A Root Rot Complex of Horseradish

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ABSTRACT

A root rot of horseradish (Armoracia rusticana) was shown to be caused by a complex of three organisms: Fusarium roseum 'Acuminatum,' Verticillium dahliae, and Pseudomonas fluorescens. Root infection by F. roseum 'Acuminatum' alone caused scattered brown lesions in the cortex and/or stele followed by dry, fibrous rot after 60 days. Infection by V. dahliae was restricted to the xylem tissue in the root, crown, and petioles, and no root rot occurred, regardless of inoculum density or incubation period in the laboratory. In the early stages of root infection, P. fluorescens was isolated from the interior of 62% of surface-sterilized roots free of both F. roseum 'Acuminatum' and V. dahliae. When horseradish was planted in soil artificially infested with different combinations of the three pathogens, the resulting preemergence losses and root disease severity were greater than with each pathogen separately. Optimal mycelial growth in culture and maximum disease severity in horseradish occurred at 24°C for F. roseum 'Acuminatum' and at 20°C for V. dahliae.

Horseradish, Armoracia rusticana Gaertn., Mey. & Scherb., is a vegetatively propagated, large-leaved, hardy perennial of the Brassicaceae family whose roots are used to make a condiment for meats and seafood (2). The areas of intense commercial cultivation in the United States are in the Mississippi River Valley near East St. Louis, IL, and Eau Claire, WI.

Root deterioration of horseradish was first observed in Germany in 1860 and described in 1899 (16), but the causal organism(s) was not identified. A Verticillium sp. was later reported to be associated with root deterioration of horseradish in Germany (9) and the United States (7). An unidentified bacterium was associated with the soft rot phase of the disease (14).

Infection of horseradish by Verticillium dahliae Kleb. alone results in the colonization of xylem tissue. Diseased plants are stunted and wilt during periods of water stress, but the root tissue does not disintegrate (8,15). Extensive root deterioration in samples from Eau Claire suggested that organisms other than V. dahliae were involved (11). The present work was undertaken to determine the organism(s) involved in the deterioration of horseradish roots in Wisconsin fields.

Each infested soil was subdivided into five aluminum trays (61 x 41 x 15 cm) and planted at a depth of 2.5 cm with 20 horseradish root sections (2.5 x 1.7 cm). Before planting, root sections were surface-disinfested with a mixture of 95% ethanol and 0.5% sodium hypochlorite (1:1, v/v) for 10 min, rinsed twice with sterile water, and drained. Trays of soil were kept at 35% WHC and incubated at 16, 20, 24, 28, and 32°C in growth chambers with a 12-hr photoperiod and light intensity of 250 µE·m⁻²·s⁻¹.

After 60 days, the plants were removed and washed, and both the root piece and stem were examined for evidence of xylem infection by V. dahliae. Plants were also observed for root rot and brown flecking in the cortex and stele caused by F. roseum 'Acuminatum.' The experiment was repeated twice.

Effect of inoculum concentration of F. roseum 'Acuminatum' and V. dahliae on disease severity. Steamed muck soil was artificially infested with either 4 x 10⁹ microconidia of F. roseum 'Acuminatum' per gram of dry soil or 250 microsclerotia of V. dahliae per gram of dry soil. After 10 days the population of F. roseum 'Acuminatum' in the infested soil was determined with a Fusarium-selective medium (12). Each of the infested soils was then diluted with steamed soil to achieve inoculum concentrations from 4 x 10⁷ to 1 x 10⁸ microconidia per gram of dry soil for F. roseum 'Acuminatum' and from 50 to 1 microsclerotium per gram for V. dahliae. The infested soils were then incubated in growth chambers for 60 days at 24°C (F. roseum 'Acuminatum') and 20°C (V. dahliae).

For both fungi each infested soil at a given inoculum concentration was subdivided into five replicate trays, and each tray was planted with 20 surface-disinfested root sections. For the controls, steam-pasteurized soil was planted without disinfested root sections.

After incubation, each plant was removed carefully from the soil, washed under running tap water, blotted, and weighed, and assigned a disease index rating (Table 1) from 0 to 4, with 0 representing healthy roots and 4 representing roots with 100% root rot symptoms.

Isolation and identification of a root rot bacterium. One hundred planting rootstocks, known as sets, with no apparent external or internal symptoms of
infection by *F. roseum* 'Acuminatum' or *V. dahliae* were washed thoroughly, surface-disinfested, rinsed in sterile distilled water, blotted dry, dipped twice in 95% ethanol, and flamed. Tissue was then removed aseptically from areas surrounding the vascular ring, plated on 15 ml of Difco nutrient-dextrose agar, and incubated at 24 C for 48 hr in the dark. Also, 100 root sections measuring 1.27 × 1.27 cm were surface-disinfested as previously described, placed into 125-ml flasks containing 50 ml of Difco nutrient-dextrose broth (NDB), and incubated in the dark on a shaker at 24 C for 5 days. After 5 days, the root sections were observed for evidence of rot. The controls were autoclaved root sections incubated in NDB.

**Interation of organisms and disease severity.** Steamed muck soil was artificially infested with *Pseudomonas fluorescens* Migula, *F. roseum* 'Acuminatum,' and/or *V. dahliae* separately and in all possible combinations at 1 × 10^6^ cells, 1 × 10^7^ microconidia, and 250 microsclerosis, respectively, per gram of dry soil. Each treatment consisted of five replicate trays, each planted with 20 surface-disinfested root sections. The trays were incubated at 24 C for 60 days in a growth chamber with a 12-hr photoperiod and a light intensity of 250 μE m⁻²s⁻¹. For the controls, noninfested steamed muck soil was planted with surface-disinfested root sections.

**Data analysis.** Regression analysis was used to describe the effect of temperature on growth of *F. roseum* 'Acuminatum' and *V. dahliae* in culture; the effect of the concentration of microconidia of *F. roseum* 'Acuminatum' on disease as measured by flecking, fibrous root decomposition, and weight; and the effect of the concentration of microsclerosis of *V. dahliae* on disease as measured by infection of roots, crowns, petioles, and leaves.

The results were used to compare overall significance of the models (P < 0.05). Coefficients of determination (r²) estimated the proportion of the variation in growth that was explained by temperature and the proportion of the variation in disease that was explained by inoculum concentration. Optimum growth temperatures for both organisms were determined by setting the first derivatives of the respective regression equations equal to zero and solving.

**RESULTS**

**Effect of temperature on mycelial growth of fungal isolates.** Growth of the *F. roseum* 'Acuminatum' and *V. dahliae* isolates was maximum at 24 and 20 C, respectively (Fig. 1). Growth of both fungi as a function of temperature showed a highly significant quadratic trend (P = 0.01).

**Effect of temperature on pathogenicity of fungal isolates.** All isolates of *F. roseum* 'Acuminatum' and *V. dahliae* tested were pathogenic on horseradish. Infection by isolates of *F. roseum* 'Acuminatum' was enhanced by increasing soil temperatures. Disease was greatest at 24 and 28 C, with 68 and 66%, respectively, of the plants infected. Horseradish growth and development at 32 C were poor, although only 10% of the plants were infected by *F. roseum* 'Acuminatum.' Infection by *V. dahliae* was greatest at the lower temperatures; 100% of the plants were infected at 20 C. Temperatures above 20 C resulted in decreased infection. In noninfested control soil, all plants were symptomless regardless of incubation temperature.

**Effect of fungal inoculum concentration on disease severity.** In steamed soil artificially infested with *F. roseum* 'Acuminatum,' the severity of root flecking and fibrous root rot in horseradish were correlated with the inoculum concentration (Fig. 2). At a concentration of 4 × 10^6^ microconidia per gram of soil, 35 and 39% of the roots showed flecking and root rot, respectively. Decreasing inoculum concentration, except between 40 and 20 microconidia per gram of soil, resulted in a reduction in both types of symptoms and in total disease, but reductions were significant only below 2 × 10^6^ microconidia per gram of soil. Average fresh weight of roots decreased with increasing concentrations of inoculum (Fig. 3). Plants in noninfested soil were free of disease symptoms, and no pathogenic organisms were isolated from control plants. The relationships of inoculum concentration to flecking, fibrous root rot, and average fresh weight were best described by the quadratic equations: y1 = 11.89 + 2.16x - 0.0409x² (r² = 0.7040); y2 = 2.59 + 3.02x - 0.0536x² (r² = 0.9415); and y3 = 5.56 - 0.2986x + 0.0049x² (r² = 0.8254), where y1 = flecking, y2 = root rot, y3 = average fresh weight, and x = inoculum concentration.

In steamed soil artificially infested with *V. dahliae*, the percentage infection of roots, crowns, petioles, and leaves was directly correlated with the concentration of microsclerosis up to 50 microsclerosis per gram of soil (Fig. 4). Average root infection ranged from 90% in soil containing 250 microsclerosis per gram to 62% in soil containing 10 microsclerosis per gram. In soil with 10 microsclerosis per gram, average infection in the crowns and petioles was severe; 62% of the plants were infected.
and 52%, respectively, of these plant parts were affected. At this same inoculum level, 53% of the leaves exhibited wilting.

Isolation and identification of a root rot bacterium in horseradish. White, shiny, smooth-margined colonies were isolated consistently from rotting horseradish sets. Characteristics on both crystal-violet-pectate medium and King's medium B and positive tests for levan formation from sucrose, oxidase production, denitrification, and ethanol utilization indicated that the organism was \( P. \) fluorescens biovar II, the group in which \( P. \) marginalis (Brown) Stevens strains are placed (3,10,16).

Five days after inoculation, 62% of the root sections were decomposed, and the bacterium could be recovered easily. Roots that were autoclaved before being incubated in NDB remained intact, with no evidence of bacterial growth. All roots taken from the field during August were found to contain \( P. \) fluorescens. An average of 44% of 200 roots taken from the field in late October in two consecutive years were infected with \( F. \) roseum 'Acuminatum' and \( V. \) dahlie in addition to \( P. \) fluorescens.

Interaction of organisms and disease severity. The effects of each pathogen and of all possible combinations thereof differed significantly (\( P = 0.05 \)) (Table 2). In muck soil containing \( P. \) fluorescens, \( V. \) dahlie, or \( F. \) roseum 'Acuminatum' alone, average emergence was 96, 96, and 65%, respectively, and 64, 66, and 82%, respectively, of the emerged roots were diseased. The combination of \( P. \) fluorescens and \( V. \) dahlie resulted in almost a 25% decrease in emergence and a 20% increase in root disease severity compared to values observed for either organism alone; emergence was 72%, and almost all roots were rotted. \( V. \) dahlie was easily isolated from the xylem of remaining root pieces. The combination of \( P. \) fluorescens and \( F. \) roseum 'Acuminatum' resulted in almost a 28% decrease in emergence, and 100% of these roots were infected with both organisms (Table 2). \( V. \) dahlie in combination with \( F. \) roseum 'Acuminatum' resulted in less emergence than with either pathogen alone, and the symptoms of \( V. \) dahlie infection were much more severe in soil infested with both pathogens; the fungus progressed from the crown throughout the root and into the petioles. When all three organisms were present, average emergence was extremely low, with only 11% of the roots producing aboveground foliage. The few roots that were not decomposed were infected by both \( V. \) dahlie and \( F. \) roseum 'Acuminatum'.

**DISCUSSION**

The root rot complex of horseradish appears to be caused by \( F. \) roseum 'Acuminatum', \( V. \) dahlie, and the bacterium \( P. \) fluorescens. \( F. \) roseum 'Acuminatum' has been reported to cause stem rot of maize and root and crown rot of legumes (1). \( P. \) fluorescens has not been reported previously to be a pathogen on horseradish.

Optimal growth and pathogenicity of \( V. \) dahlie occurred at 20°C, which is consistent with its earliest appearance in the field during late June, when soil temperatures are between 19 and 22°C. Optimal growth and pathogenicity of \( F. \) roseum 'Acuminatum' occurred at 24°C, which coincides with increasing soil temperatures later in the growing season (13). The discovery of both fungal pathogens in the symptomless sets normally used for new plantings indicates a need to examine planting material for the presence of both organisms.

\( P. \) fluorescens by itself causes rot in horseradish roots. The biochemical mechanisms within horseradish tissue that prevent the growth of peptocytic pseudomonads and rotting of the tissue have yet to be fully explained. Fluorescent peptocytic bacteria from plant soft rot roots are currently grouped as \( P. \) fluorescens biovar II or as \( P. \) marginalis (2,15,17). However, pseudomonads associated with pink rot diseases of potato tubers are generally classified as \( P. \) fluorescens (4), whereas those isolated from leafy plant parts are commonly classified as \( P. \) marginalis. In a disease similar to the root rot complex on horseradish, a Fusarium sp. was isolated with \( P. \) fluorescens in stored Kennebec potatoes infected by \( V. \) albo-atrum Reinke & Berthier (6). Also, a relationship was demonstrated between Verticillium wilt and pink eye of potato caused by \( P. \) fluorescens (5); pink eye was confined to tubers grown in fields where Verticillium wilt occurred. When the fungus was controlled with benomyl, pink eye was also reduced. In horseradish, \( F. \) roseum 'Acuminatum' may be either a primary pathogen or a secondary invader following \( V. \) dahlie in the root rot complex. It may be possible to manage the entire disease complex by using a fungicide to reduce infection by Verticillium.

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**LITERATURE CITED**