Marasmius Root Rot of Alfalfa and Khella in Egypt

H. I. SEIF EL-NASR and O. F. ABDEL-AZIM, Plant Protection Department, National Research Centre, Dokki, Cairo, Egypt, and K. T. LEATH, Research Plant Pathologist, U.S. Regional Pasture Research Laboratory, University Park, PA 16802

ABSTRACT

Seif El-Nasr, H. I., Abdel-Azim, O. F., and Leath, K. T. 1984. Marasmius root rot of alfalfa and khella in Egypt. Plant Disease 68: 906-907.

Basidiomycetes isolated from rotted roots of alfalfa (*Medicago sativa*) and khella (*Ammi visnaga*) near Giza, Egypt, were evaluated for in vitro growth rates, temperature tolerance, pathogenicity, and similarity to isolates of *Marasmiellus inoderma*, a basidiomycete pathogen of maize and sugarcane in Egypt. All fungi produced similar white, appressed colonies on potato-dextrose, corn-dextrose, and malt agar media. Colony diameters indicated that the alfalfa and khella isolates were not identical; however, they were similar to those of *M. inoderma*. Growth of the alfalfa and khella isolates was similar to that of the *M. inoderma* isolates within the temperature range of 25–35 C, but below 20 C and at 38 C, development of the alfalfa and khella isolates was significantly greater than that of either isolate of *M. inoderma*. In pathogenicity tests, the alfalfa and khella isolates performed similarly but differently from either *M. inoderma* isolate on all hosts. Characteristics of basidiocarps of the alfalfa and khella isolates agreed with those designated for the genus *Marasmius*.

Expanded irrigation has resulted in the cultivation of alfalfa (*Medicago sativa* L.) on more land and in new areas of Egypt. With this expanded growth of alfalfa has come an increased recognition of disease problems (8). During a survey of fungi associated with root diseases of various crops, nonsporulating basidiomycetes were isolated from diseased roots of alfalfa and the medicinal plant khella (*Ammi visnaga* (L.) Lam.) near Giza, Egypt, and their pathogenicity was established (7).

Because the basidiomycetes isolated from alfalfa and khella roots were similar in culture on potato-dextrose agar (PDA) to the basidiomycete *Marasmiellus inoderma* (Berk.) Sing. that causes root rots of maize (*Zea mays* L.) and sugarcane (*Saccharum officinarum* L.) in Egypt, growth characteristics and pathogenicity of the alfalfa and khella isolates were compared with those of *M. inoderma* from maize and sugarcane. Basidiocarp characteristics of these fungi were compared, and the fungus isolated from alfalfa and khella was identified.

MATERIALS AND METHODS

Basidiomycetes were isolated from rotted roots of alfalfa and khella by plating root pieces on PDA at 30 ± 1 C after surface-disinfection with mercuric chloride. Colony and hyphal characteristics were determined on PDA, and linear colony expansion was determined on PDA, corn-dextrose agar (CDA), and malt agar (MA). Colony expansion on PDA at six temperatures ranging from 8 ± 1 to 38 ± 1 C was determined in incubators. All cultures were started with 4-mm-diameter disks cut from margins of 72-hr-old cultures. Growth comparisons were terminated when the fastest-growing isolate covered the agar surface. Isolates of *M. inoderma* pathogenic on maize and sugarcane (6) in Egypt were included in several tests because the alfalfa and khella isolates resembled *M. inoderma* in culture. Data from culture and pathogenicity tests were analyzed by standard analysis of variance.

Fungal pathogenicity was tested against young plants in the greenhouse by planting alfalfa (New Valley open-pollinated variety), khella (local variety), maize (DC-67), and sugarcane (unknown variety) into steamed soil infested with one of the five fungal isolates grown on autoclaved barley seeds (8). Alfalfa, khella, and maize were seeded at a rate of 30, 10, and 10 seeds, respectively, per 0.3-m^3 pot. Sugarcane was planted at the rate of three stem internodes per pot. All seeds and stem pieces were treated with 0.1% mercuric chloride for 5 min and rinsed with water before planting. All possible combinations of isolates and host species were made, with three replicate pots of each. Seeds or stem pieces in uninfested soil served as controls. Dead plants were counted 50 days after planting. Fungi were reisolated from diseased plants.

The slant-board culture system (3) was used for additional pathogenicity tests with alfalfa (Saranac-AR). Tips and sites 2 cm above the tips on individual intact roots of 1-mo-old plants were inoculated with each isolate as described previously (4). Individual roots on a single plant were inoculated with specific isolates, and uninoculated roots on the same plant served as controls. Inoculations were replicated six times, and disease incidence was evaluated and fungal reisolations made after 1 wk. Segments from diseased roots were excised and boiled in 0.015% aniline blue lacto-phenol for whole-mount microscopic examination. Other segments were fixed, dehydrated, stained with Johansen's quadruple stain, and embedded in wax using standard procedures (1). Longitudinal sections 10 μm thick were prepared.

To induce basidiocarp formation, fungi were cultured on autoclaved barley seeds. Colonized seeds were added to steamed soil in 250-cm^3 plastic pots at a rate equal to 15% of the soil dry weight. Autoclaved alfalfa stems and wooden toothpicks were inserted in the soil. Pots were maintained in a saturated humidity chamber at 30 ± 1 C with 12 hr of incandescent and cool-white fluorescent illumination (12 μE m^{-2} sec^{-1}) daily.

RESULTS AND DISCUSSION

All fungi on PDA, CDA, and MA produced appressed, white colonies, often with thick, ropy, mycelial strands, and all hyphae were septate, binucleate, and hyaline with clamp connections. No spores were observed. Colony diameters of all isolates were similar on PDA but not on CDA and MA (Table 1). Colonies of the alfalfa and khella isolates were similar to those of *M. inoderma* within

Table 1. Colony diameters (mm) of basidiomycetes from alfalfa and khella, and of *Marasmiellus inoderma*, on different media at 30 C[y]

Fungus	Agar medium		
	Potato-dextrose	Corn-dextrose	Malt
Basidiomycete			
Alfalfa 1	88 a[z]	51 a	53 a
Alfalfa 2	90 a	57 a	58 b
Khella	90 a	61 b	52 a
M. inoderma			
Sugarcane	90 a	64 b	59 b
Maize	90 a	73 c	66 c

[y] Values are means of six replicates.
[z] Column means followed by a common letter do not differ significantly ($P = 0.05$).

Contribution 8303 of the U.S. Regional Pasture Research Laboratory, USDA-ARS, University Park, PA 16802.

Accepted for publication 23 April 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

the temperature range of 25–35 C, but below 20 C and at 38 C, development of the alfalfa and khella isolates was significantly greater than that of either isolate of *M. inoderma* (Table 2).

In cross-inoculations, isolates from alfalfa and khella were more pathogenic on those hosts than on maize or sugarcane; the reverse was also true (Table 3). Host range and degree of pathogenicity of the alfalfa and khella isolates were similar, and both were different from those expressed by either isolate of *M. inoderma*. The alfalfa and khella isolates were equally more pathogenic on khella than on alfalfa.

In the pathogenicity tests with alfalfa on slant boards, the isolates of *M. inoderma* were nonpathogenic either at the root tips or at other root inoculation sites. Rot symptoms, however, occurred after 4 days at all sites with isolates from alfalfa and khella. These roots later became completely brown and water-soaked as the fungus grew externally in ropy strands over the entire root system. Small, lateral roots showed symptoms before the main taproot, but this too was attacked. Hyphae with clamp connections typical of the basidiomycetes were observed in whole mounts of root segments, and hyphae grew intercellularly and intracellularly in epidermal and cortical cells.

Basidiocarps developed in moist-chamber cultures and were similar for the alfalfa and khella isolates. Ropy white mycelia were visible in these cultures, and small mushrooms formed on toothpicks and alfalfa stems. Mushrooms were formed by the alfalfa isolates at 30 days, by the khella isolate at 80 days, and by the *M. inoderma* isolates at 60 days. Basidiocarps produced by the alfalfa and khella isolates conformed to those described for the genus *Marasmius* by Kauffman (2) and Singer (9). Pileus color, diameter, shape, papillae, margin, and surface; lamellae color, shape, and number; stipe color, length, diameter, and attachment to pileus; and basidiospore color, shape, and size were all as described for *Marasmius*. Basidiocarps formed by the fungi from alfalfa and khella differed from those of *M. inoderma* in pileus size and margin, the number of lamellae, stipe length and diameter, and spore size range.

Based on differences in pathogenicity, growth temperature tolerance, and basidiocarp characteristics, the basidiomycetes isolated from alfalfa and khella were not forms of *M. inoderma* and belonged in the genus *Marasmius* (2,5,9). The isolates from alfalfa and khella showed similar patterns of pathogenicity. No previous report of a basidiomycete root pathogen on khella was found. *Marasmius* sp. must be considered, along with *Fusarium* spp. and the *Rhizoctonia* sp. already reported (8), as contributing to the root-rot complex of alfalfa in Egypt.

Table 2. Colony diameters (mm) of basidiomycetes from alfalfa and khella, and of *Marasmiellus inoderma*, grown on potato-dextrose agar at various temperatures[y]

	Temperature (C)					
Fungus	**8**	**20**	**25**	**30**	**35**	**38**
Basidiomycete						
Alfalfa 1	30 a[z]	48 a	69 a	86 a	90 a	41 a
Alfalfa 2	30 a	49 a	67 a	86 a	90 a	28 b
Khella	30 a	55 b	60 b	90 a	90 a	35 c
M. inoderma						
Sugarcane	0 b	39 c	67 a	90 a	90 a	0 d
Maize	0 b	43 c	70 a	90 a	90 a	0 d

[y] Values are means of six replicates.
[z] Column means followed by a common letter do not differ significantly ($P = 0.05$).

Table 3. Comparison of fungi from alfalfa and khella with *Marasmiellus inoderma* for pathogenicity to four host species[y]

	Dead plants (%)			
Fungus	**Alfalfa**	**Khella**	**Sugarcane**	**Maize**
None (control)	13 c[z]	7 c	0 a	0 d
Basidiomycete				
Alfalfa 1	45 ab	100 a	33 a	17 a
Alfalfa 2	59 b	100 a	17 a	40 b
Khella	40 a	100 a	33 a	23 a
M. inoderma				
Sugarcane	17 c	7 c	100 b	93 c
Maize	18 c	45 b	100 b	97 c

[y] Values are means for three pots, 50 days after inoculation.
[z] Column means followed by a common letter do not differ significantly ($P = 0.05$).

LITERATURE CITED

1. Johansen, D. J. 1940. Plant Microtechnique. McGraw-Hill, New York. 523 pp.
2. Kauffman, C. H. 1965. The Agaricales of Michigan. Vol. 1. Johnson Reprint Corp., New York. 924 pp.
3. Kendall, W. A., and Leath, K. T. 1974. Slant-board culture methods for root observations of red clover. Crop Sci. 14:317-320.
4. Leath, K. T., and Kendall, W. A. 1978. Fusarium root rot of forage species: Pathogenicity and host range. Phytopathology 68:826-831.
5. Pegler, D. N. 1968. Studies on African Agaricales. I. Kew Bull. 21:499-533.
6. Sabet, K. A., Ashowr, W. A., Samra, A. S., and Abdel-Azim, O. F. 1970. Root-rot of maize caused by *Marasmiellus inodermus*. Trans. Br. Mycol. Soc. 54:123-126.
7. Seif El-Nasr, H. I. 1980. A new pathogen, non-sporulating basidiomycete fungus, causing root-rot of alfalfa plants in Egypt. Proc. Egypt. Soc. Appl. Microbiol. 4:439-446.
8. Seif El-Nasr, H. I., and Leath, K. T. 1983. Crown and root fungal diseases of alfalfa in Egypt. Plant Dis. 67:509-511.
9. Singer, R. 1975. The Agaricales in Modern Taxonomy. 3rd ed. Straus and Cramer, Germany. 912 pp.