Effects of Single or Double Infections with *Helminthosporium avenae* and Barley Yellow Dwarf Virus on Yield Components of Oats

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ABSTRACT

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Field experiments were conducted over 2 yr near State College, PA, to study the effects on yield components of spring oats (cultivar Noble) of single and dual infections with *Pyrenophora avenae* (*Helminthosporium avenae*) and barley yellow dwarf virus (BYDV) (PAV-NY isolate). Oats were inoculated with BYDV 4 or 6 wk after planting and with *H. avenae* 6 or 8 wk after planting. Although the average percentage of leaf area covered with Helminthosporium leaf blotch lesions approached 14% at growth stage 11.1 and rose to 30–50% after stage 11.2, infection with *H. avenae* had no significant effect on any yield component in either year. BYDV infection reduced the number of tillers and panicles by 15%, the number of seeds per panicle by 18%, thousand-kernel weight by 5%, and total yield by 33% compared to control plants. Interactions between date of inoculation and BYDV infection were significant for seeds per panicle and total yield. Earlier inoculations were associated with larger reductions in yield. No *H. avenae*-by-BYDV interactions occurred.

Spring oats (*Avena sativa* L.) are an important feed crop in Pennsylvania: in 1990, almost 16 million bushels valued at more than $21 million were produced on 240,000 acres (15). Two of the most common diseases of oats in Pennsylvania are Helminthosporium leaf blotch and barley yellow dwarf (BYD). The severity of these diseases varies from year to year. Conditions in central Pennsylvania in late March to early April may favor both the development of leaf blotch on spring oats planted at this time and the movement of the aphid vectors of barley yellow dwarf virus (BYDV) to the young oat plants (5,20). Planting at a later date is not recommended because of the possibility of yield loss from heat stress during grain filling stages and increased incidence of BYD.

Helminthosporium avenae Eidam (syn. *Drechslera avenae* (Eidam) Scharif; teleomorph *Pyrenophora avenae* Ito & Kuribayashi) is the causal agent of Helminthosporium leaf blotch on oats (24,25). It causes leaf, stem, and seedling blights as well as root and crown rots (13). Oblong, reddish brown lesions develop on leaves of infected seedlings and may coalesce and result in plant death (25). The disease occurs worldwide and has caused large losses in some parts of Europe (25). *H. avenae* overwinters primarily on infected oat seed as mycelium or as spores; mycelium is the most important source of primary inoculum (20). Seedlings become infected from inoculum borne on the glumes and in the seed. Primary infections are most severe when low soil temperatures or low soil moisture levels hinder growth of the host. Secondary cycles of infection occur when conidia produced on conidiophores on dead tissue are blown to new leaves, which become infected through stomates (13). Grainger (7) reported that primary and secondary phases of disease development coincide with times of high carbohydrate levels in host tissues, whereas the period separating the two phases is characterized by a low carbohydrate level.

BYDV is an aphid-transmitted, phloem-restricted luteovirus characterized by five serologically distinct isolates (RPV, RMV, SGV, MAV, and PAV) (19). It can cause serious economic losses in oats (16). BYD symptoms in susceptible oat cultivars include yellowing and reddening of leaves, stunting, reduced tillering, and blasting of florets (19). Yield loss is most serious when the plant is infected at the three-leaf stage or younger; plants infected at later growth stages may not show symptoms, but their yields may be reduced (2). The effects of BYDV on oats also vary depending on the oat cultivar and the isolate of the virus (8). BYD also alters carbohydrate metabolism and accumulation in small grains (4,9,12,14).

Plant pathogens seldom occur singly on plants in nature. Viral infection of small grains during early growth stages may make plants more or less susceptible to fungal pathogens (3,11,23). Infection of small grains by BYDV is associated with either suppression or enhancement of powdery mildew (17), rusts (18), and Septoria leaf blotch (1). Because carbohydrate levels of the host influence spore production and secondary infection cycles by *H. avenae* and because BYDV infection alters host carbohydrate and nitrogen levels, we considered synergistic or antagonistic interactions between these two pathogens likely.

In a preliminary greenhouse study (21), we found that oats infected with *H. avenae,* BYDV, or both yielded 11, 30, and 38% less, respectively, than healthy oats, and significant interactions of *H. avenae* by BYDV were detected for all yield components. In this study, we inoculated Noble oats grown in the field with *H. avenae,* BYDV, or both at various growth stages and determined the effects of single or dual infection on

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yield components. In addition to providing further insight into the possible interactions between these two pathogens in oats, this information would be useful to oat breeders and in selection programs.

MATERIALS AND METHODS

Field experiments were conducted at The Pennsylvania State University Research Farm near State College, PA. The fields were plowed with a moldboard plow, disked, and harrowed. Spring oat cultivar Noble was planted at a rate of 107.8 kg/ha on 1 April 1986 and 15 April 1987 with a grain drill mounted on a crawler-type planter. Plots measured 0.9 × 3.6 m and consisted of five rows spaced 17.8 cm apart. Each oat plot was bordered by plots of barley (*Hordeum vulgare* L. 'Lud') on all four sides as a buffer. The treatments were applied to plants in one linear meter of the center row of each plot. Fertilizer (224.4 kg of 10-10-10 [N-P_2O_5-K_2O] per hectare) was incorporated into the soil at planting, and plants were topdressed with 66.2 kg of ammonium nitrate per hectare at growth stage (GS) 5 (Feekes scale [10]). Bifenox (1.08 L a.i./ha) was applied at planting to control weeds.

The experimental design was a randomized complete block with four replications. Four inoculation treatments were included: noninoculated; inoculation with BYDV only, at 4 or 6 wk after planting; inoculation with BYDV followed 2 wk later by inoculation with *H. avenae;* and inoculation with *H. avenae* only, at 6 or 8 wk after planting. Plots that were not inoculated with *H. avenae* received foliar applications of mancozeb (80 WP, 2.24 kg/ha) to prevent infection from naturally occurring inoculum. Plots that were not to be infested with aphids received foliar applications of the systemic insecticide acephate (75 WP, 0.56 kg/ha) to prevent incidental spread of BYDV. Both pesticides were applied every 14 days from GS 4 until GS 11.1 with a backpack sprayer calibrated to deliver approximately 280 L/ha at 30 psi.

Plants inoculated with *H. avenae* were sprayed with 20 ml of a conidial suspension. Inoculum was obtained from an isolate collected in 1985 from oat leaves in State College. The isolate was virulent in a previous experiment. Cultures were grown on potato-dextrose agar under fluorescent lights (16-hr days, 8-hr nights) at 21 C for about 6 wk. Conidia were scraped and rinsed from each petri dish and added to a 0.05% solution of gelatin. Concentration was adjusted to 10^6 conidia per milliliter. The conidial suspension was sprayed onto plants in the treatment area in the late afternoon. The treatment area was then covered with a tent consisting of two layers of wet 50-grade cheesecloth supported by wire hoops. The tents were used to raise the humidity in the area immediately around the plants to promote infection by the fungus. Tents were removed after 24 hr. After the initial inoculation, plants were reinoculated at 10-day intervals without tents until GS 11.1.

Aphids for field inoculations with BYDV were obtained from virus-free colonies of *Rhopalosiphum padi.* Aphids were allowed a 48-hr acquisition feeding on detached leaves of California red oats infected with the PAV-NY isolate of BYDV. About 200 aphids were placed on three 6-cm-long leaf pieces in plastic dishes with tight-fitting lids and kept at 20 C in the dark for the acquisition feeding. Another group of aphids was placed on healthy detached oat leaves as a control. Representative aphids from both groups were then allowed to feed on caged oat seedlings for 5 days. Inoculated seedlings were then fumigated with DDVP and observed in the greenhouse for 3 wk to verify the presence of PAV in the source plants. Identity of the BYDV-PAV isolate infecting source plants used for virus acquisition feedings and in subsequently infected plants in the field plots was verified by enzyme-linked immunosorbent assay (ELISA) as previously described (6).

For field inoculations, the aphid-infested leaf pieces were gently intertwined horizontally between the leaves of the plants in the meter row. The dish was then shaken over the target plants to disperse the aphids that remained in the dish. One dish was used for each meter row. The test plots were caged with fine mesh screen for 3 days. When the cages were removed, the number of aphids per 30.0 cm of row was counted. The plots then were sprayed with acephate to kill the aphids. These plants then were included in the 14-day spray schedule described previously.

Plants in the treatment areas were hand-harvested at maturity and stored in bundles until the grain was dry. The grain was then weighed, and tillers per meter, panicles per meter, and seeds per panicle were counted. All yield data were analyzed by analysis of variance (22).

A field experiment was conducted in 1987 to determine whether the pesticides acephate and mancozeb had an effect on yield. This experiment was planted as described previously. The experimental design was a randomized complete block with five replications. The treatments were applied to a 1-m-long row in the center of each plot. Plots were sprayed with acephate or mancozeb or were not sprayed (control). The treatment rows were sprayed at the same time and rate and in the same manner as described for the inoculation tests. At maturity, the plants were hand-harvested, and yield was measured.

Table 1. Severity[a] of Helminthosporium leaf blotch on Noble oats inoculated with *Helminthosporium avenae*, barley yellow dwarf virus (BYDV), or both in 1986 and 1987

Inoculation treatment	Time of inoculation	
	6 wk	8 wk
1986		
No inoculation	0.2	0.2
BYDV	0.7	2.4
H. avenae	6.4	12.4
BYDV + *H. avenae*	10.2	9.6
LSD ($P = 0.05$)	8.4	11.9
1987		
No inoculation	0.0	0.1
BYDV	0.3	3.4
H. avenae	8.4	15.8
BYDV + *H. avenae*	13.2	13.9
LSD ($P = 0.05$)	4.4	9.9

[a] Average percentage of the area of the upper three leaves covered with lesions at growth stage 11.1. Each value is the mean of four replications.

RESULTS

Aphid transmission tests verified that source plants were infected with BYDV-PAV, with at least 10 of 12 test seedlings in both years exhibiting symptoms of BYD. All seedlings infested with aphids from healthy source tissue remained healthy, indicating that the original aphid colonies were virus-free. The original virus source tissues and representative plants from field plots tested positive for BYDV-PAV in ELISA. Other BYDV types common to Pennsylvania (6) were not detected.

Applications of acephate to virus-free plots prevented infestation by the natural aphid population. No plants with symptoms of BYD were found in any of the plots not exposed to viruliferous aphids. Approximately 44 *R. padi* aphids were counted on infested plants per 30.0 cm of linear row (about 12 plants) when cages were removed. At least 80% of the plants in infested plots in both years exhibited characteristic BYD symptoms, indicating a high incidence of infection.

Results from the experiment undertaken to determine the effect of acephate and mancozeb on yield indicated that neither chemical had a statistically significant effect. The control, mancozeb, and acephate plots yielded 81.0, 85.7, and 76.7 g of grain, respectively, per linear meter of row.

The severity of Helminthosporium leaf blotch was determined as the percentage of the surface area of the upper three leaves of the plant that was covered with lesions at GS 11.1. The average severity on plants in plots not inoculated with *H. avenae* was low in both years (Table 1). Disease severity in plots inoculated with *H. avenae* both early (6 wk) and late (8 wk) was moderate but appeared to increase after growth stage 11.2 to 30–50% as plants began to senesce. Infection by *H. avenae* did not affect any of the yield components studied (*data not shown*). Although disease severity ratings for oat plants doubly inoculated

with BYDV and *H. avenae* were greater than those for plants inoculated with *H. avenae* alone at the 6-wk inoculation date, no significant differences were noted for any of the yield components for either inoculation date.

Because infection of oat plots by *H. avenae* had no significant effect on yield components, and because no fungus-by-virus interactions were detected, yield data for the BYDV plots and the dual infection plots were combined for further analysis. BYDV infection significantly reduced all variables examined in both years (Table 2). On average over both years, the number of tillers per meter was reduced 15%, the number of panicles per meter 15%, the number of seeds per panicle 18%, thousand-kernel weight 5%, and total yield 33% compared to the noninoculated control. When the *H. avenae* treatments were excluded from the analysis, the results were similar: in plots inoculated only with BYDV, the number of tillers was reduced an average of 12%, the number of panicles 12%, the number of seeds per panicle 14%, thousand-kernel weight 6%, and total yield 32% over both years compared to the noninoculated control. This result supported the conclusion that *H. avenae,* singly or in combination with BYDV, had little or no effect on yield.

The date of inoculation influenced the effects of BYDV only in 1987 and only for number of seeds per panicle and total yield (Table 3). Plants inoculated with BYDV 4 and 6 wk after planting in 1987 yielded 23 and 32 seeds per panicle, respectively. The yield components of the noninfected control plots did not differ significantly between the two dates. Total yield was 82.6 g when plants were inoculated with BYDV 6 wk after planting (14% less than the yield from healthy controls) and 51.0 g when plants were inoculated 4 wk after planting (47% less than the control); the nonvirus plots yielded about 97 g. The BYDV-by-*H. avenae* interaction was not significant for any of the yield components, and no BYDV-by-*H. avenae*-by-date interactions occurred in either year.

DISCUSSION

These experiments were conducted to determine whether BYDV influenced the susceptibility of oat plants to Helminthosporium leaf blotch and whether this fungus-virus combination influenced yield components. Although we observed interactions between *H. avenae* and BYDV in our greenhouse study (21), no interaction was detected under field conditions. This difference may have been due to differences in environmental conditions in the field and greenhouse. Susceptible leaf tissue from greenhouse-grown plants would be expected to differ physiologically from field plants. In addition, stresses on field plants, such as exposure to high summer temperatures and inadequate moisture, may affect yield components more than either disease.

Although the disease severity ratings in Table 1 indicate moderate levels of Helminthosporium leaf blotch at GS 11.1, *H. avenae* did not affect yield components and did not significantly interact with BYDV. The high leaf blotch severity observed after GS 11.2 probably did not influence yield components, because grain fill is no longer occurring after this time.

We did not formally rate BYDV severity, but the observed reductions in yield components and the estimated incidence of symptomatic plants in the meter row of over 80% indicate adequate infection by BYDV. Oats were inoculated with BYDV 4 wk after planting because this time most closely represents the earliest stages of insect activity in Pennsylvania. The 6-wk inoculation date corresponds to the period when aphids appear to be most active. Inoculations with *H. avenae* were made 2 wk later to allow time for BYDV to become distributed throughout the seedlings. Because acephate was sprayed on plots that were inoculated with BYDV, it is unlikely that plants were infected with isolates other than PAV, and none were detected in random samples tested by ELISA.

Date-by-BYDV interactions were significant in 1987 but not in 1986. This may be because BYD was less severe in 1986 as a result of cooler temperatures during the first infestation at 4 wk.

We did not attempt to duplicate naturally occurring aphid populations in these studies. Aphid population density in the experiment was much higher than what normally occurs in nature in Pennsylvania. A survey of natural aphid populations in Pennsylvania in June 1986 found fewer than one aphid per 0.3 m of row on oats (6) and correspondingly few plants with leaf symptoms of BYD. In this study, aphid population density averaged more than 40 aphids per 0.3 m of row, and more than 80% of the BYDV-treated plants developed BYD leaf symptoms.

Depending on environmental conditions and predators, aphids normally feed on oats for several weeks. In this experiment, aphids were killed 3 days after being placed on the oats. If feeding had been allowed to continue and more plants had become infected, the effect of BYD on yield might have been greater.

In summary, we observed no interactions between *H. avenae* and BYDV-PAV, two common endemic pathogens, in field tests with the spring oat cultivar Noble. Our results differ from those reported for *Erysiphe* (17), *Puccinia* (18), and *Septoria* (1) spp. on BYDV-infected small grains. However, our study examined only one isolate each of *H. avenae* and BYDV and only one oat cultivar, and the results might be different with different fungal pathogens, variants of BYDV, or cultivars.

Table 2. Effects of barley yellow dwarf virus (BYDV) infection of Noble oats on yield components in 1986 and 1987[a]

Inoculation[b]	Tillers/m (no.)	Panicles/m (no.)	Seeds/panicle (no.)	TKW[c] (g)	Yield (g)
1986					
No BYDV	132.7	128.7	22.3	31.4	89.3
BYDV	112.1*	107.6*	17.8*	30.1*	57.0*
1987					
No BYDV	91.0	91.0	32.5	32.9	96.5
BYDV	80.5*	80.8*	27.3*	30.3*	66.8*

[a] Data were analyzed by analysis of variance. An asterisk indicates that the means are significantly different ($P = 0.05$).

[b] No significant differences in yield components were found from any of the *Helminthosporium avenae* treatments. Therefore, the no-BYDV inoculation data include both the control plots and the *H. avenae*-only plants. The BYDV inoculation data include both the BYDV plots and the dually inoculated plants. Analysis of data from only the noninoculated control plots and the plots inoculated only with BYDV gave results similar to those shown here, which verifies that *H. avenae*, in the presence or absence of BYDV, had no significant effect on yield.

[c] Thousand-kernel weight.

Table 3. Effect of time of inoculation of Noble oats with barley yellow dwarf virus (BYDV) on yield components in 1987[a]

Time of inoculation	Tillers/m (no.)	Panicles/m (no.)	Seeds/panicle (no.)	TKW[b] (g)	Yield (g)
4 wk after planting	76.8	76.1	22.7	29.5	51.0
6 wk after planting	85.3	85.3	32.0*	31.1	82.6*
Control	91.0	91.0	32.5	32.9	96.5

[a] Means represent both BYDV and dual inoculation (BYDV plus *Helminthosporium avenae*) treatments combined. An asterisk indicates that the means are significantly different by analysis of variance ($P = 0.05$).

[b] Thousand-kernel weight.

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