

Soil pH Effects on Nematode Populations Associated with Soybeans¹

NANCY C. BURNS²

Abstract: 'Amsoy' soybeans were grown for 2 months in nonsterilized Jackson silt loam amended to pH 4.0, 6.0, and 8.0. Nematodes were extracted biweekly from soil and roots. The greatest numbers of *Pratylenchus alleni* colonized soybean roots at pH 6.0. *Hoplolaimus galeatus* and members of the Tylenchinae-Psilenchinae survived best at pH 6.0, while numbers of the Dorylaimoidea were greatest at both pH 6.0 and 8.0. The non-stylet nematodes were recovered in greater numbers from pH 8.0 soil. Potassium, manganese, and phenols were highest in soybean plants grown in pH 4.0 soil, the pH at which there were the fewest nematodes. A thicker suberized outer layer of root tissue occurred in plants grown at pH 4.0. **Key Words:** *Pratylenchus alleni*, pH, Soybeans.

Little information is available concerning the influence of soil pH on plant endoparasitic nematodes, and some of the early work is contradictory, especially with *Meloidogyne* and *Heterodera* (5, 12, 20). Early work with *Pratylenchus* spp. caused maximum "coarse root" on tobacco at pH 5.2-6.2 (8). *Pratylenchus penetrans* (Cobb) Filipjev and Schurmans-Stekhoven reached maximum numbers in soil at pH 6.2 and *Pratylenchus crenatus* Loof was found in greater numbers in acidic soils (3). An increase in nematodes was correlated with a pH increase with *Criconemoides xenoplax* Raski (24), *Tylenchorhynchus maximus* Allen (23), and *Tylenchulus semipenetrans* (27, 28, 29).

The purposes of this study were: (i) to determine the effect of soil pH on the invasion and colonization of soybean roots by *Pratylenchus alleni* Ferris and on population levels of other nematodes associated with soybeans, and (ii) to study plant mineral composition changes in soybean plants resulting from parasitism by nematodes under different pH regimes.

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² Department of Botany and Plant Pathology. Present address: 57 Mountain View Ave., Long Valley, New Jersey 07853. Appreciation is extended to Dr. B. Rojas, Department of Statistics, Iowa State University, Ames, Iowa 50010 for statistical assistance.

MATERIALS AND METHODS

Jackson silt loam containing a large population of *Pratylenchus alleni* was obtained from a soybean field in Keokuk County, Iowa. The soil analysis was: 14.5% sand, 66.7% silt, 18.8% clay, and pH 6.0. Fertility analysis indicated 40, 36, and 20 pounds/acre of nitrifiable -N, available -P, and available -K, respectively.

After the soil was thoroughly mixed, dilute sulfuric acid or concentrated calcium hydroxide was added to separate portions to adjust the pH to either 4.0 or 8.0, as required. A 1-week adjustment period was allowed for the soil pH to stabilize. The soil, as collected, was used for pH 6.0. Similar tests using steam-sterilized soil adjusted to corresponding pH values served as controls.

The soil was placed in sterile 4-inch clay pots and three 1-day-old germinating 'Amsoy' soybeans seeds were evenly planted in each pot. Three replications, consisting of three plants/treatment, were maintained at 25 C and a 12 hr photoperiod in a growth chamber. Eighteen pots, representing three replications of six treatments, were sampled for nematode numbers, and plant and soil characteristics every 2 weeks for 2 months.

EXTRACTION OF NEMATODES: Nematodes were extracted from the soil essentially by the Christie and Perry method (4). To extract *Pratylenchus alleni* from the roots, one

root system from each pot at each sampling was cut into 1–2 mm segments and barely covered with water in petri dishes. The emerged nematodes were collected and the water replaced weekly for 28 days. The roots were oven-dried, and the cumulative total of *P. alleni* per g of dried root was calculated. Data were taken on the following nematodes or groups of nematodes: nonstylet-bearing nematodes, Dorylaimoidea (hereafter “dorylaims”) excluding *Xiphinema americanum* Cobb, *Pratylenchus alleni*, *Hoplolaimus galeatus* (Cobb) Thorne; and the Tylenchinae-Psilenchinae, principally *Tylenchus* and *Basiroides*.

ROOT INVASION: The location of nematodes in the root was studied after the first sampling. In the earlier samplings an entire root system from each replication was stained with cotton blue in lactophenol. Because root systems were large at later samplings, 2-g of randomly selected portions from each replication were cut into 2.5-cm segments. These were examined for invasion and colonization by nematodes.

MEASUREMENT OF SOIL PROPERTIES: The soil pH in each pot was measured (21), and soluble salts were determined with a conductivity bridge and compared against a standard 0.01 N KCl solution (2) at every sampling. The nitrifiable -N, available -P and K, and a particle size analysis using the modified pipette method (26) were determined prior to the beginning of the experiment.

PLANT TISSUE ANALYSIS: All roots and shoots were dried and ground separately in a Wiley Mill using a 20-mesh sieve. A 0.5 g random portion from each pot at each sampling was analyzed after 4 and 6 weeks for

total elemental N by the micro-Kjeldahl method (6). Compositated plant shoots from three replications of the third and fourth samplings of all treatments were spectrographically analyzed for K, P, Ca, Mg, Mn, Fe, B, C, Zn, Al, Sr, Co, Mo, Si, and Ba by the Ohio State University Spectrographic Laboratory. Total polyphenols contained in a random sample of dried root tissue from each pot were determined spectrophotometrically after each sampling (22).

SUBERIZED ROOTS: The upper 5-mm portion of a tap root from each pot was fixed, dehydrated, and embedded in paraffin (7). Tissues were sectioned at 15 μ , stained with safranin and fast green (14), and the thickness of the outer suberized layer of root measured.

The experiments and tests were repeated using Hagener loamy fine sand containing 80.5% sand, 14.3% silt, and 6.16% clay. The nitrifiable -N, available -P and K at the beginning of the experiment were 24, 43, and 65 pounds/acre, respectively. Once a week, 100 ml/20-20-20 fertilizer was applied to each pot in 0.5 g/L concentration. Determinations for N, P, and K were made for each treatment at 6 and 8 weeks.

The *F-test* and Student's *t* distribution were used to measure the level of statistical significance in all tests. Points on graphs were calculated using the equation:

$$y = B_0 \pm B_1 (pH) \pm B_2 (\text{weeks})$$

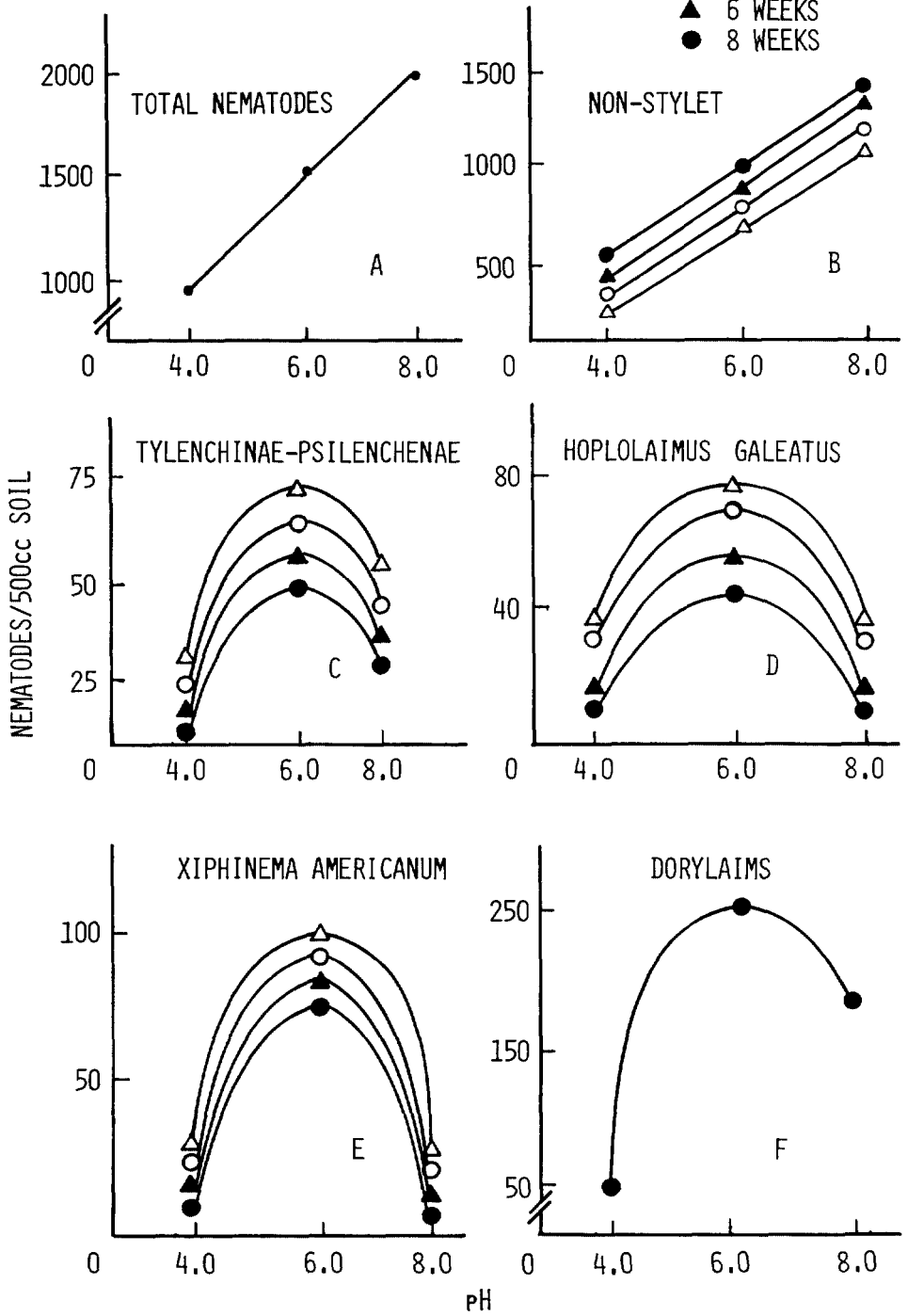
where the coefficient (B) is multiplied by its variable. Subscripts designate B as separate coefficients.

RESULTS

The soil pH values (Fig. 1–2) represent the statistical analysis of the raw data and

FIG. 1. Statistically analyzed data showing numbers of nematodes associated with greenhouse-grown 'Amsoy' soybeans in 500 cc of Jackson silt loam soil at three soil pH regimes for two months. Significant difference levels are: A. $P = 0.01$; B. $P = 0.05$; C. $P = 0.01$; D. $P = 0.01$; E. N.S.; F. $P = 0.01$.

- △ 2 WEEKS
- 4 WEEKS
- ▲ 6 WEEKS
- 8 WEEKS



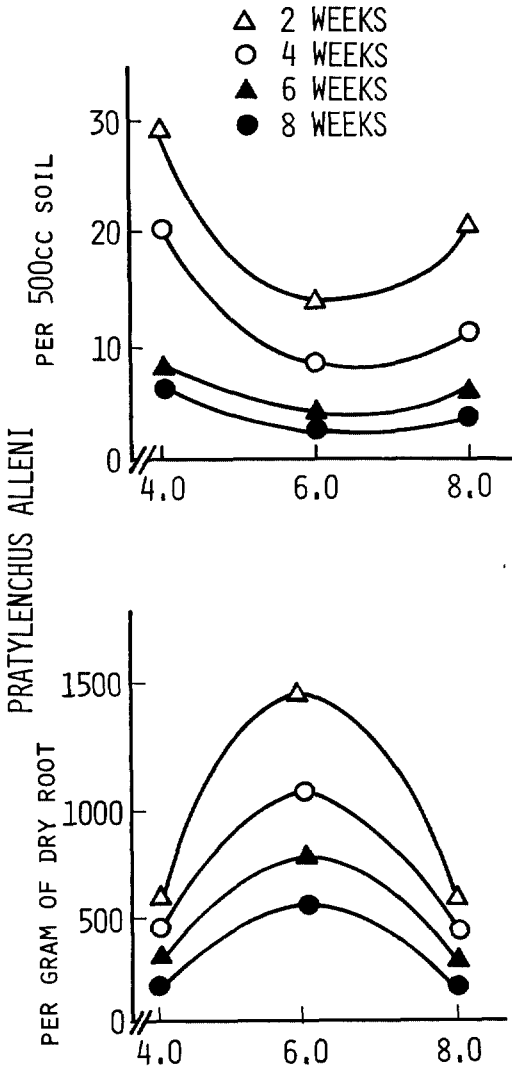


FIG. 2. Statistically analyzed data of numbers of *Pratylenchus alleni* associated with 'Amsoy' soybeans grown for two months at soil pH 4.0, 6.0, or 8.0. Significant difference levels are: Soil, $P = N.S.$, Root, $P = 0.01$.

are those of the originally adjusted soil. There was a shift from the extremes of pH toward pH 6.0 as the experiment progressed, but this did not exceed 0.5 of a pH unit.

More ($P = 0.01$) total nematodes were recovered from the soil at pH 6.0 or 8.0 than at pH 4.0 (Fig. 1A). The nonstylet nema-

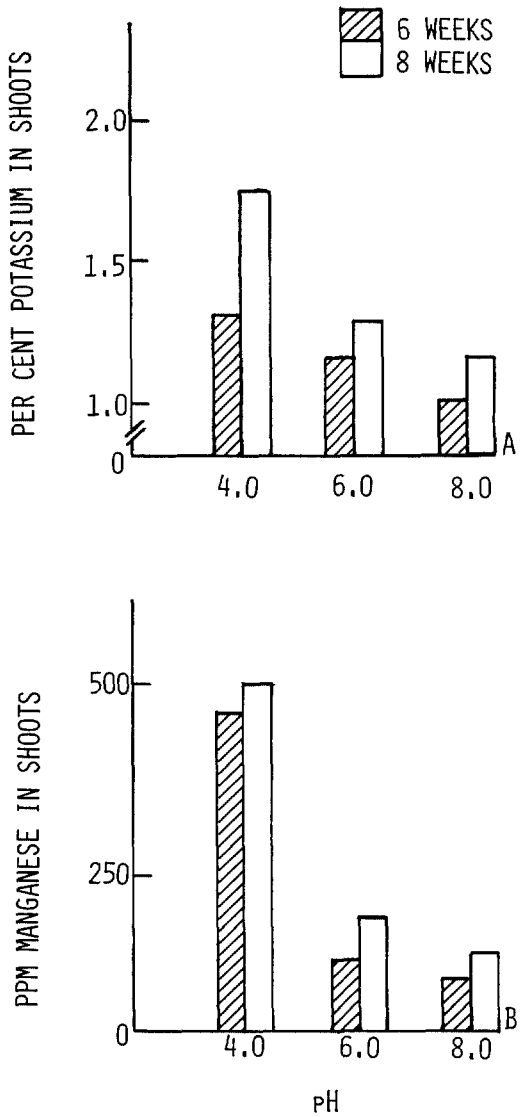


FIG. 3. Spectrographically determined potassium (A) and manganese (B) in 6 and 8-week-old 'Amsoy' soybean shoots grown in Jackson silt loam soil adjusted to three soil pH regimes. Significant difference levels are: A. $P = 0.01$; B. $P = 0.01$.

todes, consisting mainly of Rhabditidae and Cephalobidae, were recovered in significantly ($P = 0.05$) greater numbers at pH 8.0 soil than at pH 4.0 or 6.0 (Fig. 1B). Significantly ($P = 0.01$) more Tylenchinae-Psilen-

chinae and *H. galeatus* were recovered at pH 6.0 than at pH 4.0 or 8.0 (Figs. 1C, D). *Xiphinema americanum* was recovered in the greatest numbers from soil at pH 6.0 and least numbers were recovered from the extreme pH regimes (Fig. 1E). Dorylaims were recovered in the greatest numbers ($P = 0.01$) from the pH 6.0 and 8.0 soil (Fig. 1F). *Pratylenchus alleni* was recovered in greatest numbers from soybean roots grown in pH 6.0 soil. The numbers of *P. alleni* recovered from soils exhibited an inverse relationship ($P = 0.01$) to those recovered from the roots at corresponding pH levels (Fig. 2). Generally, there was no increase in numbers of nematodes over the 2-month period and, thus, the data may reflect survival as much as reproduction. Fresh whole weights and plant lengths were significantly ($P = 0.01$) less at the pH 4.0 regime compared with the more basic soil treatments. Spectrographic analysis of elements in the shoots revealed that K and Mn were significantly higher ($P = 0.01$) in plants in the pH 4.0 regime than in the more basic soil treatments (Fig. 3). Other elements that showed significantly ($P = 0.01$) higher trends were Fe and Al, in the pH 8.0 regime. This occurred only in the steamed soil with Fe and Al, however, and probably resulted from conversion of these elements to a more available form as a result of the steaming process. The other elements analyzed did not show consistent or significant trends. The total phenolic compounds in plant roots were significantly ($P = 0.01$) higher in pH 6.0 nonsteamed soil than the same pH of steamed soil (Fig. 4). Plant roots grown in pH 4.0 soil had a significantly ($P = 0.05$) thicker suberized layer on the surface of the root than either of the more basic treatments (Figs. 5A, B, C). The amounts of soluble salts in the different soil treatments did not differ significantly.

Although a sandier soil was used when the

experiments were repeated, the changes in nematode numbers in the different pH regimes were statistically similar.

DISCUSSION

Tannins and other phenolic compounds, such as chlorogenic acid, are believed to be important in disease resistance (10, 11). Some investigators have compared the amount of phenolics in plants with colonization by *Pratylenchus*. MacDonald (13) found that Sudan grass, a poor host for *P. penetrans*, had 4.7 to 6.1 times more phenol than winter vetch, a good host. Pitcher et al. (19) found that cortical tissues of apple roots, which were tolerant to *P. penetrans*, were relatively free of phenols. The dermal and endodermal layers, which were not tolerant to the nematode and underwent rapid necrosis, contained high concentrations of phenolic compounds. Peach roots had high concentrations of phenols in all extensively colonized tissues. In the present study, the amount of phenols in plant tissues was independent of the number of nematodes (Fig. 4), indicating that phenols may not be important in inhibiting colonization.

Several investigators have reported correlations of increased numbers of nematodes or damage with decreased K content of the host (9, 17, 18, 25). Results of the present study are consistent with these previous findings in that the least amount of invasion by *P. alleni* occurred when there was the greatest amount of K in the plants, which was at the pH 4.0 regime. It was also at pH 4.0 that the thickest outer epidermal walls occurred (Fig. 5). It is known that deficiencies of K decrease the amount of carbohydrates stored in plants causing the plants to be more susceptible to disease (15). This can result from decreased outer epidermal cell wall thickness or by decreased development of the cork cambium (1, 16). Results in the present study indicate that soil

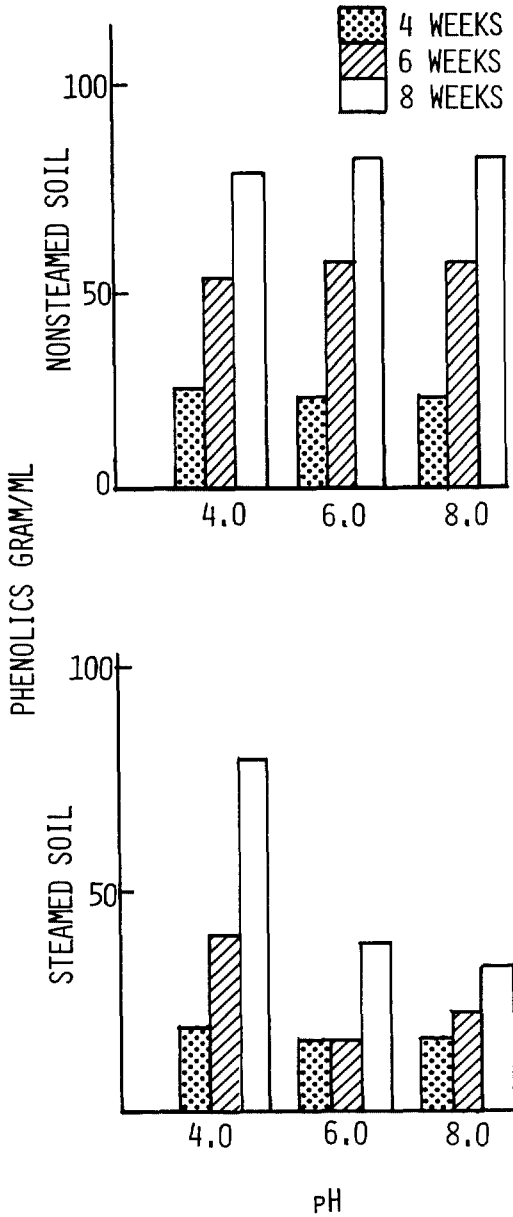
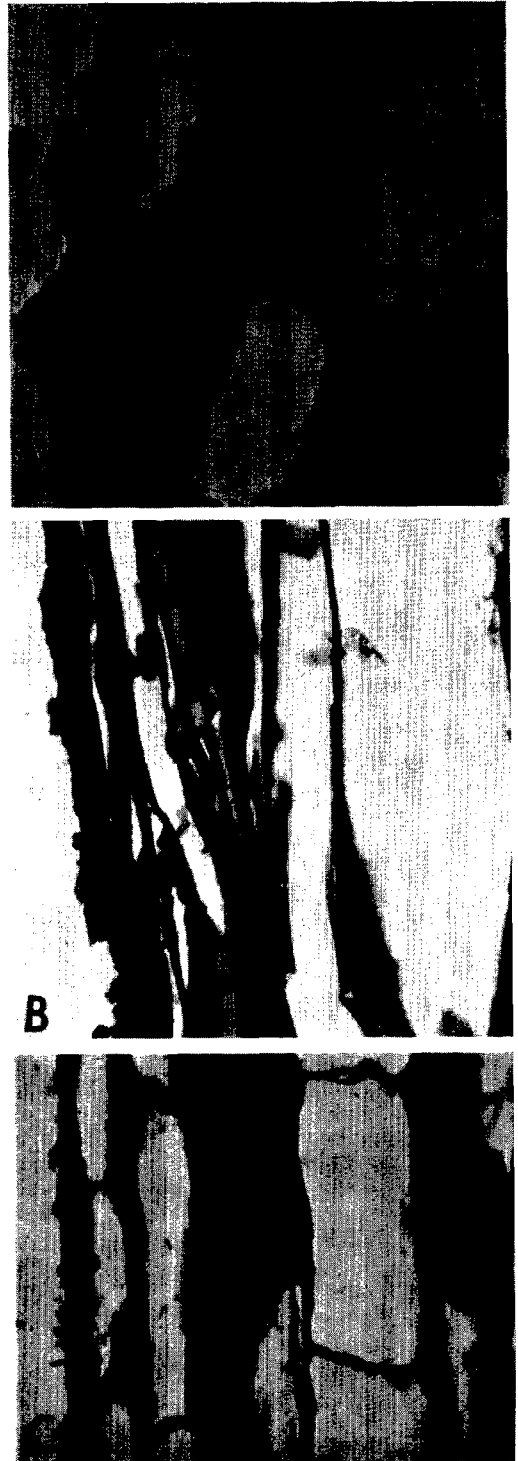


FIG. 4. Phenolic compounds (g/ml) in 4, 6, and 8-week-old 'Amsoy' soybeans grown in Jackson silt loam adjusted to three soil pH regimes. Data are calculated from spectrophotometric absorption (610 m μ) readings on plant shoot extracts. Significant difference level— $P = 0.01$.

FIG. 5. Photomicrograph of the suberized epidermal layer of 'Amsoy' soybean root tissue grown in Jackson silt loam soil. A. pH 4.0; B. pH 6.0; C. pH 8.0. Epiderm thickness differences significant at $P = 0.05$.



pH affects mineral uptake in soybeans (Fig. 3). The resulting morphological changes such as the thickened epidermal cell walls (Fig. 5), could affect the degree of invasion by *P. alleni*. Since most other elements did not show consistent trends at different pH levels, it is possible that the ultimate effects could be due to K or Mn. Less is known concerning the role of Mn than of K in disease resistance, however.

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