

Effect of Leaf Miner Feeding Activity on the Incidence of *Alternaria* Leaf Blight Lesions on Muskmelon Leaves

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ABSTRACT

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In laboratory experiments, exposure of muskmelon (*Cucumis melo*) leaves to adult female leaf miners (*Liriomyza trifolii*) before inoculation with *Alternaria cucumerina* significantly increased the incidence of *Alternaria* leaf blight lesions. Number of lesions increased with the number of female *L. trifolii* present, exposure time, and increased leaf wetness hours. Linear regression analyses indicated a high degree of significance ($r^2 = 0.80$) when the number of leaf miner punctures per leaf was regressed on the number of lesions per leaf. Regression of the number of punctures on leaf wetness hours did not provide as good a fit for the data ($r^2 = 0.35$).

Alternaria leaf blight, incited by *Alternaria cucumerina* (Ellis & Everh.) J. A. Elliot, is an important disease of muskmelon, *Cucumis melo* L., in the southeastern United States (12). Early disease symptoms appear as yellow or brown flecks, about 0.5 mm in diameter, surrounded by light green halos. Lesions then develop distinct concentric zones as they enlarge to 5–20 mm and become light to dark brown in color (5). As older leaves die, developing fruit are exposed to direct sunlight resulting in loss of mature fruit attributable to sunscalding (2,7). Higher temperatures of exposed fruit cause increased respiration with the corresponding loss of soluble solid content and the resultant decrease in fruit quality (1).

Mycelia of *A. cucumerina* that survive in dead plant material provide the primary inoculum during the muskmelon season (2,5). Martin (8) and Van Haltern (15) suggest that planting muskmelon in fields previously containing diseased melons can lead to rapid disease development. Middleton and Whitaker (9), Jackson (5), and Van Haltern (15) also suggest that seedborne conidia are

important in early infection of cotyledon and crown leaves, as well as in introducing the pathogen into new areas. Thomas (12) noted that significant numbers of lesions were not present until at least 10 hr of continuous leaf wetness had occurred. Effects of insect or mechanical damage of leaf surfaces on the infection process are not currently known.

Liriomyza trifolii (Burgess) (Diptera: Agromyzidae) is a significant insect pest of muskmelon (3). Adult female leaf miners puncture the upper leaf surface with their ovipositor. These puncture sites are then used for either feeding or oviposition. The puncture wounds kill localized groups of cells, resulting in chlorotic depressions in the leaf (14), and reduce the photosynthetic capacity of the leaf (6,11). Wounds average 0.35 mm in diameter (10). Adult leaf miners have been associated with plant virus transmission because of their feeding habits (4,16). However, nothing is known about the effect of adult leaf miner feeding behavior on fungal disease incidence. The purpose of this study was to determine the effect of *L. trifolii* puncture wounds on the incidence of *A. cucumerina* lesions on muskmelon leaves.

MATERIALS AND METHODS

Effect of *L. trifolii* populations on infection of muskmelons by *A. cucumerina*. Seed of muskmelon cultivar Perlita were planted in Jiffy Mix in 5.0-cm-square

plastic pots in an insect-free greenhouse. When plants had developed to the two-expanded-leaf stage, they were brought into the laboratory. Groups of five muskmelon plants were placed into 46 × 76 × 46 cm wooden cages fitted with glass tops and muslin cloth-covered side and back openings for ventilation. *L. trifolii* adult females were released inside each cage at the density of either 0, 1, 5, or 10 per plant. For each female release density, exposure time was set at either 24, 48, or 72 hr, for a total of three cages (one per exposure time) for each population density.

After the designated exposure times, all plants were removed from the cages. The adaxial surfaces of leaves one and two were then inoculated with 5×10^3 conidia per milliliter of *A. cucumerina* with a DeVilbiss No. 15 spray atomizer. Inoculum was prepared as described by Thomas (13). Inoculated plants were then placed in a high humidity (100%) chamber for 18 hr. The plants were subsequently removed from the chamber, placed on laboratory benches under fluorescent lights, and monitored for development of lesions. After removal from the chamber, leaf miner punctures were counted on the second expanded leaf of each plant. Seven days after inoculation, the second expanded leaf was removed from each plant and the number of *Alternaria* leaf blight lesions was counted. The laboratory was maintained at 24 ± 2 C and $50 \pm 5\%$ RH, and 12:12 light-dark photoperiod. The experiment was repeated 10 times.

Effect of leaf wetness hours on infection of *L. trifolii*-punctured muskmelon by *A. cucumerina*. Muskmelon plants were grown as described earlier. When plants were brought into the laboratory, 20 were exposed to five *L. trifolii* females per plant (100/cage) for 24 hr in a 46 × 76 × 46 cm cage. After leaf miner exposure, plants were removed from the cage and inoculated with 5×10^3 conidia per milliliter of *A. cucumerina*. An additional 20 unexposed plants were also

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inoculated. A control group of 20 plants was not inoculated and was not exposed to leaf miners.

All plants were then placed into the high humidity chamber. Groups of five plants from each of the three treatment groups (punctured and inoculated, inoculated only, and control) were removed after either 2, 4, 8, or 16 hr. Plants were placed on laboratory benches under fluorescent lights, and *L. trifolii* puncture counts were made on the second expanded leaf of each plant. After 7 days, the number of Alternaria leaf blight lesions on the second expanded leaf of each plant was counted. The experiment was repeated six times.

Photo documentation of *A. cucumerina* infection. Eight Perlita plants were grown as described, and four of the plants were caged and exposed to 10 *L. trifolii* adult females per plant for 24 hr. Plants were then removed from the cage and all eight were inoculated with 5×10^3 conidia per milliliter of *A. cucumerina* as described. Plants were placed into the high humidity chamber for 18 hr. After initial removal of the plants from the chamber, two 1-cm-diameter disks were cut from the second expanded leaf of a single leaf miner punctured and non-punctured plant. These disks were then placed into separate jars containing a 50-50 (v/v) solution of 95% ethanol and glacial acetic acid. This procedure was repeated at 42, 66, 90, and 114 hr post-inoculation. During night hours, plants were returned to the high humidity chamber. Leaf disks were then stained with either cotton blue, acid fuchsin, or Calcofluor to examine the disks for histological evidence of the enhancement of infection after leaf miner exposure. Photographs were taken to document the infection process.

Statistical analysis. Means were calculated for all data and an analysis of variance was conducted where appropriate. Means were separated using Duncan's multiple range test. Regression analyses ($Y = a + bX$) of the length of leaf miner exposure times vs. the number of lesions and punctures per leaf, the number of female leaf miners per plant vs. the number of lesions per leaf, the number of punctures vs. the number of lesions per leaf, and the number of leaf wetness hours vs. the number of lesions per leaf were conducted.

RESULTS

Effect of *L. trifolii* populations on infection of muskmelon by *A. cucumerina*. Regression analysis showed that the mean number of Alternaria leaf blight lesions per leaf increased ($Y = -3.40 + 0.72X$, $r^2 = 0.073$, $F = 4.60$, $P > F = 0.036$) when inoculation was preceded by a 72-hr exposure period to *L. trifolii* females compared with 48- and 24-hr exposure periods (Table 1). Leaf miner puncturing per leaf also increased ($Y =$

$-21.97 + 1.15X$, $r^2 = 0.079$, $F = 5$, $P > F = 0.029$) as the exposure period increased (Table 1). Although both regression equations exhibited a poor data fit (as noted by the low r^2 values), a definite linear increase in lesion formation and leaf miner puncturing was clearly evident.

A linear regression analysis of the number of female leaf miners per plant vs. the number of leaf blight lesions per leaf indicated that lesions increased as the number of females per plant increased ($Y = 13.26 + 4.45X$, $r^2 = 0.114$, $F = 7.46$, $P > F = 0.008$) (Table 1). Seven to eight times more lesions per leaf were present on plants exposed to densities of 10 and five female *L. trifolii* than on plants that were not exposed to leaf miners. Plants exposed to one *L. trifolii* female per plant had about three times as many leaf blight lesions per leaf as plants not exposed to leaf miners.

A linear regression analysis of the number of punctures per leaf vs. the total number of leaf blight lesions per leaf resulted in a highly significant relationship ($Y = 9.32 + 0.48X$, $r^2 = 0.798$, $F = 277.24$, $P > F = 0.0001$). This indicates that as the number of punctures per leaf increases, the incidence of Alternaria leaf blight lesions increases. The number of female leaf miners per plant and the length of exposure of the plant to the female leaf miners were not as important in explaining increased lesion incidence. Only slight improvement in the regression equation ($Y = 3.60 + 0.48X_1 +$

$0.26X_2 + 0.11X_3$, $r^2 = 0.800$, $F = 90.90$, $P > F = 0.0001$) was achieved when regressing total number of leaf miner punctures per leaf, number of female leaf miners per plant, and length of exposure time on total number of lesions per leaf.

Effect of leaf wetness hours on infection of *L. trifolii*-punctured muskmelon by *A. cucumerina*. For each leaf wetness period, the mean number of Alternaria leaf blight lesions per leaf was greater ($P \leq 0.05$) on plants punctured by leaf miners than on leaves of plants not exposed to leaf miner activity (Table 2). Leaf miner punctures per leaf were also significantly greater ($P \leq 0.05$) for the same comparisons (Table 2). No lesions were present on plants that were neither inoculated nor punctured.

A linear regression analysis of the number of leaf wetness hours on the number of lesions formed per leaf on plants exposed to leaf miners did not give a clear linear relationship between the two variables. Regression statistics ($Y = -9.80 + 6.39X$, $r^2 = 0.35$, $F = 11.860$, $P > F = 0.0023$) indicated a poor fit of the data (as noted by the low r^2 value) in spite of a significant test value (F). However, as the data in Table 2 indicate, leaf blight lesions increased in number as leaf wetness hours increased. Lesion numbers were about three to eight times greater with a 16-hr exposure than on plants exposed to the other three leaf wetness periods. In addition, the number of lesions formed on leaves after 8 hr of wetness was two to three times greater

Table 1. Mean number of Alternaria leaf blight lesions and leaf miner punctures after exposure to *Liriomyza trifolii* females and effect of female density on lesions per leaf of muskmelon plants (10 tests)

Exposure duration (hr)	Effect of exposure time		Effect of female density	
	Lesions/leaf	Punctures/leaf	Females/plant	Lesions/leaf
72	46.7	65.0	10	46.6
48	21.7	24.1	5	41.8
24	16.8	10.0	1	18.8
			0	6.3

Table 2. Effect of leaf wetness hours and number of leaf miner punctures on the incidence of *Alternaria cucumerina* lesions on muskmelon leaves that were either exposed or not exposed to leaf miners before inoculation with the pathogen (six tests)

Leaf wetness (hr)	Treatment	Leaf miner punctures/leaf (mean no.)	Alternaria lesions/leaf (mean no.)
2	Punctured + inoculated	122 a ²	11 a
	Inoculated only	0 b	2 ab
	Control	0 b	0 b
4	Punctured + inoculated	130 a	14 a
	Inoculated only	0 b	3 b
	Control	0 b	0 b
8	Punctured + inoculated	130 a	30 a
	Inoculated only	0 b	18 ab
	Control	0 b	1 b
16	Punctured + inoculated	165 a	98 a
	Inoculated only	0 b	24 b
	Control	0 b	0 b

²Numbers in a column per hour of leaf wetness followed by the same letter are not significantly different according to Duncan's multiple range separation procedure ($P > 0.05$).

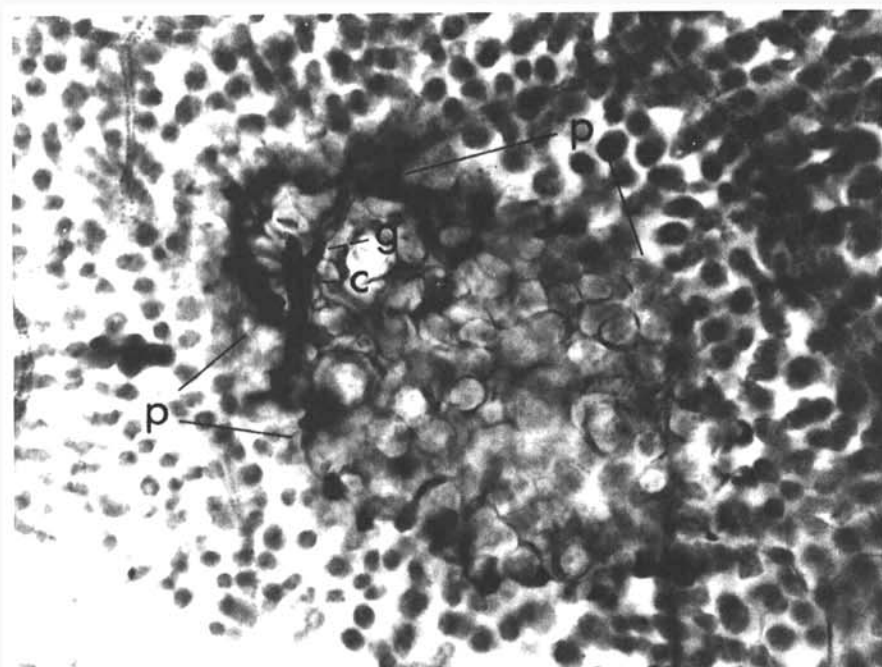


Fig. 1. Germ tube (g) from *Alternaria cucumerina* conidium (c) growing into leaf miner puncture (p) on adaxial surface of muskmelon leaf.

than the number of lesions formed on leaves in the 2- and 4-hr wetness periods. A similar linear relationship was noted with plants not exposed to *L. trifolii* but inoculated with *A. cucumerina* ($Y = -0.93 + 1.70X$, $r^2 = 0.17$, $F = 4.451$, $P > F = 0.0465$).

Photo documentation of *A. cucumerina* infection. Observations of the infection process by *A. cucumerina* on muskmelon leaves punctured by leaf miners did not indicate any taxic response of germ tubes toward the puncture sites. Germ tubes from the conidia ramified over the surface of the leaf. The degree of ramification increased as leaf wetness hours increased. When a germ tube traversed a puncture site, it grew into that site, infection was established, and a lesion formed (Fig. 1). Puncture sites appeared to merely present an opportunistic site for the pathogen to penetrate the leaf.

DISCUSSION

The data presented here clearly show the significance of *L. trifolii* puncture wounds on increased incidence of infection of *C. melo* by *A. cucumerina*. Our experiments indicate that any increase in the number of leaf miner punctures, caused by either increased numbers of *L. trifolii* adult females or by the length of time plants were exposed to *L. trifolii* adult females, results in significant increases in incidence of *A. cucumerina*

lesions. Although it is well established that muskmelon leaves can be infected by *A. cucumerina* without the presence of leaf miner punctures (5), these studies demonstrate that these punctures provide wound sites that enhance the infection process. The puncturing activity of *L. trifolii* females results in an increased incidence of *Alternaria* leaf blight lesions per unit of leaf area, thus increasing the severity of the disease.

Our data also confirm the role of leaf wetness hours in *Alternaria* leaf blight incidence on muskmelons and document the effect of leaf wetness hours in conjunction with *L. trifolii* infestations on muskmelon. As previously noted by Thomas (12), an increase in the number of *A. cucumerina* lesions on muskmelon leaves occurred after plants received a minimum of 10 hr of leaf wetness. The current studies demonstrated a substantial increase in lesion formation after 8 hr of leaf wetness on plants that were inoculated but not punctured. The addition of leaf miner punctures further increased the number of lesions formed, but 8-10 hr of leaf wetness were still necessary for significant levels of infection by *A. cucumerina*.

The management of *L. trifolii* populations in field-grown muskmelons is important to the maintenance of peak levels of crop productivity. Leaf miners alone may not always inflict severe

economic damage to a muskmelon crop, but the enhancing effect of leaf miner punctures on infection of leaves by *A. cucumerina* and the resultant increase in the severity of the disease would increase leaf loss with a corresponding decrease in marketable fruit attributable to sunscald. For this reason, muskmelon growers should practice management strategies that emphasize both insect and disease control. Monitoring leaf wetness hours and leaf miner activity in muskmelon fields should further aid growers in the management of *Alternaria* leaf blight.

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