Distribution of Nothanguina phyllobia and Its Potential as a Biological Control Agent for Silver-leaf Nightshade

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Abstract: The nematode *Nothanguina phyllobia* Thorne was found within large foliar galls on the perennial weed *Solanum elaeagnifolium* Cav. in west Texas. A two-year survey of a 6400-sq-km area in west Texas showed extensive distribution of the nematode. No hosts other than *S. elaeagnifolium* were observed. Densities of juvenile nematodes in the soil were high. *N. phyllobia* spread rapidly after small numbers of infective juveniles were applied in a foliar spray to an *S. elaeagnifolium* population. The host plant declined in vigor and frequently died. Artificial inoculation of an *S. elaeagnifolium* population with large numbers of the nematodes by broadcasting infected plant tissue resulted in high infection incidence. *Key Words:* leaf gall nematode, biological weed control.

Solanum elaeagnifolium Cav. (silver-leaf nightshade) is an erect herbaceous perennial with deep running rootstocks (1). It is a troublesome weed in every county of the Texas southern high plains, infesting more than 1.2 million hectares of crop land. Weed scientists consider it one of the most important weeds in the area's agriculture. Smith (5) found silver-leaf nightshade infestations to be associated with yield reductions in cotton and grain sorghum of 5–14% and 4–10%, respectively. Orr (4) suggested that *Nothanguina phyllobia* Thorne, a nematode which causes large foliar galls on silver-leaf nightshade (6), should be evaluated as a biological control agent for the weed. This research investigated the distribution, virulence, and biology of *N. phyllobia*.

MATERIALS AND METHODS

Geographic distribution of N. phyllobia: A 6400-sq-km area surrounding Lubbock, Texas, was scouted for 2 consecutive years to ascertain the distribution of *N. phyllobia*. Six 80-km transects were traveled during August 18–20 in 1975 and 1976. Scouts stopped every 8 km to record dominant weeds and the severity of nematode galling on silver-leaf nightshade. Stem and leaf samples of crop and weed species from areas with high *N. phyllobia* populations were stained with acid fuchsin-lactophenol (3) and examined for nematodes.

Distribution in the soil: Twenty-one soil samples were examined for *N. phyllobia*. Samples came from four silver-leaf nightshade populations near Lubbock. Vertical distributions of *N. phyllobia* to a depth of 62.5 cm were determined in seven samples by subsampling vertically in 2.5-cm increments. Nematodes were extracted from soil by sieving and sugar flotation (2). Silver-leaf nightshade roots excavated with each sample were stained with acid fuchsin-lactophenol and examined for nematodes.

To verify that nematode larvae escape abscised galled leaves and enter the soil, two 1,000-ml graduated cylinders (6 cm diam) were filled with steam-sterilized Olton loam soil (55% sand, 21% silt, and 24% clay), with percent moisture adjusted to field capacity. A dry galled leaf was placed on the soil surface in each cylinder and thoroughly wetted. Cylinders were covered with clear plastic to retain moisture. After 7 days, soil was removed in 2.5-cm increments and examined for nematodes.

Inoculation with crushed foliar galls: A 136-sq-m plot was staked in a cultivated field with a high population of silver-leaf nightshade in September 1975. Nematode inoculum was prepared from foliar galls dried to less than 10% moisture, crushed, and sifted through a 2.35-mm sieve. *N. phyllobia* was introduced by hand-broadcasting 404 g of nematode inoculum evenly over the plot. This achieved a mean nematode density of 8-16 viable larvae/cm^2 soil surface. The plot was then disked and chiseled and thereafter farmed with the rest of the field.

Inoculation with nematodes in a foliar spray: A 75-sq-m plot was staked in a population of silver-leaf nightshade in June 1974. The plants initially showed no signs of *N. phyllobia* infection. Nematodes were intro-

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duced by spraying all leaves of 4 randomly selected silver-leaf nightshade plants to the point of runoff with infective larvae in a water suspension (ca. 2,000 larvae per plant). These plants were monitored for foliar galls during the next 10 weeks. Leaf samples were removed at biweekly intervals and stained with acid fuchsin-lactophenol to evaluate tissue penetration. Large annual plant species were carefully removed to reduce competition; otherwise, the plot was left undisturbed.

Observations were continued in 1975 and 1976. All plants in the plot were staked individually and data were collected on 12 dates. Plant height, crown area, leaf morphology, and numbers of flowers and berries were recorded. Severity of infection was evaluated by assigning a numerical gall-size rating and multiplying that by the percentage of leaves galled on each plant.

RESULTS

Geographic distribution: Silver-leaf nightshade plants were present at 95% of the locations observed. The populations of silver-leaf nightshade were heaviest and infections with the nematode were severest in the southwest sector of the surveyed area (Fig. 1). Nematode galls were found at 42% of the locations in 1975 and 64% in 1976. The increase in number of locations infected and ratings on the severity of infection at each location indicated twice as much *N. phyllobia* leaf galling in 1976 as in 1975.

The most common weed species were silver-leaf nightshade, pigweed (*Amaranthus* spp.), Johnsongrass (*Sorghum halapense* (L.) Pers.), Texas blueweed (*Helianthus ciliaris* DC.) and kochia (*Kochia scoparia* (L.) Schrad.). Crop species included cotton (*Gossypium hirsutum* L.), sorghum (*Sorghum bicolor* (L.) Moench.), corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and sunflower (*Helianthus annuus* L.). Weed and crop plants never showed infection even though they were often tangled with heavily galled silver-leaf nightshade leaves. Laboratory examinations occasionally revealed small numbers of *N. phyllobia* on leaves and stems of these plants but no evidence of tissue penetration or nematode reproduction was found.

Distribution in the soil: Fifteen of the 21 soil samples contained infective-stage larvae of *N. phyllobia.* No other juvenile stages or adults were found. Six of the vertically graduated soil-sample series contained 100–9,000 individuals each. Larvae were concentrated at the soil surface (maximum density, 8.0 larvae/cc) and at the 15-cm depth (maximum density, 2.3 larvae/cc). In one sample, 1.4 larvae/cc were found 33 cm below the soil surface. *N. phyllobia* were never found in root tissue. Final vertical distributions in laboratory tests were similar to those in the field (Fig. 2).

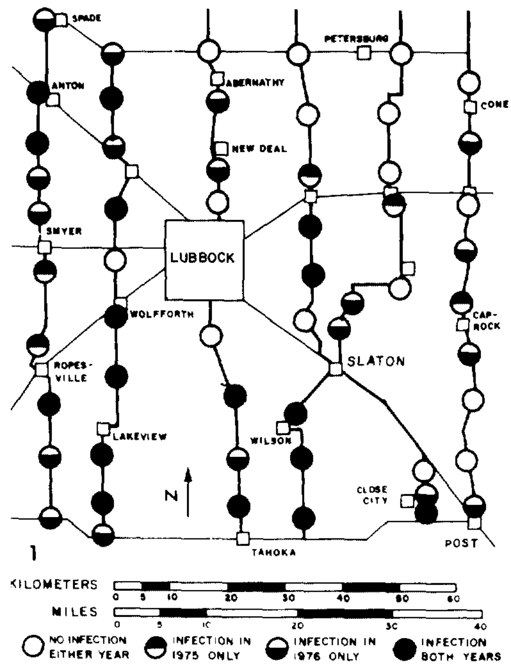
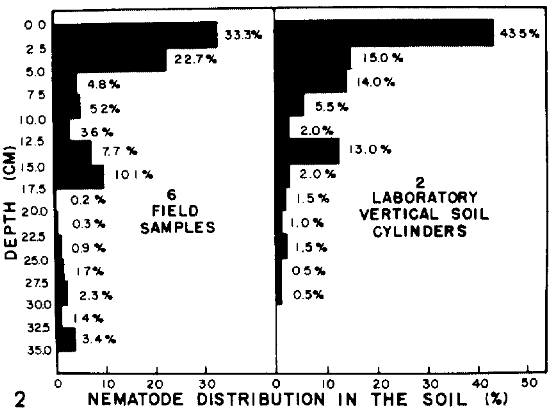


FIG. 1. Occurrence of *S. elaeagnifolium* infected by *N. phyllobia* in the Lubbock area.

Inoculation with crushed foliar galls: In 1975, late-fall weather prevented regrowth of silver-leaf nightshade. The spring and early summer months of the following year, 1976, were dry (13 cm total precipitation), followed by heavy rainfall in July (20 cm). Prior to July, little infection was observed. On July 30, silver-leaf nightshade plants throughout the plot showed extreme infection with *N. phyllobia.* Infected plants were generally smaller than uninfected plants, and were frequently prostrate from the weight of galled leaves. Nematode infections on silver-leaf nightshade continued for 18 m east of the plot. This area was



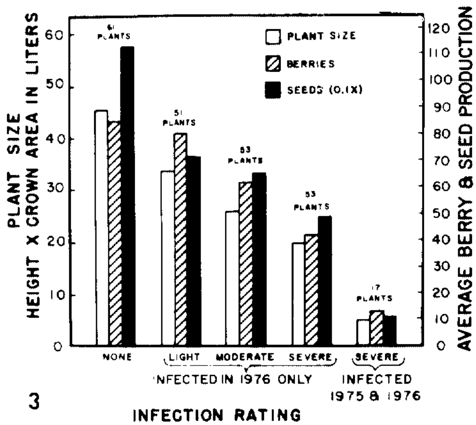


FIG. 2. Mean vertical distribution of infective larvae of *N. phyllobia*.

FIG. 3. The observed relationship of infection severity to the size and fruiting of host plants in a population of *S. elaeagnifolium*.

slightly downhill and in the direction of the prevailing winds.

Inoculation with a foliar spray: Inoculation in 1974 was followed by 6 weeks of dry weather, during which *N. phyllobia* larvae, apparently in a quiescent state, were found only on leaf surfaces. After the 6 dry weeks, 2 weeks of rainy weather followed, and two inoculated plants developed foliar galls containing *N. phyllobia* larvae. The number of galled silver-leaf nightshade plants in the plot increased from 2 in 1974 to 17 in 1975, and to 157 in 1976. Fifty-nine percent of the plants infected in 1975 died; only 6% of the uninfected plants died. The seven plants infected in 1975 which resprouted in 1976 were 90% smaller than uninfected plants; fruiting was similarly reduced. Approximately equal partitioning of all plants in 1976 into four infection categories (none, light, moderate, or severe, based on numerical ratings) showed that reductions in plant size and fruiting were correlated with the degree of infection (Fig. 3). Moist conditions in 1976 increased nematode symptoms. The most active leaf galling of the year occurred after substantial precipitation and continuously high relative humidity (Fig. 4). In April and early May (3 cm precipitation), foliar galls were

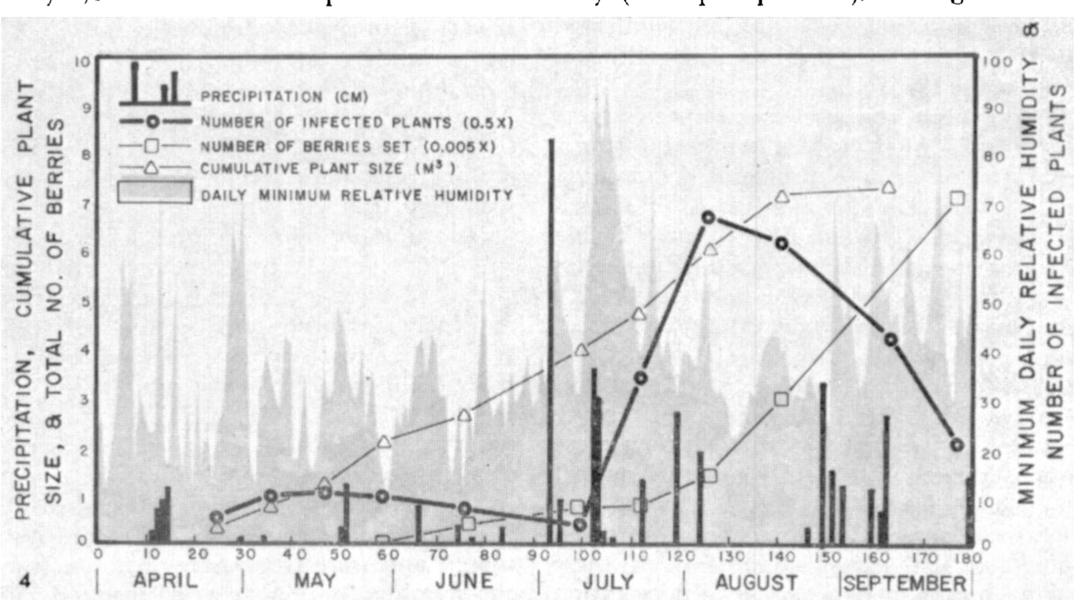


FIG. 4. Effect of rainfall and humidity on the development of nematode symptoms in a population of *S. elaeagnifolium*.

observed on 22 plants. All of these plants showed galling within 10 days of emergence. During late May and June (3 cm precipitation), many galled leaves abscised, and no additional plants developed galls. Intense new gall formation occurred in July and early August (22 cm precipitation), and the number of galled plants increased to 135 (Figs. 5, 6, 7).

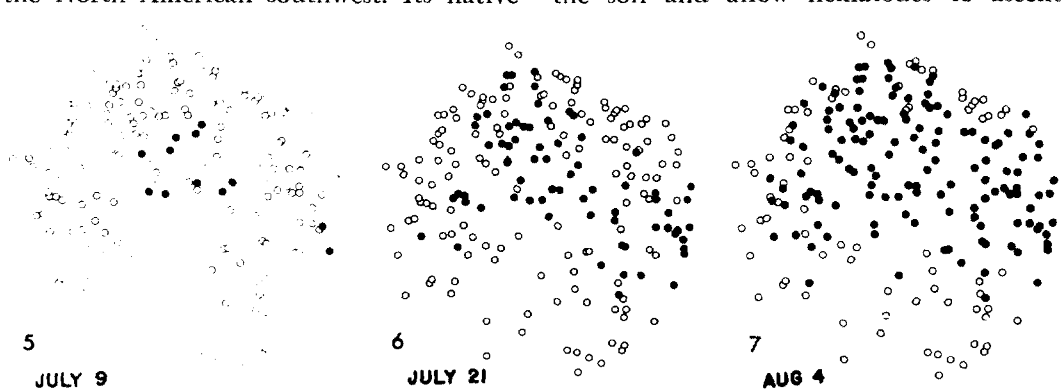
DISCUSSION

Our observations contribute the following information on the life history of *N. phyllobia*. Adults and preinfective juveniles live and develop only within the moist microhabitat of foliar galls; when galls abscise, adults and preinfective juveniles die. Infective-stage larvae within drying galls enter a quiescent state, in which they may remain viable for years and from which they revive to an active state within several hours when rewetted. During sustained moist conditions, these larvae exit abscised galled leaves clinging to the host plant and travel in moisture films on stem surfaces to new infection sites, which are generally actively growing young tissues. If the dead galled leaf from which larvae escape is on the soil surface, larvae enter the soil and infect preemergent shoots of silver-leaf nightshade. Infective larvae overwinter both in the soil and in galled leaves; the latter are especially subject to dispersal by wind and water. A high biotic potential (often hundreds of thousands of individuals in a single gall) contributes to rapid dispersal by these largely random means.

Silver-leaf nightshade is indigenous to the North American southwest. Its native range extends from Missouri southward to Louisiana and westward to Arizona and adjacent Mexico (1). *N. phyllobia* was first reported from central Arizona, at the west end of this range, and from the lower Rio Grande Valley of Texas 1,600 km to the east (6). Our report of the nematode on the Texas high plains, midway between these points, strongly suggests that the geographic distributions of *N. phyllobia* and silver-leaf nightshade coincide.

N. phyllobia is host-specific for silver-leaf nightshade. In close examinations of numerous plant species from areas with high *N. phyllobia* populations, no evidence of tissue penetration or nematode reproduction was found. Direct inoculation of several plant species in the field (4) failed to produce disease symptoms, while nematode galling developed on silver-leaf nightshade control plants. Pronounced host specificity and coincident geographic distributions suggest that *N. phyllobia* and *S. elaeagnifolium* are highly coevolved, minimizing the possibility that *N. phyllobia* would ever switch hosts if populations were augmented in a biological control program.

We have demonstrated that large numbers of infective larvae of *N. phyllobia* can be readily introduced into silver-leaf nightshade populations. Once introduced, the nematodes spread rapidly, significantly reducing top growth of the host and reproducing prolifically as long as the host plant is present. We speculate that the development of nematode symptoms would be accelerated under aerial irrigation. The added moisture would facilitate activation of infective larvae within dead galls or in the soil and allow nematodes to ascend



FIGS. 5, 6, 7. Spread of nematode disease in a 10-m-diam plot of *S. elaeagnifolium* during a rainy 4-wk period (uninfected plants = white circles; infected plants = black circles).

plant stems. Silver-leaf nightshade populations can be reduced by dispersing the nematode artificially, modifying irrigation regimes and similar manipulations.

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