Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in Causing Red Root Rot of Corn

W. Mao, Former Graduate Research Assistant, R. B. Carroll, Professor, and D. P. Whittington, Research Associate, Department of Plant and Soil Sciences, University of Delaware, Newark 19717-1303

ABSTRACT

Mao, W., Carroll, R. B., and Whittington, D. P. 1998. Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in causing red root rot of corn. Plant Dis. 82:337-342.

Greenhouse and field tests were conducted in 1992 and 1993 to determine the causal pathogen(s) of red root rot (RRR) of corn. Corn hybrids Dekalb DK 522, DK 572, DK 677, and DK 582 were utilized. *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* were used alone or in combination to infest potting mix in greenhouse tests or soil in field tests. Results indicated that *P. terrestris* is the primary pathogen in the RRR complex of corn in Delaware. When *P. terrestris* and *Pythium irregulare* were associated, the disease progressed faster and was more severe, resulting in significantly higher ($P \leq 0.01$) root rot, basal stalk rot, and wilt. *F. acuminatum* played a minor role in causing the disease, with little additional symptom development when combined with *P. terrestris* or *Pythium irregulare*.

Red root rot (RRR), also named Pyrenochaeta rot, has been destructive in the past several years in the Delmarva region, where many corn hybrids have exhibited premature senescence late in the growing season (1). The disease may develop so rapidly that within 10 days, entire corn plants wilt and stalk lodging occurs, resulting in severe yield loss (1,2,19,26). Symptoms of RRR include dark reddish discoloration and necrosis on rotted roots and basal stalks accompanied by greenish gray discoloration on wilted leaves. These symptoms occur late in the growing season. The disease may be more severe when corn is densely planted (2,8,14,15,29).

Phoma terrestris E.M. Hans. (=Pyrenochaeta terrestris (E.M. Hans.) Gorrenz, J.C. Walker, & R.H. Larson) is recognized as a widespread saprophyte and weak parasite on the underground parts of a wide variety of hosts (16,24). In addition to colonizing corn, it attacks the roots of many other cereals, vegetables, and grasses (10,23,24). Pink root of onion is an economically destructive disease and the most widely studied one caused by this pathogen (21). *P. terrestris* occurs in many types of soils, and populations increase rapidly with successive host crops (9,12). The fungus spreads mainly by movement of infested soil, diseased plant debris, water movement, and mechanical cultivation (5). There is a lack of information about the

survival role of pycnidia, the asexual reproductive structure of the fungus (5); however, the fungus can survive in soils for years.

Under field conditions, RRR of corn is seldom caused by *P. terrestris* alone, but is generally associated with several soilborne fungal pathogens that cause a root rot complex (15,17). Species of *Pythium*, *Fusarium*, *Helminthosporium*, *Rhizoctonia*, and *Phialophora* have been implicated in the disease complex (14,15,19,26). Among these pathogens, *Pythium* spp. may be highly destructive, causing damping-off and root rot, especially under cold, wet soil conditions in the early spring and again in the late summer (18,26). *Pythium irregulare* Buisman, *P. arrhenomanes* Drechs., and *P. aphanidermatum* (Edson) Fitzp. were most frequently recovered from lesions on roots of 11- to 15-week-old corn grown in irrigated fields (3,26). These fungi were moderately virulent in causing root rot of corn. *Fusarium* spp. also have been suggested as the cause of damping-off, root rot, and stalk rot (3,6,7,19,20). However, it is unclear whether these species of *Fusarium* are primary or secondary pathogens (13).

RRR of corn received little attention until the first report by Campbell et al. (2) in 1991, and only a few studies on RRR have been published. The work reported here focuses on the etiology of RRR of corn, including the association and the causal relationship among *P. terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* Ellis & Everh. with regard to disease development.

MATERIALS AND METHODS

Preparation of inocula. *P. terrestris*, *Pythium irregulare*, and *F. acuminatum*

were isolated from diseased corn roots collected from a field with a history of RRR in Seaford, Delaware. These isolates were maintained on potato dextrose agar (PDA) (Difco) at 24°C. Inocula of *P. terrestris* and *F. acuminatum* were prepared on a semisolid wheat seed and water (1:1 vol/vol) medium autoclaved twice for 1 h on each of 2 consecutive days. Flasks with the medium were inoculated with mycelial disks (1 cm diameter) of each fungus, incubated for two weeks at $23 \pm 2^\circ\text{C}$, air-dried for 5 days, milled in a blender to pass through a 3.6-mm screen, and stored at 4°C until used. The inoculum contained 10^4 to 10^5 CFU/g as determined by dilution plating on rose bengal medium + pentachloronitrobenzene (PCNB). For infesting field soil, inoculum of *P. irregulare* was prepared on a semisolid medium of 660 ml of vermiculite (Therm-O-Rock, Inc., New Eagle, PA), 120 ml of V8 juice, 210 ml of distilled water, and 0.5 g of CaCO_3 . Flasks were autoclaved for 1 h on each of 2 consecutive days before inoculation with mycelial disks (1 cm diameter) of the fungus and were incubated for 2 weeks at $23 \pm 2^\circ\text{C}$. The inocula contained approximately 10^4 CFU/g, as determined by dilution plating on rose bengal medium + PCNB. For the production of zoospores, soil-water extract solution was made by adding 100 g of dry soil (from a corn field) to a flask with 1 liter of distilled water. The soil suspension was allowed to settle for 1 day, then the solution was separated from the soil by filtering through Whatman no. 4 filter paper. One hundred mycelial plugs (1 cm^2) from 5-day-old V8 agar cultures of *Pythium irregulare* were placed in a 200-ml beaker with 100 ml of the soil extract solution and incubated at 15°C for 2 days, followed by incubation at 4°C for 30 to 60 min, and finally incubating at $23 \pm 2^\circ\text{C}$ for 1 h. Zoospores were collected by pouring the suspension through sterile cheesecloth. The zoospore concentration was determined with a hemacytometer and was diluted with sterile distilled water to a concentration of 8×10^4 zoospores per ml. The adjusted spore suspension was applied to pots immediately.

Corn hybrids and parameters measured. Based on prior field tests (1), corn hybrids used for the 1992 greenhouse test were DK 522 (susceptible) and DK 677 (resistant), and those for the 1993 greenhouse test and 2-year field tests were DK 572 (susceptible) and DK 582 (resistant).

Current address of W. Mao: Biocontrol of Plant Diseases Laboratory, USDA-ARS, Beltsville, MD 20705; E-mail: wmao@asrr.arsusda.gov

Accepted for publication 13 November 1997.

All seeds (captan-treated) were provided by Dekalb Plant Genetics (Dekalb, IL). Severities of root rot, stalk rot, and wilt were determined using a disease rating index based on the percentage of discoloration, necrosis, and rotting of roots and basal stalks, or wilting of leaves. The scale was: 1 = healthy plant, 2 = 3 to 30% (slight disease), 3 = 31 to 60% (moderate disease), 4 = 61 to 90% (severe disease), and 5 = dead plant. In the greenhouse test, wilt evaluation was performed before harvest (120 days after planting [DAP]). Following harvest, root samples were removed from the basal stalk and washed twice with tap water, then evaluated for discoloration and decay. In field tests, destructive sampling to evaluate root rot and wilt was conducted on 52, 80, 108, and 128 DAP. Samples were collected at random with three plants per treatment and evaluated as described above. Basal stalk rot was evaluated at harvest with 30 plants per treatment. Stalks were collected as described for root samples and were split lengthwise from the third internode to the soil level and then rated according to the above index.

Greenhouse tests. Treatments consisted of infesting potting mix with the three fungi, alone or in combination, and a non-infested control. Seeds of the two hybrids for each year were sown in seed-germination trays (45 × 28 × 6 cm) containing

potting mix (Redi-Earth, Scotts-Sierra Horticultural Products Co., Marysville, OH) and watered twice a day. Two-week-old seedlings were transplanted into pots (36 cm diameter) with one seedling per pot containing 15 liters of potting mix infested (2 days before transplanting) with 8 g of *P. terrestris* inoculum, 8 g of *F. acuminatum* inoculum, or 100 ml of *Pythium irregulare* zoospore suspension for the treatments. A randomized complete block design with five replicates was used. Osmocote slow-release fertilizer (21-5-20, Scotts-Sierra Horticultural Products Co., Marysville, OH) was applied twice at the recommended rate during a 120-day growth period (April to August), and plants were watered daily. Greenhouse temperature ranged from 22 to 28°C.

Field tests. Field tests were conducted on the University of Delaware farm in Newark in 1992 and 1993. Treatments used were the same as the greenhouse tests. A split plot design with three replicates per treatment was utilized, where the main plot was the hybrid and the split plot was the treatment. Each main plot consisted of six rows with eight subplots (4.6 × 6.1) with seven border rows (4.6 × 3.0) between each of the sub plots. There was a six-row border between each main plot and surrounding the entire plot. Field soil was fumigated with Busan 1020 (a.i. 33%

metham-sodium, Buckman Laboratory, Memphis, TN) at 187 liters/ha 2 weeks before planting. Inoculum was applied by hand into rows at 300 g per treatment per row with each isolate alone or in combination. Corn seeds were sown mechanically into the infested rows on the same day, and the planter was washed thoroughly with a 10% solution of bleach (5.25% NaOCl) between each of the treatments. Field plots were not irrigated during the growing season.

Statistical analyses. Analyses of variance were conducted using the general linear models procedures of the SAS ver. 6.08 (SAS, Cary, NC) to evaluate the effect of different treatments on the development of root rot, stalk rot, and wilt of the corn hybrids in 1992 and 1993 greenhouse and field tests. Analyses were also conducted to determine interactions between or among the factors of treatment, hybrid, sampling time, and year. Duncan's multiple range test was used to determine the mean separation of the measurements. To characterize the relationship of the progress of root rot and wilt over time among years, hybrids, and treatments of the field tests, analyses were carried out using regression.

RESULTS

Greenhouse test. In general, significant differences ($P \leq 0.01$) in severity of root rot and wilt were found among treatments and between corn hybrids, with interactions between treatments and hybrids in both years (Table 1). There was less ($P \leq 0.01$) root rot and wilt on the resistant hybrids DK 677 and DK 582 than on the susceptible hybrids DK 522 and DK 572 (Table 2). Most treatments with the fungi, alone or in combination, resulted in higher ($P \leq 0.01$) root rot and wilt than the noninfested control (Table 2). Treatments with each fungus alone, as well as the combined inocula of *Pythium irregulare* + *F. acuminatum*, resulted in similar disease ratings, which were less than those with other

Table 1. Analysis of variance (ANOVA) for root rot and wilt incidence on corn in 1992 and 1993 greenhouse tests

Sources ^y	df	Root rot		Wilt	
		1992	1993	1992	1993
Treatment	7	++ ^z	++	++	++
Replicate	4	—	—	—	—
Hybrid	1	++	++	++	++
Treatment × hybrid	7	++	++	++	++

^y Treatments included infesting potting mix with the three fungal isolates, alone or in combination, and a noninfested control. There were five replicates per treatment. Hybrids used were Dekalb DK 677 (resistant) and DK 522 (susceptible) in 1992, and DK 582 (resistant) and 572 (susceptible) in 1993.

^z ++ = *F* test significant at $P \leq 0.01$, — = not significant.

Table 2. Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* in the development of root rot and wilt of corn in 1992 and 1993 greenhouse tests

Treatments	1992				1993			
	Root rot ^x		Wilt		Root rot		Wilt	
	DK 522	DK 677	DK 522	DK 677	DK 572	DK 582	DK 572	DK 582
Ph. + Py. + Fu. ^y	4.4 a ^z	3.6 a	5.0 a*	4.4 a	4.0 a*	3.6 a	5.0 a	4.5 a
Ph. + Py.	4.1 ab*	3.4 ab	5.0 a*	4.0 ab	4.0 a*	3.4 ab	5.0 a*	4.4 a
Ph. + Fu.	3.6 c*	3.0 bc	4.4 b*	3.8 b	3.3 b*	2.8 bc	4.3 b*	3.7 b
Py. + Fu.	3.0 d*	2.5 cd	4.2 bc*	3.0 cd	3.0 bc*	2.4 c	4.0 bc*	3.2 bc
Ph.	3.2 cd*	2.8 c	4.0 bc*	3.4 c	3.2 bc*	2.4 c	3.6 c	3.4 bc
Py.	2.9 d*	2.4 cd	4.2 bc*	3.0 cd	2.5 c	2.3 c	3.8 bc*	3.0 c
Fu.	2.6 de*	1.9 d	3.6 c*	3.2 c	2.2 cd*	1.8 cd	3.2 cd	2.8 cd
Noninfested Ctrl.	1.3 e	1.1 e	2.8 d	2.6 d	1.4 e	1.1 d	2.4 d	2.1 d

^x Evaluation for root rot and wilt was based on a disease rating index where 1 = healthy plant, 2 = 3 to 30% (slight disease), 3 = 31 to 60% (moderate disease), 4 = 61 to 90% (severe disease), and 5 = dead plant.

^y Ph. = *P. terrestris*, Py. = *Pythium irregulare*, Fu. = *F. acuminatum*.

^z Means within columns followed by the same letter are not significantly different ($P \leq 0.01$) from each other. According to Duncan's multiple range test, means followed by an asterisk (*) are significantly greater ($P \leq 0.01$) than the corresponding means of each treatment for the other corn hybrid.

combination treatments. Combined treatments that included *P. terrestris* and *Pythium irregulare* generally had higher ($P \leq 0.01$) root rot and wilt indices than any other treatment on both hybrids in each year.

Field tests. According to the analyses of variance (ANOVA) for the 2-year field tests, significant differences ($P \leq 0.01$ or $P \leq 0.05$) were found between years, sampling times, hybrids, and among treatments (Table 3) for severities of root rot, wilt, and stalk rot. Significant interactions ($P \leq 0.01$ or $P \leq 0.05$) for these parameters were found between year and hybrid, treatment and sampling time, treatment and hybrid, and hybrid and sampling time. In general, hybrid DK 572 was more susceptible than hybrid DK 582, resulting in faster disease progression and significantly higher ($P \leq 0.01$) root rot, wilt, and basal stalk rot. Differences ($P \leq 0.01$) between the two growing seasons on each of the parameters were also found. The disease progressed significantly faster and was more severe in 1993 than in 1992, where similar severities of root rot or wilt were found on each hybrid 1 month earlier.

Root rot and wilt. For both hybrids, root rot was present at the first sampling time (52 DAP) in each year (Fig. 1), and wilt symptoms appeared 80 DAP in 1992 and 50 DAP in 1993 (Fig. 2). A linear increase in severities of root rot and wilt was observed for each treatment (Figs. 1 and 2). Root rot and wilt progressed faster and were more severe ($P \leq 0.01$) in most infested treatments, alone or in combination, than in the noninfested control. These symptoms progressed fastest in the combined treatments where *P. terrestris* and *Pythium irregulare* were included, resulting in significantly greater ($P \leq 0.01$) ratings of root rot and wilt than most other treatments. However, progress was slowest in the treatment with *F. acuminatum* or *Pythium irregulare* alone.

Both root rot and wilt progressed faster and were more severe on DK 572 than on DK 582 in each year's test (Figs. 1 and 2). Differences in severity ratings of root rot and wilt between the two hybrids became significant ($P \leq 0.01$) in most corresponding infested treatments at the third sampling time (108 DAP) in 1992 and at the second sampling time (80 DAP) in 1993. Due to the variations of environmental factors between the two growing seasons, root rot and wilt progressed faster and were more severe ($P \leq 0.01$) on both hybrids in 1993 than in 1992. From the second sampling time (80 DAP) on DK 572 and the third sampling time (108 DAP) on DK582, significantly higher ($P \leq 0.01$) ratings of root rot and wilt were found in most infested treatments in 1993 compared with those corresponding treatments in 1992 (Figs. 1 and 2).

Basal stalk rot. Differences ($P \leq 0.01$) in severity of basal stalk rot on each hybrid

were found between the two growing seasons (Table 4) and between the two hybrids in most infested treatments for each growing season. Similarly, infested treatments,

alone or in combination, resulted in more severe ($P \leq 0.01$) basal stalk rot than did the noninfested control. Combined treatments including *P. terrestris* and *Pythium*

Table 3. Analysis of variance (ANOVA) for incidence of root rot, wilt, and stalk rot on corn hybrids DK 572 and DK 582 in 1992 and 1993 field tests in Newark, Delaware

Sources ^x	df	Root rot ^y	Wilt	Stalk rot
Year	1	+ ^z	++	+
Sampling time	3	++	++	na
Treatment	7	++	++	++
Replicate	2	—	—	—
Hybrid	1	++	++	++
Year × sampling time	3	—	—	na
Year × treatment	7	—	—	—
Year × hybrid	1	+	++	+
Treatment × sampling time	21	++	++	na
Treatment × hybrid	7	++	++	++
Hybrid × sampling time	3	++	++	na
Year × sampling time × treatment	21	—	—	na
Year × treatment × hybrid	7	—	—	—
Sampling time × treatment × hybrid	21	—	—	na

^x Years = 1992 and 1993. Sampling for root rot and wilt was conducted at 52, 80, 108, and 128 days after planting. Treatments included soils infested with the three fungal isolates, alone or in combination, and a noninfested control. There were five replicates per treatment. Hybrids Dekalb DK 582 (resistant) and DK 572 (susceptible) were tested.

^y Evaluation for root rot, wilt, and stalk rot was based on a disease rating index where 1 = healthy plant, 2 = 3 to 30% (slight disease), 3 = 31 to 60% (moderate disease), 4 = 61 to 90% (severe disease), and 5 = dead plant.

^z + = *F* test significant at $P \leq 0.05$; ++ = significant at $P \leq 0.01$; na = not applicable, only done at harvest; and — = not significant.

Fig. 1. Effect of *Phoma terrestris* (Ph.), *Pythium irregulare* (Py.), and *Fusarium acuminatum* (Fu), alone or in combination, on the progression of root rot of hybrids Dekalb DK 582 (resistant) and DK 572 (susceptible) over the growing seasons of 1992 and 1993. Evaluation for root rot severity was based on a disease rating index where 1 = healthy plant, 2 = 3 to 30% (slight disease), 3 = 31 to 60% (moderate disease), 4 = 61 to 90% (severe disease), and 5 = dead plant. The coefficient values for treatments of Ph. + Py. + Fu., Ph. + Py., Ph. + Fu., Py. + Fu., Ph., Py., Fu., and noninfested control for DK 572 in 1992 were $R^2 = 0.99, 0.99, 0.98, 0.98, 0.99, 0.98, 0.99, 0.80$, respectively; those for DK 582 in 1992 were $R^2 = 0.96, 0.92, 0.91, 0.94, 0.99, 0.96, 0.95$, and 0.84, respectively; those for DK 572 in 1993 were $R^2 = 0.98, 0.99, 0.98, 0.99, 0.99, 0.98, 0.95$, and 0.84, respectively; and those for DK 582 in 1993 were $R^2 = 0.99, 0.99, 0.98, 0.97, 0.98, 0.99, 0.99$, and 0.83, respectively.

irregulare resulted in significantly higher ($P \leq 0.01$) ratings of basal stalk rot than most other treatments except for the combined treatment with *P. terrestris* and *F. acuminatum* on DK 572 in 1992 and on DK 582 in 1993. The combined treatment

of *P. terrestris* and *F. acuminatum* developed greater basal stalk rot than with *Pythium irregulare* or *F. acuminatum* alone on both hybrids. Infested treatments with each inoculum alone or with *Pythium irregulare* + *F. acuminatum* resulted in similar basal stalk

rot ratings on both hybrids, except on DK 572 in 1992. In that year, treatments with *Pythium irregulare* alone developed higher ($P \leq 0.01$) basal stalk rot than *F. acuminatum* alone, but both were less ($P \leq 0.01$) than other treatments.

Table 4. Association of *Phoma terrestris*, *Pythium irregulare*, and *Fusarium acuminatum* on the development of stalk rot of corn in 1992 and 1993 field tests in Newark, Delaware

Treatments	Stalk rot ratings ^x			
	1992		1993	
	DK 572	DK 582	DK 572	DK 582
Ph. + Py. + Fu. ^y	2.56 a * ^z	1.16 a	3.50 a *	2.60 a
Ph. + Py.	2.33 ab *	1.08 a	3.41 a *	2.53 ab
Ph. + Fu.	1.80 bc *	0.86 b	3.03 b *	2.33 b
Py. + Fu.	1.50 c *	0.73 bc	2.80 bc *	2.10 c
Ph.	1.56 c *	0.60 c	2.88 bc *	2.16 bc
Py.	1.13 d *	0.53 c	2.60 c *	2.03 c
Fu.	0.83 e	0.56 c	2.54 c *	2.06 c
Noninfested Ctrl.	0.30 f	0.23 d	1.70 d	1.41 d

^x Evaluation for stalk rot was based on a disease rating index where 1 = healthy plant; 2 = 3 to 30% (slight disease), 3 = 31 to 60% (moderate disease), 4 = 61 to 90% (severe disease), and 5 = dead plant.

^y Ph. = *P. terrestris*, Py. = *Pythium irregulare*, Fu. = *F. acuminatum*.

^z Means within columns followed by the same letter are not significantly different ($P \leq 0.01$) from each other. According to Duncan's multiple range test, means in each row with an asterisk (*), are significantly greater ($P \leq 0.01$) than the corresponding means of the treatments for the other corn hybrid. All means for 1993 stalk rot ratings were significantly greater ($P \leq 0.01$) than the corresponding means of all treatments for each hybrid in 1992.

Fig. 2. Effect of *Phoma terrestris* (Ph.), *Pythium irregulare* (Py.), and *Fusarium acuminatum* (Fu), alone or in combination, on the progression of wilt of hybrids Dekalb DK 582 (resistant) and DK 572 (susceptible) over the growing seasons of 1992 and 1993. Evaluation for wilt severity was based on a disease rating index where 1 = healthy plant, 2 = 3 to 30% (slight disease), 3 = 31 to 60% (moderate disease), 4 = 61 to 90% (severe disease), and 5 = dead plant. The coefficient values for treatments of Ph. + Py. + Fu., Ph. + Py., Ph. + Fu., Py. + Fu., Ph., Py., Fu., and noninfested control for DK 572 in 1992 were $R^2 = 0.90, 0.89, 0.84, 0.83, 0.84, 0.81, 0.78$, and 0.68 , respectively; those for DK 582 in 1992 were $R^2 = 0.86, 0.83, 0.77, 0.75, 0.68, 0.68, 0.70$, and 0.60 , respectively; those for DK 572 in 1993 were $R^2 = 0.93, 0.96, 0.98, 0.92, 0.95, 0.95, 0.93$, and 0.85 , respectively; and those for DK 582 in 1993 were $R^2 = 0.97, 0.97, 0.93, 0.91, 0.89, 0.89, 0.90$, and 0.85 , respectively.

DISCUSSION

Typical symptoms of RRR of corn, dark reddish discoloration of rotted roots and basal stalks and greenish gray discoloration of wilted foliage, were observed with the susceptible hybrid DK 572 in both greenhouse and field tests. They were identical to the symptoms expressed with the same corn hybrid in commercial fields with a history of RRR. RRR of corn is a late season disease (8,14,26). Root rot precedes wilt and stalk rot, which become evident as the plant nears maturity (17,31). Results from these field experiments are in agreement with this chronology. Symptoms of root rot or wilt became evident about the same time each year and progressed in a similar sequence from slight to moderate to severe. There was variance in severity due to differing environmental conditions between the two seasons. Symptoms of root rot progressed slowly for both hybrids until the second sampling time (80 DAP) in the 2 years. Root rot severities ranged from >2% to <30% on plants in most infested treatments 50 to 80 DAP. Then symptoms progressed much faster, especially on the susceptible DK 572, where root rot severities ranged from >30 to >90% among the infested treatments. Progression of root rot became associated with rapid development of discoloration and wilting of foliage 108 DAP in 1992 and 80 DAP in 1993. Similarly, root rot severity was associated with the development of basal stalk rot on both hybrids. The higher the RRR ratings, the more severe the wilt and basal stalk rot.

Hornby and Ullstrup (8) suggested that factors controlling susceptibility to root rot and stalk rot in corn do not fully account for the differences observed among genotypes during the vegetative phase of host development. The results from our field tests confirm this concept. Similar patterns for symptom development on roots were observed in the early-growth stage (vegetative phase: 1 to 60 DAP) for both resistant and susceptible hybrids. However, from the middle to late growing season, root rot progressed significantly less on the resistant hybrid DK 582 than on the susceptible DK 572. This might be attributed to the maintenance of physiological vigor and protracted senescence (8), which resulted in a relatively high proportion of new healthy roots formed during this period (data on root mass measurements not shown). Leopold (11) suggested that senescence takes place in the roots during the vegetative phase, and the living cell is the main factor in resistance to pathogens. Organ senescence might be a physiological signal accounting for the differences be-

tween resistant and susceptible genotypes (8). The basal portion of the stalk is important for translocation of water and sugars from the stalk to leaves and kernels (4), which suggests that basal stalk rot may play an important role in wilt development. Results from our field tests showed that DK 582 was more resistant to basal stalk rot than DK 572 and had significantly lower wilt severity.

Previous studies have shown that severity of root rot, stalk rot, and wilt of plants can be greatly influenced by environmental factors such as soil moisture and temperature (19,25,26). Dodd (4) reported that any factor increasing plant stress may lead to increased stalk rot and early senescence. Differences in the response of hybrids and treatments for root rot, wilt, and basal stalk rot between the two growing seasons might be attributed to differences in environmental conditions. There was only a 0.25-cm difference in total rainfall from 1 May through 30 September (period of the field experiments) between the two seasons. However, in 1993, the average daily temperature was 1.17°C higher. Christensen and Wilcoxson (3) reported that temperature, rainfall, and humidity have a marked influence on infection and development of root rot and stalk rot because these diseases are favored by high temperature and excess moisture from middle to late growing season. Symptoms of root rot, wilt, and basal stalk rot developed faster and were more severe in 1993 than in 1992.

Previous studies indicated that *P. terrestris* is predominant late in the growing season (8,19,27) but is relatively avirulent in causing root damage on corn (19,26). In our studies, *P. terrestris* alone produced red lesions on roots in the mid-season and induced 40 to 55% of RRR on the susceptible hybrid late in the season. This might indicate that colonization by *P. terrestris* increases as aging corn undergoes root senescence, losing resistance to the pathogen (8). Some roots might also be wounded or killed by environmental stress factors, and/or by soil insects and nematodes, resulting in more colonization by the organisms used to infest soil or by other secondary invaders (19,27). On the other hand, in the late growing season, root rot severity ranged from 80 to >90% on DK 572 in combined treatments of *P. terrestris* and *Pythium irregulare* compared with 35 to 55% of root rot in treatments with each inoculum alone or 60 to 70% with other combined inocula. Similarly, wilt developed fastest in the combined treatments of *P. terrestris* and *Pythium irregulare*, where wilt severities on DK 572 was >90% at harvest in both years compared with 35 to 65% wilt among most other infested treatments. These data indicate that *Pythium irregulare* could be a primary root pathogen of corn (19,25,26), which damages roots in the early season and makes

it easier for *P. terrestris* to later invade and colonize root and basal stalk tissues. Damage on roots was only about 35 to 45% when *Pythium irregulare* was inoculated alone. Results also demonstrated that when *P. terrestris* and *Pythium irregulare* were combined, much more basal stalk rot occurred on DK 572. Severities of basal stalk rot were 15 to 25% higher than with each fungus alone. These results indicate the association of *P. terrestris* and *Pythium irregulare* results in faster development and greater severity of the disease.

F. acuminatum has been reported as a secondary invader associated with root and stalk rot of corn (13,22,30), and it can produce T-2 toxin (trichothecene) in infected corn tissues, which can inhibit plant growth (18,28). In our studies, *F. acuminatum* produced yellow-brown lesions in the midseason and developed ratings of 10 to 30% necrosis of roots accompanied by 30 to 50% wilt and <2 to 15% basal stalk rot on DK 572 at the end of the season. Even when combined with other pathogens, *F. acuminatum* contributed little or no additive effects on disease development. This indicates that *F. acuminatum* is a weak pathogen and does not significantly contribute to RRR.

This study demonstrated the causal relationship among the three fungi on the development of RRR. Further research is required to gain information relating to hybrid resistance, potential chemical or biological control measures, and cultural practices for the reduction of the disease. Such information would be valuable for seed and other agrichemical companies and farmers in formulating disease management strategies for control of RRR.

ACKNOWLEDGMENTS

We thank John Pesek, programmer/analyst, University of Delaware, for his timely assistance on data analysis, Dekalb Plant Genetics for providing corn seed, and Buckman Laboratory for providing Busan 1020.

LITERATURE CITED

- Campbell, K. W. 1991. Evaluation of maize genotypes for red root rot resistance in the Delmarva region. M.S. thesis. University of Delaware, Newark, DE.
- Campbell, K. W., Carroll, R. B., Veitenheimer, E. E., Hawk, J. A., and Whittington, D. P. 1991. Red root rot, a new disease of maize in the Delmarva Peninsula. Plant Dis. 75:1186.
- Christensen, J. J., and Wilcoxson, R. D. 1966. Stalk rot of corn. APS Press, Worcester, MA.
- Dodd, J. L. 1980. The role of plant stresses in development of corn stalk rots. Plant Dis. 64:533-537.
- Flota, J. E. 1976. The identification and quantification of *Pyrenochaeta terrestris* (Hans.) Gorenz, Walker and Larson, propagules in soil and onion roots. M.S. thesis. Oregon State University, Corvallis.
- Futrell, M. C., and Kilgore, M. 1969. Poor stands of corn and reduction of root growth caused by *Fusarium moniliforme*. Plant Dis.

- Rep. 53:213-215.
- Gilbertson, R. L., Brown, W. M., Jr., and Ruppel, E. G. 1985. Prevalence and virulence of *Fusarium* spp. associated with stalk rot of corn in Colorado. Plant Dis. 69:1065-1068.
- Hornby, D., and Ullstrup, A. J. 1967. Fungal population associated with maize roots. Quantitative rhizosphere data for genotypes differing in root rot resistance. Phytopathology 57:76-82.
- Klinger, A., and Pontis-Vedela, R. E. 1961. Pink root of onions in Mendoza, Argentina. Plant Dis. Rep. 45:235.
- Kreutzer, W. A. 1941. Host-parasite relationships in pink root of *Allium cepa* L. II. The action of *Phoma terrestris* on *Allium cepa* and other hosts. Phytopathology 31:907-915.
- Leopold, A. C. 1961. Senescence in plant development. Science 134:1727-1732.
- Lima, M. D. 1984. *Pyrenochaeta terrestris* on tomato in Brazil. Pesquisa Agro. Bras. 19:307-309.
- McGee, D. C. 1993. Diseases that are not seedborne or seed transmitted. Pages 100-103 in: Maize Diseases-A Reference Source for Seed Technologists. Part 3. American Phytopathological Society, St. Paul, MN.
- McKeen, W. E. 1952. Preliminary studies of root and basal stalk rot of maturing corn in Ontario. Can. J. Bot. 31:132-141.
- Miller, R. E. 1963. A root rot complex of field corn: Symptomatology sequence of fungi involved and the effect of corn root exudate on these fungi. Phytopathology 24:3921.
- Morgan-Jones, G., and White, J. F. 1983. Studies in the genus *Phoma*. III. *Paraphoma*, a new genus to accommodate *Phoma radicina*. Mycotaxon 18:382-392.
- Pappelis, A. J. 1965. Relationship of seasonal changes in pith condition ratings and density to *Gibberella* stalk rot of corn. Phytopathology 55:623-626.
- Rabie, C. J., Sydenham, E. W., Thiel, P. G., Lubben, A., and Marasas, W. F. O. 1986. T-2 Toxin production by *Fusarium acuminatum* isolated from oats and barley. Appl. Environ. Microbiol. 52:594-596.
- Rao, B., Schmitthenner, A. F., Caldwell, R., and Ellet, C. W. 1978. Prevalence and virulence of *Pythium* species associated with root rot of corn in poorly drained soil. Phytopathology 68:1557-1563.
- Schieber, B. C., and Muller, A. S. 1968. A leaf blight of corn (*Zea mays*) incited by *Fusarium moniliforme*. Phytopathology 58:554.
- Shannon, E. L. 1968. Onion pink root. New Mexico State Univ. Crop Ext. Serv. 3:7.
- Shurtleff, M. C., ed. 1980. Compendium of Corn Disease. 2nd ed. American Phytopathological Society, St. Paul, MN.
- Sparace, S. A., Shahin, E. A., and Mudd, J. B. 1986. Accumulation of the onion pink root pathogen *Pyrenochaeta terrestris* and some observations on their phytotoxicities. Physiol. Mol. Pathol. 28:381-392.
- Sprague, R. 1944. *Pyrenochaeta terrestris* on gramineae in Northern great plains. Phytopathology 34:129-131.
- Sumner, D. R., and Dowler, C. C. 1983. Herbicide, planting date, and root disease interactions in corn. Plant Dis. 67:513-517.
- Sumner, D. R., Gascho, G. J., Johnson, A. W., Hook, J. E., and Threadgill, E. D. 1990. Root disease, populations of soil fungi, and yield decline in continuous double-crop corn. Plant Dis. 74:704-710.
- Vakil, N. G. 1985. Mycoparasitic fungi associated with potential stalk rot pathogens of corn. Phytopathology 75:1201-1207.
- Visconti, A., Mirocha, C. J., Logrieco, A., Bottalico, A., and Solfrizzo, M. 1989. Mycotoxin produced by *Fusarium acuminatum*.

- Isolation and characterization of Acuminatin: A new Trichothecene. J. Agric. Food Chem. 37:1348-1351.
29. Wall, R. E., and Mortimore, C. G. 1965. The growth pattern of corn in relation to resistance to root and stalk rot. Can. J. Bot. 43:1277-1283.
 30. Warren, H. L., and Kommedahl, T. 1973. Prevalence and pathogenicity to corn of *Fusarium* species from corn roots, rhizospheres, residues, and soil. Phytopathology 63:1288-1290.
 31. Whitney, N. J., and Mortimore, C. G. 1957. Root and stalk rot of field corn in southwestern Ontario. I. Sequence of infection and incidence of the disease in relation to maturation of inbred lines. Can. J. Plant Sci. 37:342-346.