DAMPING-OFF ON ALFALFA CAUSED BY A BINUCLEATE *RHIZOCTONIA* IN MÉRIDA, VENEZUELA

ABSTRACT

Cedeño, L. y Quintero, K. 2006. Damping-off on alfalfa caused by a binucleate *Rhizoctonia* in Merida, Venezuela. Fitopatol. Venez 19(1):2-4.

It was determined that a binucleate *Rhizoctonia* sp. was the cause of damping-off disease observed on alfalfa (*Medicago sativa*) grown at Jají, Merida State, Venezuela. Each culture was identified using the criteria of hyphal morphology, cellular nuclear number in young hyphae close to the tip, septal pore configuration and width of the main runner hyphae. Any of the numerous obtained isolates anastomosed with referential cultures (testers) of anastomosis groups AG-I and AG-K which have been associated with infected alfalfa roots. During pathogenicity tests, the investigated *Rhizoctonia* sp. produced pre-emergence and post-emergence damping-off on alfalfa variety Gimboa.

Additional key words: Alfalfa, Damping-off, lucerne, *Medicago sativa*, *Rhizoctonia*

RESUMEN

Cedeño, l. y Quintero, K. 2006. Damping-off en alfalfa causado por una *Rhizoctonia* binucleada en Mérida, Venezuela. Fitopatol. Venez 19(1):2-4.

Se determinó que una especie de *Rhizoctonia* binucleada fue la causa del damping-off observado en alfalfa (*Medicago sativa*) cultivada en Jají, estado Mérida, Venezuela. La identificación se realizó en función de la morfología y el diámetro hifal, la configuración de la estructura septal y el número de núcleos presentes en células somáticas próximas al ápice de hifas jóvenes. Ninguno de los numerosos aislamientos obtenidos hizo anastomosis con cultivos referenciales (patrones) de los grupos de anastomosis AG-I y AG-K, los cuales han sido asociados con raíces infectadas de alfalfa. En pruebas de patogencicidad, la *Rhizoctonia* sp. investigada produjo "Sancocho"(Damping-off) pre-emergente y post-emergente en alfalfa variedad Gimboa.

Palabras clave adicionales: Alfalfa, lucerne, *Medicago sativa*, *Rhizoctonia*, "Sancocho"

INTRODUCTION

The genus *Medicago* is a member of the leguminous family of plants, which includes perennial and annual species (22). About 15 species are used for diverse purposes, such as animal feed, soil improvement, cover crops, human nutrition, and as ornamentals (2). *Medicago sativa* L., commonly known as alfalfa or lucerne, also called the "Queen of the Forages", is a perennial species and is the most widely grown forage crop around the world (2, 24). It is adapted to various ecological conditions, energy efficient, and produces higher protein yield per acre (26). Alfalfa hay is used primarily as animal feed for dairy cattle but also for horses, beef cattle, sheep, turkeys and other animals (2). In the highlands of Mérida State, Venezuela, alfalfa is usually employed as food complement to increase the productivity of dairy cattle.

Many factors may contribute to the decline in productivity of an alfalfa stand, including poor management practices, unpredictable weather or problems associated with pests (26). Alfalfa seedlings are very susceptible to infection by soil fungi that cause damping-off, such as *Aphanomyces*, *Fusarium, Pythium, Phytophthora* and *Rhizoctonia* (16). Seedlings may die before o after emergence, and accordingly, the disease is named pre-emergence or post-emergence damping-off (15). Pre-emergence damping-off rots the sprouting seed before it breaks through the soil. Post-emergence damping-off is the rotting or wilting of seedlings soon after they emerge from the soil.

In 2002, pre-emergence damping-off was observed on seedlings of the alfalfa variety Gimboa grown in Jají (Municipality of Campo Elías), Mérida State, Venezuela. Commonly the seedlings were seen fallen over the ground showing rotting on the basal portion of the stem. The disease killed 15% of the seedlings from a stand of 4.5 ha located in Jají.

The aim of the present study was to determine the cause of the Damping-off disease on alfalfa seedlings and to evaluate its pathogenicity in both pre-emergence and post-emergence developmental stages.

MATERIALS AND METHODS

Pathogen isolation and identification. Diseased stem tissues were immersed for 1 min in 0.5 % sodium hypochlorite solution, washed three times in sterile distilled water (SDW), dried on sterile filter paper, and placed on 2 % water agar plates amended to pH 4.0 with lactic acid (WAA). The plates were incubated at 25± 2 ºC in the dark. Hyphae from the margin of each developing colony were transferred to new WAA and later to potato–dextrose agar (Difco) plates. The isolates were preserved under refrigeration on PDA slants. Each culture was identified using the cytomorphological criteria of cellular nuclear

number in young hyphae, configuration of septal pore apparatus, and width of the main runner hyphae (23). All identification tests were done on plates containing 4.5 ml of water agar 2.4 % plus 0.39 % PDA (Difco). The cultures selected for determining the nuclear condition were previously treated for at least 2 h with 4% formalin and the nuclei stained for 1 min with 0.025 % acid fuchsin in 85% lactic acid. The fungus was paired with culture tester of the anastomosis groups named AG-I and AG-K, which were kindly supplied by Dr. C. Eken (Department of Plant Protection, Faculty of Agriculture, Atatürk University, Erzurum, Turkey). Mycelial plugs (5mm in diameter) were taken from either actively growing cultures of AG testers and isolates of the investigated binucleate *Rhizoctonia*, and placed 2 cm apart in 9-cm diameter Petri dishes which were incubated for 48-72 hours at room temperature in the dark. When margins of the colonies made contact, agar blocks (3 x 1 cm^2) were removed from the overlapped area, placed on clean slide, stained with lactofuchsin and examined under a light microscope.

Pre-emergence pathogenicity tests. One isolate was selected for determining the fungus pathogenicity on seedlings of alfalfa var. Gimboa introduced from Israel. All the tests were done in plastic trays (38 holes) containing a sterile mixture (1:1) of sand and peat.

Inoculum was produced by growing the fungus in sterile sand and cornmeal (23) for 1 week at room temperature (22 ºC). Washed quartz sand (96 g) was passed through a sieve, mixed with 4 g of corn meal plus 20 ml of water in an Erlenmeyer flask (250 ml). The mixture was autoclaved for 60 min and when cooled, inoculated with the fungus. Eight alfalfa seeds were planted in each hole of the tray of which substrate was inoculated at a 2 % rate (w/w). Two hundreds forty seeds were deposited in contaminated substrate and sixty four seeds were planted in substrate amended with sterile sand cornmeal mixture without the fungus. The substrate was watered with SDW and the trays incubated under greenhouse conditions.

Post-emergence pathogenicity tests. Seeds were pre-germinated on moist filter paper placed in Petri dishes incubated at room temperature. The seedlings were transplanted to plastic trays containing sterile substrate and six days later were inoculated with barley grains colonized by the fungus. The inoculum was produced by growing the fungus in test tubes (25 x 150 mm) containing barley grains. A layer (2-3 cm) of sterile cotton was placed at the bottom of each tube, and over it, barly grains were deposited on top of the cotton to a height of 2 cm. Then, SDW was applied to moisten the cotton and barley grains. The tubes were sealed with cotton, protected with gauze and sterilized twice for 20 min at 121 ºC, with an interval of 24 h. Each tube was inoculated with four disks (6-mm-diam) of mycelium removed from 5 days-old culture grown on PDA. The tubes were incubated at room temperature (22 ºC) and under 12:12 h (light:darkness) regime for 2 weeks. Thirty four seedlings were inoculated by gently removing the soil mixture around the stem base, placing 6 colonized barley grains in direct contact with it, and covering the

inoculum with soil mixture. Control seedlings were inoculated in a similar manner by applying sterile barley grains without the fungus. Reisolations were performed from experimentally infected seedlings to confirm Koch's postulates.

RESULTS

Pathogen isolation and identification. The fungal isolates obtained from diseased alfalfa seedlings produced similar fungal colonies of which hyphae showed the morphological characteristics of *Rhizoctonia*. On PDA the isolates produced colonies that initially were white colored and later changed to tan. Two types of mycelium were apparent: dense surface mycelium growing as concentric rings when grown under fluorescent light, and abundant aerial mycelium that frequently touched the Petri dish lid. Few or no sclerotia developed. In old cultures abundant runner hyphae were observed. The runner hyphae averaged 5.6 (5.0- 8.0) μm in diameter. In 100 somatic cells, the number of nuclei varied between 1 and 4 (7% uninucleate, 75% binucleate, 14% trinucleate and 4 % tetranucleate). The septal structure showed the typical dolipore configuration.

Based on the hyphal cytomorphological features (growth pattern, hyphal diameter, septal structure and number of nuclei per hyphal cell), the fungus was identified as a binucleate *Rhizoctonia* species. The fungus did not anastomose with the AG-I and AG-K referential testers. Only these AGs of binucleate *Rhizoctonia* have been associated with alfalfa worldwide (8).

Pre-emergence pathogenicity tests. Five days after planting, 98.2 % and 66.2 % of the seeds planted in uncontaminated and contaminated substrate, respectively, produced seedlings. This means that 32.0 % of the seedlings were killed by the fungus before their emergence. In the tray containing contaminated substrate, 75% of the emerged seedlings died in 2 weeks, whereas the other 25% died three days later. The seedlings used as control remained asymptomatic throughout the test.

Post-emergence pathogenicity tests. Twenty five days after inoculation, 58.8% of the inoculated seedlings were killed by the fungus. All diseased seedlings showed stem and root rot. The other seedlings died 2 weeks later.

On both tests, the diseased seedlings showed root and stem rot. At first, lesions on the stems appeared tan colored and later changed to dark brown, and a binucleate *Rhizoctonia* was consistently isolated from them.

DISCUSSION

This study demonstrated that the damping-off disease detected on alfalfa grown in Jají was caused by a binucleate *Rhizoctonia* species. The fungus produced pre-emergence and post-emergence damping-off on seedlings of alfalfa var. Gimboa inoculated experimentally.

Rhizoctonia species infect seeds, roots, leaves, stems and fruits of different plant species worldwide (18, 23). On alfalfa it has been associated with damping-off, root rot, root canker, crown and bud rot, stem blight and foliage blight (1,8,10-14, 21,25).

Based on the number of nuclei present in young hyphal cells close to the tips and the width of main runner hyphae (23), *Rhizoctonia* species have been divided into two major groups known as multinucleate and binucleate. *R. solani* Kühn [teleomorph= *Thanathephorus cucumeris* (Frank) Donk] is the best known and the most important multinucleate species, whereas the binucleate species are commonly recognized as binucleate *Rhizoctonia* and their teleomorphic stages belong to the genus *Ceratobasidium* Rogers (23). Based on *in vitro* tests of hyphal fusion reaction among isolates, both multinucleate and binucleate groups have been separated in numerous anastomosis groups (AGs). *R. solani* is conformed by 14 AGs designed AG-1 through AG-13 and bridging isolates AG-BI (3, 17,19,23). The binucleate isolates are divided consecutively from AG-A to AG-S (20,23).

R. solani is the most common *Rhizoctonia* species that has been related with alfalfa diseases (1,13,14,6,8,9,25). However, strains of binucleate *Rhizoctonia* have been isolated from infected alfalfa rootlets in Turkey (6,8,9). Regardless of geographical location, most damage caused by *R. solani* occurs during periods of high temperature and high soil moisture (10). *R. solani* causes both pre-emergence and post-emergence damping-off on alfalfa seedlings, but the latter damage is more common (10).

The *R. solani* strains isolated from alfalfa belong to AG-1-IB, AG-2-1, AG-3, AG-4, AG-5, AG-6 y AG-10 (1,4,6,8,9,25). All these groups, except AG-3 and AG-10, have shown to be pathogenic. Moreover, AG-5 isolates were highly virulent (8).

Only AG-I and AG-K of all of the AGs groups corresponding to binucleate *Rhizoctonia* have been associated with infected alfalfa roots (8). AG-K isolates were moderately virulent (8), but AG-I isolates were not pathogenic to forage leguminous species, including alfalfa. AG-K isolates have also been associated with barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) (5) and with Johnsongrass (*Sorghum halapense*) (Demirci *et al*., 2002), but only the latter demonstrated to be pathogenic on their host.

During the present investigation it was not possible to get representative culture testers of the others recognized AGs of binucleate *Rhizoctonia;* that is why the hyphal fusion tests only were made with the AG-I and AG-K isolates. However, we are looking for other sources of the missing testers because we consider that it is important to further establish other potential anastomosis relationships among our isolates and appropriate testers.

To our knowledge, in Venezuela this is the first report about damping-off disease caused by a binucleate *Rhizoctonia* on alfalfa, and it is also the second report in the world (8). In 1994, Cedeño and Carrero (4) identified a strain of *R. solani* AG-4 causing damping-off on alfalfa grown in Santa Rosa, Mérida, Venezuela.

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LITERATURE CITED

1. Anderson, J. R., Bently, S., Irwin, J. A. G., McKie, J. M., Neate, S., and Pattemore, J. A. 2004. Characterization of *Rhizoctonia solani* isolates causing root canker of lucerne in Australia. Australasian Plant Pathology 33: 241-247.

2. Bauchan, G., and Greene, S. 2000. Report on the status of *Medicago* germplasm in the United States-October 2000. 58pp. *http://www.ars-grin.gov/npgs/cgc_reports/alfalfa/alfalfacgc2000.htm#table1* *(retrieved: March 2005).*

3. Carling, D. E., Baird, R. E., Kuninaga, S., and Gitaitis, R. D. 1999. Characterization of anastomosis group (AG) 13 of *Rhizoctonia solani*. Phytopathology 89: S11

4. Cedeño, L. y Carrero, C. 1996. "Sancocho" en alfalfa causado por *Rhizoctonia solani* AG-4. Universidad de Los Andes, Instituto de Investigaciones Agropecuarias, Mérida, Venezuela. Boletín Divulgativo, Año 21, Nº 1-4. pp. 16.

5. Demirci, E. 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. Plant Pathology 47: 10-15.

6. Demirci, E., and Döken, M. T. 1995. Anastomosis groups of *Rhizoctonia solani* Kühn and binucleate *Rhizoctonia* isolates from various crops in Turkey. Journal of Turkish Phytopathology 24: 57-62.

7. Demirci, E., Eken, C., and Zengin, H. 2002. First report of *Rhizoctonia solani* and binucleate *Rhizoctonia* from Johnsongrass in Turkey. Plant Pathology 51:391.

8. Eken, C., and Demirci, E. 2003. Identification and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* anastomosis groups isolated from forage legumes in Erzurum, Turkey. Phytoparasitica 31: 76–80.

9. Eken, C., and Demirci, E. 2001. Erzurum ilinde yonca bitkilerinde saptanan fungal etmenlerin yayilislari ve patojeniteleri. [The distribution and pathogenicity of fungal pathogens determined from alfalfa plants in Erzurum province]. Atatürk Univ. Ziraat Fak. Derg. 32: 143-150. (English abstract).

10. Erwin, D. C. 1990. Diseases caused by *Rhizoctonia*. *In*: Compendium of Alfalfa Diseases. D. L. Stuteville, and D. C. Erwin (Eds.). American Phytopathological Society, St. Paul, Minnesota. pp. 24–26.

11. Hancock, J. G. 1982. Infection of lateral roots of alfalfa by plant pathogenic fungi. (Abstr.). Phytopathology 72: 949.

12. Hancock, J. G. 1983. Seedlings diseases of alfalfa in California. Plant Disease 67: 1203–1208.

13. Hancock, J. G. 1985. Fungal infection of feeder rootlets of alfalfa. Phytopathology 75: 112-1120.

14. Hancock, J. G. 1993. Fungal rootlets colonization and forage yields of alfalfa in fungicide-treated field plots. Plant Disease 77: 601-608.

15. Horst, R. K. 2001. Westcott's Plant Disease Handbook. Sixth Edition. *Kluwer Academic Publishers*, Boston/Dordrecht/London. pp. 215-216.

16. Munkvold, G.1997. Alfalfa seedlings diseases. Iowa State University, Department of Plant Pathology, Integrated Crop Management. http://www.ipm.iastate.edu./ipm/icm/1997/4-21-1997/alfalfa.html *(retrieved: February 2005).*

17. Ogoshi, A.1996. Introduction –The genus *Rhizoctonia in:* Sneh B, Janji-Hari S., Neate S and Dijst G [Eds.] *Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control*. Kluwer Academic Publishres, Dordrecht, The Netherlands. pp. 1-9.

18. Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annual Review of Phytopathology 25: 125-143.

19. Ogoshi, A.1976. Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stage of the groups. Bulletin of National Institute of Agricultural Sciences, Japan, Ser. C 30: 1-63.

20. Ogoshi, A., Oniki, M., Araki, T., and Ui, T. 1983. Studies on the anastomosis groups of binucleate Rhizoctonia and thier perfect states. Journal of Faculty of Agriculture, Hokkaido University, Japan, 61: 244-260.

21. Rhodes, L. H. 2001. Diseases of Alfalfa (*Medicago sativa* L.) *in:* Common Names of Plant Diseases. Plant Pathology Online. American Phytopathological Society. 6pp. *http://www.apsnet.org/online/common/names/alfalfa.asp (retrieved: July 2002)*

22. Small, E., and Jomphe, M.1989. A synopsis of the genus *Medicago* (Leguminosae). Canadian Journal of Botany 67: 3260-3294.

23. Sneh, B., Burpee, L., and Ogoshi, A.1991. Identification of *Rhizoctonia* species. American Phytopathological Society, St. Paul, Minnesota. 133 pp.

24. Stuteville, D. L., and Erwin, D.C. (Eds.).1990. Compendium of Alfalfa Diseases. American Phytopathological Society, St. Paul, Minnesota. 84 pp.

25. Vicenlli, P. C., and Herr, L. J.1992. Two diseases of alfalfa caused by *Rhizoctonia solani* AG-1 and AG-4. Plant Disease 76:1283.

26. Watkins, J. E., Gray F. A, and Anderson, B.1996. Alfalfa crown and root rots and stand longevity. Cooperative Extension, Institute of Agriculture and National Resources, University of Nebraska-Lincoln. *http://www.ianr.unl.edu/pubs/plantdisease/g912.htm (retrieved: January 2005)*