Blackpatch of forage legumes

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In 1975 blackpatch, a disease of forage legumes caused by ***Rhizoctonia leguminicola,*** was found at Lacombe, Alberta, for the first time in Canada. Naturally infected red clover ***(Trifolium pratense),*** sainfoin ***(Onobrychis viciifolia),*** and cicer milkvetch ***(Astragalus cicer)*** have been found in the field, and five other legume species have proven susceptible in inoculation tests; O. ***viciifolia*** and ***A. cicer*** are new host records. The toxicity of fungus mycelium and culture filtrate to mice was examined but results were inconclusive.

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La "plaque noire", maladie des legumineuses fourrageres causee par ***Rhizoctonia leguminicola,*** a été signalée pour la premiere fois au Canada dans la region de Lacombe, en Alberta. Le trefle rouge ***(Trifolium pratense),*** le sainfoin ***(Onobrychis viciifolia)*** et l'astragale pois chiche ***(Astragalus cicer)*** infestés naturellement on ete trouves dans le champ, et cinq autres espèces de legumineuses se sont revelees sensibles lors des essais d'inoculation; ***O. viciifolia*** et ***A. cicer*** ont été identifiees comme etant de nouvelles plantes hôtes. On a étudié la toxicité du mycelium fongique et du filtrat de culture sur les souris, et les resultats obtenus n'ont pas ete concluants.

The blackpatch fungus, previously unreported in Canada, was isolated at Lacombe, Alberta, from lesions on leaves of red clover and sainfoin in 1975, and of cicer milkvetch in 1976. The disease is suspected to occur in British Columbia on white clover (D. J. Ormrod, personal communication, 1975). Blackpatch was first reported on red and white clovers in 1933 from Kentucky (1). The name refers to the appearance of affected patches in a field, not to symptoms on individual plants. Smith ***(8)*** in Wisconsin gave a detailed description of the disease, the host range, and the fungus. Weimer (9) described blackpatch on soybeans, cowpea, kudzu, and blue lupine, while Wells (10) reported the blackpatch fungus causing blight of leaves and stems of big trefoil ***(Lotus uliginosis*** Schkuhr.) in Georgia. The fungus was described as ***Rhizoctonia leguminicola*** by Gough and Elliott (5), who reviewed its distribution in the eastern U.S.A. Symptoms of its toxicity to animals have been described as excessive salivation, loss of appetite, frequent urination and defecation, piloerection and lacrimation (3, 6). and the active principle (slaframine) has been characterized (2, 4, 7).

Materials and methods

The host range of the blackpatch fungus was tested with an isolate obtained from sainfoin leaf lesions at Lacombe, Alberta, in 1975. Cultures of the fungus were grown in flasks of malt-yeast broth for 10 days; the mycelium was drained, ground in a Waring blender with distilled water, and sprayed onto potted greenhouse grown plants of the legumes cicer milkvetch ***(Astragalus cicer*** L.), red clover ***(Trifolium pratense*** L.), sainfoin ***(Onobrychis viciifolia*** Scop.), alfalfa ***(Medicago sativa*** L.), alsike clover ***(Trifolium hybridum*** L.), white clover ***(Trifolium repens*** L.), sweet clover ***(Melilotus alba*** Desr.), and birdsfoot trefoil ***(Lotus corniculatus*** L.). The inoculated plants were kept in a dew chamber at near saturation for 2.5 days at 22°C. Symptoms were described and severity estimated 7 days after inoculation. Detached leaflet inoculations were made by placing small pieces of fungus from a potato dextrose agar (PDS) culture onto four detached leaflets of each species supported on moist filter paper in petri dishes. Severity was estimated 9 days after inoculation.

Animal toxicity tests were carried out by mixing equal parts by weight of dried fungus mat with regular feed, or by moistening feed with culture filtrate, 0.5 ml/g. Injections were prepared using twenty-one 200 ml flasks containing a total of 750 ml of malt-yeast broth. The flasks were inoculated with the blackpatch fungus and incubated at room temperature for 2 weeks. The fungal mats were then ground wet and extracted in a separatory funnel with chloroform by a method similar to that used by Rainey et al. (7). The residue remaining after evaporation of the chloroform layer was suspended in 2.0 ml of sterile distilled water. Two test mice were given 0.5 ml, and two were given 0.25 ml intraperitoneal injections of fungal extract. Three control mice were injected with 0.5 ml distilled water and one was not injected.

Results and discussion

In PDA culture the colonies of the blackpatch fungus are at first white, becoming dark brown to black, coarse, and forming small sclerotia-like bodies. On leaves incubated under moist conditions in petri dishes, long dark aerial hyphae spread over the leaves and the supporting filter paper (Figure 1). We have not encountered other fungi that form such obvious dark aerial hyphae and consider this diagnostic for blackpatch.

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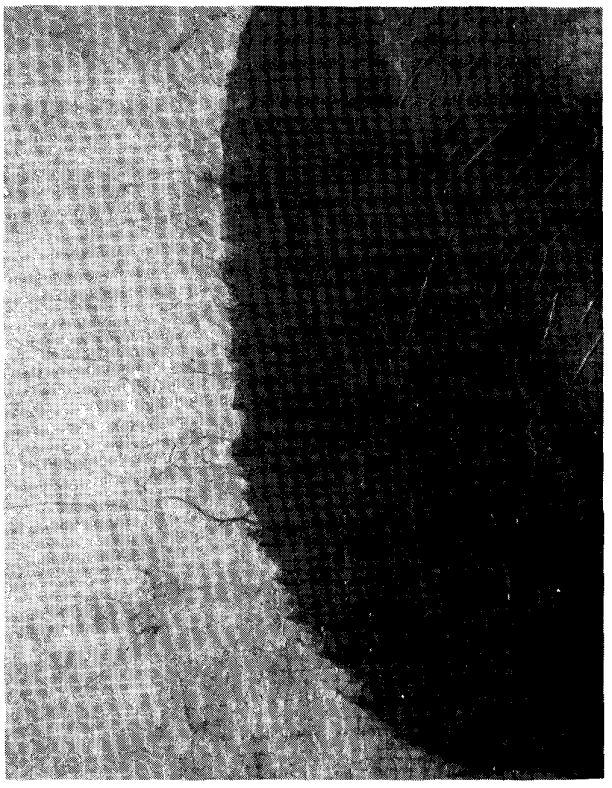

Figure 1. Detached white clover leaf inoculated with the blackpatch fungus and incubated on moist filter paper in a petri dish, showing dark hyphal strands, X 7.

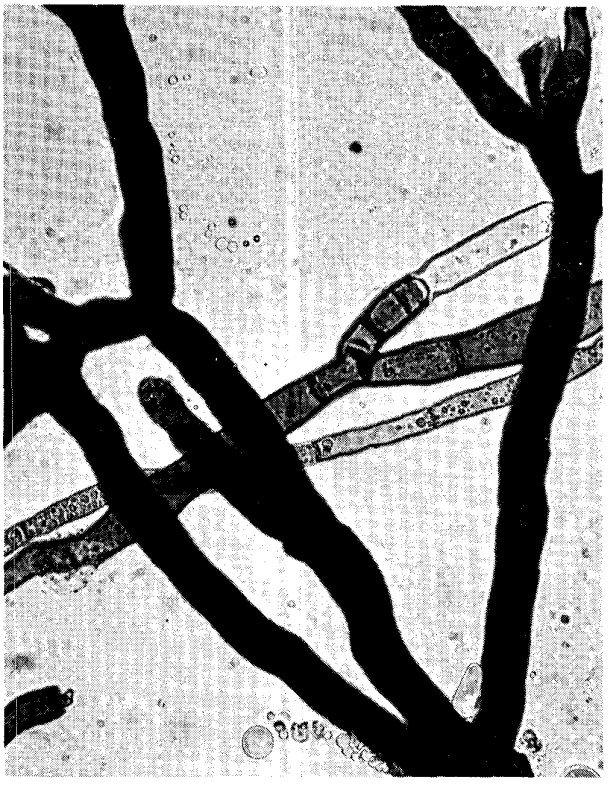

Figure 2. Hyphae of the blackpatch fungus, potato dextrose agar culture, X 330.

The hyphae (Figure 2) vary from 7 to 15 μm in diameter, some being straight walled and some constricted at the septa. The color ranges through hyaline, amber, dark brown, to black, but no green color was noted. Occasionally large, up to 45 μm diameter, swollen chlamydospore-like cells without thickened walls were found in the hyphae.

Sclerotia-like bodies are composed of loose branching hyphae with short swollen cells, and are not differentiated into layers. The older, highly vacuolate hyphae usually break smoothly at the septations, but occasionally roughly torn breaks are found between septa. Except for the pseudosclerotia, the hyphae are usually dichotomously branched. The fungus resembles the descriptions of *R. leguminicola* by Smith *(8)* and Gough and Elliott (5). except that it lacks green pigment and the hyphae are slightly smaller in diameter. According to R. A. Shoemaker and L. K. Weresub (personal communication), this fungus does not belong in the genus *Rhizoctonia.* However the name *R. leguminicola* is used here because a more appropriate name is not available at present. A specimen of the fungus from *Astragalus cicer* has been deposited in the National Mycological Herbarium, Ottawa, Ont., as DAOM 155489.

In the host range test all of the legumes tested were affected to varying degrees. Table 1 shows severity ratings on hosts, as well as symptoms produced by artificial inoculation. Naturally infected plants in the field did not show the light gray areas seen on inoculated plants in the greenhouse. In the field, diseased areas on leaves were dark brown and difficult to distinguish from the symptoms caused by *Stemphylium sarcinaeforme,* as mentioned by Smith *(8).* Birdsfoot trefoil was infected artificially in the greenhouse, but was not found to be infected in the field. However sainfoin and cicer milkvetch were found to be infected in the field and these are new host records for blackpatch.

The addition of fungal mats and filtrate to feed and the force feeding of ground cultures or filtrate to mice resulted in inconsistent and indistinct symptoms. Intraperitoneal injections of aqueous suspensions of the residue from the chloroform extract of fungal mats resulted in mild symptoms of griping and piloerection albout 0.5 h after injection. The treated mice recovered after 4 h and no fatalities occurred. Excessive salivation symptoms were not observed, and mice may be similar to rats, which do not show this symptom (4).

Table 1. Disease severity on inoculated detached leaflets and entire plants, and description of symptoms caused by the blackpatch fungus in greenhouse tests

Host and cultivar	Severity*		Symptoms on entire plants spray-inoculated in greenhouse
	Detached leaflets	Entire plants (avg of 3)	
Alfalfa, Beaver	4	3.0	Pale tan patches with dark margins
Alsike clover, commercial	4	2.7	Small circular brown lesions ("pepperspot") with chlorotic halos, to larger brown spots containing concentric rings
Cicer milkvetch, Oxley	4	4.3	Gray to brown irregular patches, some with dark margins; also dark brown very small spots; occasional young shoot "blasted"
Birdsfoot trefoil, Leo	3	2.0	Very small, brown, circular spots
Red clover, Altaswede	2	2.7	Gray circular patches containing concentric rings ("target" spots); also some very small dark brown spots
Sainfoin, Melrose	5	2.7	Numerous small brown spots
White clover, commercial	2	2.7	Gray to light brown spots varying in size from small to quite large
Sweet clover, Arctic		2.7	Gray to light brown circular patches often with darker brown centers; some with chlorotic halos

* Disease rating: 1 (healthy) to 5 (severely diseased).

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