# Effects of Density of Helicotylenchus dihystera and Pratylenchus vulnus on American Boxwood Growing in Microplots[1]

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*Abstract:* American boxwood, *Buxus sempervirens* var. *globosum*, was tolerant of *Helicotylenchus dihystera* [in field microplots] as measures of plant growth were similar to the control and nematode densities were maintained at high levels (1,705-1,810/500 cm³ soil after 29 months). Boxwood was intolerant of *Pratylenchus vulnus* at initial densities of 163, 281, or 475 nematodes per 500 cm³ soil. In comparisons with those of controls, vigor ratings of boxwood after 14 months were much lower at all densities of this nematode. Nematode density was not directly related to vigor rating. However, initial nematode density was directly proportional to growth suppression of boxwood as measured by the difference of the product of final plant height x width or by the difference of the plant surface area determined from a standardized photograph as compared to those of controls. A nematode density of 160/500 cm³ of soil was found to suppress growth by 50%. Populations of *P. vulnus* declined, according to a linear function, with time after reaching over 7,100 nematodes/500 cm³ of soil taken from the root zones of boxwoods. Ninety-five percent of the *P. vulnus* population died between 15 and 20 months after soil infestation. *Key Words:* semilogarithmic transformation, *Buxus sempervirens* var. *globosum*.

American boxwood, *Buxus sempervirens* var. *globosum* L., is an ornamental widely used for landscape plantings in the eastern United States. Previously, the etiology of boxwood decline has been attributed to an unfavorable environment involving either infertile soil, and/or extreme fluctuations in moisture or temperature. Since 1950, parasitic nematodes and root-rot fungi, including *Phytophthora parasitica* and *Paecilomyces buxi*, in combination with the previously mentioned factors have been associated with boxwood decline (3, 4, 6).

In 1950, *Pratylenchus* spp. were reported to be associated with boxwood in field plantings at the Plant Industry Station at Beltsville, Maryland (9). Haasis et al. (4) reported an association of *Pratylenchus vulnus* Allen & Jensen with decline of American and English boxwood, *B. sempervirens* var. *suffruticosa* L., in the coastal plain, piedmont, and mountain regions of North Carolina. In 1962, Osborne and Jenkins (7) demonstrated the pathogenicity of *P. vulnus* to American boxwood in the greenhouse. Symptoms of decline in

Received for publication 26 February 1976.

[1] Paper No. 4938 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N. C.

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the greenhouse and field include bronzing of foliage, stunting, and root necrosis. A density of ca. 700 *P. vulnus*/500 $cm^3$ soil caused a severe decline of boxwood in the greenhouse (7). In mixed populations of several parasitic species, as many as 4,300 *P. vulnus* specimens/500 $cm^3$ soil were recovered from the root zone of declining American and English boxwood in field plantings (4). In the same study, as many as 9,100 *Helicotylenchus* spp. specimens per 500 $cm^3$ soil were found in association with American and English boxwood (4). The pathogenicity of *Helicotylenchus* spp. on boxwood has not been reported in the literature, however.

The present study reports the influence of density of *H. dihystera* (Cobb) Sher and *P. vulnus* on growth of boxwood.

## MATERIALS AND METHODS

Field microplots were established at the Central Crops Research Station, Clayton, North Carolina by installing fiberglass cylinders (0.3 cm thick x 60.0 cm high x 80 cm diam) 50 cm deep. The tops of the cylinders extended 10 cm above field level to prevent recontamination by soil microflora and fauna in surface waters. The Appling loamy soil in each microplot was amended with 10 liters of peat moss and fumigated (1.0 $kg/m^2$, methyl bromide) 9 days prior to soil infestation with either *H. dihystera* or *P. vulnus.*

Inoculum of *H. dihystera* was increased for 84 days on *Glycine max* L. cv. 'Lee' in the greenhouse. Inoculum of *P. vulnus* was increased for 141 days on *B. sempervirens* var. *globosum* or on peach [*Prunus persica* (L.) Batsch] growing in 20-cm pots of fumigated sand:soil mixture (1:1, v/v) in the greenhouse. Supplemental light was provided to induce vegetative growth. To minimize differences in microflora and nutrient levels, host plants also were grown in noninfested soil. Sufficient soil from these healthy plants was added to the respective control plots and plots receiving low or medium densities of either nematode species to bring the total amount of soil added/plot to 14 liters. Inoculum was thoroughly mixed into a depression (40 cm diam by 20 cm deep) in the center of each microplot. Four replications of each inoculum density, plus eight control plots, were used in a randomized, complete block design.

Fifteen-month-old uniformly rooted plants of *B. sempervirens* var. *globosum* growing in a sand:soil:peat mixture (1:1:1, v:v:v) were planted singly into each microplot the day after soil infestation (27 April 1973). Five days after soil infestation, 10 soil cores/microplot were taken from the boxwood root zone. Nematodes in soil were extracted by centrifugal flotation (5), and *P. vulnus* in roots were extracted over a 2-week period in a mist chamber (8). Initial densities ($P_i$) of *H. dihystera* ranged from 125 to 1,325/500 $cm^3$ of soil. The $P_i$ for *P. vulnus* ranged from 75 to 650/500 $cm^3$ of soil.

Plants were fertilized each spring with 100 gm of a slow release fertilizer (18-9-13). Malathion and dimethoate were used as needed for foliage insect control. Nematode densities were determined in the fall of 1973, and the following spring and fall of 1974 and 1975. Plant growth and vigor were rated on a 0-10 scale where 0 = dead plant, 10 = most vigorous plant.

The experiment was terminated after 29 months. Plant height and width were measured and each plant was photographed. A 35-mm single-lens reflex camera (utilizing a 50-mm lens) was positioned 0.87 m above the ground at a distance of 2.13 m from the plant for each photograph. A 10.2 x 12.6 cm black and white print was made from each negative. A standard enlarging distance (0.56 m) with a 135-mm enlarging lens was used. A planimeter was utilized to measure the area of the plant in the photographs for statistical analysis. Correlation between height x width values and plant surface area was highly significant ($r = 0.97$), a fact which indicates that the physical measurement (HxW) was strongly related to the photographic measurement (surface area).

## RESULTS

*Buxus sempervirens* var. *globosum* was tolerant of *H. dihystera* when measures of growth for inoculated plants were compared to those of controls; there were no statistical differences.

Density of *H. dihystera* increased by several-fold after soil infestation (Fig. 1-A).

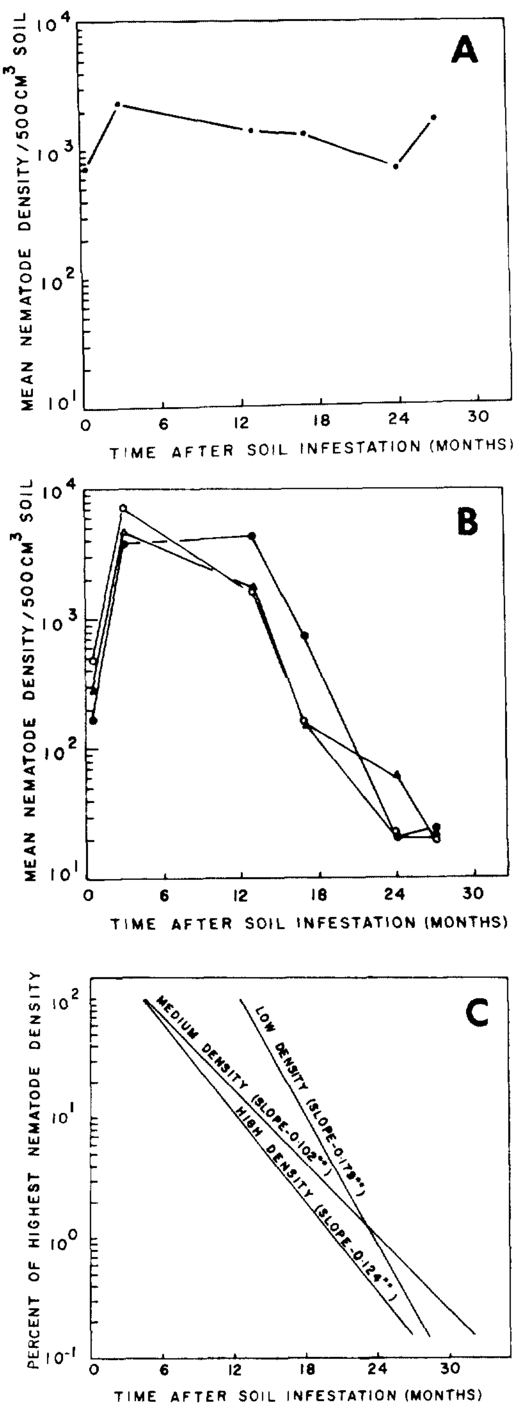


FIG. 1-(A-C). *A)* Semilogarithmic plot of *Helicotylenchus dihystera* population on *Buxus sempervirens* var. *globosum* over a 29-month sampling period (data are means of all initial densities, ranging from 125 to 1,325/500 cm³ of soil). *B)* Semilogarithmic plot of *Pratylenchus vulnus* on *Buxus sempervirens* var. *globosum* over a 29-month sampling period: 0 = high initial density, 475 nematodes/500 cm³ soil; △ = medium initial density, 281 nematodes/500 cm³ soil; and ● = low initial density, 163 nematodes/500 cm³ soil. *C)* Semilogarithmic transformation of Fig. 1-B. The correlation coefficients are all highly significant ($P \leq 0.01$).

Tolerance of boxwood to *H. dihystera* was demonstrated when nematode density remained high (regardless of initial density) throughout the sampling period and when measures of growth for inoculated plants were similar to those of control plants.

*Buxus sempervirens* var. *globosum* inoculated with *P. vulnus* failed to make perceptible growth. Vigor ratings at 14 months were significantly less than those of the controls, regardless of initial nematode density (Table 1). Above-ground symptoms included a failure to establish growth, bronzing of existing foliage, and partial defoliation. Two of 12 infested plants died by the end of the third growing season. The remaining 10 plants were severely stunted.

*Pratylenchus vulnus* density increased rapidly during the 3 months (regardless of initial inoculum density) following soil infestation (Fig. 1-B). Density of *P. vulnus* declined during the second growing season and through the final sampling date (29 months later) when only 20 nematodes per 500 cm³ of soil were recovered. Final population density did not depend on initial inoculum density. When the semi-logarithmic transformation (2) was applied, survival curves with slopes ranging from −0.102 for the medium density to −0.179 for the high density were found (Fig. 1-C). The regression line for each initial nematode density was used to interpolate the $ED_{95}$ value (time required for 95% of the nematode population to die). This value at $y = 5$ was 14.7 months for the high density, 17.3 months for the medium density, and 19.7 months for the low density. By extrapolation, the regression model ($\log \hat{y} = 2.5 - 0.124\,x$) predicts that, after 36 months, less than 1 nematode/500 cm³ of soil would be found on boxwood at the high-density treatment. Similar results were found at the other initial densities. Coefficients of correlation (r) were significant ($P = 0.01$).

*Pratylenchus vulnus* density (Fig. 1-B) and vigor rating (Table 1) were not

TABLE 1. Comparisons of *Buxus sempervirens* var. *globosum* growth rate, surface area, and height x width product as affected by *Pratylenchus vulnus* over a 29-month growing period in field microplots.

| Nematode density[w] | Boxwood vigor rating[x] Months after soil infestation[y] 5 | 14 | 17 | 25 | 29 | Plant Growth[y] Surface area ($cm^2$)[z] | Height width ($cm^2$) |
|---|---|---|---|---|---|---|---|
| High density | 2.3 a | 1.8 a | 1.5 a | 0.9 a | 0.5 a | 0.37 a | 126.0 a |
| Medium density | 2.8 ab | 2.3 a | 1.9 a | 1.5 a | 1.1 a | 0.64 a | 281.0 a |
| Low density | 3.8 ab | 3.0 a | 2.3 ab | 1.8 a | 1.0 a | 0.88 a | 374.0 a |
| Control-1 | 7.8 b | 8.5 b | 8.6 b | 9.4 b | 9.5 b | 6.18 b | 2,035.3 b |
| Control-2 | 6.8 ab | 8.0 b | 8.5 b | 9.4 b | 9.9 b | 7.02 b | 2,041.3 b |
| Tukey's HSD $P = 0.05$ | 5.1 | 2.4 | 6.4 | 1.6 | 1.2 | 2.6 | 795.3 |

[w]Plants were inoculated at either a high density = 475 nematodes/500 $cm^3$ soil, a medium density = 281 nematodes/500 $cm^3$ soil, or a low density = 163 nematodes/500 $cm^3$ soil. There were eight replicated control plants (no nematodes) split into two groups of four plants each in the experimental design.
[x]Vigor rating based on scale: 10 = most vigorous plant, 0 = dead plant.
[y]Numbers followed by the same letter are not significantly different ($P = 0.05$).
[z]Surface area computed (after 29 months) by a planimeter from a 10.2 x 12.6 cm photograph of the plant printed with a 135-mm lens at 0.56 m. A camera utilizing a 50-mm lens and 35-mm film was positioned 0.87 m above the ground at a distance of 2.13 m from the plant to make the photograph.

significantly correlated on four of five sampling dates. Consequently, other measures of host response to nematode density were evaluated. Boxwood was intolerant of *P. vulnus* even at the low initial density (Fig. 2-A). When the initial nematode density was compared to plant growth (surface area) by linear regression analysis, a highly significant ($P<0.01$) relationship was found (Fig. 2-B). Interpolation from the regression line demonstrated that a 50% suppression of growth would be expected with an initial nematode density of 160/500 $cm^3$ of soil. Theoretical extrapolation of the linear regression line demonstrated that no growth would be expected with a $P_i$ of 460 nematodes/500 $cm^3$ of soil. Similar results were found at other sampling dates. For instance, a nematode density of 14/500 $cm^3$ of soil at 29 months after soil infestation would suppress growth by 50% (Fig. 2-B). A theoretical *P. vulnus* density of only 49/500 $cm^3$ of soil at 29 months after soil infestation would be expected to suppress growth completely.

## DISCUSSION

Microplots have several advantages over field plantings for nematode research. These include the isolation of test plants from surrounding soil microflora and fauna, the ease of adding soil amendments to a relatively small volume of soil, and the ability to control nematode inoculations carefully. The use of fiberglass in microplot construction permits easy assembly and installation as well as reuse for future experiments. One problem encountered with the microplots was that of recolonization by unwanted parasitic nematodes in the later stages of the experiment. Higher rates of fumigant and greater care in preventing reintroduction of debris to the treated area might alleviate this problem.

The host reaction to a given nematode species cannot be predicted from the mere association of a plant-parasitic nematode and a given host. For instance, *H. dihystera* is frequently associated with *B. sempervirens* var. *globosum* in North Carolina, but this study demonstrated the tolerant nature of boxwood to this nematode. Even though high nematode densities were measured over the 29-month sampling period, infected plants had growth equal to that of the controls.

Field studies of the pathogenicity of *P. vulnus* on boxwood have not used controlled nematode densities (4, 9). Greenhouse studies using ca. 700 nematodes/500 $cm^3$ soil showed significantly less growth for inoculated plants as compared to that of the control plants after

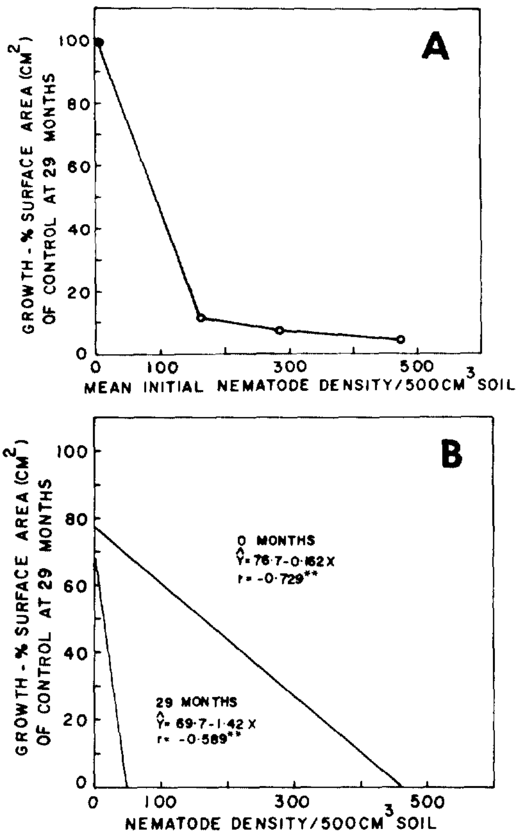


FIG. 2-(A-B). The effects of initial density of *Pratylenchus vulnus* on growth of *Buxus sempervirens* var. *globosum: A*) arithmetic plot; *B*) linear regression analysis of either initial or final nematode density on growth [percent surface area (cm²) of control at 29 months]. The correlation coefficients are all highly significant ($P = 0.01$).

318 days (7). In the present study, a very low initial nematode density (163/500 cm³ soil) resulted in severe inhibition of growth during 3 growing seasons. Although lower inoculum densities could be used, these data indicate that any density of *P. vulnus* will damage *B. sempervirens* var. *globosum*.

The direct relationship between initial *P. vulnus* density and boxwood growth was demonstrated clearly by the highly significant regression coefficient. This direct relationship is well known for field crops where only one growing season is involved, but it is not as obvious in certain perennial ornamentals when few inoculum levels are used (1).

A greenhouse study of the pathogenicity of *P. vulnus* on boxwood showed that an initial nematode density of 700/500 cm³ soil increased to an average of 9,000 nematodes/500 cm³ over a 318-day period (7). In the present study, the high density of *P. vulnus* reached only a maximum of 7,100 nematodes/500 cm³ soil before numbers declined. In addition, the *P. vulnus* population declined (as a linear function) with time on the intolerant boxwood root system according to the semilogarithmic transformation. As part of our state nematode advisory service, recommendations for control measures are now commonly made when very low densities of *P. vulnus* are encountered on American boxwood.

## LITERATURE CITED

1. AYCOCK, R., K. R. BARKER, and D. M. BENSON. 1976. Susceptibility of Japanese holly to Criconemoides xenoplax, Tylenchorhynchus claytoni and certain other parasitic nematodes. J. Nematol. 8:26-31.
2. DIMOND, A. E., and J. G. HORSFALL. 1965. The theory of inoculum. Pages 404-415, *in* K. F. Baker and W. C. Snyder, eds., Ecology of soil-borne plant pathogens. Univ. Calif. Press, Berkeley.
3. HAASIS, F. A. 1961. Phytophthora parasitica, the cause of root rot, canker, and blight of boxwood. Phytopathology 51:734-736.
4. HAASIS, F. A., J. C. WELLS, and C. J. NUSBAUM. 1961. Plant parasitic nematodes associated with decline of woody ornamentals in North Carolina and their control by soil treatment. Plant Dis. Rep. 45:491-496.
5. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
6. LAMBE, R. C., and W. H. WILLS. 1975. Decline of English boxwood in Virginia. Plant Dis. Rep. 59:105-108.
7. OSBORNE, W. W., and W. R. JENKINS. 1962. Pathogenicity of Pratylenchus vulnus on boxwood, Buxus sempervirens var. arborescens. Plant Dis. Rep. 46:712-714.
8. SEINHORST, J. W. 1950. Debetekenis van de toestand van de grond voor het optreden van de aantasting door het stengelaaltje (Ditylenchus dipsaci Kühn Filipjev). Tijdschr. Plziekt. 26:289-348.
9. TARJAN, A. C. 1950. Investigations of meadow nematodes attacking boxwood, and the therapeutic value of sodium selenate as a control. Phytopathology 49:1111-1124.