RHIZOPHYDIUM GRAMINIS (CHYTRIDIALES): MORPHOLOGY, HOST RANGE, AND TEMPERATURE EFFECT

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Abstract

Rhizophydium graminis, an obligate parasite of root cells was found in soil from most winter wheat growing areas of Ontario. In laboratory tests an isolate from wheat grew abundantly on barley (Hordeum vulgare), Agropyron repens, Bromus japonicus, Cigitaria sanquinalis, Elymus canadensis, Lolium perenne, and Zea mays but poorly or not at all on oats (Avena sativa), 13 other species of Gramineae and 9 commercial plants in other families. R. graminis was also found in Ontario on six nongraminaceous hosts, Two isolates from Plantago major failed to infect wheat. The morphology of R. graminis is discussed and a non-sexual origin of the resting spores is suggested. The fungus grew between 5 and 21 C with optimum about 15-19 C.

Introduction

The chytridiaceous fungus Rhizophydium graminis was first reported by Ledingham (3) on roots of wheat and Panicum from Ontario and Massachusetts. More recently, MacFarlane (4) found this fungus on cereals, grasses, Chenopodium, Stellaria, and tobacco in England. In Ontario, R. graminis was one of four zoosporic fungi commonly found associated with wheat spindle streak mosaic virus (1). The possibility of it being a vector of WSSM virus prompted further investigation on morphology, distribution in Ontario, host range, and temperature effect.

Methods

Rhizophydium graminis Ledingham was found on the roots of winter wheat seedlings grown in soil from various fields at 15-18 C for 3 to 4 weeks. To increase the number of fruiting bodies and to make roots easier to examine microscopically, the roots of seedlings removed from the soil were rinsed in tap water, the young plants potted in sand and watered with half-strength Hoagland's solution. The fruiting bodies were usually abundant after a further 2 to 3 weeks at 15-18 C. Previous failure to find the fungus on roots of plants grown in sand (1) can be attributed to temperature which occasionally exceeded 20.C.

The fungus was transmitted to other seedlings by growing seedlings around an infected wheat plant in sand in a 4-inch pot for 2-3 weeks. The test seedlings were then repotted in sand. The fungus has been maintained on wheat in this manner for over 4 years. Host range and temperature experiments were done with an isolate from a mixture of soils collected from wheat fields near Brantford, Ontario.

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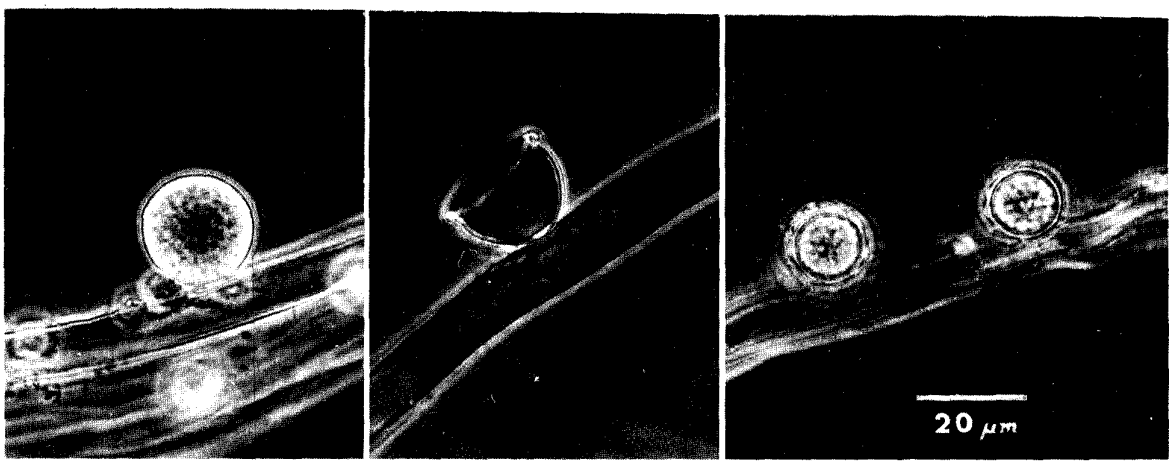
Observations and results

Morphology

Rhizophydium graminis occurs on root hairs and epidermal cells of wheat, barley, and certain grasses and weeds. It is recognized by spherical sporangia on the surface of root cells (Fig. 1), or by the cup-shaped walls of empty sporangia following zoospore release (Fig. 2). The host cell is penetrated by a fine germ tube which grows into a sparingly branched rhizoid (Fig. 2) while the external zoospore cyst enlarges and becomes a sporangium. The empty sporangia and rhizoids clearly distinguish the fungus from the protozoa or from Pythium sporangia which are at times superficially similar to the globular, external sporangia of R. graminis.

Sporangia of all isolates that I have examined from Ontario were typically spherical, 6-36 um diam and occasionally ovate. Larger sporangia, reported up to 100 um diam (3), were not seen on either freshly collected plants or on plants grown in sand. At maturity sporangia release zoospores by a sudden bursting of a thin apical portion of the sporangium wall. The zoospores, which swim away immediately, vary from spherical, 2-2.5 µm diam to elliptical, 2X3-1.5X4 µm and have one lipid body, 0.5-1.5 µm diam, and one posterior flagellum. The flagellum lengths of five isolates differed as follows: 10-12, 12.5-14, 13-15, 14-15.5, and 15-16 µm. The measurements include the very fine and difficult-to-see whiplash end 1.5 µm in length.

Resting spores are 10-18 µm diam and have a hyaline or very pale brown outer wall 0.5-



Figures 1-3. *Rhizophydium graminis* on root hairs of wheat. 1) Mature sporangium; 2) Empty sporangium following discharge of zoospores, note the rhizoids inside the root hair; 3) Two thick-walled resting spores. All figures at the magnification shown in Fig. 3.

1.0 µm thick and a hyaline inner wall 0.5 µm. The outer wall is at first smooth but becomes roughened when mature (Fig. 3). I have not been able to confirm earlier observations (3) that resting spores result after fusion of rhizoids. Although root hairs usually are infected by many large and small thalli with interlacing rhizoids, I believe resting spores are asexual because often a solitary resting spore was seen without any sign of fusion to an adjacent cyst or rhizoid.

Temperature effect on growth

The maximum temperature measured among the roots in sand culture was 20-21 C; the estimated optimum growth, based on the abundance of sporangia, was between 15 and 19 C. The fungus also grew at 5 C which was the lowest temperature tested. Resting spores occurred at 5 to 21 C and were exceedingly abundant at 20-21 C.

Survival

The fungus has survived 9 years, the longest period tested, in air-dried soil stored in a greenhouse in which the temperature varied from about 15 to 30 C.

Distribution

In 1971 R. graminis was recovered from 15 out of 19 soil samples collected in wheat fields in southern Ontario, including the following counties: Elgin, Haldimand, Huron, Kent, and Lincoln. It has been recovered from soil at the Central Experimental Farm, Ottawa, and at Amherst Island in Lake Ontario. It is therefore probably present in all wheat growing areas of Ontario. In addition, it was found in muck soil in which vegetable crops had been grown for many years from the Bradford Marsh, Simcoe County.

Host range

Plants were recorded as susceptible, lightly infected or resistant to an isolate of R. graminis from wheat.

Susceptible - Susceptible species were infected at least as severely as *cv.* Kent winter wheat: mature sporangia were abundant on epidermal cells and root hairs and the fungus was able to subsist on the test plant after removal of the infected wheat plant from the pot. Susceptible plants included winter wheat (Triticum aestivum L. cv. Kent), durum wheat (T. durum Desf. cv. Ramsey), barley (Hordeum vulgare L. cv. Vantage), field corn (Zea mays L. cv. Dekalb), sweet corn (Zea mays var. rugosa Bonsf.), perennial rye grass (Lolium perenne L.), Agropyron repens (L.) Beauv., Bromus japonicus Thumb., Digitaria sanguinalis (L.) Scop., and Elymus canadwnsis L.

Light infection - Lightly infected plants had a few mature sporangia on roots and possibly only succumbed to R. graminis because of the favorable test conditions. The fungus either did not survive or was barely detectable on the test plants when the infected wheat plant was removed from the pot. Species included in this category were cabbage (Brassica oleracea var. capitata L. cv. Golden Acre), cress (Lepidium sativum L.), rice (Oryza sativa L. cv. Blue Bonnet), lettuce (Lactuca sativa L. cv. Grand Rapids), Agropyron trachycaulum (Link) Malte., Avena byzantina K. Koch, A. sterilis L., Bromus inermis Leyss., Festucn rubra L., Holcus lanatus L., and Hordeum bulhosum.

Resistant - Plants were considered resistant if encysted zoospores failed to develop into mature sporangia. It is interesting to note that within the genus Avena, wild oats (A. fatua and A. barbata) and the cultivated varieties Stormont and Garry were completely resistant, whereas on cv. Clintland 60 a few aborted sporangia were seen with their germ tubes walled-off by host plug material. In contrast, the wild

grasses A. byzantina and A. sterilis were lightly infected. Resistant plants included oats (Avena sativa L. cv. Stormont, Garry, and Clintland 60), beet (Beta vulgaris L.), green bean (Phaseolus vulgaris L. cv. Harvester), parsley (Petroselinum crispum (Mill.) Mansf. cv. Moss Curl), Avena barbata Brot., Avena fatua L., Dactylis glomerata L., and Poa pratensis L.

In addition to the host range tests recorded above, R. graminis has been found on a number of occasions on plants other than wheat. In a sample of muck (organic) soil from the Bradford Marsh, R. graminis grew abundantly on barnyard grass (Echinochloa crus-galli (L.) Beauv.), purslane (Portulaca oleracea L.), and common groundsel (Senecio vulgaris L.), which grew from inadvertently collected with the soil. The fungus grew on wheat grown in this soil but not on cabbage, carrot (Daucus carota L.), celery (Apium graveolens L.), lettuce, onion (Allium cepa L.), parsley, tomato (Lycopersicum esculentum Mill.), or Oenothera biennis L.

On two occasions in 1973 R. graminis was found on plantain (Plantago major L.) from Vineland and Port Colborne. On each occasion attempts to transmit the fungus to wheat were unsuccessful.

R. graminis was found on five other occasions in 1973 on the following grasses and weeds collected in Ontario: Agropyron repens, redroot pigweed (Amaranthus retroflexus L.), shepherd's purse (Capsella bursa-pastoris (L.) Medic.), wild strawberry (Fragaria virginiana Duchesne) and Polysonum aviculare L.

These findings suggest there are host specific strains of R. graminis because the isolate from wheat severely infected only graminaceous plants whereas the fungus occurred naturally on non-graminaceous weeds from several locations. Moreover, two isolates from Plantago major failed to infect wheat under favorable test conditions in the laboratory.

A search of the literature shows that apart from the rather scarce reportings of R. graminis (1, 3, 4), a species of Rhizophydium has been found parasitic on Erica gracilis in Switzerland (5), and in Germany R. patellariurn was reported parasitic on cortical cells of cabbage (2). However, most species of Rhizophydium occur on algae in aquatic habitats or have been isolated on pine pollen or other baits from soil. Several have been grown in pure culture on chemically defined media but all attempts by the author to grow R. graminis on algae, pine pollen, or in pure culture have, so far, failed.

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Literature cited

1. Barr, D. J. S., and J. T. Slykhuis. 1970. Zoosporic fungi associated with wheat spindle streak mosaic in Ontario. Can. Plant Dis. Surv. 49:112-113.

2. Gaertner, A. 1960. Einiges zur Ernahrungsphysiologie von Rhizophydium patellarium Scholz. Arch. Mikrobiol. 36:46-50.

3. Ledingharn, G. A. 1936. Rhizophydium graminis n. sp., a parasite of wheat roots. Can. J. Res., C., 14:117-121.

4. MacFarlane, I. 1970. Laqena radicicola and Rhizophydium graminis, two common and neglected fungi. Trans. Brit. Mycol. Soc. 55: 113-116.

5. Stalder, L., and F. Schutz. 1957. Untersuchungen uber die kausalen Zusammenhange des Erikawurzelsterbens. Phytopathol. Z. 30: 117-148.