A Root Rot Complex of Horseradish

J. A. PERCICH, Associate Professor, and D. R. JOHNSON, Research Assistant, Department of Plant Pathology, University of Minnesota, St. Paul 55108

ABSTRACT

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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.

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MATERIALS AND METHODS

Effect of temperature on the growth of *Fusarium roseum* 'Acuminatum' and *V. dahliae*. Mycelial disks (6 mm in diameter) of three horseradish isolates each of *F. roseum* Link:Fr. emend. Snyd. & Hans. 'Acuminatum' and *V. dahliae* were removed from the margins of 3- and 6-day-old colonies, respectively, growing on 15 ml of Difco potato-dextrose agar (1:19, v/v) in petri dishes (100 × 15 mm) at 24 C. Disks of each fungus were then transferred separately to 250-ml flasks containing 100 ml of potato-dextrose broth. Cultures were incubated on a shaker at 8, 16, 20, 24, 28, and 32 C for 10 and 21 days for *F. roseum* 'Acuminatum' and *V. dahliae,* respectively. Five replicate flasks of each fungus were grown at each temperature. Mycelial mats were obtained by filtration, dried at 50 C for 24 hr, and weighed. The experiment was repeated twice.

Effect of temperature on pathogenicity of *F. roseum* 'Acuminatum' and *V. dahliae*. Three isolates of *F. roseum* 'Acuminatum' and three isolates of *V. dahliae* isolated from horseradish were each mixed separately into equal lots of steamed muck soil at 15% moisture content (35% water-holding capacity [WHC]). Populations of *F. roseum* 'Acuminatum' and *V. dahliae* were adjusted to 4×10^4 microconidia and 200 microsclerotia per gram of dry soil, respectively, by dilution with noninfested steamed soil and were stored in closed containers at 15% moisture content (35% WHC). Inoculum concentration was determined by counting the number of propagules in 1 g of soil using a hemacytometer.

Each infested soil was subdivided into five aluminum trays (61 × 41 × 15 cm) and planted at a depth of 2.5 cm with 20 horseradish root sections (2.5 × 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 $\mu E \cdot m^{-2} \cdot s^{-1}$.

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 4×10^4 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 4×10^4 to 1×10^3 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 with surface-disinfested root sections.

After incubation, each plant was removed carefully from the soil, washed under running tap water, blotted, 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.

Interaction 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, 1×10^4 microconidia, and 250 microsclerotia, 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 $\mu E \cdot m^{-2} \cdot s^{-1}$. 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 microsclerotia of *V. dahliae* on disease as measured by infection of roots, crowns, petioles, and leaves.

The *t* statistics were used to compare overall significance of the models ($P < 0.05$). Coefficients of determination (r^2) 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 4×10^4 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 2×10^4 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 $y_1 = 11.89 + 2.16x - 0.0409x^2$ ($r^2 = 0.7040$); $y_2 = 2.59 + 3.02x - 0.0536x^2$ ($r^2 = 0.9415$); and $y_3 = 5.56 - 0.2986x + 0.0049x^2$ ($r^2 = 0.8254$), where y_1 = flecking, y_2 = root rot, y_3 = 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 microsclerotia up to 50 microsclerotia per gram of soil (Fig. 4). Average root infection ranged from 90% in soil containing 250 microsclerotia per gram to 62% in soil containing 10 microsclerotia per gram. In soil with 10 microsclerotia per gram, average infection in the crowns and petioles was severe; 62

Table 1. Disease severity scale for evaluating symptoms caused by *Verticillium dahliae* or *Fusarium roseum* 'Acuminatum' in horseradish root cross sections

V. dahliae (vascular ring)	Severity value	*F. roseum* 'Acuminatum' (nonvascular)
None	0	None
Trace of ring flecking	1	Flecking in stele
Entire ring flecked	2	Flecking in stele and cortex
Entire ring blackened	3	Entire stele and cortex discolored
Root decomposed	4	Dry, fibrous root rot

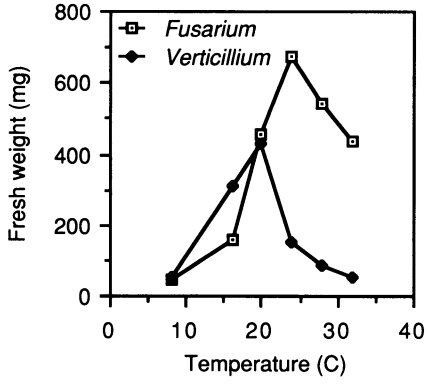

Fig. 1. Effect of temperature on weight of mycelium (mg/100 ml of potato-dextrose broth) of *Fusarium roseum* 'Acuminatum' and *Verticillium dahliae* after 10 and 21 days, respectively.

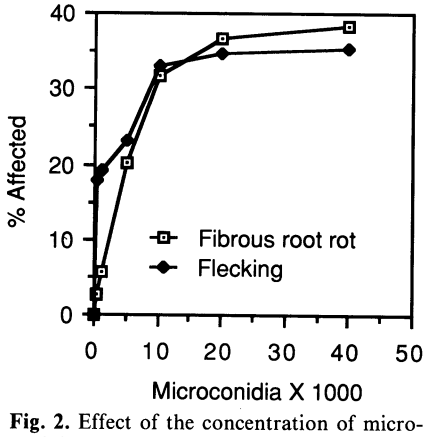

Fig. 2. Effect of the concentration of microconidia of *Fusarium roseum* 'Acuminatum' on fibrous root rot and cortical flecking of horseradish roots.

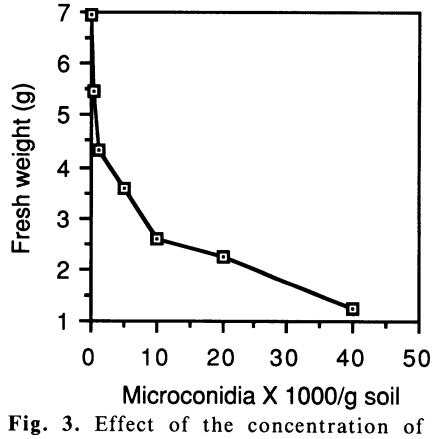

Fig. 3. Effect of the concentration of microconidia of *Fusarium roseum* 'Acuminatum' on fresh weight of horseradish roots.

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. dahliae* 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. dahliae,* 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. dahliae* 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. *Verticillium* 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. dahliae* in combination with *F. roseum* 'Acuminatum' resulted in less emergence than with either pathogen alone, and the symptoms of *V. dahliae* 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. dahliae* and *F. roseum* 'Acuminatum.'

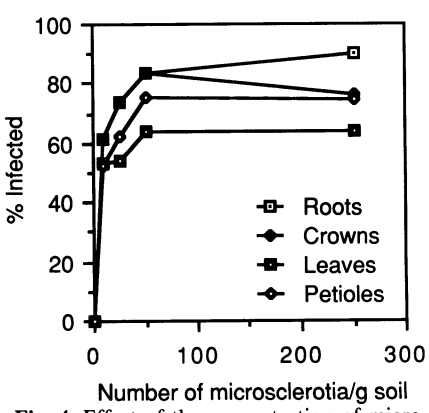

Fig. 4. Effect of the concentration of microsclerotia of *Verticillium dahliae* on horseradish root, crown, petiole, and leaf infection.

DISCUSSION

The root rot complex of horseradish appears to be caused by *F. roseum* 'Acuminatum,' *V. dahliae,* 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. dahliae* 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 pectolytic pseudomonads and rotting of the tissue have yet to be fully explained. Fluorescent pectolytic bacteria from plant soft rots are currently grouped as *P. fluorescens* biovar II or as *P. marginalis* (2,15,17). However, pseudomonads associated with pink eye 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. dahliae* in the root rot complex. It may be possible to manage the entire disease complex by using a fungicide to reduce infection by *Verticillium.*

Table 2. Emergence and disease incidence in horseradish seedlings grown in soil infested with *Pseudomonas fluorescens (Pf), Verticillium dahliae (Vd),* and *Fusarium roseum* 'Acuminatum' (*Fra*) alone or in combination

Pathogens present	Emergence[z] (%)	Disease incidence[z] (%)
Pf	96 e	64 b
Vd	96 e	66 b
Fra	65 d	82 c
Pf + *Vd*	72 d	98 d
Pf + *Fra*	26 b	86 c
Vd + *Fra*	58 c	99 d
Pf + *Vd* + *Fra*	11 a	100 d
Uninoculated	98 e	0 a

[z]Means in each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

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LITERATURE CITED

1. Booth, C. 1971. The Genus *Fusarium.* Commonwealth Mycological Institute, Kew, Surrey, England. 273 pp.
2. Couter, J. W., and Rhodes, A. M. 1969. Historical notes on horseradish. Econ. Bot. 23:156-164.
3. Doudoroff, M., and Palleroni, N. J. 1974. Gram-negative aerobic rods and cocci. Pages 217-243 in: Manual of Determinative Bacteriology, 8th ed. R. E. Buchanan and N. E. Gibbons, eds. Waverly Press, Baltimore, MD. 1246 pp.
4. Folsom, D., and Friedman, B. A. 1959. *Pseudomonas fluorescens* in relation to certain diseases of potato tubers in Maine. Am. Potato J. 36:90-97.
5. Frank, J. A., Webb, R. E., and Wilson, D. R. 1973. The relationship between Verticillium wilt and pink eye disease on potato. Am. Potato J. 50:431-439.
6. Friedman, B. A., and Folsom, D. 1953. Storage behavior of Kennebec potatoes infected by *Verticillium albo-atrum.* (Abstr.) Phytopathology 43:108.
7. Heald, F. D., Jones, L. K., and Huberty, G. A. 1938. Plant disease survey. Wash. Agric. Exp. Stn. Bull. 354:56-57.
8. Kadow, K. J., and Anderson, H. W. 1940. A study of horseradish diseases and their control. Univ. Ill. Bull. 469. 26 pp.
9. Kroff, G., and Bonning, K. 1934. Die Meerrettichschwärze und ihre Bekämpfung. Prakt. Bl. Pflanzenschutz 11:273-277.
10. Lelliot, R. A., Bellong, E. A., and Hayard, A. C. 1966. A determination scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
11. Mueller, J. P., Percich, J. A., and Mitchell, J. E. 1982. Root deterioration associated with Verticillium wilt of horseradish. Plant Dis. 66:410-414.
12. Nash, S. M., and Snyder, W. C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52:567-572.
13. Percich, J. A., Mitchell, J. E., and Mueller, J. P. 1976. Root deterioration of horseradish in Wisconsin. (Abstr.) Proc. Am. Phytopathol. Soc. 3:287-288.
14. Poole, R. F. 1922. Investigation of the horseradish root rot. N.J. Agric. Exp. Stn. Annu. Rep. 33:260-261.
15. Sands, D. C., and Hankin, L. 1975. Ecology and physiology of fluorescent pectolytic pseudomonads. Phytopathology 65:921-924.
16. Sorauer, P. 1899. Kernfaule und Schwarzwerden des Meerrettichs. Z. Pflanzenkrankh. 9:132-137.
17. Stainer, R. Y., Palleroni, N. J., and Doudoroff, M. 1966. The aerobic pseudomonads: A taxonomic study. J. Gen. Microbiol. 43:159-271.