PATHOGENICITY OF FUSARIUM SPECIES FROM RED CLOVER 1/

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Abstract

The pathogenicity of 36 isolates of Fusarium from red clover was tested on 'Lakeland' red clover seedlings in liquid culture in test tubes. Isolates differed greatly in virulence. On the average of all isolates tested, F. solani was most virulent, followed by F. oxysporum and F. roseum in that order. Among the five varieties of red clover used for greenhouse studies, 'Lakeland' and 'Dollard' were relatively susceptible, 'Kenland' was relatively resistant, and 'Chesapeake' and 'Pennscott' were intermediate in their reaction to the Fusarium species. All of the legume hosts used, except Phaseolus vulgaris, became diseased but none of the species in Cruciferae, Gramineae and Solanaceae showed any evidence of infection. None of the Fusarium species from cabbage, tomato and pea produced any Infection on the clovers.

Introduction

Red clover, Trifolium pratense L., is an important forage legume in eastern Canada, although its usefulness is limited by a lack of persistence of stands. A root and crown rot complex is one of the main factors in this problem. Fusarium species probably are the most prevalent and important pathogens in association with this disease complex (4). The destructiveness of Fusarium species during seasons favorable for disease development is a serious threat to the production of clover. It is known that variations in virulence exist between isolates of Fusarium. It has also been suggested (13) that some Fusarium isolates are specific in host range, i.e. their virulence is restricted to a single species of host or to closely related species. Little work has been done on the pathogenicity of isolates of Fusarium from red clover on other plant species. Since such information is a subject which has been of increasing interest to plant breeders in general and to plant pathologists in particular, relevant data are presented in this paper.

Materials, Methods and Results

Tests in nutrient solution

Thirty-six isolates of Fusarium obtained from red clover with root canker, stem canker, and crown bud necrosis were tested in liquid culture in test tubes (1.7 x 15 cm) in a laboratory. Each tube contained a glass wool platform (2) and 10 ml of Hoagland's nutrient solution (9). The top of each platform was flat and extended to the upper surface of the solution in the tube. Prepared tubes were plugged, autoclaved, and cooled to room temperature. Than, five 3-day-old 'Lakeland' red clover seedlings, started on moist filter paper in petri plates, were placed aseptically in each tube. Ten replicates, totalling 50 seedlings for each isolate were inoculated in each of three tests and the same number of seedlings were left uninoculated in each case as a control. Inoculation consisted of placing six 5 mm discs of 7-day-old inoculum which had been increased on potato-dextrose agar (PDA) in petri plates, on top of the glass wool platform. Six sterile PDA discs were placed in each control. A few drops of sterile potato-dextrose broth were dispensed over each platform with a pipette to facilitate rapid and uniform spread of the inoculum. Tubes were arranged on a large rack to expose them to constant light and to facilitate daily examination. Temperature was 22-24°C. Final notes were recorded 10 days after inoculation.

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Of 14 isolates of F. oxysporum Schlecht, emend. Snyd. & Hans. tested, 3 were highly or very highly pathogenic, 3 were non-pathogenic and the others were intermediate. Of 10 isolates of F. roseum (Lk.) emend. Snyd. & Hans., 4 were highly pathogenic, 1 was non-pathogenic, and 5 were intermediate. With F. roseum, most of the damage to the seedlings was a "smothering" effect as the seedlings were overgrown with the fungus mycelium. Of the 12 isolates of F. solani (Mart.) Appel & Wr. emend. Snyd. & Hans. tested, 5 were very highly pathogenic and 7 were slightly pathogenic. On the average, of all isolates tested, F. solani was most virulent, followed by F. oxysporum, and F. roseum in that order.

Tests in sand or sand and soil

1. Pathogenicity on 5 red clover varieties.

One virulent isolate each of F. oxysporum, F. roseum, and F. solani, selected on the basis of the laboratory tests, were used for greenhouse studies. Hosts were 'Chesapeake', 'Dollard', 'Kenland', 'Lakeland', and 'Pennscott' red clovers. The tests were made under conditions favorable for infection and disease development. Temperature was 24°C. Two kinds of substrata were used for comparison. In the first experiment, a soil mixture of 3 parts loam to 2 parts brown sand was used; in the second, white silica sand provided the substrate. The white sand was steamed once for 3 hours at 15-pounds pressure; the soil mixture was steamed for 1 hour at 15-pounds pressure on each of 2 consecutive days. The pH of the white sand was 6.0; of the soil mixture, 6.2. Wooden flats, 14 x 20 inches, containing 30 pounds of substrate were used. Inoculum was increased in potato-dextrose broth on a shaker and was 10 days old when used. Inoculation consisted of mixing 500 ml of a spore suspension containing approximately 100,000 cells per ml in the soil in each flat and also in dipping the roots of the seedlings in the spore suspension prior to transplanting. Control plants were treated similarly except that sterile potato-dextrose broth was used instead of the spore suspensions. Sixty-four 2-week-old seedlings were transplanted to each flat. Distilled water was used in the soil mixture. Hoagland's nutrient solution was used for the sand cultures. All experiments were terminated 65 days after transplanting. The plants were lifted, washed, and critically examined for disease symptoms. Pathogenicity was measured in terms of disease severity ratings. The two experiments were made at different times; each was repeated twice.

The results of the 2 experiments were very similar. They were averaged together and are given in Table 1. F. solani was clearly the most virulent and F. roseum the least virulent. These results agreed with those obtained in the laboratory tests. 'Lakeland' and 'Dollard' were relatively susceptible, 'Kenland' was relatively resistant, and the other varieties were intermediate in their reaction to the Fusarium species

2. Pathogenicity of the red clover Fusarium isolates on hosts other than red clover.

Two experiments were conducted to obtain more information on the range of host specificity of the species of Fusarium from red clover. The same methods were used as previously described, except that surface-sterilized seeds of the hosts to be tested were sown directly in the soil mixture or white sand which had previously been infested with pure cultures of F. oxysporum, F. roseum or F. solani. The tested seeds were soaked in a spore suspension for 24 hours prior to seeding. Nineteen species in 4 families, including Cruciferae, Gramineae, Leguminosae and Solanaceae were tested as possible hosts. The results obtained are given in Table 2. 'Lakeland' red clover was used as a check on the disease ratings.

All of the legume hosts, except Phaseolus vulgaris, became diseased, but none of Brassica oleracea L., Raphanus sativus L. in Cruciferae, Avena sativa L., Hordeum vulgare L., Secale cereale L., Triticum vulgare Vill., and Zea mays L. in Gramineae and Lycopersicum esculentum Mill. in Solanaceae showed any evidence of infection. All uninoculated seedlings remained healthy.

3. pathogenicity of Fusarium species from hosts other than clover on red clover

In 2 experiments similar to those described above, isolates of F. oxysporum f. conglutinans (Wr.) Snyd. & Hans., F. oxysporum f. lycopersici (Sacc.) Snyd., & Hans., and F. oxysporum f. pisi (Linford) Snyd. & Hans. race 1 were tested for virulence on 'Lakeland' and 'Dollard' red clovers.

None of these isolates of Fusarium produced any infection on the clovers, whereas they produced good infection on 'Penn State Ballhead' cabbage, 'Bonny Best' tomato, and 'Davis Perfection' peas, respectively.

Table 1. - Virulence of 3 Fusarium isolates on 5 varieties of red clover seedlings as indicated by disease severity ratings and effect on stand

Isolate and Variety	Disease severity rating[a]	Percent plants killed
F. solani		
Lakeland	4-5	62.5
Dollard	3-4	57.0
Chesapeake	2-4	42.2
Pennscott	2-3	19.5
Kenland	2-3	16.4
F. oxysporum		
Lakeland	3-5	57.8
Dollard	2-4	26.6
Chesapeake	3-4	23.4
Pennscott	2-3	21.1
Kenland	1-3	6.3
F. roseum		
Dollard	1-3	21.9
Lakeland	3	19.5
Chesapeake	2-3	18.8
Pennscott	2-3	16.4
Kenland	1-2	3.1
Control		
Lakeland	1-2	7.6
Pennscott	1	3.1
Chesapeake	1-2	2.0
Kenland	1	2.0
Dollard	1	0.0

a/ Range in disease severity based on surviving plants only. 0 = no disease or root discoloration; 1 = a trace of root browning or an occasional lesion, mostly on secondary roots; 2 = slight to moderate root browning, considerable necrosis on secondary roots; 3 = moderately severe rotting of secondary roots and tap roots; 4 = severe rotting of entire root system; 5 = very severe rotting; plant killed.

b/ Percent plants killed in final recording, based on 384 plants per treatment per red clover variety.

Discussion

Fungi, bacteria, insects, nematodes, and winter injury have all been implicated in the deterioration of red clover stands. Several species of fungi have been reported to incite wilts and root rots, but Fusarium species have been among the fungi most commonly associated with diseased red clover roots (4, 10). Some workers (6, 14) have concluded that Fusarium species are, at the most, only weakly pathogenic on red clover although

Table 2. - Reaction of 11 species of plants to 3 isolates of Fusarium species from red clover

Host	Common Name	Fusarium isolate	Disease[a] Reaction
Lotus corniculatus L.	Birdsfoot trefoil	F. solani	++
		F. oxysporum	++
		F. roseum	+
Medicago sativa L.	Alfalfa	F. solani	++
		F. oxysporum	++
		F. roseum	+
Melilotus alba Desr.	White sweet clover	F. solani	++
		F. oxysporum	++
		F. roseum	+
M. officinalis (L.) Lam.	Yellow sweet clover	F. solani	++
		F. oxysporum	++
		F. roseum	t
Phaseolus vulgaris L.	Bean	F. solani	-
		F. oxysporum	-
		F. roseum	-
Pisum arvense L.	Field pea	F. solani	++
		F. oxysporum	++
		F. roseum	-
Soja max (L.) Piper	Soybean	F. solani	+
		F. oxysporum	+++
		F. roseum	-
Trifolium hybridum L.	Alsike	F. solani	+++
		F. oxysporum	+++
		F. roseum	+
T. incarnatum L.	Crimson clover	F. solani	++
		F. oxysporum	++
		F. roseum	+
T. pratense L.	Red clover	F. solani	+++
		F. oxysporum	++
		F. roseum	+
T. repens L.	Ladino clover	F. solani	+++
		F. oxysporum	+++
		F. roseum	++

[a] - = no infection; +, ++, and +++ = alight, moderately severe, and severe infection, respectively.

it has been clearly demonstrated that they can infect young seedlings (1). In the field, clovers are often weakened by winter injury, drought, insects, mineral deficiencies and other factors (8). There axe, therefore, many opportunities for even weak pathogens like species of Fusarium to produce severe damage in red clover.

As was expected, isolates of Fusarium from red clover differed in virulence and specificity in the host range they can attack. On the average of all isolates tested, F. solani was most virulent, followed by F. oxysporum and F. roseum. Kilpatrick et al (11), Fulton and Hanson (7), and Ostazeski and Gerdemann (12) have reported similar results. The specificity in host range of Fusarium spp. from red clover suggests that cereals and grasses, as well as solanaceous and other non-legume crops, might be grown in crop rotations to reduce the incidence of Fusarium species harmful to legumes. This specificity in host range is in contrast to the nonspecific character of the enzymatic activity produced by the Fusarium species (3).

'Lakeland' and 'Dollard' are both promising red clover varieties in North America, but they are both relatively susceptible to Fusarium spp. Crall (5) has also observed resistance in 'Kenland'. It should be pointed out that since these tests were made with pure cultures under artificial conditions, they do not necessarily indicate what happens in the field where many factors interact to influence the virulence of fungi and the susceptibility of varieties to diseases. It is well known that root rots are more severe where the plants have been injured (8) so that the resistance of a variety to such factors may influence its susceptibility to diseases. Fulton and Hanson (7) showed that too frequent clipping of red clover predisposed the plants to root rot.

Literature cited

1. Chi, C.C., W.R. Childers, and E.W. Hanson 1964. Penetration and subsequent development of three Fusarium species in alfalfa and red clover. Phytopathology 54: 434-437.
2. Chi, C.C., and E.W. Hanson, 1962. Interrelated effects of environment and age of alfalfa and red clover seedlings on susceptibility to Pythium debaryanum.
3. Chi, C.C., and E.W. Hanson, 1964. Mechanism of wilting incited by Fusarium in red clover. Phytopathology 54 [illegible]
4. Chi, C.C., and W.G. Montgomery, 1963. Root rots - a problem in producing red clover in eastern Canada. Forage Notes 9: 19-22.
5. Crall, J.M. 1951. Wilt of red clover seedlings (Abstr.). Phytopathology 41:7.
6. Fergus, E.M., and W.D. Valleau, 1926. A study of clover failure in Kentucky. Kentucky Agr. Exp. Sta. Res. Bull. 269: 143-210.
7. Fulton, N.D., and E.W. Hanson, 1960. Studies on root rots of red clover in Wisconsin. Phytopathology 50: 541-550.
8. Hanson, E.W., 1953. Relative prevalence and severity of the diseases of forage legumes in Wisconsin, 1946-1952. Plant Disease Reptr. 37: 467-472.
9. Hoagland, D.R., and W.C. Snyder, 1933. Nutrition of strawberry plant under controlled conditions: (a) Effect of deficiencies of boron and certain other elements; (b) Susceptibility to injury from sodium salts. Amer. Soc. Hort. Sci. Proc. 30: 288-294.
10. Kilpatrick, R.A., E.W. Hanson, and J.G. Dickson, 1954. Root and crown rots of red clover in Wisconsin and the relative prevalence of associated fungi. Phytopathology 44: 252-259.
11. Kilpatrick, R.A., E.W. Hanson, and J.G. Dickson, 1954. Relative pathogenicity of fungi associated with root rots of red clover in Wisconsin. Phytopathology 44: 292-297.
12. Ostazeski, S.A., and J.W. Gerdemann, 1957. Effect of methods of soil infestation on the pathogenicity of three fungi associated with red clover root rot. (Abstr.). Phytopathology 47: 26.
13. Walker, J.C., 1957. Plant Pathology. 2nd Ed. McGraw Hill Book Co., New York, 707 p.
14. Young, W.J., 1924. An investigation of clover root rot (Abstr.). Phytopathology 14: 63.