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Seasonal Fluctuations of Soil and Tissue Populations of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* in Alfalfa

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Abstract: Population dynamics of *A. ritzemabosi* and *D. dipsaci* were studied in two alfalfa fields in Wyoming. Symptomatic stem-bud tissue and root-zone soil from alfalfa plants exhibiting symptoms of *D. dipsaci* infection were collected at intervals of 3 to 4 weeks. Both nematodes were extracted from stem tissue with the Baermann funnel method and from soil with the sieving and Baermann funnel method. Soil moisture and soil temperature at 5 cm accounted for 64.8% and 61.0%, respectively, of the variability in numbers of both nematodes in soil at the Big Horn field. Also at the Big Horn field, *A. ritzemabosi* was found in soil on only three of the 14 collection dates, whereas *D. dipsaci* was found in soil on 12 dates. *Aphelenchoides ritzemabosi* was found in stem tissue samples on 9 of the 14 sampling dates whereas *D. dipsaci* was found on all dates. Populations of both nematodes in stem tissue peaked in October, and soil populations of both peaked in January, when soil moisture was greatest. Numbers of *D. dipsaci* in stem tissue were related to mean air temperature 3 weeks prior to tissue collection, while none of the climatic factors measured were associated with numbers of *A. ritzemabosi*. At the Dayton field, soil moisture plus soil temperature at 5 cm accounted for 98.2% and 91.4% of the variability in the soil populations of *A. ritzemabosi* and *D. dipsaci*, respectively. *Aphelenchoides ritzemabosi* was extracted from soil at two of the five collection dates, compared to extraction of *D. dipsaci* at three dates. *Aphelenchoides ritzemabosi* was collected from stem tissue at six of the seven sampling dates while *D. dipsaci* was found at all sampling dates. The only environmental factor that was associated with an increase in the numbers of both nematodes in alfalfa stem tissue was total precipitation 1 week prior to sampling, and this occurred only at the Dayton field. Numbers of *A. ritzemabosi* in stem tissue appeared to be not affected by any of the environmental factors studied, while numbers of *D. dipsaci* in stem tissue were associated with cumulative monthly precipitation, snow cover at time of sampling, and the mean weekly temperature 3 weeks prior to sampling. Harvesting alfalfa reduced the numbers of *A. ritzemabosi* at the Big Horn field and both nematodes at the Dayton field.

Key words: alfalfa, alfalfa stem nematode, *Aphelenchoides ritzemabosi,* chrysanthemum foliar nematode, climate, distribution, *Ditylenchus dipsaci, Medicago sativa,* nematode, sampling, seasonal fluctuations.

The alfalfa stem nematode (ASN), *Ditylenchus dipsaci* (Kühn) Filipjev, and the chrysanthemum foliar nematode (CFN), *Aphelenchoides ritzemabosi* (Schwartz) Steiner & Buhrer, are widespread cohabitants of alfalfa plants in the western United States (Gray et al., 1994). Symptoms of stem nematode parasitism in alfalfa are stunted and swollen stems, stem necrosis, white flagging of leaves and stems (Griffin, 1990), crown rot, and stand decline. Researchers have previously reported high numbers of *D. dipsaci* in the crown and upper root of alfalfa plants (Griffin, 1990). Symptoms are exacerbated by low temperature, resulting in winterkill (Boelter et al., 1985). Seedlings show swelling in the cotyledonary node region as well as severe stunting and distortion. Symptoms of chrysanthemum foliar nematode parasitism on mature alfalfa plants consist of inhibition of apical growth and stunting of shoots. Seedlings develop cotyledonary lesions and may die (Dropkin, 1989; Grundbacher and Stanford, 1962; Krusberg, 1961; Gray et al., 1986). Tissue swelling, however, does not occur. Under field conditions, few alfalfa plants have been reported with only *A. ritzemabosi* parasitism. Most plants appear to be parasitized with both *A. ritzemabosi* and *D. dipsaci* (Gray et al., 1994).

Several studies have addressed the relationship between the soil environment and soil populations of ASN. However, neither soil populations of CFN nor concomitant populations of the two nematodes have been studied in alfalfa fields. Lewis and Mai (1960) found most *D. dipsaci* in the top 6 cm of fallowed soil, while Wallace (1962) observed *D. dipsaci* to a soil depth of 10 cm.

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Wallace (1962) found that the number of *D. dipsaci* in an oat field increased greatly after rain but decreased following a dry period. Tseng et al. (1968) reported that the largest number of *D. dipsaci* were recovered from the top 10 cm of soil in an alfalfa field in Utah. They found that the number of nematodes in soil at all depths were markedly affected by the temperature deviation from 15 °C.

Our objective was to study the effects of environmental factors on soil and tissue populations of *D. dipsaci* and *A. ritzemabosi* in Wyoming alfalfa fields.

Materials and Methods

Two fields were selected for sampling in north-central Wyoming, one near Big Horn and the other near Dayton.

Big Horn field: The field near Big Horn was a 3-year-old stand, approximately 6 ha of alfalfa ('Fortress'), rated as highly resistant (>50% resistant plants) to ASN. This field was selected because a previous stem-bud tissue sample contained populations of both *A. ritzemabosi* and *D. dipsaci*. The soil was classified as a silty clay, pH 7.5, containing 5.2% organic matter. Although previously sprinkler-irrigated, the field was not irrigated during the study. Alfalfa hay was harvested once on 9 July 1990. No pesticides were applied to the field. Samples were collected at 3- to 4-week intervals on 14 dates between April 1990 and April 1991. Samples were taken of both stem-bud tissue and root-zone soil from ASN-symptomatic plants at 20 collection sites in the field. Since the field was not irrigated during the study, collection sites were selected in the lower areas of the field where soil remained relatively moist throughout the sampling period. Size of the sites ranged from 1.0 m^2 to approximately 1.0 × 2.0 m.

At each collection site, one symptomatic stem bud was collected from each of five plants. The stem-bud tissue was bulked and refrigerated until nematodes were extracted. Symptomatic stem-bud tissue was chopped with a sterile razor blade and placed on a Baermann funnel for 5 hours. Live nematodes were collected on a 45-µm-pore sieve, collected in 50 ml sterile-distilled water, and refrigerated at 4 °C until counts were made. After thorough mixing of each sample, a 1-ml aliquot was placed on a Peters 1-ml eelworm counting slide (Hawksley, London, UK). Nematodes were killed by passing the slide over an open flame and observed at ×100. Due to the nematodes' rapid movement, heat fixing was necessary to distinguish *A. ritzemabosi* from *D. dipsaci* accurately. both nematodes were identified according to Commonwealth Institute of Helminthology descriptions (Hooper, 1972; Siddiqi, 1974) and a key to genera (Mai and Mullin, 1996). Nematodes from three separate 1-ml aliquots per sample were counted and averaged. The dry weight of chopped stem-bud tissue, nematode density (total number of *A. ritzemabosi* and *D. dipsaci* per gram (dry stem-bud tissue), and present *A. ritzemabosi* were determined for each sample.

A soil sample (single core, 2-cm diam. to a depth of 15 cm) was obtained from the root zones of the five symptomatic plants at each site. Soil from each site was bulked and thoroughly mixed, and a 250-ml subsample was processed with a sieving method. The soil was placed in a 10-liter plastic bucket and 7 liters of water added. The water and soil were stirred, allowed to stand for 30 seconds, then slowly poured through a nested series of three sieves (500, 150, and 45-µm-pore size). Nematodes and fine soil particles caught on the 45-µm-pore screen were placed on a Baermann funnel. Nematodes were collected and counted as described for tissue samples, and the number of *D. dipsaci* and *A. ritzemabosi* per 100 g of the dry soil was determined.

To obtain information on overwintering, whole plants were collected from 10 sites during January, February, and March 1991. Symptomatic stem-bud tissue and necrotic tissues in the crown and upper root were processed separately. The primary objective was to determine if *A. ritzemabosi* could be found in the crown and upper root tissue. Two separate soil samples, one from the root zone and a second from the rhizosphere, were also obtained from each of the

sites. Root zone soil was obtained using the 15-cm-depth soil plug as previously described; rhizosphere soil was collected from the alfalfa root surface after plants were removed.

Dayton field: Collections were made from a 0.5-ha area within a 4-year-old alfalfa ('Arrow') field approximately 30 ha in size on the Padlock Ranch near Dayton, known to have both nematodes. Arrow is rated as moderately resistant (15–30% resistant plants) to ASN. The field was sprinkler-irrigated on 10 July and 30 August 1990. Hay was harvested on 12 June and 3 August 1990. No pesticides were applied. The study was terminated in September 1990 when the crop was plowed due to declining plant stand. The soil was a loam with pH 6.9 and 5.4% organic matter content.

During 1990, symptomatic stem-bud tissue collections were made from 10 sites at 3 to 4-week intervals from mid-April to mid-September (seven collections), and soil samples were collected from mid-June to mid-September (five collections). Samples were collected and processed using the method previously described for the Big Horn field.

Environmental data: Local climatological data for Big Horn and Dayton were obtained from the National Oceanic and Atmospheric Administration (NOAA) office located in Sheridan, Wyoming, 9.7 km from the Big Horn field and 33.8 km from the Dayton field. These data consisted of daily minimum and maximum temperatures and precipitation. Moisture content of soil samples was calculated with the formula: (1 – [dry weight ÷ wet weight]) × 100. Soil samples were dried at 105 °C for 24 hours. Soil temperature at the 5 and 15-cm soil depths were measured with a soil thermometer at the time of collection.

Data analysis: Single and multiple regression analyses were conducted on nematode populations and environmental data. Soil nematode populations were regressed against soil temperatures, soil moisture, and the presence or absence of snow cover at the time of collection. Numbers of nematodes in tissue were regressed against weekly ambient temperatures prior to each collection, weekly and multiweek precipitation prior to each collection, and snow cover at the time of collection.

Results

Big Horn Field: Aphelenchoides ritzembosi was found in soil on only three dates: mid-October 1990, late January 1991 when the population peaked at approximately 50 nematodes/100 g soil, and early April 1991 when the population was approximately 20 nematodes/100 g soil (Figure 1A). *Ditylenchus dipsaci* was extracted from soil on all but two sampling dates (early April 1990 and late August 1990). *Ditylenchus dipsaci* had two population peaks—one in mid-June to mid-August (40 nematodes/100 g soil), which was interrupted by the harvest, and another larger peak in late January (600 nematodes/100 g soil) corresponding to the peak for *A. ritzemabosi*. Soil populations of *D. dipsaci* throughout the year ranged from 0 to 572/100 g of dry soil compared to *A. ritzemabosi* with populations that ranged from 0 to 54/100 g of dry soil. Soil temperature peaked in mid-July 1990, while soil moisture peaked in early February 1991, corresponding to the peak populations of both nematodes (Fig. 1B).

Soil population densities of the two nematodes were correlated ($r = 0.875$, $P \leq 0.01$). Soil population density of each nematode was closely related with soil conditions. Soil moisture had a greater effect on *D. dipsaci* ($r^2 = 0.61$, $P \leq 0.01$) than on *A. ritzemabosi* ($r^2 = 0.59$, $P \leq 0.01$). Soil temperature at 15 cm similarly appeared to have a greater effect on *D. dipsaci* ($r^2 = 0.42$, $P \leq 0.01$) than on the *A. ritzemabosi* ($r^2 = 0.22$, $P \leq 0.01$), as did soil temperature at 5 cm ($r^2 = 0.40$, $P \leq 0.01$ for *D. dipsaci* and $r^2 = 0.21$, $P \leq 0.01$ for *A. ritzemabosi*). Multiple regressions for soil moisture and average soil temperature (mean of data for 5 and 15-cm depths) with soil nematode populations were $R^2 = 0.61$ at $P \leq 0.01$ for *D. dipsaci* and $R^2 = 0.64$, $P \leq 0.01$ for *A. ritzemabosi*. The greatest relationship achieved with soil populations of *D. dipsaci* and environmental factors was obtained

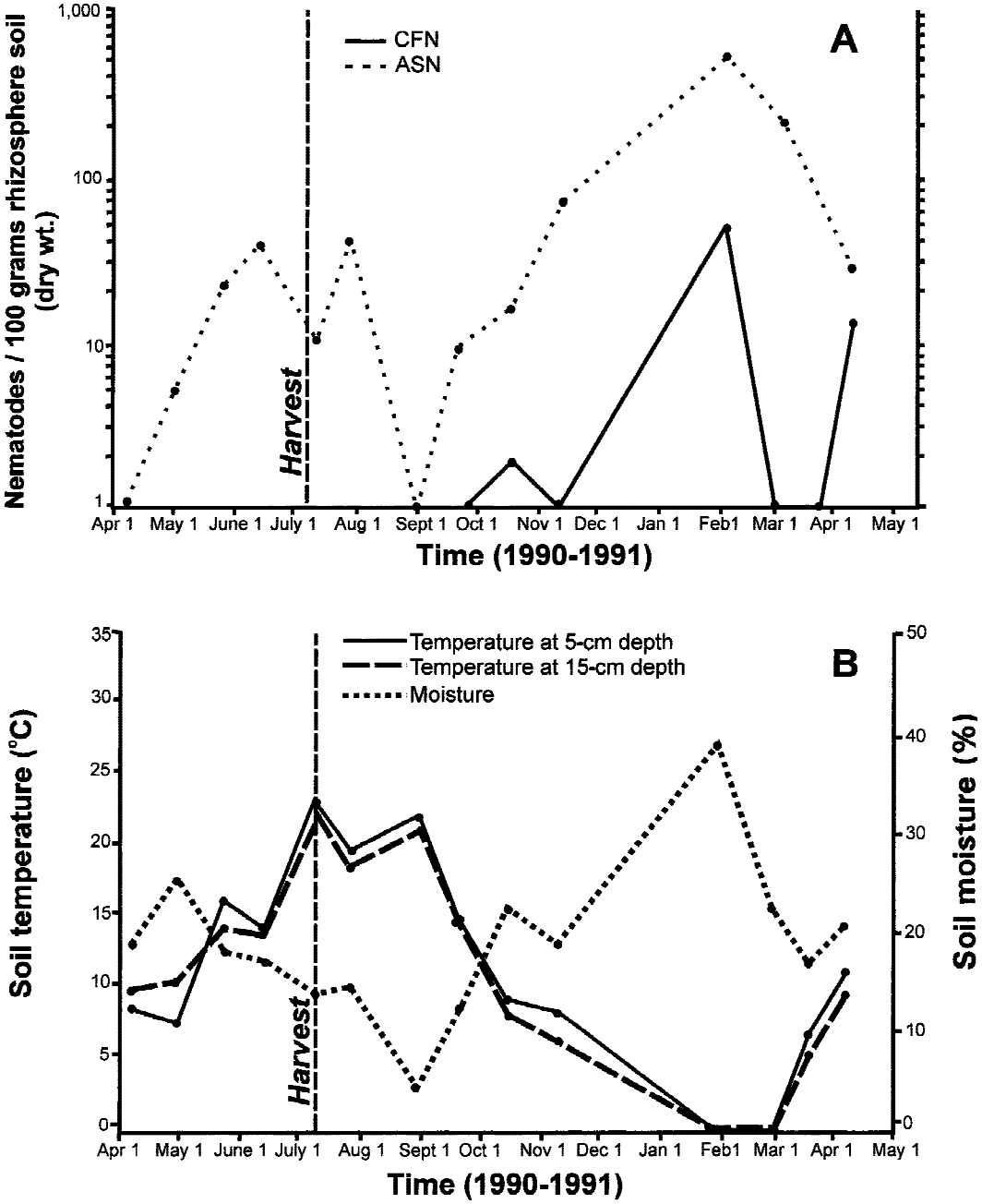

FIG. 1. Relation of *Ditylenchus dipsaci* (ASN) and *Aphelenchoides ritzemabosi* (CFN) populations in root-zone soil to soil parameters, Big Horn field. A) Nematode population dynamics. B) Soil temperature and moisture.

with the combination of soil moisture, mean soil temperature, and snow cover ($R^2 = 0.67$, $P \leq 0.01$). Soil populations of *A. ritzemabosi* were closely associated with soil moisture and soil temperature at 5 cm as well as with soil moisture, mean temperature, and snow cover ($R^2 = 0.65$, $P \leq 0.01$). All multiple environmental factors resulted in significant relationships with soil populations of both nematodes.

High population densities of *D. dipsaci* were found at all sampling dates in stem tissue (Figure 2A). The most were detected in mid-September (8,411 nematodes/g dry

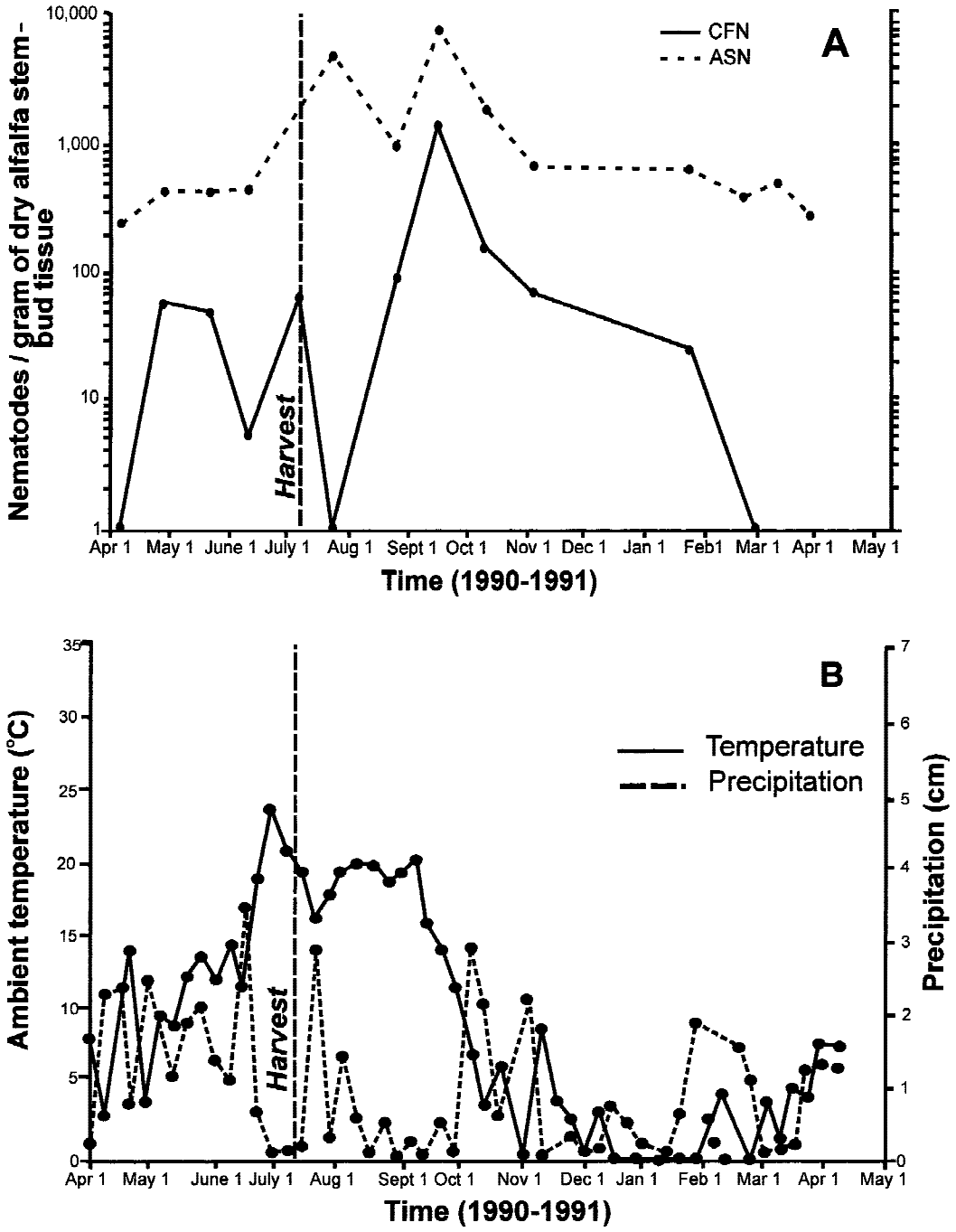

FIG. 2. Relation of *Ditylenchus dipsaci* (ASN) and *Aphelenchoides ritzemabosi* (CFN) populations in symptomatic stem-bud tissue to atmospheric parameters, Big Horn field. A) Nematode population dynamics. B) Ambient temperature and precipitation.

stem tissue). *Aphelenchoides ritzemabosi* was found on 9 of the 14 sampling dates. Many of the ASN-infected plants had rot of the crown and pith of the upper root. The stem tissue population of *A. ritzemabosi* peaked in late September with 1,466 nematodes/g dry stem tissue. Harvest in July 1990 occurred at a period of high temperatures and low pre-

cipitation (Figure 2B). Populations of *A. ritzemabosi* showed an immediate and severe decline following harvest and removal of the plant canopy, whereas the tissue population of *D. dipsaci* declined only slightly and only after a 4-week period. Both nematode populations eventually recovered. *Aphelenchoides ritzemabosi* in stem tissue was undetectable on 1 March 1991, apparently due to the extremely cold and dry weather conditions that occurred in January 1990. The ambient air temperature was highest from early July to late September 1990 (Fig. 2B). Precipitation consisted of frequent showers of up to 3.5 cm, which included both rain and snow.

The stem tissue populations of *D. dipsaci* and *A. ritzemabosi* were highly correlated ($r = 0.84$, $P \leq 0.01$). However, regression analysis of these nematode populations revealed few significant relationships with environmental factors for *D. dipsaci* (13 of 44 regressions significant at $P \leq 0.05$), and none were significant for *A. ritzemabosi*. The greatest relationship between *D. dipsaci* and a single environmental factor was shown with the third-week mean temperature ($r^2 = 0.43$, $P \leq 0.01$). Most of the significant multiple regressions combined several weeks of temperature and total precipitation, while one combined snow cover and cumulative weekly temperature. The strongest relationship for multiple environmental factors occurred with the third-week mean temperature + the 4-week total precipitation prior to sample collection ($R^2 = 0.49$, $P \leq 0.05$).

There were always more *D. dipsaci* in stem tissue than in crown tissue except in the samples of 28 February, which had similar numbers in both tissues (Table 1). Numbers of *D. dipsaci* in rhizosphere soil were much larger than in root-zone soil at all three dates (Table 2). *Aphelenchoides ritzemabosi* was extracted from crown tissue on two dates when it was not found in stem tissue (Table 1) and was found in stem tissue at one date when it could not be found in the crown tissue. *Aphelenchoides ritzemabosi* was identified from rhizosphere soil at all three dates but not from root zone soil (Table 2).

Dayton field: Aphelenchoides ritzemabosi was found in soil on two of the five collection dates (Fig. 3A). Neither nematode was recovered on 9 July or 30 August 1990—two dates that correspond to periods when soil moisture was lowest and soil temperature highest (Fig. 3B). Soil populations of *A. ritzemabosi* ranged from 0 to 21/100 g dry soil. Soil populations of *D. dipsaci* ranged from 0 to 24/100 g dry soil.

Aphelenchoides ritzemabosi was collected from stem tissue on six of the seven collection dates (Figure 4A). Stem tissue populations ranged from 0 in April to 1,202/g dry stem tissue in September 1990. *Ditylenchus dipsaci* also was recovered from stem tissue at six collection dates. It was not recovered on 14 June, immediately after the first harvest. Populations ranged from 0 in June to 6,817/g dry stem tissue in September 1990. Populations of both nematodes declined in mid-June after harvest, in association with a decrease in precipitation and increase in ambient temperature (Fig. 4B). Nematode

TABLE 1. Numbers of *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* found in symptomatic stem-bud and necrotic crown-upper root tissues in alfalfa at Big Horn, Wyoming, in 1991.

Collection date	Nematodes per gram dry tissue			
	D. dipsaci		*A. ritzemabosi*	
	Stem	Crown	Stem	Crown
31 January	684	77	25	0
28 February	420	417	0	0
19 March	544	24	0	97
6 April	293	0	0	80

Data shown are sums of nematodes found in 10 samples.

TABLE 2. *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* found in root-zone soil and rhizosphere soil in an alfalfa field at Big Horn, Wyoming.[a]

Collection date	Nematodes per gram dry soil			
	D. dipsaci		*A. ritzemabosi*	
	Root zone[b]	Rhizosphere[c]	Root zone	Rhizosphere
28 February	234	945	0	32
19 March	73	331	0	15
6 April	31	289	0	17

[a] Data shown are sums of nematodes found in 10 samples.
[b] Root zone soil was soil greater than 2 cm from the alfalfa root.
[c] Rhizosphere soil was soil within 2 cm of the alfalfa root.

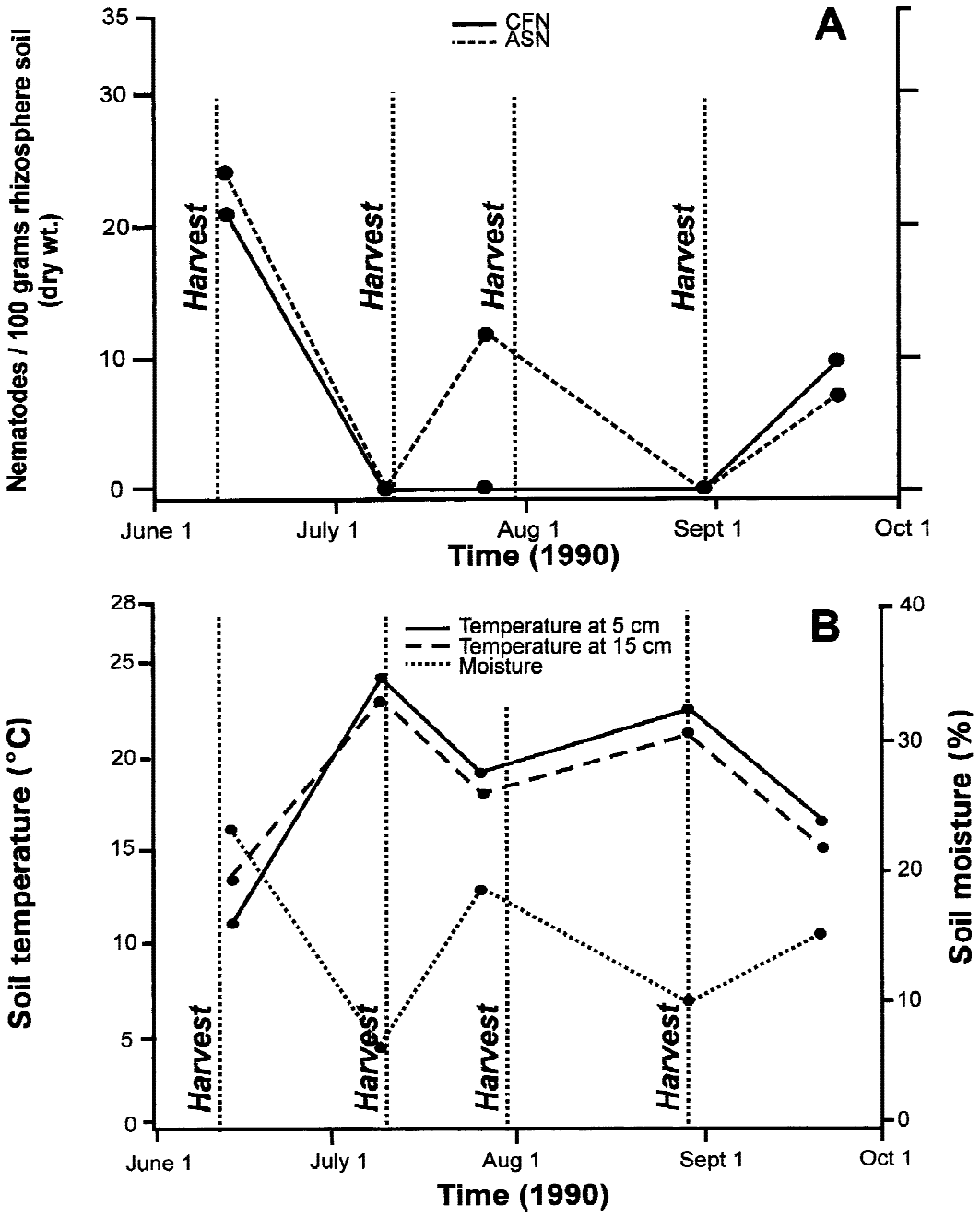

FIG. 3. Relation of *Ditylenchus dipsaci* (ASN) and *Aphelenchoides ritzemabosi* (CFN) populations in root-zone soil to soil parameters, Dayton field. A) Nematode population dynamics. B) Soil temperature and moisture.

population densities in both soil and stem tissue were increasing at the end of the season when the crop was plowed under.

Soil populations of the two nematodes were correlated ($r = 0.82$, $P \leq 0.10$). There was a significant relationship between soil moisture and soil population densities of *D. dipsaci* ($r^2 = 0.89$, $P \leq 0.01$) but not *A. ritzemabosi*. Soil temperatures of 5 and 15-cm depths had a major influence over soil population densities of both nematodes at Dayton and Big Horn. The strongest rela-

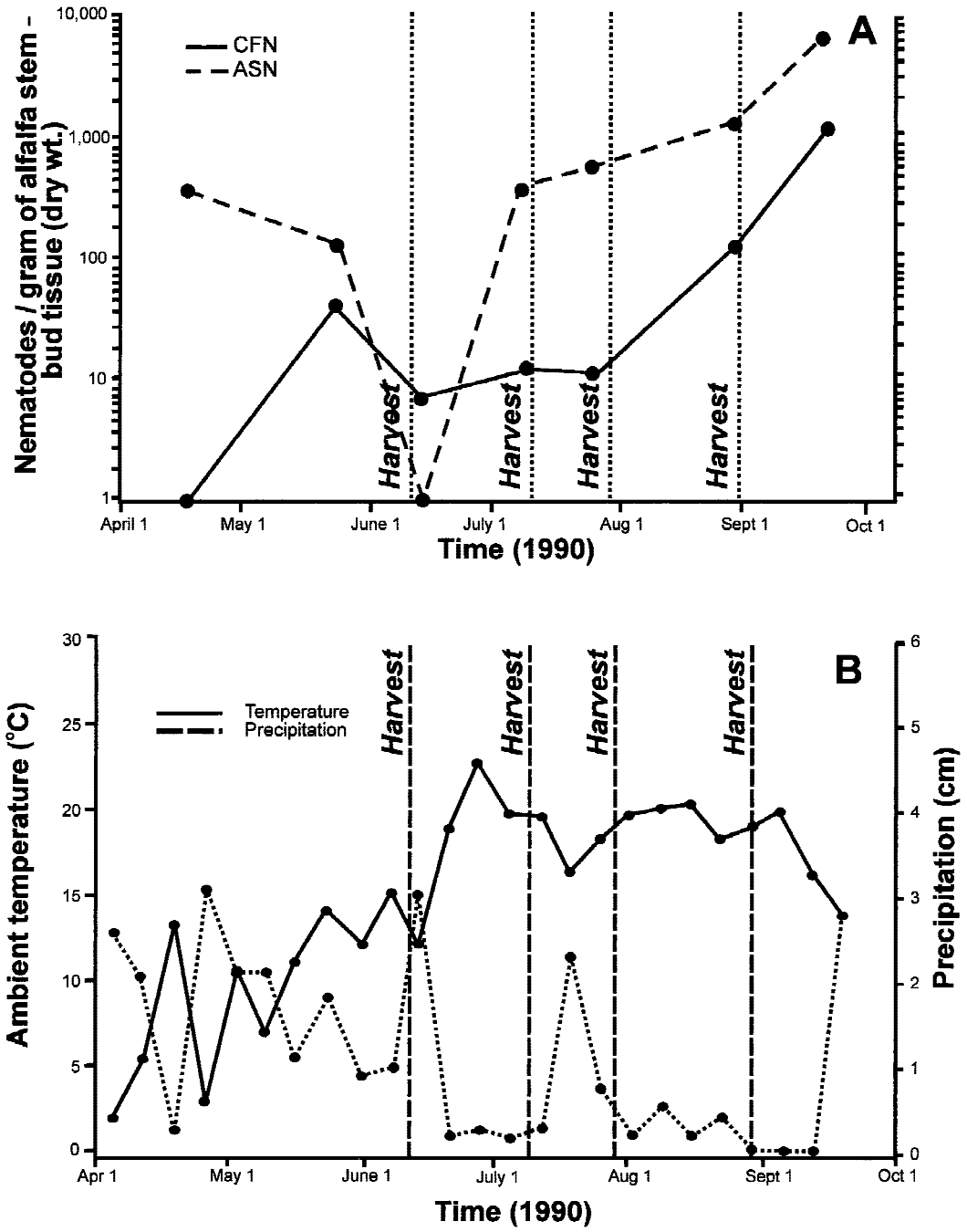

FIG. 4. Relation of *Ditylenchus dipsaci* (ASN) and *Aphelenchoides ritzemabosi* (CFN) populations in symptomatic stem-bud tissue to atmospheric parameters, Dayton field. A) Nematode population dynamics. B) Ambient temperature and precipitation.

tionship for soil populations of *A. ritzemabosi* was achieved with the combination of percent soil moisture and the soil temperature at the 5-cm depth ($R^2 = 0.98$, $P \leq 0.05$). These soil environmental factors also have the strongest relationship with soil populations of *D. dipsaci* ($R^2 = 0.91$, $P \leq 0.01$).

Numbers of the two nematodes in stem

tissue were highly correlated (r = 0.99, $P \leq 0.01$). The only significant relationship for stem tissue populations of both nematodes and associated environmental factors was for total precipitation 1 week prior to collection ($r^2 = 0.48$, $P \leq 0.05$ for both *D. dipsaci* and *A. ritzemabosi).*

Discussion

We previously reported the distribution of *D. dipsaci* and *A. ritzemabosi* in alfalfa fields in the western United States (Gray et al. 1994). Of the 40 samples collected from symptomatic stem-bud tissue in 10 states, 73% had both nematodes, 27% contained *D. dipsaci* only, and no sample contained *A. ritzemabosi* only. The number of *D. dipsaci* ranged from 26 to 8,027/g tissue compared to 0 to 1,100/g tissue for *A. ritzemabosi.* In our current study, symptomatic stem-bud tissue populations of both nematodes were monitored in two separate alfalfa fields in Wyoming. Of the 20 samples collected over the course of a year, 14 (70%) had both nematodes, 5 (25%) had *D. dipsaci* only, and 1 (5%) contained *A. ritzemabosi* only. The number of *D. dipsaci* ranged from 0 to 8,411/g tissue compared to 0 to 1,466/g stem tissue for *A. ritzemabosi.* These data are in close agreement with our previous findings. Although *A. ritzemabosi* was found only in association with *D. dipsaci* in our previous study, it was found alone in one sample from the Dayton field.

Time of collection has an important effect on detection of these two nematodes in stem tissue. Numbers of both nematodes in stem tissue at both fields peaked in the fall (late September to early October) when ambient temperature was near 20 °C. Ambient temperature, precipitation, irrigation, and removal of the plant canopy with harvesting all influenced stem tissue populations of both nematodes. The environmental factors that most affected nematode populations in the stem tissue were total monthly precipitation and mean temperature 3 weeks prior to sampling. Previous reports have shown stem tissue populations of *D. dipsaci* to be reduced by insecticides (Elgin and Evans, 1975 Elgin and Gray, 1971). Control of *A. ritzemabosi* in chrysanthemum is commonly obtained with foliar-applied insecticides (Thorne, 1961). Tissue populations of *D. dipsaci* and *A. ritzemabosi* were both reduced in alfalfa stems in England with soil application of carbofuran (Whitehead, 1994). Therefore, insecticide application, alfalfa cultivar, climatic conditions, soil moisture and temperature, and irrigation all will have an impact on the stem tissue and soil populations of these two nematodes.

Although both nematodes were extracted from soil in the alfalfa plant root-zone, populations were, for the most part, relatively small. Since both are endoparasites of the plant shoot, low numbers would be expected. Soil moisture was the predominant environmental factor influencing soil populations of both nematodes. The combination of soil moisture and soil temperature at the 5-cm depth showed the strongest relationship with population densities of both nematodes. The nematode population dynamics in the soil appear to be related to those in the stem tissues. As stem tissue populations increase, soil populations increase, indicating that nematodes may be moving, perhaps washing down from the stems into the rhizosphere soil. This scenario agrees with previous reports for *D. dipsaci* (Wallace, 1962) and for *A. ritzemabosi* (Dropkin, 1989). Rhizosphere soil was found to have much larger populations of both nematodes than root-zone soil. Had samples been collected from the rhizosphere at all sampling dates, we undoubtedly would have had a much higher rate of detection, especially for *A. ritzemabosi.*

Tseng et al. (1968) reported that the soil population of *D. dipsaci* in an alfalfa field in Utah was greatest in late September to early October. Our results show that both nematodes peaked somewhat later, in January, when soil moisture was greatest. However, soil moisture was greater in the Utah field (greater than 100% of field capacity), particularly during November through March, and possibly suppressed the soil population of *D. dipsaci* (Sayre and Mountain, 1962).

Although the Big Horn field was not irrigated during the 1990 growing season as was

the Dayton field, peak stem tissue and soil populations at both fields were similar. The similarity of nematode populations was somewhat unexpected. However, soil temperature and moisture from mid-June to mid-September were surprisingly similar at both locations, as were ambient temperature and precipitation. Although the Big Horn field was not irrigated during the study, it received more frequent rain and snow showers than the Dayton field.

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