Population Fluctuations and Observations of the Life Cycle of *Xiphinema americanum* Associated with Cottonwood (*Populus deltoides*) in South Dakota[1]

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The American dagger nematode, *Xiphinema americanum* Cobb, is one of the most commonly encountered nematodes in South Dakota soils (Thorne and Malek, 1968). Occurring in greatest abundance around tree roots, it is thought to be a factor in stunting and premature decline of shelterbelt trees (Malek, 1968). Because of difficulties in maintaining *X. americanum* populations in laboratory or greenhouse, demonstrations of its pathogenic capabilities have been infrequent and often inconclusive. However, pathogenic relationships with certain tree species have been reported by White (1955), Ruehle and Sasser (1962), Griffin and Epstein (1964), and Krebill et al. (1967).

Although the influence of environmental conditions on *X. americanum* has been studied under controlled conditions (van Gundy et al., 1962; Lownsbery and Maggenti, 1963; Griffin and Barker, 1966), the biology of this nematode remains poorly understoood. Ecological studies of *X. americanum* on alfalfa in Iowa (Norton, 1963) and on spruce in Wisconsin (Griffin and Darling, 1964) have revealed possible host- or climate-influenced differences in population fluctuations.

As part of a broad study of the relationship of *X. americanum* to unthriftiness of South Dakota shelterbelt trees, the present investigation was undertaken to determine its population fluctuations around the roots of a commonly planted tree species under the climatic conditions of the upper Great Plains. In addition, further knowledge of the field biology of this nematode was needed to develop practical techniques for its study in the laboratory and greenhouse.

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Materials and Methods

A weed-free planting of 8-year-old cottonwood (*Populus deltoides* Marsh.) known to sustain dagger nematodes and located on the Plant Pathology Research Plots, Brookings, was chosen as the study site. The sample area consisted of 30-foot trees, 14 feet apart and in rows of eight on a level Vienna loam soil. In November, 1964, one tree from the inner six in each of six alternate rows was randomly selected as a sample tree. Preliminary sampling at that time revealed nematode populations consisting of 90–98% *X. americanum* with only trace numbers of *Psilenchus hilarulus, Boleodorus thylactus, Eudorylaimus* spp., *Nygolaimus brachyurus* and *Tylencholaimellus* sp.

Sampling was resumed in mid-April, 1965, when the sample horizon had thawed and, except for the period of December through March when the horizon was frozen, was continued through mid-November, 1966. At biweekly intervals through August and at 4-week intervals thereafter until mid-November, approximately one liter of soil was removed from the rhizospore in the 5–25 cm profile just inside the dripline of each sample tree. Beginning at a randomly chosen point under each tree, consecutive samples were taken from undisturbed soil until the tree had been encircled at the end of the season. This procedure was repeated in 1966, except that samples were taken from an adjacent tree in the row. Soil temperatures during the sampling period were measured at the 15 cm depth by a recording thermograph, and soil moisture percentages in the sample horizon were determined at each sampling date.

Samples were collected in the late morning and processed within 4 hours. After each sample was thoroughly mixed and the large roots were discarded, a 400 cc portion was removed for processing. Free-living stages of *X. americanum* were extracted by a modification of the method of Christie and Perry (1951). Residues were collected on a 270-mesh sieve and allowed to remain on a Baermann funnel overnight. During 1966, eggs were extracted from 100 cc of a sample soil composite by the centrifugal flotation method of Caveness and Jensen (1955), collected on a 325-mesh sieve, and backwashed into a petri dish.

Extracted nematodes were observed and counted in a Syracuse watch glass with a subdivided bottom. Because of seasonal temperature variations in the extraction room and their effect on extraction efficiency, numbers of live nematodes remaining in the residues were determined by the aliquot method. The two figures were then combined. Total numbers, as well as numbers of adults and gravid individuals, were recorded. The presence of dagger nematode eggs at sample dates was noted but no counts were made.

Results and Discussion

Seasonal population fluctuations of *X. americanum* around roots of cottonwood at Brookings are presented in Figure 1. Nematode numbers were lowest in February and highest in June and July. Following a decline during the remainder of the summer, another population peak occurred in early autumn. These results agreed with those of Griffin and Darling (1964) but were dissimilar to those of Norton (1963), who reported population peaks in early spring and late summer on alfalfa in Iowa.

Population fluctuations from July through November in 1965 and 1966 were similar but the spring trends were markedly different. Soil beneath sample trees in November, 1964, showed a mean value of 2,850 nematodes per 400 cc of soil, while numbers in April, 1965, were 53% lower. Average monthly air temperatures for the period of December through March of 1964–65 were 6, 5, 5, and 7 C, respectively, below the normal of –7, –10, –8, and –1 C. Thus, the severity of the winter evidently resulted in a high mortality of dagger nematodes. During the following winter (1965–66), subnormal average temperatures occurred in January and February alone, and the April population level in 1966 was only 6% lower than that (3,400) in November, 1965. Another disparity in spring trends was the absence in 1965 of the substantial population increase in May and early June, 1966. Despite the differences in population levels of *X. americanum* during the spring of 1965 and that of 1966, primary population peaks were the same for both years (ca. 4,600).

Adult population fluctuations, though less obvious, were similar to those of the total population. Molting of preadults was observed from April to late July and again in September or October. Dead transparent females were common in residues in June and again in August and September. Griffin and Darling (1965) observed that adults often outnumbered larvae. In the present study, larvae always were predominant, even though extraction loss of any stage during soil settling was less than 15%. This difference may have been due to a longer life cycle and a high mortality of larvae under South Dakota conditions.

Gravid females first appeared in early May in 1965. In the following spring, soil temperatures rose more slowly and reproduction began 3 weeks later. Oocyte development was noted 7–10 days before the appearance of gravid females and at a soil temperature of 10–15 C. Norton (1963) found reproduction to be most intense during the late stages of the cycle, which continued into late August in Iowa. In the present study gravid females were most abundant in May and early June. These early individuals, which were thought to be overwintered adults, were transparent except for eggs or had only sparsely granulated intestines. Reproduction increased slightly in late June and terminated in late July. These late egg-bearing females had densely granulated intestines, which obscured eggs, and may have passed the winter as third or fourth stage larvae. Adult numbers increased again in autumn but no evidence of gonad activity was observed. Dagger nematode populations were periodically observed in 1965 in an adjacent field of alfalfa (*Medicago sativa* L.) and in 1967 in a planting of American elm (*Ulmus americana* L.) in sandy loam soil and in the cottonwood plot. In all cases, the reproductive cycle was similar to that shown in Figure 1. This evidence that reproduction in the field is limited to late spring and early summer in South Dakota is in contrast to the findings of Griffin and Darling (1964), who observed a second period of reproduction in late autumn in Wisconsin.

Eggs of *X. americanum* were found at all

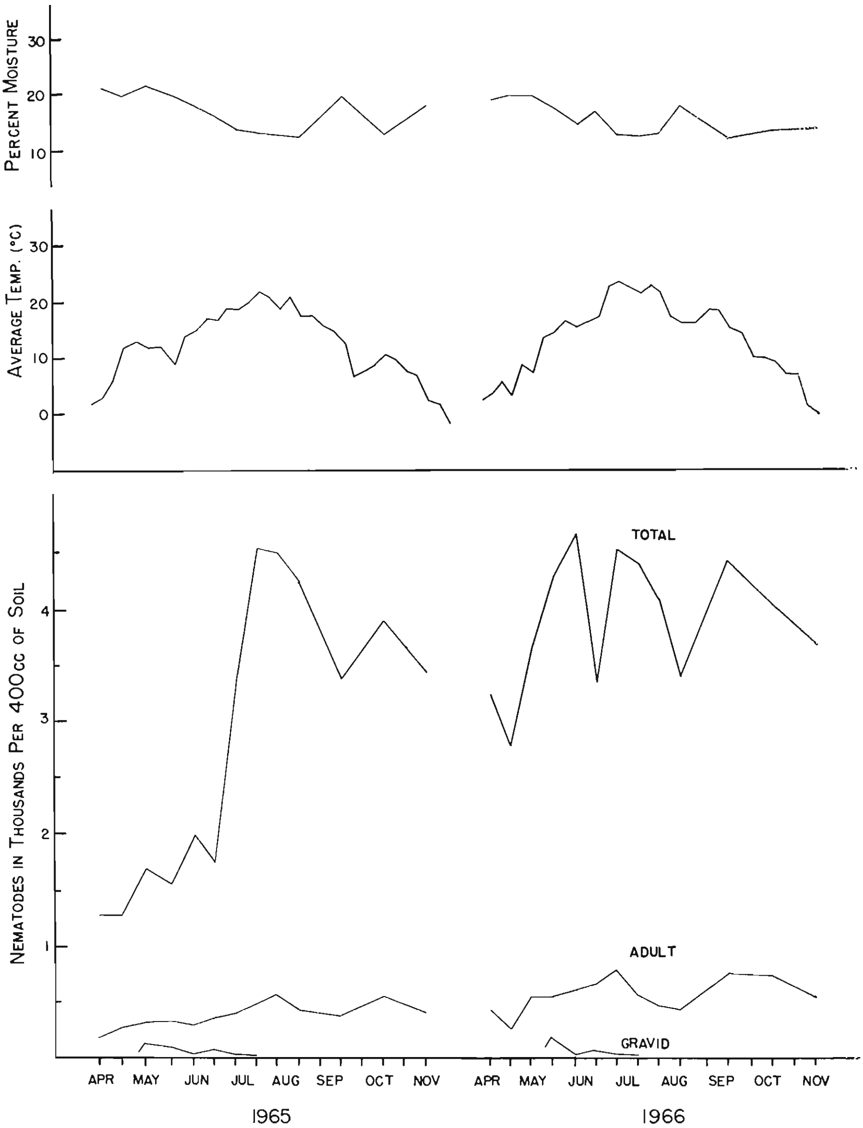

Figure 1. Population fluctuations of *Xiphinema americanum*, average soil temperature at 15 cm, and soil moisture around cottonwood roots. Plant Pathology Research Plots, Brookings.

sampling dates during 1966. However, hatching apparently occurred in spring and early summer and again in early autumn, since free-living first stage larvae were present only when total populations were increasing. These larvae were comparable in size and appearance to those artificially hatched by rupturing fully developed eggs with an eye knife. Eggs and first stage larvae were seen prior to the reproductive period in 1966, indicating that eggs may remain in the soil up to a year before hatching.

In late June, 1966, there was a temporary decline in total numbers of nematodes and a disappearance of first stage larvae in extracts, which was associated with an increase in dead individuals in residues. This mortality may have resulted from failure of newly hatched larvae to find feeding sites. Many small root branches die during the summer months, and eggs deposited in a region of healthy rootlets were therefore likely to hatch some distance from a source of food. Furthermore, adults were more active in extracts than larval forms; first stage larvae seldom exhibited motion, suggesting that newly hatched larvae may be incapable of migrating any distance to live roots. First stage larvae reappeared in mid-July, 1966, and the resultant secondary population peak closely corresponded with the primary peak in 1965. Since both occurred near the end of the reproductive cycle and nearly two months after its inception, July peaks were attributable to hatch of eggs deposited in the respective seasons. It would seem that 6 to 8 weeks of incubation were necessary before hatching could occur, and eggs deposited late in the reproductive cycle may not hatch until the following autumn or spring when moisture and/or temperatures were favorable. First stage larvae were scarce in the spring of 1965; the absence of a substantial late spring population increase similar to that of 1966 suggests that a near total egg-mortality occurred during the preceding winter.

Samples taken from the frozen cottonwood rhizosphere in mid-March, 1967, revealed approximately the same ratio of adults to immature forms (ca. 1:8) as did samples taken 4 weeks later and in November, 1966. Furthermore, the ratios between second, third and fourth stage juveniles did not appear to change noticeably and eggs were present in the soil on all three occasions. In general, intestines of larvae were densely granulated, while those of adults were sparsely granulated to transparent. However, tesselated or transparent females were seen in May with eggs in the uteri. Markedly subnormal temperatures occurred only in February; averages for the remaining months were near or slightly above normal. Apparently, no one stage of *X. americanum* was most capable of overwintering under normal South Dakota conditions.

Growth and development of larval stages were confined to relatively short periods of the year. Molting of second, third, and fourth stages began approximately 2 weeks before the appearance of gravid females, when soil temperatures rose to 5–10 C. Molting ceased in early July, but resumed briefly in early autumn. First stage larvae, which were absent after population peaks were attained, probably molted soon after hatching if feeding sites were accessible. Dead second, third and fourth stage larvae were present in residues throughout the sampling season, indicating continuous mortality, but their numbers were generally lower during these periods of activity. The cyclic nature of nematode activity may have been directly attributable to temperature and moisture effects, but tree root growth patterns, which closely correspond with periods of nematode activity, may have been a factor as well.

It could not be determined whether the egg to egg cycle could be completed within a single season. However, considering the restricted reproductive period and the length of time eggs may lie dormant, it is thought that *X. americanum* may require as least one year to complete its life cycle in the upper Great Plains.

Differences between the results of this study and those of Norton (1963) and Griffin and Darling (1964) are apparently related to host and climatic influences on the biology of *X. americanum* and emphasize the need for more extensive research on the comparative ecology of nematodes in their native habitats.

Summary

Population fluctuations of *Xiphinema americanum* around cottonwood roots in South Dakota are described and the life cycle of the nematode under field conditions is discussed. Nematode numbers were lowest in April and highest in June and July. A population decline in August and September was followed by an early autumn peak. In consecutive years, there was a 61% difference in initial spring population levels, but maximum numbers at the primary peaks were the same. Adult population peaks occurred at the same time as those of the total population, but reproduction was limited to May through July. Egg-hatch, growth, and development apparently were confined to spring and early summer and a brief period in mid-autumn. Except for first stage larvae, which were not present during the winter months, all stages were capable of overwintering in South Dakota, but eggs ap-

peared to be most affected by subnormal winter temperatures. The restricted reproductive period and long dormancy of many eggs suggest that the life cycle of *X. americanum* may require at least one year for completion in the upper Great Plains. Differences between the results of this and similar studies were ascribed to host and climatic influences on the biology of the nematode.

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Parasites of the Pygmy Whitefish, *Prosopium coulteri* (Eigenmann and Eigenmann) and Mountain Whitefish *Prosopium williamsoni* (Girard) from Western Montana

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The parasite fauna of the pygmy whitefish *Prosopium coulteri* (Eigenmann and Eigenmann), to our knowledge, has never been reported. In addition, the mountain whitefish *Prosopium williamsoni* (Girard) whose parasite fauna has been studied in other areas (Skinker, 1931; Wardle, 1932; Smedley, 1933; Bangham, 1951; Bangham and Adams, 1954; and Fritts, 1959) has not been studied extensively in western Montana. The pygmy whitefish *P. coulteri*, which appears to have a disjunct distribution, has been recorded from Lake Superior (Eschmeyer and Bailey, 1954), Columbia River drainage in Washington, Montana and British Columbia (Weisel and Dillon, 1954) and from the Fraser, Skeena, Yukon, and Mac-