Nematode Angular Leaf Spot of Dry Bean in Wyoming

G. D. Franc, C. M.-S. Beaupré, F. A. Gray, and **R. D. Hall,** Department of Plant, Soil and Insect Sciences, University of Wyoming, P.O. Box 3354 University Station, Laramie, WY 82071-3354

ABSTRACT

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A new foliar disease of dry bean, nematode angular leaf spot (NALS), is caused by *Aphelenchoides ritzemabosi*. The disease is typified by numerous dark, angular lesions on leaves and occasionally a superficial necrosis on the upper surface of the petiole. Diagnosis of NALS is based on the presence of foliar symptoms and recovery of nematodes from symptomatic tissue. Infested fields were infrequent in a field survey of 7,175 ha in Wyoming conducted during a 3-year period. The field survey represented 17% of the crop harvested during those years. Therefore, it is unlikely that NALS will cause economic yield loss unless conditions for nematode survival and foliar parasitism are unusually favorable. The nematode is also known to parasitize alfalfa foliage in Wyoming and other western states. Infested dry bean fields found during the survey had a recent history of alfalfa production. Therefore, alfalfa and dry bean crop rotation provides a potential mechanism for nematode survival. Parasitic nematodes persisted in air-dried bean leaf tissue for at least 27 months, which suggests that persistence of NALS inoculum in crop debris is likely to occur in the field.

Additional keywords: chrysanthemum foliar nematode, *Phaseolus vulgaris*

Dry bean (*Phaseolus vulgaris* L.) is an important field crop in Wyoming. Approximately 17,000 ha are grown annually. *Aphelenchoides ritzemabosi* (Schwartz, 1911) Steiner & Buhrer, 1932, the chrysanthemum foliar nematode, recently was discovered in Wyoming (4). The incidence and potential impact of this disease, herein named nematode angular leaf spot (NALS), on dry bean production in Wyoming was not known. This report summarizes the results of surveys to detect NALS in Wyoming production areas, identifies characteristics of NALS symptomatology, and outlines the procedure used for NALS diagnosis.

MATERIALS AND METHODS

Field surveys for NALS detection were conducted in Wyoming during 1992, 1993, and 1994. Certified dry bean fields were surveyed during the mid-portion of each growing season, after full canopy development and before plant senescence. Preliminary NALS diagnosis was based on observation and collection of plants with dark, angular lesions on middle to upper leaves (Fig. 1). Presumptive NALS plants were placed in plastic bags and kept cool microscopic examination of symptomatic tissue for the presence of nematodes, followed by nematode recovery and inoculation of healthy plants for subsequent proof of plant parasitism.

Nematodes were recovered from diseased tissue by a modified Baermann funnel technique (3). Tissue was macerated with the aid of a disinfested razor blade. Macerated tissue was placed in approximately 200 ml of sterile deionized water in a Baermann funnel lined with tissue paper. After 24 h, nematodes were collected in approximately 50 ml of liquid. Nematodes were concentrated by centrifugation for 7 min at 1,750 × *g*, and most of the supernatant was discarded. Inoculum was immediately prepared from the concentrated suspension, which was combined with an equal volume of 1% aqueous carboxymethyl cellulose. The inoculum density was not determined; however, microscopic observation readily revealed the presence of living nematodes. Distilled water was misted onto 15- to 25-cm-tall host plants (*P. vulgaris*, pinto cv. Othello) to wet the foliage and inoculum was applied with a plants were covered with plastic bags for several days to maintain leaf wetness and humidity. Immediately after inoculation,

Fig. 1. Foliar symptoms of nematode angular leaf spot in dry bean foliage (trifoliolates) collected during field surveys in Wyoming.

Corresponding author: G. D. Franc
E-mail: francg@uwyo.edu

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all plants were placed in a growth chamber at 22°C and 12 h of light per day and observed daily for up to 5 weeks for symptom development.

Symptomatic foliage collected during the field survey, and foliage harvested from inoculated plants, was air-dried and stored at room temperature for 27 to 30 months. Inoculum was prepared from stored tissue by the procedure described, and plants were inoculated to determine the survival of plant parasitic nematodes in plant tissue.

RESULTS

Approximately 7,175 ha were inspected during the 3-year survey period. Two fields, representing a total of 38.4 ha, contained plants (cv. Othello) with NALS symptoms. The incidence of plants expressing symptoms in infested fields was less than 1%. Both fields were found during the 1992 survey, and no additional fields were found during the 1993 and 1994 surveys. Microscopic examination revealed that *A. ritzemabosi* was associated with symptomatic tissue. Proof of plant parasitism was determined with inoculum recovered from tissue collected at both field sites. Nematode specimens were deposited at the USDA Nematode Collection, Beltsville, MD.

Inoculated unifoliolate and trifoliolate leaves developed angular lesions after approximately 11 days at 22°C (Fig. 2). Lesions were similar to those observed in the field. The discoloration associated with angular lesions became more obvious 14 to 20 days after inoculation, with increased leaf senescence as incubation time increased. Expansion of individual lesions was limited by leaf veins, with most lesions ranging in size from several millimeters to approximately 1 cm. Occasionally, large sections of leaflets, or entire inoculated leaflets, became chlorotic or necrotic within 24 days after inoculation. Symptom development also readily occurred with inoculum prepared after two serial transfers through artificially inoculated plants. Inoculum prepared from symptomatic tissue that had been air-dried and stored at room temperature for at least 27 months was infectious, and symptoms developed within 3 weeks after inoculation.

Fig. 2. Foliar symptoms of nematode angular leaf spot in artificially inoculated unifoliolate leaves of dry bean plants 21 days after inoculation.

DISCUSSION

Foliar symptoms and the presence of *A. ritzemabosi* are used to diagnose NALS. Presence of the nematode is quickly determined by placing several small pieces (1 to 2 mm^2 each) of symptomatic tissue in a drop of water on a microscope slide, waiting several minutes for nematode extraction, heat fixing, and viewing at 100 to 400×. A summary of characteristics used for identification of *A. ritzemabosi* is readily available (8). These diagnostics differentiate symptoms of NALS from similar lesion morphology associated with symptoms of angular leaf spot caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris (7).

We have shown that general characteristics of disease development reported for chrysanthemum and other hosts of *A. ritzemabosi* are similar to NALS development in dry bean. Infection of the host is enhanced by moisture, because the nematode can swim in the water film on the plant surface (1,8). When favorable conditions exist, the nematode can feed on the epidermis and also invade leaves through stomata, where it subsequently feeds and reproduces (1). The presence of the nematode in leaves initiates lesion development and eventual breakdown of the mesophyll (1). Larger leaf veins initially limit lesion expansion; however, with advanced stages of parasitism, widespread necrosis across leaf veins can occur (1). The postinoculation time required for initial appearance of NALS lesions in our experiments (approximately 11 days) closely corresponded to generation times of 10 to 13 days estimated for *A. ritzemabosi* (9).

Parasitism of dry bean by *A. ritzemabosi* may cause yield loss because photosynthetic leaf area is destroyed during nematode feeding and reproduction. However, fields with parasitized plants were rarely found in Wyoming, and the number of affected plants was small. Therefore, it is unlikely that NALS will cause economic yield loss unless circumstances for nematode survival and foliar parasitism are unusually favorable. It is likely that cool, wet environmental conditions increase the risk of NALS development and the probability of observing symptoms in the field.

A. ritzemabosi readily parasitizes alfalfa and is routinely found in association with the alfalfa stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev in Wyoming and other western states (5). Fields in which NALS was detected either had volunteer alfalfa plants present or had alfalfa present during the year immediately before NALS was detected. Because production areas overlap in Wyoming, alfalfa and dry bean crop rotation may permit the nematode to survive on alternate host plants. Fields in which NALS was detected had been planted with the bean cultivar Othello. Therefore, a recent history of alfalfa production and, perhaps, the cultivar planted may be associated with NALS development. Future studies should include a wide range of bean germ plasm to determine varietal responses.

Parasitic nematodes persisted in air-dried leaf tissue held at room temperature for at least 27 months. The nematode is known to undergo anhydrobiosis (6) and will survive 3 years in dried chrysanthemum foliage (8). Therefore, persistence of NALS inoculum in dried bean foliage and associated crop debris between growing seasons is also likely. Long distance spread may occur when parasitized leaf debris contaminates seed lots.

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