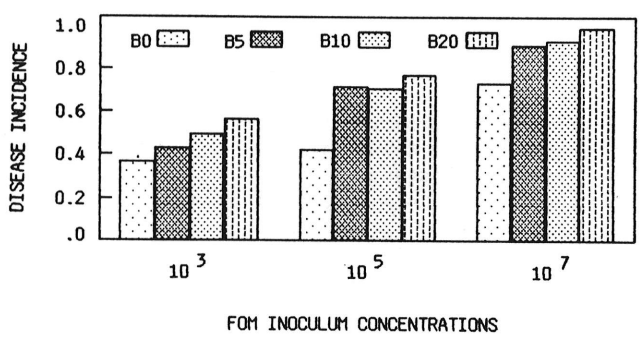

Fig. 2. Fusarium wilt incidence for four levels of infestation with striped cucumber beetle (STCB) larvae and three concentrations of *Fusarium oxysporum* f. sp. *melonis* (FOM) microconidia. Incidence is expressed as the proportion of seedlings exhibiting wilt symptoms. No disease was observed where the inoculum concentration of FOM was 0. Infestation levels of STCB are designated as B0, B5, B10, and B20 for 0, 5, 10, and 20 larvae, respectively, per seedling,

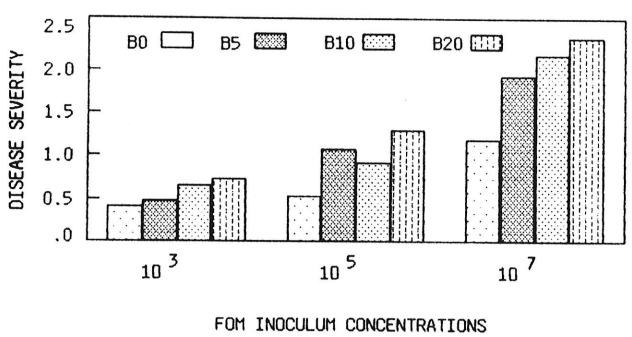

Fig. 3. Fusarium wilt severity for four levels of infestation of striped cucumber beetle (STCB) larvae and three concentrations of microconidia of *Fusarium oxysporum* f. sp. *melonis* (FOM). Severity scores were: 0 = no symptoms, 1 = wilted or chlorotic cotyledons, 2 wilted or chlorotic true leaves, and 3 = dead seedling. No disease was observed where the inoculum concentration of FOM was 0. Levels of STCB are designated as B0, B5, B10, and B20 for infestations with 0, 5, 10, and 20 larvae per seedling, respectively.

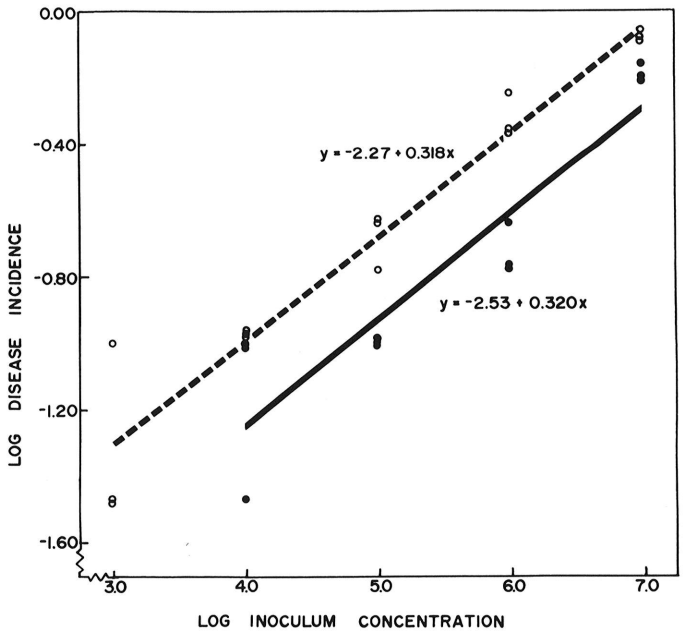

Fig. 4. Regression of log disease incidence on log inoculum concentration for treatments infested (- - - - -) and uninfested (——) with striped cucumber beetle larvae. The *y*-intercept values were significantly different and the slopes were equal.

others) (F = 49.80 and F = 42.98 for incidence and severity, respectively), but revealed no real differences between infestation levels B20, B10, and B5 (Table 1).

The mean FOM × STCB interaction for disease incidence was not statistically significant (F = 1.35) (Table 1). However, the "F7, F5 versus F3 × B0 versus others" contrast was significant (F = 4.45) (Table 1). This demonstrates that real differences in wilt incidence were observed between STCB-infested (B20, B10, and B5) and uninfested (B0) treatments, and that incidence increased significantly at inoculum levels F7 and F5 of FOM (Fig. 2).

Analysis of variance for wilt severity data resulted in a highly significant FOM × STCB interaction (F = 3.08) (Table 1). Further partitioning of sums of squares into independent comparisons showed that the greatest contribution to the interaction variance was made by the "F7, F5 versus F3 × B0 versus others" contrast (F = 11.30) (Table 1). This describes significantly greater wilt severities in Fig. 3 for STCB-infested treatments (B20, B10, and B5) than in uninfested treatments (B0), especially at higher levels of inoculum of FOM (F7 and F5).

For the experiment which included two levels of STCB infestation (infested and uninfested) and six inoculum concentrations of FOM (0, 10^3, 10^4, 10^5, 10^6, and 10^7), no disease resulted at the zero level of inoculum FOM. Mean disease incidence for STCB-infested treatments ranged from 0.05 to 0.85 for inoculum concentrations 10^3–10^7 of FOM. No disease occurred in uninfested treatments at the 10^3 inoculum level of FOM. Mean disease incidence for STCB-uninfested treatments ranged from 0.07 to 0.65 for inoculum concentrations 10^4–10^7 of FOM.

Regression lines describing disease response to inoculum levels of FOM for STCB-infested and -uninfested treatments resulted in a good linear fit and an acceptable residual pattern after log transformation of the variables. Coefficients of determination for regression lines representing STCB-infested and -uninfested treatments were R^2 = 93.9% and R^2 = 84.4%, respectively. The log-log transformed inoculum concentration-disease incidence relationship differed between STCB-infested and -uninfested treatments (Fig. 4). Slopes of regression lines (0.318 and 0.320) for STCB-infested and -uninfested treatments, respectively) were not significantly different. The intercept value for STCB-infested treatments (−2.27) was significantly greater than that of the uninfested treatment intercept (−2.53).

TABLE 1. Analysis of variance[a] for Fusarium wilt incidence and severity assessments on seedlings of muskmelon cultivar Gold Star treated with four levels of striped cucumber beetle (STCB) egg infestation and with three levels of inoculum of *Fusarium oxysporum* f. sp. *melonis* (FOM)

		Incidence		Severity	
Source of variation	df[b]	MS	*F*	MS	*F*
FOM	2	0.752	99.08**	7.59	118.09**
F7 vs F5[c]	1	0.495	65.22**	7.02	110.03**
F7, F5 vs F3	1	1.009	132.94**	8.15	127.74**
STCB	3	0.143	18.84**	1.03	16.18**
B20 vs B10[d]	1	0.025	3.29	0.20	3.20
B20, B10 vs B5	1	0.024	3.16	0.15	2.38
B0 vs others	1	0.378	49.80**	2.74	42.98**
FOM × STCB	6	0.010	1.35	0.19	3.08**
F7 vs F5	3	0.006	0.77	0.12	1.91
F7 vs F5 × B20 vs B10	1	0.001	0.16	0.07	1.10
F7 vs F5 × B20, B10 vs B5	1	0.003	0.36	0.06	0.88
F7 vs F5 × B0 vs others	1	0.014	1.78	0.24	3.75
F7, F5 vs F3 × STCB	3	0.015	1.93	0.27	4.25**
F7, F5 vs F3 × B20 vs B10	1	0.001	0.16	0.09	1.39
F7, F5 vs F3 × B20, B10 vs B5	1	0.009	1.19	0.01	0.07
F7, F5 vs F3 × B0 vs others	1	0.034	4.45*	0.72	11.30**
Error	36	0.0076		0.0638	

[a] Asterisks: * significant F-test, P = 0.05; and ** highly significant F-test, P = 0.01.
[b] df = degrees of freedom and MS = mean square.
[c] Levels of FOM inoculum were designated as F7, F5, and F3 for inoculum concentrations of 10^7, 10^5, and 10^3 microconidia per milliliter, respectively.
[d] Levels of STCB infestation were designated as B20, B10, B5, and B0 for infestations of 20, 10, 5, and 0 eggs per plant, respectively.

DISCUSSION

Injury sustained by root-feeding of STCB larvae significantly increased both the incidence and severity of Fusarium wilt development. This corresponds to reports of increased Fusarium wilt on plants injured by nematode feeding (1,13). The increases reported here were especially great where inoculum concentrations of FOM were high (10^5 and 10^7) (Figs. 2 and 3). This suggests that larger populations of FOM might be better able to take advantage of the effects of larval feeding. Root-feeding by beetle larvae also lowered the apparent threshold for infection by FOM as determined by symptom expression. The lowest inoculum concentration at which disease symptoms were observed was 10^4 with FOM alone, but was 10^3 for STCB-infested treatments (Fig. 4). Assuming that increased wilt incidence results from increased root wounding, it is possible that other root-feeding insects such as the spotted cucumber beetle (*Diabrotica undecimpunctata howardi* (Barber)), the banded cucumber beetle (*Diabrotica baleteata* (LeConte)), and the western striped cucumber beetle (*Acalymma trivittata* (Mann)) as well as tillage practices might also increase disease development.

The demonstration that root-feeding of the STCB larvae may increase the incidence and severity of Fusarium wilt of this crop places increased emphasis on the effective management of larval and adult stages of this insect. At present, the adult stage is controlled with repeated applications of foliar insecticides to reduce the incidence of bacterial wilt of muskmelon and other susceptible cucurbits. Incorporation of a systemic insecticide into soil at transplanting or when plastic mulch is applied should inhibit most beetle larvae feeding. These results provide further reason to control striped cucumber beetles on muskmelon and suggest that protection against root-feeding larvae may reduce losses to Fusarium wilt.

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Resistance

Virus Content as an Index of Symptomatic Resistance to Barley Yellow Dwarf Virus in Cereals

Mani Skaria, Richard M. Lister, John E. Foster, and Gregory Shaner

First, second, and fourth authors, graduate research assistant, professor, and professor, respectively, Department of Botany and Plant Pathology; third author, associate professor, Department of Entomology and research entomologist USDA, ARS, Purdue University, West Lafayette, IN 47907, USA.

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ABSTRACT

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Barley yellow dwarf virus (BYDV) content in extracts from cereals grown in the greenhouse, growth chamber, or field was measured by enzyme-linked immunosorbent assay. The results showed that, for some of the virus/host combinations tested, symptomatic "resistance" to BYDV as previously determined by plant breeders was associated with reduced virus productivity in infected plants. Although this effect was cultivar-specific and virus isolate-specific, it could be a valuable adjunct in breeding for BYDV resistance and management in cereals and deserves more extensive investigation. Suggested procedures for this are outlined.

In breeding cereals for resistance to barley yellow dwarf virus (BYDV), selection is based on symptoms such as leaf discolorations, plant dwarfing, and yield reduction (13). However, these are often difficult to distinguish from other effects and to assess accurately. They may also vary with seasonal and environmental influences. Moreover, because of technical difficulties and the scale of selection programs, little attention has been given to possible differences in reactions to different BYDV isolates (14). There is a need for a more precisely defined basis for comparing the response of breeding lines to BYDV and following the genetics of resistance.

For some viruses, symptomatic resistance has been associated with reduced virus production and such correlation has been suggested for some BYDV/host combinations (6). Virus content may, therefore, provide a basis for assessing resistance to BYDV. It has also been suggested (6) that some cultivars symptomatically resistant to BYDV are relatively poor sources of virus for transmission by vectors because of reduced virus content. If so, reduced virus content is in itself an attribute worth selecting for in breeding programs.

Enzyme-linked immunosorbent assay (ELISA) now provides a convenient measure for BYDV in tissue extracts (5,7). We report here on the use of ELISA to quantify virus content over time for three different isolates of BYDV in cultivars of oats (*Avena sativa* L.), barley (*Hordeum vulgare* L. em. Boden), and wheat (*Triticum aestivum* L. em. Thell.) previously assessed by plant breeders as symptomatically "resistant" or "susceptible." We wished to see if virus content followed these characteristics.

MATERIALS AND METHODS

Virus isolates. Isolates of three of the BYDV types distinguished

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