An Environmentally Controlled Experiment to Monitor the Effect of Aphanomyces Root Rot and Rhizomania on Sugar Beet

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Accepted for publication 4 August 1993.

ABSTRACT

Harveson, R. M., and Rush, C. M. 1993. An environmentally controlled experiment to monitor the effect of Aphanomyces root rot and rhizomania on sugar beet. Phytopathology 83:1220-1223.

An experiment was initiated to determine how sugar beets are affected by *Aphanomyces cochlioides* and beet necrotic yellow vein virus (BNYVV), the causal agents of Aphanomyces root rot and rhizomania, respectively. The experiment was conducted in a controlled temperature box maintained at 27 ± 2 C. Treatments consisted of soil infested with *Aphanomyces*, BNYVV, both pathogens combined, and an untreated control. At 8 and 12 wk after planting, leaf and petiole weights and leaf areas were taken. At harvest, tops were removed, and the soil cores were divided into three 15-cm segments and washed. After 8 wk, leaf weights and leaf areas for the control treatment were significantly higher than those for the pathogen treatments. However, after 12 wk, significant reductions in infected beets were seen only when both pathogens were present. Simultaneous infection with both pathogens caused a greater reduction of dry top weight and taproot weight than either pathogen alone. Although the root-rot rating for *A. cochlioides* was more severe than for BNYVV, there was less weight reduction in the taproot. No differences were detected between the three pathogen treatments regarding the number of roots recovered from the bottom segment of the soil profile, but infected beets had significantly fewer roots than plants grown in noninfested soil.

Aphanomyces root rot (ARR) and rhizomania are two economically important diseases of sugar beets (*Beta vulgaris* L.) in the Texas Panhandle (24). Both diseases have been recognized in Texas for several years (10,23) and are familiar to industry personnel. ARR is not commonly recognized as a major disease problem in most sugar beet-production areas of the United States; however, it does cause significant losses annually in Texas (24). ARR is caused by *Aphanomyces cochlioides* Drechs., an oomycete belonging to the family Saprolegniaceae (22). The same organism also induces black root, the most commonly observed seedling disease of sugar beets in Texas (25,26). Rhizomania is a virus disease caused by beet necrotic yellow vein virus (BNYVV) and is transmitted by the plasmodiophorid fungus *Polymyxa betae* Keskin. In Texas, rhizomania has failed to spread as rapidly or cause losses as severe as it has in California (24). Nevertheless, it is one of the most feared sugar beet diseases and is currently the major research focus of the plant pathology program at Bushland, TX.

Although induced by different pathogenic entities, the two diseases have several similarities. The infective units for both diseases are zoospores. Both are soilborne and require warm temperatures (20–28 C) and water-saturated soils for infection to take place (2,3,11,21). *P. betae* has been shown to infect plants in soils −0.3 bars or wetter, but not at −0.4 bars (14). Rush and Vaughn concluded that zoospore movement for *A. cochlioides* was negligible at <100 J kg^{-1} (26).

Although both pathogens require similar conditions for disease development, their modes of action and effects on plants are different. The distinctive water-soaked tissue associated with ARR is a result of the activity of hydrolytic enzymes produced by the fungus during infection. Under optimal conditions for disease development, these pectic enzymes break down the middle lamellae, causing cell collapse and facilitating rapid spread of the attacking fungus (21). Only primary tissues such as cortical and epidermal cells are susceptible to *P. betae*. When the fungus infects these tissues, its zoospores can either acquire the virus or transmit BNYVV if already viruliferous (1,16). If conditions are conducive, multiple infections can occur throughout the season with every zoospore release. Excessive yield loss from rhizomania is due to root-tip death. This results in loss of apical dominance of taproot and increased secondary root proliferation (13), which is a characteristic symptom of this disease. Damage to plants is considered to be due primarily to BNYVV, although *P. betae* alone has been shown to cause necrosis of small rootlets, stunting, and reductions of dry leaf and root weights (6,13).

It has been difficult to estimate losses due to both ARR and rhizomania because they commonly occur in a mixture of other soilborne pathogens and are rarely found individually (17). Therefore, because they are often found together, a greater understanding of the interdependency of these two diseases would aid in designing and evaluating control strategies. This study was initiated to determine the damage caused to sugar beet by rhizomania and ARR, alone and in combination.

MATERIALS AND METHODS

The experiment was performed in the greenhouse in tubes made from 10-cm PVC pipe. Each tube, 50 cm in length, was sealed at one end, filled with soil, and placed in a controlled temperature box. Temperature was regulated by refrigerated air and a heating coil in the bottom of the box. The top 5 cm of each tube extended above the top of the box, allowing foliage to be exposed to ambient greenhouse temperatures, which ranged from 20 to 30 C. The rhizosphere temperature in the tubes was held constant at 27 ± 2 C to assist infection by both diseases.

Inoculum preparation. Rhizomania inoculum was obtained by pulverizing dried roots of sugar beets infected with viruliferous cystosori of *P. betae*. The infected plants were inoculated with a California isolate of BNYVV-infected *P. betae* obtained originally from J. S. Gerik and grown in the greenhouse. Cultivar HH39 sugar beet seeds were coated with a slurry consisting of the powdered roots mixed with 2% methyl cellulose as the carrier at 1:10:10 (w/v/w, roots/methyl cellulose/seed) (18). Oospores used as inoculum for ARR were prepared as previously described (22,25,27). Agar disks from an actively growing culture were grown in 0.5% oatmeal broth. After incubation in the dark for 1 mo, mycelial mats containing mature oospores were rinsed and ground in a blender. Oospores were counted with a hemacytometer and added to sterile field soil. They were later diluted to the desired concentration, ~500 oospores per gram of soil.

Four treatments, consisting of soil infested with *Aphanomyces*, BNYVV, BNYVV and *Aphanomyces*, or uninfested controls were

employed. Eighteen replications of each treatment (one tube per replication) were completely randomized in the box. Each PVC tube was filled two-thirds full with unsterilized Pullman clay loam field soil. The top third consisted of sterilized soil infested with oospores at a final concentration of ≈500 *Aphanomyces* oospores per gram of soil for the two treatments with *Aphanomyces*. The treatment with BNYVV alone and the control had only sterilized soil. BNYVV was introduced into the experiment via the *P. betae*-infested seeds described above.

To insure a uniformly wet profile and good emergence, each tube was flooded twice daily for two days prior to seeding. Seeds (15–20) were planted in each tube and covered with a thin layer of sand. After emergence, plants were watered twice daily, 3 days a week, for 2 wk to insure adequate infection and were eventually thinned to one plant per tube. After 4–5 wk, when plants were established, the watering schedule was reduced to once a day, 3 days a week. They were fertilized once every 2 wk and sprayed twice weekly with insecticidal soap to control aphids and spider mites.

Data collection. Leaf and petiole weights were taken 8 and 12 wk after planting. Leaf area was also measured with an LI-3100 area meter (LI-COR Inc., Lincoln, NE). Leaf numbers 12 and 17, the youngest fully expanded leaves for the first and second sample, respectively, were excised from each plant for weight and area measurements. The experiment was harvested after 14 wk and repeated once.

Water was withheld 1 wk prior to harvest to allow the soil profile to dry. Tops were removed, and the soil core was pushed out one end. Cores were divided into approximately 15-cm segments and washed individually to determine root mass from each segment. Plant tops and roots recovered from each segment were dried and weighed. A root-rot rating was made on each taproot with a 0–4 scale. This scale was based on root size and percentage of roots with necrosis. A disease-free taproot was rated 0, and a dead root was rated a 4. A rating of 1, 2, or 3 represented taproot necrosis of 1–25, 26–50, and 51–75%, respectively. Samples also were taken from the two treatments infested with BNYVV for analysis by ELISA.

Data analysis. All data were subjected to analysis of variance, means separated by Duncan's multiple range test, and variance between the two tests was analyzed for homogeneity. Variance between the two tests for all parameters, except root weight in the top third of the soil cores, was homogenous and no treatment–test interactions existed. Therefore, for simplicity and ease of presentation, data from both tests were combined.

RESULTS

Test plants were readily infected with the two pathogens. By flooding the profile several days prior to seeding, enough moisture was available for seedlings to emerge. Heavy watering the first 2 wk and a temperature of 27 C promoted early infection (6–8). Very few plants did not become infected. The number of plants that escaped infection by BNYVV, *Aphanomyces*, or both pathogens were 3, 0, and 1, respectively. Only diseased plants from the three pathogen treatments were included in the analysis. One control plant was contaminated and infected with BNYVV and was not included in the analysis. This presumably happened as a result of splashing from an adjacent BNYVV treatment while the experiment was being watered.

Eight weeks after planting, weight and area of leaves from plants grown in uninfested soil were significantly greater than leaves from plants grown in the infested soil (Table 1). Based on leaf weight and area, disease effects on plants infected with BNYVV were less than on plants with both diseases but were no different than those infected with *Aphanomyces*. At the second reading, made shortly before harvest, there was no difference between controls and the treatments containing BNYVV or *Aphanomyces* by themselves. Leaf weight and area were significantly different from the controls only with both pathogens present. Results for total top weight taken at the end of the experiment were similar to the leaf weight and area data. The combined pathogen treatment caused greater reduction of dry top weight than did either *Aphanomyces* or BNYVV alone (Table 2). Not surprisingly, significant differences were seen between the controls and the three pathogen treatments with regard to root-rot rating. Ratings indicated that BNYVV-infected plants were less affected than those infected by *Aphanomyces* alone or both pathogens. However, no significant difference was observed between the *Aphanomyces* and pathogen-combination treatments (Table 2).

Data concerning dried root tissue from each segment of the soil cores are presented in Table 3. The amount of root recovered from the top third of the soil profile was statistically different among all four treatments. Combined pathogens caused the most root-weight reduction, followed by BNYVV, *Aphanomyces*, and

TABLE 1. Effect of *Aphanomyces cochlioides* and beet necrotic yellow vein virus (BNYVV), alone and in combination, on sugar beet leaf size and weight

Treatment	Leaf weight (g)[x]		Leaf area (cm^2)	
	Reading 1[y]	Reading 2	Reading 1	Reading 2
Control	7.1 a[z]	6.0 a	113.9 a	85.0 a
A. cochlioides	5.4 bc	5.3 ab	94.6 b	76.2 ab
BNYVV	5.9 b	5.9 a	99.8 b	86.9 a
A. cochlioides + BNYVV	4.6 c	4.7 b	81.4 c	70.1 b

[x] Includes leaf and petiole.
[y] Reading 1 was taken on leaf 12 and reading 2 on leaf 17, 2 and 3 mo after planting, respectively. Leaves were fully expanded when harvested.
[z] Values represent the mean of 35, 36, 33, and 35 replications (one plant per replication) for control, *Aphanomyces*, BNYVV, and combined pathogen treatments, respectively. Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 2. Effect of *Aphanomyces cochlioides* and beet necrotic yellow vein virus (BNYVV), alone and in combination, on root-rot severity and top weight of sugar beet at final harvest

Treatment	Root-rot rating[x]	Top weight[y] (g)
Control	0.2 a[z]	26.3 a
A. cochlioides	2.6 c	19.5 b
BNYVV	2.1 b	19.1 b
A. cochlioides + BNYVV	2.8 c	14.7 c

[x] Represents a scale of 0–4, 4 being most severe.
[y] Includes leaves, petioles, and crown.
[z] Values represent the mean of 35, 36, 33, and 35 replications (one plant per replication) for control, *Aphanomyces*, BNYVV, and combined pathogen treatments, respectively. Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

TABLE 3. Effect of *Aphanomyces cochlioides* and beet necrotic yellow vein virus (BNYVV), alone and in combination, on root weights of sugar beet at three depths[y]

Treatment	Top (g)	Middle (g)	Bottom (g)
Control	16.6 a[z]	0.64 a	0.34 a
A. cochlioides	10.7 b	0.23 c	0.13 b
BNYVV	8.5 c	0.45 b	0.13 b
A. cochlioides + BNYVV	6.5 d	0.21 c	0.08 b

[y] Each depth represents a 15-cm segment of the soil core.
[z] Values represent the mean of 35, 36, 33, and 35 replications (one plant per replication) for control, *Aphanomyces*, BNYVV, and combined pathogen treatments, respectively. Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

control. The middle segment of the profile showed no difference between *Aphanomyces* and the combined pathogens. More roots were recovered from BNYVV-infested plants in this segment than from the other two pathogen treatments, but fewer roots were found in plants infected with BNYVV than in control plants. In the bottom third of the soil cores, no differences were recorded between the pathogen treatments, but the uninfested control plants had significantly more roots.

DISCUSSION

Numerous studies have demonstrated the effects of viral and fungal pathogens simultaneously infecting crops (4,9,12,28,29). Some suggest that infection by a virus may offer resistance or protection against subsequent infection by fungi (9,29). However, most studies indicate that viral infection increases the plant's susceptibility to root-rotting fungi, causing greater damage with both pathogens than with either one alone. It has been hypothesized that this observation may result from increased leakage of electrolytes, carbohydrates, and amino acids from virus-infected plants, which may provide an added nutrient source that stimulates germ tube formation and colonization by parasitic fungi and other rhizosphere organisms (4,12,28). Another study indicates that virus-infected seedlings are less able to lignify and wall-off infection courts for subsequent attacks by root-rot fungi (12). It is not known whether similar phenomena are occurring in the interaction between ARR and rhizomania, because none of the previously mentioned studies involve a soilborne, root-infecting virus such as BNYVV.

However, agronomic characteristics such as leaf weight, leaf area, and top weight (Tables 1 and 2) were more severely affected when subjected to both pathogens than when subjected to either one alone. In the first reading for leaf area and weight, significant differences were seen between the control and all other treatments. However, by the end of the experiment when the second reading was taken, these differences were not evident. In fact, only plants exposed to both pathogens were significantly different from uninfested controls. We have observed similar trends in the field. Often, near harvest plants can appear deceptively healthy based on foliage appearance but produce poor root yields when harvested. If plants become infected early in the season, the small root system is insufficient to maintain optimum top growth, and the plants appear small and stunted. However, later in the season, if conditions favoring the host instead of the pathogen are present, plants can recover by producing many small secondary roots. Even though the taproot size and total yield is reduced, there are enough roots to support vigorous top growth.

The root-rot rating indicated that *A. cochlioides* is a more severe pathogen than BNYVV. However, this is misleading because in the top 15 cm of the soil cores, BNYVV caused a greater reduction in the size of roots than did *Aphanomyces*. This portion of the profile contains most of the taproot, which is of major importance in sugar beet production. Rhizomania is caused by a soilborne pathogen that is primarily confined to the root system and seldom becomes systemic. It can kill the young taproot, resulting in excessive secondary root formation. Although *Aphanomyces* induces a visibly more severe rot, BNYVV can cause more damaging effects if infection occurs early. This is because ARR usually occurs later in the season, and although the root tip may be killed, the uninfested portion of the root continues to grow.

BNYVV also produced a greater amount of roots in the middle segment of the profile than did the other two pathogen treatments. This can be explained by the secondary proliferation of small roots after taproot infection occurs. The bottom third of the profile showed no difference among the three pathogen treatments, illustrating the fact that both pathogens adversely affect the plants enough to limit root formation and extension into deep soil. Reduced rooting can result in water stress, further reducing root yield.

There have been numerous attempted methods to control rhizomania in the United States since its discovery in 1983. These have included fumigation (20), cultural practices (16,24), breeding for resistance (5,15,30), and testing soils for BNYVV prior to planting (19). Efforts to control ARR have not been as extensive. There are no current fungicides approved for use against ARR in the United States (24). A limited number of cultivars with tolerance to ARR are available (31), but they are not adapted for conditions in Texas, nor do they have resistance to rhizomania. Breeding for resistance is a promising option for managing rhizomania because resistance is controlled by a single dominant gene, and there are now several cultivars available with tolerance to BNYVV (5,15). However, they have little or no resistance to ARR (17).

Rhizomania and ARR are members of a complex of soilborne diseases affecting sugar beets in the Texas Panhandle. This combination is apparently unique to this area because there are no other reports implicating BNYVV and *A. cochlioides*. This study has demonstrated that both diseases are capable of causing serious losses by themselves. If resistance is to be a viable option in situations in which both diseases are present, it must be incorporated for both diseases because of the similarity in disease-development conditions. No cultivar like that is available. Until dual resistance is available to Texas growers, they must continue to manage these diseases with an integrated approach, using early planting, judicious water usage, and long rotations between sugar beet plantings.

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