Susceptibility of American Beachgrass and Other Dune Plants to Marasmiellus mesosporus

Tyler B. Warren and L. T. Lucas

Former Graduate Student and Associate Professor, respectively, Department of Plant Pathology, North Carolina State University, Raleigh 27607.

Journal Series Paper No. 4357 of the North Carolina Agricultural Experiment Station, Raleigh.

Supported by the N. C. Sea Grant Program of the Office of Sea Grant, NOAA, Department of Commerce, Grant Nos. GH-78 and GH-103.

Accepted for publication 25 January 1975.

ABSTRACT

American beachgrass, *Ammophila breviligulata*, inoculated with *Marasmiellus mesosporus* under greenhouse, aseptic, and field conditions died and exhibited characteristic signs and symptoms of Marasmius blight. Other species of dune plants that were susceptible to the fungus under greenhouse conditions were *Cakile edentula*, *Eragrostis spectabilis*, *Erigeron canadensis*, and *Panicum amarulum*. *Hydrocotyle bonairensis*, *Spartina patens*, and *Uniola paniculata* were not affected in greenhouse tests. A *Chloris* sp. and *Panicum amarum* were chlorotic 8 weeks after inoculation, but were not different from controls after 16 weeks. *Marasmiellus mesosporus* was reisolated from all plants that died after inoculation with the fungus.

Phytopathology 65:690-692

Additional key words: Marasmius blight, *Ammophila breviligulata*.

Previous reports (3, 6, 7) have described the disease, Marasmius blight, that causes dying-out of American beachgrass (*Ammophila breviligulata* Fernald) along the Outer Banks of North Carolina. The disease is characterized by circular areas of dead plants on sand dunes along the beach. Basidiocarps associated with the disease have been recently described as a new species and named *Marasmiellus mesosporus* Singer, sp. nov. (5).

The purpose of this study was to determine the pathogenicity of *Marasmiellus mesosporus* on the other plants that grow on the sand dunes and the effect of different amounts of inoculum on the development of Marasmius blight on American beachgrass under laboratory, greenhouse, and natural conditions.

MATERIALS AND METHODS.—Two isolates of *M. mesosporus* were selected for pathogenicity tests. Isolate M2 was obtained from a surface sterilized basidiocarp and isolate P1 was isolated from a diseased beachgrass plant. Inoculum used in all experiments was grown on sterilized oat seeds (25 ml water and 50 g of dry oat seeds) in 500 ml flasks. Mycelial disks from actively growing cultures of isolates M2 or P1 were used to inoculate the media in each flask. Inoculated seeds were incubated for 2 weeks at room temperature (27 C). Noninoculated sterile oat seeds were used as controls.

American beachgrass seedlings used in greenhouse inoculations were obtained by treating seeds with 70% ethanol for 30 seconds and 1.3% sodium hypochlorite for 15 minutes before subjecting the seeds to the special procedures needed for germination of the seeds (4). Small seedlings were transferred to large sterile containers and grown for one month with exposure to sunlight. One-month-old seedlings were transplanted in the greenhouse

in either vermiculite or dune sand that was collected from an area of healthy beachgrass. The seedlings were watered daily as needed and fertilized monthly with Hoagland's solution No. 1 (2).

Three-month-old beachgrass plants in the greenhouse were inoculated by placing 1, 2, 5, 10, 25, 50, or 100 infested oat seeds 1- 5 cm below the surface of the sand or vermiculite beside the roots and stems, or with 100 infested oat seeds on the surface of the sand or vermiculite near the stems. Control plants received an equal number of noninfested oat seeds. Disease development was recorded at weekly intervals for 9 weeks after inoculation.

In laboratory studies, aseptic beachgrass seedlings were obtained by treating scarified seeds with 31.3% hydrogen peroxide to break dormancy to promote rapid germination and to sterilize the seeds before germinating on potato dextrose agar (PDA) (6). Seedlings that showed no bacterial or fungal growth on PDA after 4 weeks were planted 2.5 cm deep in 1.9-liter Mason jars. Each jar contained 650 ml of sterile 246 μm (60-mesh) quartz sand or fine dune sand 177 μm (approx. 80-mesh) plus 350 ml of Hoagland's solution No. 1. The jars were equipped with tops that had a piece of glass tubing (12 mm in diameter and 70 mm long) inserted through a hole and fastened with silicone rubber sealant. The tubing was plugged with cotton and covered with aluminum foil. The jars were autoclaved at 121 C for 30 minutes and reautoclaved at 121 C for 30 minutes 48 hours later. After autoclaving the aluminum foil caps were removed to allow exchange of gases through the cotton.

Plants in jars were inoculated two weeks after transplanting by placing 1, 5, or 10 infested oat seeds 2.5 cm deep next to the beachgrass plants. An equal number of sterile oat seeds were used in control jars. The plants were incubated for 3 weeks at 26-27 C with room light and sunlight from nearby windows.

For dune inoculations, 15 random sites of healthy American beachgrass on foredunes on Ocracoke Island were inoculated by placing 1, 5, 10, 25, or 50 infested oat seeds 1- 5 cm deep near the stems of mature healthy plants. Disease development was recorded at monthly intervals for 4 months after inoculation.

Pathogenicity tests were conducted in the greenhouse on transplants of the following species found on the dunes at Ocracoke, North Carolina: American beachgrass, horseweed (*Erigeron canadensis* L.), pennywort (*Hydrocotyle bonairensis* Lam.), purple love grass [*Eragrostis spectabilis* (Pursh) Steudel], running beachgrass (*Panicum amarum* Ell.), saltmeadow cordgrass [*Spartina patens* (Aiton) Muhl.], sea oats (*Uniola paniculata* L.), sea rocket [*Cakile edentula* (Biglelow) Hooker], and a *Chloris* sp. Two strains of silver bunchgrass (*Panicum amarulum* Hitchc. & Chase) were collected from dunes near Duck, North Carolina (1).

Healthy plants of the above species were collected and transplanted into 20-cm diameter pots in the greenhouse containing beach sand collected from an area of healthy beachgrass on Ocracoke Island. Three weeks after transplanting, four plants of each species were inoculated by placing 10, 25, 50, or 75 infested oat seeds 1- 5 cm below the soil line adjacent to the roots and stems. The same number of sterile oat seeds were placed beside control plants. Disease development was recorded at weekly intervals for 8 weeks.

Isolations were made from inoculated and noninoculated plants used in greenhouse, laboratory and dune experiments. Pieces of plant tissues were surface sterilized in 0.525% sodium hypochlorite solution for 10 minutes, rinsed in sterile distilled water, and placed on acidified PDA, PDA, and water agar.

RESULTS.—Infection and death of beachgrass plants grown in sand and inoculated with infested oat seeds in the greenhouse occurred at all rates of inoculum tested. There were no apparent differences in virulence of the two isolates used.

In the greenhouse, wilting was evident and mycelium was present on the stems of American beachgrass at the soil line 1 week after plants in sand were inoculated with 1, 2, 5, or 10 infested oat seeds. At this time plants inoculated with 25, 50, or 100 infested oat seeds showed no symptoms, and no disease was evident in plants inoculated with 100 infested oat seeds on the sand surface. Two weeks after inoculation plants inoculated with 2, 5, or 10 infested oat seeds were dead and plants inoculated with other amounts of inoculum were wilted. Nine weeks after inoculation all of the beachgrass plants inoculated with 2, 5, 10, or 25 infested oat seeds and 80% of the plants inoculated with 1, 50, or 100 infested oat seeds below the sand surface were dead. Only 40% of the plants inoculated with 100 infested oat seeds on the surface of the sand were dead after 9 weeks.

Wilting of plants grown in vermiculite occurred only with 1, 2, 5, or 10 infested oat seeds, and symptoms and signs developed slower than with sand-grown plants. Dead plants were not observed in vermiculite until 3 weeks after inoculation and then only with plants inoculated with five infested oat seeds per plant. Nine weeks after inoculation of vermiculite-grown plants, all of the plants inoculated with five infested oat seeds and 60% of the plants inoculated with 10 infested oat seeds were dead. None of the other plants inoculated in vermiculite died.

In the laboratory, all seedlings inoculated with either isolate M2 or P1 in Mason jars were killed and were covered with white mycelium within 2 weeks after inoculation in dune sand or quartz sand. White mycelium was visible on the basal stems 3 days after inoculation and had surrounded the bottom half of the plants in 1 week. Seedlings inoculated with five or ten infested oat seeds were dead within 1 week after inoculation. Seedlings inoculated with one or two infested oat seeds were dead within 2 weeks. None of the control plants were diseased.

On the dunes, 70% of the beachgrass plants inoculated with isolate M2 and 60% of those inoculated with isolate P1 were dead or showing symptoms 2 months after inoculation. Within 3 months 90% of the M2-inoculated and 93% of the P1-inoculated plants were dead. At this time all of the beachgrass plants inoculated with 25 or more infested oat seeds with either isolate M2 or P1 were dead, and 80 and 89% of the plants inoculated with 10 or fewer oat seeds infested with isolates M2 and P1, respectively, were dead. The fungus spread to and killed adjacent plants as observed in naturally diseased areas. Plants on the dunes treated with sterile oat seeds remained healthy.

Five of the 10 species including *A. breviligulata*, *C. edentula*, *E. spectabilis*, *E. canadensis*, and *P. amarulum* that were grown in the greenhouse and inoculated with

M. mesosporus were dead within 2 weeks after inoculation with both isolates at all inoculum rates. The five surviving species in order of decreasing vigor at all inoculum rates were: *H. bonairensis*, *U. paniculata*, *S. patens*, *Chloris* sp., and *P. amarum*. *Hydrocotyle bonairensis*, *U. paniculata*, and *S. patens* appeared to be resistant to the fungus. Chlorosis was observed on *Chloris* sp. and *P. amarum* 8 weeks after inoculation, but after 16 weeks the plants had recovered and did not appear different from the controls. All of the control plants treated with sterile oat seeds remained healthy throughout the experiment except for some of the *C. edentula* plants that did not survive transplanting.

Marasmiellus mesosporus was reisolated from all diseased plants that had been inoculated with the fungus. Other fungi infrequently isolated from plants inoculated with *M. mesosporus* in the greenhouse and on the dunes were species of *Aspergillus*, *Fusarium*, and *Trichoderma*. *Marasmiellus mesosporus* was the only organism isolated from inoculated seedlings grown aseptically in jars.

DISCUSSION.—Results from pathogenicity tests in greenhouse, aseptic, and natural conditions confirm a previous report (3) that *M. mesosporus* is a pathogen of American beachgrass and is the cause of Marasmius blight of American beachgrass on the Outer Banks of North Carolina. Vermiculite was not a satisfactory substrate for pathogenicity tests. It may have been too wet for the fungus to grow and cause disease. Less moisture and better aeration would exist in the sand, which is the natural substrate of both the plant and the pathogen.

Plants inoculated with the larger amounts of inoculum under greenhouse conditions developed symptoms and signs more slowly. This suggests that the additional substrate from the oat seeds may have enhanced the saprophytic growth of the pathogen, reducing its ability to cause disease. Also, saprophytic microorganisms in the beach sand could have been stimulated by the larger amounts of oat seeds and could have affected the growth of the pathogen and disease development.

The fungus was pathogenic to certain other dune plants under greenhouse conditions. Although most plants tested do not grow as well as American beachgrass on newly formed dunes, they are important in maintaining older dunes. Since some of the native dune plants are susceptible, they may serve as a source of inoculum whenever American beachgrass is transplanted into new areas. Also, the resistant or tolerant plants found in these tests, particularly *U. paniculata*, *S. patens*, and *P. amarum*, may be useful in stabilizing areas where American beachgrass is dying from Marasmius blight.

LITERATURE CITED

1. AHLES, H. E., C. R. BELL, and A. E. RADFORD. 1968. Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill. 1183 p.
2. HOAGLAND, D. R., and D. I. ARNON. 1938. The water-culture method for growing plants without soil. California Agric. Exp. Stn. Circ. 347. 32 p.
3. LUCAS, L. T., T. B. WARREN, W. W. WOODHOUSE, JR., and E. D. SENECA. 1971. Marasmius blight, a new disease of American beachgrass. Plant Dis. Rep. 55:582-585.
4. SENECA, E. D. 1969. Germination response to temperature and salinity of four dune grasses from the Outer Banks of North Carolina. Ecology 50:45-53.
5. SINGER, R., L. T. LUCAS, and T. B. WARREN. 1973. The Marasmius-blight fungus. Mycologia 65:468-473.
6. WARREN, T. B. 1972. Marasmius blight, a new disease of American beachgrass on the Outer Banks of North Carolina. M. S. Thesis, North Carolina State University, Raleigh. 55 p.
7. WARREN, T. B., and L. T. LUCAS. 1973. Histopathology of Marasmius blight of American beachgrass. Phytopathology 63:725-728.