DISTRIBUTION AND BIOLOGY OF *DITYLENCHUS DIPSACI* AND *APHELENCHOIDES RITZEMABOSI* IN ALFALFA GROWN IN COLORADO

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ABSTRACT

M.C. Milano de Tomasel, and G. A. McIntyre 2001. Distribution and biology of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* in alfalfa grown in Colorado. Nematropica 31:11-16.

A survey was conducted in 14 major alfalfa producing counties of Colorado (USA) to determine the distribution of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi*. Both nematodes were found in six counties while neither was found in three. *D. dipsaci* occurred exclusively in three counties, and only *A. ritzemabosi* was found in two others. In addition to the survey, the response of four cultivars of alfalfa (Pioneer 5312, Pioneer 5364, Pioneer 5454, and Ranger 5-1153) to both nematodes was evaluated in greenhouse experiments, where a mixture of both nematodes was used as innoculum. Under these conditions, the four entries showed a high percentage of symptomatic plants, with symptoms ranging from slight to severe stem swelling and distortion. A field study designed to evaluate the effect of Furadan 4F as a means of nematode control showed that this pesticide is ineffective on established alfalfa.

Key words: alfalfa, *Aphelenchoides ritzemabosi*, chrysanthemum foliar nematode, *Ditylenchus dipsaci*, nematode control, stem nematode.

RESUMEN

M. C. Milano de Tomasel and G. A. McIntyre 2001. Distribución y biología de *Ditylenchus dipsaci* y *Aphelenchoides ritzemabosi* en alfalfa de Colorado. Nematrópica 31:11-16.

Se realizó un estudio en 14 de los más importantes condados productores de alfalfa de Colorado (USA) para encontrar la distribución de *Dytilenchus dipsaci* y *Aphelenchoides ritzemabosi*. Se encontraron ambos nemátodos en seis condados, mientras que en otros tres no se encontró ninguno de los dos. *D. dipsaci* apareció en forma exclusiva en tres condados, mientras que en otros dos condados sólo se encontró *A. ritzemabosi*. Además de este estudio, se evaluó la respuesta de cuatro variedades de alfalfa (Pioneer 5312, Pioneer 5364, Pioneer 5454, y Ranger 5-1153) a la presencia de ambos nemátodos. Para ello, se realizaron experimentos de invernadero donde se inocularon plantas de estas variedades con una mezcla de ambos nemátodos. Bajo estas condiciones, las cuatro variedades mostraron un gran porcentaje de plantas con síntomas que variaban desde ligeras hasta severas tumefacciones. Finalmente, se realizó un experimento de campo para evaluar el efecto de Furadan 4F como agente de control de nemátodos en alfalfa. Este estudio mostró que este pesticida es inefectivo cuando se lo aplica a alfalfa ya establecida.

Palabras clave: alfalfa, *Aphelenchoides ritzemabosi*, control de nemátodos, *Ditylenchus dipsaci*, nemátodo del tallo, nemátodo de la hoja del crisantemo.

INTRODUCTION

Crop losses from plant-parasitic nematodes in the USA were estimated by the United State Department of Agriculture (USDA) at about $4.0 billion per year (Pimentel, 1981). For alfalfa, *Trifolium repens* L., losses from nematodes amount to about 3-5% of the national production (Hanson, 1972). In Colorado, there are over 344 000 ha of alfalfa with an annual production of 2 971 000 T worth approximately 300 million dollars. The most severe disease problem for alfalfa in Colorado is the alfalfa stem nematode (ASN), *Ditylenchus dipsaci* (Kuhn) Filipjev. In spite of its importance, only limited data are available on the extent on nematode infestations in the major alfalfa producing areas. *Ditylenchus dipsaci* frequently parasitizes alfalfa in combination with the chrysanthemum foliar nematode (CFN), *Aphelenchoides ritzemabosi* (Schwartz) Steiner and Buhrer (Brown, 1957; Soh, 1986). Infection by ASN plus CFN was observed in plants collected from several states (Soh, 1986; Gray, 1994), but this dual infection was noted in only three soil samples in Colorado. The presence of a second nematode in association with ASN is of importance for the development of new alfalfa cultivars with resistance to nematodes.

The present work reports the distribution of both ASN and CFN in major alfalfa producing counties of Colorado. It also summarizes the results from greenhouse experiments conducted to study the response of four different cultivars of alfalfa to dual infestation, and reports field experiments where the effect of Furadan 4F as a means of nematode population control was evaluated.

MATERIALS AND METHODS

Nematode survey: The first survey to determine nematode occurrence was conducted from May to October 1995, and the second from June to September 1996. A total of 69 samples, 47 for the first year and 22 for the second, were collected from 14 counties (Fig. 1). The samples were randomly chosen and consisted of four crowns plus stems. Within 24 h of arrival at our laboratory, all the stems, buds and leaves in each sample were cut into small pieces, placed in a bag and mixed. A 15-g subsample from each sample was used for nematode extraction using the Baermann funnel (Agrios, 1988) for 3 days. An aliquot of 20 ml containing the extracted nematodes was collected in a beaker daily and stored at 4°C. Each daily collection was combined to bring the final sample to 60 ml. Nematode samples were then refrigerated at 4°C for 24 h to allow nematodes to settle to the bottom of the beaker. The next day samples were decanted to 20 ml, transferred to vials, chilled at 5°C, and formaldehyde (37%) was then added to each vial. The final concentration of formalin was 3% and vials were chilled for 2-3 days at 5°C. Prior to counting, solutions containing nematodes were agitated thoroughly and 3 ml withdrawn and placed in a counting dish. Samples were observed at 100× magnification using an inverted microscope. When necessary, higher magnifications (400× and 1000×) were used to identify nematodes to genus.

Host plant damage assessment: Four cultivars of alfalfa with different reactions to ASN; Pioneer 5364 (resistant), Pioneer 5454 (moderately resistant), Pioneer 5312 (susceptible), and Ranger 5-1153 (susceptible) were evaluated under greenhouse conditions. On July 1 1996, 100 seeds/flat were planted in Metromix 200 (Scotts Co., Marysville, OH, USA) soil mix contained in plastic flats 28 cm × 43 cm × 16.5 cm height following a 10 by 10 matrix. The experimental design was completely random with four replicates, each containing five flats to be inoculated and one uninoculated flat per cultivar (a total of 600 plants/rep). All flats within each replicate were arranged randomly and rotated four times during the experiment to minimize variation in the greenhouse. The temperature was maintained at 24 ± 1°C. Plants were grown in natural light and no supplemental lighting was used. The first inoculation was made with field inoculum on July 25, 2 weeks after emergence, using the technique described by Grundbacher and Stanford (1962). Inoculum was collected from a field in Fort Col-

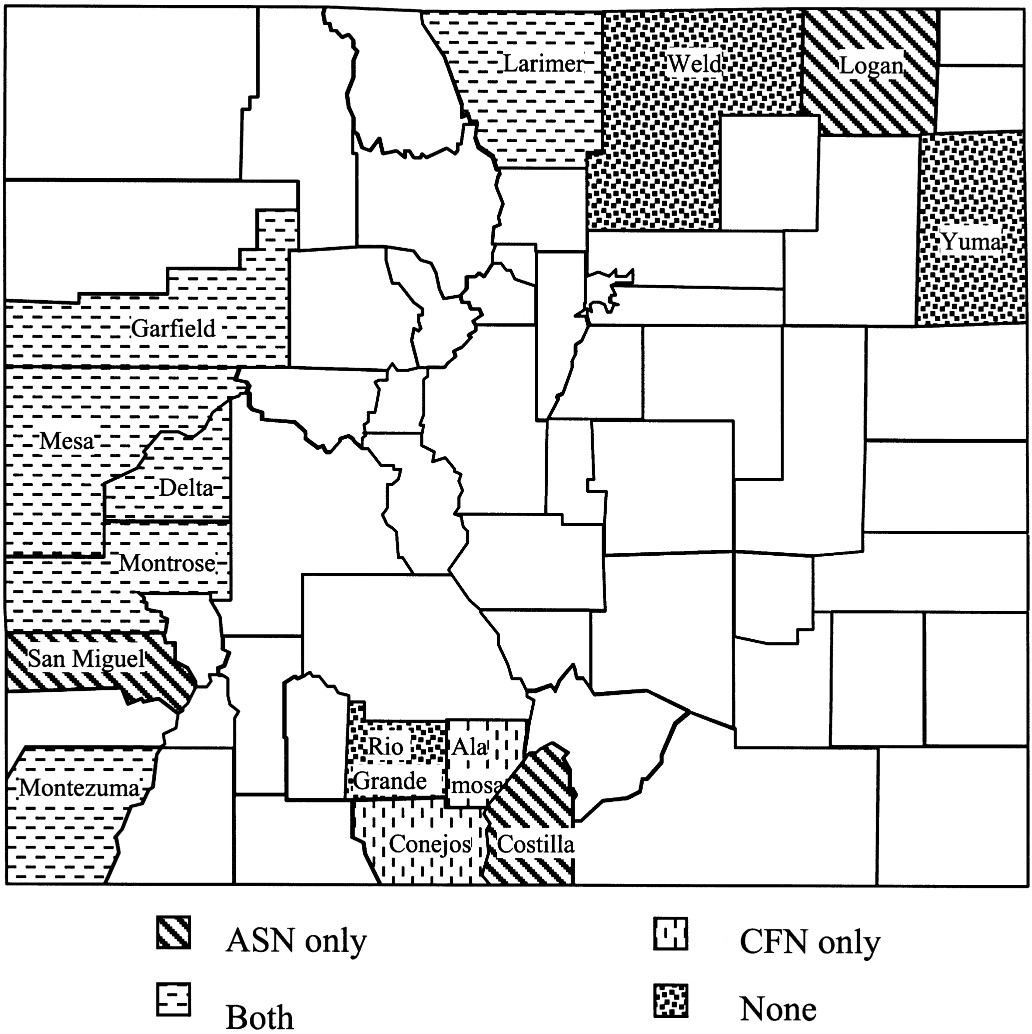

Fig. 1. Distribution of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* as revealed by a 1995-96 survey in Colorado.

lins, CO, and contained a mixture of 82% *D. dipsaci*: 18% *A. ritzemabosi*. One hundred grams of plant tissue were chopped into 0.5-cm pieces and spread on each of the five flats reserved for inoculation within each replication. Flats were then covered with vermiculite until the end of the experiment to reduce the rate of soil desiccation. Control flats received only vermiculite.

A second inoculation was made on August 7. Axenic tissue cultures of *D. dipsaci* provided by Pioneer Hi-bred International, Inc. were used. Plants in each of the inoculated flats were sprayed with 60 ml of solution containing approximately 9 000 nematodes. To decrease evaporation, inoculations were made late in the afternoon and each flat was covered with a plastic bag until the next morning.

Harvest was conducted from November 11 through December 4. Ten plants from each flat were selected randomly and evaluated for severity of swelling and distortion of the cotyledonary node using the following rating scale (Elgin, 1984): 1 = no swelling or distortion, 2 = slight swelling but no distinct symptoms, 3 = moderate swelling and distortion, 4 = severe swelling and distortion, and 5 = severe necrosis and death. All remaining above ground tissue in each flat was harvested, cut using scissors, placed in a plastic bag, shaken to mix thoroughly and weighed. Nematodes were extracted using the Baermann funnel technique. Three funnels, each containing 15 g tissue, were used per flat. The remaining plant tissue was used to determine shoot biomass. Both tissue and roots were oven-dried at 65°C for 48 h to calculate dry biomass.

Data sets for shoot biomass, root biomass, number of plants per flat, and number of nematodes/gram of dry tissue were subjected to ANOVA. When F values were significant ($P = 0.05$), means were separated according to Fischer's protected Least Significant Differences (LSD) test.

Population response to management: This study was conducted to evaluate the effect of Furadan 4F on nematode population. Furadan 4F is a carbofuran-based insecticide/nematicide routinely used in alfalfa weevil control. A completely random design with a total of 12 plots of 4.5 m by 7.5 m was set up in a field sown with alfalfa (Dekalb 120), with a history of ASN infestation located in Fort Collins, CO. In summer 1996, two Furadan 4F (1.1 kg of active ingredient per ha) treatments were applied. They differed from each other in the date of nematicide application: Treatment 1 was applied on May 6, before the first alfalfa cutting, and Treatment 2 was applied July 10, ten days after the first cutting. Both treatments consisted of four replications each, and the remaining four plots were used as controls. The pesticide was applied following the general recommendations for alfalfa: it was applied with a CO_2 sprayer calibrated to deliver 188 l/ha at 2.1 atm through six 8004 (LF4) nozzles mounted on a 3-m-long boom 45 cm above the canopy.

Alfalfa tissue samples containing five stems plus leaves from five different plants in each plot were collected randomly each month from April to September in 1996. Samples were collected in plastic bags and held at 4°C prior to processing. All samples were processed within 24 h of collection, following the same methodology described for the nematode survey.

RESULTS AND DISCUSSION

Nematode survey: From the 69 random samples collected during the summers of 1995 and 1996, only *D. dipsaci* was found in 17 samples. *A. ritzemabosi* was present alone in four samples. Both nematodes were found in 20 samples. Twenty eight samples had neither *D. dipsaci* or *A. ritzemabosi.* Table 1 summarizes the results of the survey showing both location and counts for each of the nematodes averaged by county. Both *D. dipsaci* and *A. ritzemabosi* were found in six counties, while samples from 3 of the 14 counties contained neither nematode. Samples from three other counties contained only occurrence of *D. dipsaci* and in two counties only *A. ritzemabosi* was found (Fig. 1). Data from individual samples show that the number of *D. Dipsaci* ranged from 0 to 938/g dry tissue, whereas population densities of *A. ritzemabosi* ranged from 0 to 147/g dry tissue. The highest total nematode population, 938/g dry tissue, was found in a sample from Logan county which contained only *D. dipsaci.* In those samples where both nematodes were present, the percentage of *A. ritzemabosi* in the population ranged from 1.2% to 87%.

Host plant damage assessment: Effects of mixed inoculation were evaluated in terms of the disease rating and the value of the dry shoot and dry root biomass per flat.

Table 1. Population densities of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* in alfalfa tissue of plants collected from 14 counties in Colorado.

	Nematodes/g dry tissue (averaged by county)	
Location	*D. dipsaci*	*A. ritzemabosi*
Alamosa (8)[z]	0	<1
Conejos (2)	0	2
Costilla (1)	67	0
Delta (7)	158	10
Garfield (4)	13	6
Larimer (1)	180	11
Logan (6)	157	0
Mesa (21)	45	29
Montezuma (3)	145	27
Montrose (5)	91	3
Rio Grande (4)	0	0
San Miguel (2)	84	0
Weld (2)	0	0
Yuma (3)	0	0

[z]Number of fields sampled.

At the time of harvest, the four entries showed a high percentage of symptomatic plants (above 90%). The symptoms ranged from slight to severe swelling. The values of the disease ratings for cultivars P5454, P5312, and P5364 were not significantly different (2.5, 2.6, and 2.7, respectively), in spite of the difference in their rated resistance to *D. dipsaci.* Ranger, chosen as a susceptible control, was observed to have the lowest ($P = 0.05$) rating (1.7). The total number of nematodes ranged from 9-14.5/g dry shoot biomass. Although the total number of nematodes per plant at termination was lower in cultivars with lower disease ratings, the difference was not significant ($P > 0.05$). On average, less than 3% of the total number of recovered nematodes were *A. ritzemabosi.*

Dry shoot biomass (DSB) and dry root biomass (DRB) were measured to evaluate the potential effect of nematodes on biomass production. The DSB per flat of both inoculated and uninoculated plants for the different cultivars was not significantly different, with a general average of about 15 g/flat. The same was true for the DRB per flat, which averaged 18 g/flat. These results indicate that the incidence of nematode attack on alfalfa yield was not noticeable in young, recently infested plants.

Population response to management: Population densities of *D. dipsaci* and *A. ritzemabosi* in plots treated with Furdan 4F were not significantly different from those in the control plots at any time after treatments were applied, indicating that the Furadan 4F application on established alfalfa had no suppressive effect on nematode populations. Nematode densities per gram of dry shoot biomass following the May treatment ranged from 261-893 for ASN and 267-2231 for CFN. Following the July treatment, density ranges declined to 9-107 and 9-44 for ASN and CFN, respectively.

In summary, the survey reported herein constitutes the first comprehensive documentation of the distribution of alfalfa stem nematode *D. dipsaci* and chrysanthemum foliar nematode *A. ritzemabosi* in Colorado. According to the results of the survey, both nematodes are widely distributed in the state. The chrysanthemum foliar nematode, which was reported to occur with the ASN in other areas of the United States (Gray *et al.*, 1994), was found in 6 of 14 counties in association with the ASN. The most effective strategy to control the increase of *D. dipsaci* population is the use of crop rotation followed by the planting of resistant cultivars. However this strategy is problematic given the wide host range of CFN. The results of our greenhouse experiments suggest that resistant cultivar P5364 may be more susceptible than the commercial rating indicates. The differences found between the results reported here and the existing classification for the alfalfa cultivars used in our experiment could be the consequence of several factors. The nematodes used for the first inoculation were collected in the field from infected alfalfa. The resistance of a given alfalfa cultivar is reported to vary greatly among different stem nematode populations (Whitehead, 1987). The CFN was used in combination with ASN in this study, and the role that CFN may have in altering alfalfa resistance to the ASN remains unknown. Additional time for greater nematode population increase may also produce different results.

An alternative strategy to control the ASN is the use of a nematicide, although none are currently labeled for nematode control on established alfalfa. Furadan 4F was chosen for the present study because it is routinely applied to control alfalfa weevil. Although carbofuran has been shown to be effective when applied at sowing (Whitehead and Tite, 1988; Whitehead, 1994), spraying of established alfalfa did not significantly reduce populations of *D. dipsaci* or *A. ritzemabosi*. Other insecticides/nematicides with systemic activity may be more efficient in the control of this endoparasite.

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