Effect of Black Plastic Mulch and Nitrogen Side-Dressing on Verticillium Wilt of Eggplant

WADE H. ELMER, Assistant Plant Pathologist, and FRANCIS J. FERRANDINO, Assistant Scientist, Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, Box 1106, New Haven 06504

ABSTRACT

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Eggplants were grown in soils naturally infested with *Verticillium dahliae* and in soils that had been planted to rye for more than 15 yr. Plants were grown using four cultural treatments with or without black plastic mulch, and either side-dressed by hand with nitrogen (112 kg N/ha) 35–45 days after planting or not side-dressed. The date when visual symptoms first appeared, the percentage of chlorotic and/or wilted leaves, plant size, and yield were monitored over the season. Colonization of vascular tissue was assessed at final harvest by sampling nodes from the main stem of each plant and isolating *V. dahliae* on a selective medium. Fifty percent disease incidence occurred on mulched plants 13 days sooner than plants grown on bare ground; nitrogen supplements had no effect on 50% disease incidence. Area under the disease progress curve was not significantly affected by any treatment despite the early onset of visual symptoms in mulched plants. However, plants were larger in mulched plots. Systemic colonization of eggplant stems by *V. dahliae* was variable and not affected by any treatment or combination of treatments. Compared with yields from diseased plants that were not mulched and did not receive supplemental nitrogen, fruit weight was significantly greater when both treatments were used together. In 1990, Verticillium wilt caused a three-fold decrease in yield when compared with asymptomatic plants growing in adjacent plots that had previously been cropped to rye. These asymptomatic plants had greater yield than untreated plants only when they were mulched and not treated with nitrogen. Yield was correlated with the area under the plant growth curve ($r = 0.74$, $P = 0.001$) and less correlated with AUDPC ($r = 0.55$, $P = 0.01$).

Verticillium wilt is a destructive disease of eggplant (*Solanum melongena* L.) caused by *Verticillium dahliae* Kleb. Symptoms can appear anytime but commonly appear around anthesis and include unilateral wilting, chlorosis, and defoliation. It is not uncommon for growers to experience more than 50% loss in yield when symptoms appear early in the season on highly susceptible cultivars (17).

V. dahliae can survive as microsclerotia in soil for as long as 8 yr in the absence of susceptible hosts (16). Because all commercially grown cultivars of eggplants are susceptible (12), recommendations for disease management suggest 5- to 10-yr rotations, which are often not feasible, and fumigation, which is undesirable in environmentally sensitive areas. Although short-term (2–4 yr) rotation is still an important strategy in eggplant culture, rotating fields out of eggplant and other susceptible hosts for this period may not reduce populations of the fungus below thresholds necessary to prevent crop loss (17). In these situations, cultural controls, such as mulching, may provide some disease suppression (4,8).

Guba (4) found that eggplants grown in soil infested with *Verticillium* showed more vigor and initial growth if mulched with black paper mulching. The growth enhancement was attributed to the effect of increased soil temperature on plant growth. Interestingly, these larger plants grown with mulch also exhibited symptoms of Verticillium wilt earlier and more severely than did plants grown on bare ground. Although Guba (4) reported no yield data, Moorman (8) demonstrated that yield from black plastic-mulched eggplants was greater than that from plants grown on bare ground. His studies indicated that the apparent infection rate, defined as the slope of the transformed $\log(x/1 - x)$ proportion of diseased plants (x) over time, was unaffected by mulching.

Many conflicting reports have emerged as to the benefit of nitrogen applications in suppressing Verticillium wilt diseases (13). Sivaprakasam and Rajagopalan (18) reported that urea applications were associated with increased Verticillium wilt in eggplant and that increasing rates of urea were correlated with increased symptom development; however, the effect on yield was not studied. In contrast, growers are recommended to provide sufficient nitrogen during anthesis to ensure optimal fruit production (17). Experiments on other crops have demonstrated suppression of Verticillium wilt with increased nitrogen availability (1, 2,13,15).

The objective of this paper was to determine the effect of black plastic mulch and supplemental nitrogen alone, and in combination, on yield, growth, disease, and colonization of eggplants by *V. dahliae*. A preliminary report has been published (3).

MATERIALS AND METHODS

Site history. The experiments were conducted at the Lockwood Farm in Hamden, CT, in 1989 and 1990. The soil was a Cheshire fine sandy loam (57% sand, 33% silt, and 10% clay) with a pH of 5.8. At the time of planting, soil fertility was determined from a composite soil sample containing 16 randomly sampled soil cores that were removed from a depth of 15 cm. Soil fertility was 25 μg/g of NO_3·N, 12 μg/g of NH_4·N, 70 μg/g of PO_3, 160 meq/g of soil-exchangeable K, 800 meq/g of soil-exchangeable Ca, and 67.2 meq/g of soil-exchangeable Mg as determined by the

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methods of Lunt et al (7). Plots had been cropped to potatoes, tomatoes, and eggplants in 1986, 1987, and 1988, respectively. Verticillium wilt disease incidence on the eggplants grown in 1988 was 100% at the end of the season and the disease severe. The field was rototilled in 1989 at right angles before planting to disperse infested soil evenly within the plots.

Plot establishment and experimental design. Seeds of eggplant (cv. Agway Super Hybrid No. 1, Agway, Inc., Syracuse, NY) were germinated in plastic trays filled with potting mix (Promix BX, Premier Brand, New Rochelle, NY) at 25–30 C. Seedlings were transplanted 12 days later into 10-cm-diameter plastic pots containing the same potting mix and kept in a greenhouse (18–25 C) for 1 mo. Plants received weekly applications of 100 ml of Agway Sol-U-Green 20-20-20 soluble fertilizer at 1.0 g/L.

The experiments were designed each year in randomized block factorial designs (two mulch × two nitrogen treatments). Each treatment consisted of four plants arranged in a 0.9-m-square array and replicated four times. Plants were transplanted into soil mulched with 4-mil black plastic or into bare ground. The mulch extended 0.5 m from the plot and plots were spaced 1.8 m apart. At 35–45 days (period of anthesis) after field planting (DAP), one-half of the mulched plots and one-half of the bare-ground plots were randomly chosen to be side-dressed by hand with 28 g of NH_4NO_3 (equivalent to 112 kg of N per hectare). The other plots were left untreated. Insects and weeds were removed by hand. At the end of the season in 1989, a composite soil sample containing one soil core (depth 15 cm) from each plot per site was assayed for nematodes by pan extraction (11). Because 10–17 lesion nematodes (*Pratylenchus penetrans* (Cobb) Filip. & Shuur.-Stek.) per 50 cm^3 of soil were found and because they are known to exacerbate Verticillium wilt (9), phosphoramidate (Nemacur 2E, Mobay Corp., Kansas City, MO) was incorporated into the soil in 1990 before planting at 6.25 L (1.4 kg a.i) per hectare.

In addition to these plots, a portion of field that was immediately adjacent to these plots and that had been planted to rye for more than 15 yr was plowed and planted to eggplant in 1990. The soil fertility of this soil was similar to the infested sites. The treatments and experimental design used in infested soils were used in this field. No phytopathogenic nematodes were found in soil samples that were assayed as described earlier.

Growth and yield measurements. Plant size was estimated every week beginning 2 wk after after transplanting (14 DAP). Two methods were used during the first 40 DAP. The first method involved classifying every leaf on a plant into one of six leaf area categories with a size key. Cardboard was cut into shapes of eggplant leaves that measured 0.1, 0.4, 0.9, 1.4, 1.9, 2.4, or 2.9 dm^2. The total number of leaves in a class was multiplied by the averaged leaf area for that class, and the total leaf area for each plant was summed and expressed as square meters. Initially, all leaves were counted, and it was determined that leaves smaller in length than 4 cm contributed less than 5% of the total leaf area. Therefore, these leaves were not counted in later measurements. Plant size was also determined from an estimate of the plant canopy that intercepted sunlight using the formula $\pi(a \times b)/4 \times f$, where a and b were the largest and smallest diameters of that plant's canopy (f is a visual estimate of the fraction of the canopy that intercepted sunlight).

Marketable fruits were individually weighed every 3–5 days beginning approximately 50 DAP, and the foliar fresh weights were measured at final harvest on 104 or 95 DAP for 1989 and 1990, respectively. Fruits less than 0.2 kg were not included in the final yield.

Disease assessment. The day that visual symptoms appeared on a plant was recorded. The DAP when 50% of the plants in a replicate (two out of four) became symptomatic was computed. Occasionally, this value was estimated by interpolating between two points. Disease severity, determined when growth measurements were taken, was expressed as the percentage of wilted or chlorotic leaves per plant. All disease estimates were made on attached foliage. The area under the disease progress curve (AUDPC) was computed by the equation: $\Sigma\ (S_i + S_{i+1})/2 * (t_{i+1} - t_i)$, where S_i is the disease severity at that time (t_i).

Host colonization assays. To confirm the presence of *V. dahliae* in the symptomatic leaves when the disease first appeared, petioles were disinfested in 0.5% NaOCl, rinsed in distilled water, and placed onto ethanol-streptomycin agar (ESA) (10). At final harvest, the main stem was separated from all branching stems and surface-disinfested by twice dousing and flaming the stem with 95% ethanol. Wedge-shaped pieces of stem were cut from areas just above every node, and the vascular tissue pieces were arranged in consecutive order on ESA (10). Between 30 and 40 nodes from each plant were sampled (five to six pieces per plate). Plates were incubated in the dark for 10–14 days at 20–24 C and then scored positive or negative for *Verticillium* spp. The data were expressed as the percentage of stem segments colonized in the basal or apical stem region (total nodes divided by two). Fifty-two isolates were subcultured onto potato-dextrose agar (PDA), identified as *V. dahliae* by presence of microsclerotia and absence of other melanized structures, and stored in 15% glycerol at −40 C.

Pathogenicity tests. Thirty *V. dahliae* isolates were chosen from the stored cultures, transferred to PDA, and grown for 2 wk. The colonized agar was homogenized in a blender with 100 ml of sterile distilled water. The roots from two 8-wk-old eggplant seedlings of the same cultivar were dipped into each fungal-agar homogenate, transplanted into 10-cm-diameter plastic pots filled with Promix, and grown in the green-

Table 1. Effect of black plastic mulch and supplemental nitrogen on the time of 50% disease incidence, area under the disease progress curve (AUDPC), and the colonization of stems of eggplants grown in soils infested with *Verticillium dahliae*

		1989				1990			
		50%	AUDPC	% Stem colonization		50%	AUDPC	% Stem colonization	
Black plastic	NH_4NO_3[a]	DAP[b]	(days)	Basal	Apical	DAP[b]	(days)	Basal	Apical
−	−	46	2.2	44	37	51	10.1	44	33
−	+	48	2.3	49	36	44	13.3	66	64
+	−	29	2.8	56	39	35	11.6	51	22
+	+	39	3.0	61	40	34	10.4	44	32
LSD		13	NS[c]	NS	NS	11	NS	NS	NS
Linear contrast (P)									
Mulch + vs. mulch −		0.001	NS	NS	NS	0.001	NS	NS	NS
Nitrogen + vs. nitrogen −		NS	NS	NS	NS	NS	NS	NS	NS

[a]Nitrogen applied on DAP 45 at 112 kg/ha.
[b]50% DAP refers to the day after planting when 50% of the plants had symptoms of Verticillium wilt.
[c]NS = nonsignificant.

house for 6 wk at 18–25 C. Roots from seedlings were also dipped into sterile PDA homogenates to serve as uninoculated controls. Plants were rated for disease based on a scale of 1–5 where 1 = no wilt and 5 = severe wilt. *V. dahliae* was recovered from discolored vascular tissue on ESA (10).

Leaf analyses. Dried leaves from the four replicated plants within each treatment were bulked and ground in a Wiley mill. Tissue was assayed for total N, P, and K by atomic absorption.

Statistical procedures. All data were subjected to an analysis of variance (ANOVA). Means were compared by Student's least significant difference test at $P = 0.05$. Linear contrasts for mulch and nitrogen were also computed when interaction terms were not significant.

RESULTS

Symptoms of Verticillium wilt were first observed as early as 25 DAP in 1990. Symptoms first appeared as wilted leaves with small aggregated chlorotic patches on the leaf. Leaves withered and dropped off within a week or became turgid and developed necrotic and mottled margins.

AUDPC was much greater in 1990 than in 1989. In both years, when averaging across the nitrogen treatment, symptoms of Verticillium wilt appeared in 50% of the mulched plants 13 days before symptoms were observed in 50% of plants on bare ground (Table 1). However, AUDPC in mulched plants was not significantly greater than in plants grown on bare ground.

Fungal colonization of primary stems was variable and not affected by either treatment or the combination of treatments (Table 1). Between 44 and 66% of the basal nodes and 22 and 66% of the apical nodes sampled gave rise to *V. dahliae* colonies. Nodes from eggplants grown in the adjacent site previously planted to rye were not colonized by *V. dahliae*.

When 30 *V. dahliae* isolates from infected stem pieces were tested for pathogenicity on eggplant seedlings, 29 isolates incited severe to mild wilting in eggplant seedlings within 2 wk. *V. dahliae* isolates were recovered from the stems of 21 inoculated eggplants.

Both methods used for measuring plant size gave similar results from 14 DAP until 40 DAP ($r = 0.75–0.85$). After 40 DAP, leaves began to overlap, therefore, plant size was measured only by the method that estimated sunlight intercepted by the plant canopy. The area under the plant growth curve (AUPGC) (meters squared multiplied by days) of each plant was computed in the same manner as the AUDPC. AUPGC was not affected by combining the use of black plastic mulch and nitrogen side-dressing; however, averaging across nitrogen treatments revealed a 26% increase in the AUPGC on black plastic mulch in 1989, a 16% increase in 1990, and 6% increase for asymptomatic plants grown in adjacent plots that were previously cropped to rye (Table 2). Foliar weights at harvest were not altered by any treatment or combination of treatments.

In both years, eggplants grown in infested soil with black plastic mulch and nitrogen side-dressing had significantly larger yields than eggplants grown without this combination of treatments (Table 3). Fruit number was also significantly increased by the combination of treatments in 1990. During this year, infected eggplants produced roughly half the yield that was produced in 1989, but eggplants grown in adjacent plots that had been previously cropped to rye yielded more than three times the yield than from the infected eggplants and were asymptomatic.

Leaf analyses from plants at final harvest were not affected by any treatment. Eggplants averaged from 3.0 to 3.3, 0.9 to 1.2, and 3.3 to 3.7% by weight for N, P or K, respectively.

Table 2. Effect of black plastic mulch and supplemental nitrogen on the area under the plant growth curve (AUPGC) and final fresh weights of eggplant grown in soils infested with *Verticillium dahliae* and soils previously cropped to rye

		Infested soils				Soil cropped to rye	
		1989		1990		1990	
Black plastic	NH_4NO_3[a]	AUPGC[b]	Foliar wt (kg)	AUPGC[b]	Foliar wt (kg)	AUPGC[b]	Foliar wt (kg)
−	−	24.3	0.7	19.2	0.5	44.6	2.5
−	+	26.1	0.8	23.9	0.7	44.7	1.7
+	−	35.4	1.1	25.0	0.6	47.4	2.2
+	+	32.5	1.0	25.6	0.7	47.6	2.1
LSD		7.0	NS[c]	NS	NS	NS	NS
Linear contrast (P)							
Mulch + vs. mulch −		0.001	0.001	NS	NS	NS	NS
Nitrogen + vs. nitrogen −		NS	NS	NS	NS	NS	NS

[a]Nitrogen applied on DAP 45 at 112 kg/ha.
[b]AUPGC = meters squared multiplied by days.
[c]NS = nonsignificant.

Table 3. Effect of black plastic mulch and supplemental nitrogen on yield of eggplants grown in soils infested with *Verticillium dahliae* and in soils previously cropped to rye

		Infested soils				Soils cropped to rye	
		1989		1990		1990	
Black plastic	NH_4NO_3[a]	kg	Fruit (no.)	kg	Fruit (no.)	kg	Fruit (no.)
−	−	3.0[b]	6.8	1.6	3.5	8.6	16.3
−	+	3.3	8.8	1.6	3.9	9.2	16.8
+	−	4.2	8.8	1.9	4.9	11.5	20.0
+	+	5.2	10.7	3.0	6.7	10.6	18.8
LSD		2.1	NS[c]	1.2	3.0	1.9	NS
Linear contrast (P)							
Mulch + vs. mulch −		...[d]	NS	...	...	0.02	NS
Nitrogen + vs. nitrogen −		...	NS	...	...	NS	NS

[a]Nitrogen applied on 35–45 days after planting at 112 kg/ha.
[b]Values represent the means of 16 plants.
[c]NS = nonsignificant.
[d]Linear contrast not appropriate.

DISCUSSION

When both practices were combined, black plastic mulch and nitrogen increased eggplant yield in soils naturally infested with *V. dahliae*. However, black plastic mulch alone had greater influence on the growth and the onset of disease. The interaction between these cultural treatments on fruit weight was greatest when disease was greatest (1990), tended to have less influence in 1989 when disease was less severe, and was even less important when disease symptoms were absent. This pattern logically suggests that these treatment combinations affect yield by influencing disease development. However, we could not measure any reduction in Verticillium wilt (AUDPC) over the season in response to any treatment. Instead, the onset of disease was earlier on black plastic mulch. Nitrogen did not affect the time symptoms appeared or the progress of disease but did interact with the use of mulch to produce higher yielding plants. Others have reported that applications of urea enhance Verticillium wilt of eggplant (18), but we observed no detectable effect on disease expression in field studies with NH_4NO_3 applied at midseason. Possibly the form of nitrogen, time of application, and differences between greenhouse and field environments may have influenced these findings.

Fifty percent of the eggplants developed symptoms in mulched plots an average of 13 days before 50% of plants on bare ground became symptomatic. Moorman (8) saw no enhancement of Verticillium wilt symptoms on mulched eggplants, but Guba (4) found the onset of visual symptoms and plant growth were increased by mulching. Guba suggested mulching increased the soil temperature to levels optimal for infection and growth. The optimal soil temperature for Verticillium wilt of eggplant was later reported by Ludbrook (6) to be between 20 and 24 C. The effect of mulching on soil temperatures and on Verticillium wilt needs further study.

Symptoms of Verticillium wilt are most striking around anthesis and fruit-set (17). If anthesis influences the onset of symptoms, the larger mulched plants may flower earlier than smaller plants and develop symptoms of Verticillium wilt sooner. However, mulched and nonmulched plants both produce their first fruits at the same relative time in early August, so the time of flowering was probably not different. Another possibility is that the environment created by black plastic fosters larger root systems than plants grown on bare ground. This may allow larger volumes of soil to be permeated by young roots which, in turn, increases the probability that root tips, the infection court, intercept microsclerotia of *V. dahliae* and become infected.

In the present study, the AUDPC estimated disease development over the season but did not reveal differences between treatments. Similarly, Moorman (8) found poor correlation between the apparent infection rate and yield from diseased eggplants. However, AUPGC was well correlated with yield and with the effects of mulch on yield, whereas AUDPC was more variable and was less correlated with yield. Therefore, AUPGC was a better yield predictor than AUDPC. Its measurement also is less labor intensive than AUDPC and other disease measurements, such as area under the green leaf area curve (AUGLAC) (5) and healthy area days (HAD) (20), which require measuring symptomatic and total leaf area.

Talboy (19) stated that the severity of Verticillium wilt depended on the quantity of vessels colonized by the fungus. We did not determine vessel colonization but did find that fungal colonization of nodes was highly variable and not affected by any treatment or combination of treatments. If the fungus colonizes eggplant stems discontinuously, as has been observed in other Verticillium diseases (14), this may explain the variability in this particular assay. Because the increased yield with black plastic mulch and nitrogen was not related to a reduction in stem colonization in these plants, these treatments may have increased yield without affecting the ascent of the fungus into the stems.

When growers cannot rotate out of susceptible crops and/or fumigate, the reduction in eggplant yield attributable to Verticillium wilt may be lessened by using black plastic mulch with applications of nitrogen at midseason. Applying nitrogen under black plastic may be achieved by injecting soluble nitrogen under the mulch at periods before anthesis.

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