DISTRIBUTION AND SAMPLING OF ROOT WEEVIL LARVAE IN YOUNG ORNAMENTAL CONIFER PLANTATIONS

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Abstract

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We determined the distribution of root weevil larvae feeding on the roots of young ornamental conifers in field studies using a new non-destructive sampling technique. Most root weevil larvae fed on the roots during summer, fall, and late spring with significantly more larvae found at 10 cm than at 20 and 30 cm and more at 20 cm than at 30 cm. Larvae moved down into the soil to overwinter and moved closer to the surface (<15 cm) in the spring. Soil samples predicted the population density of larvae under individual trees. Four samples (0.0076 m^3), 9 cm in diameter by 30 cm long, taken equidistantly 20 cm from the tree stem provided the best estimate. The equation, $T = 64.04M$, where T is the total number of larvae beneath a tree, and M is the mean number of root weevil larvae from four soil samples, described the linear relationship between the number of larvae in soil samples and the total population of larvae beneath a tree. Implications for the timing and location of sampling and control measures are discussed.

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Résumé

Nous avons étudié la répartition des larves de charançons dans les racines de jeunes conifères ornementaux au moyen d'une technique d'échantillonnage non perturbante. La plupart des larves se nourrissaient de racines durant l'été, l'automne et à la fin du printemps et il y avait significativement plus de larves à 10 cm qu'à 20 ou à 30 cm et plus à 20 qu'à 30 cm de profondeur. Les larves s'enfonçaient dans le sol pour passer l'hiver et revenaient vers la surface (<15 cm) au printemps. Des échantillons de sol ont servi à faire des prédictions sur la densité des larves au pied d'arbres en particulier. Le prélèvement de quatre échantillons (0,0076 m^3) de 9 cm de diamètre et de 30 cm de longueur à équidistances de 20 cm du tronc est la stratégie qui a donné les meilleures estimations. L'équation $T = 64{,}04M$, où T est le nombre total de larves sous un arbre et M, le nombre moyen de larves de charançons des racines dans quatre échantillons de sol, décrit la relation linéaire entre le nombre de larves dans les échantillons de sol et la population totale de larves sous un arbre. Le moment idéal et le site optimal d'échantillonnage et les mesures de lutte à utiliser contre les charançons font l'objet d'une discussion.

[Traduit par la Rédaction]

Introduction

Total Ontario sales from the nursery industry were $196 million in 1990 (Ontario Ministry of Agriculture and Food 1992). Root weevils such as *Otiorhynchus ovatus* (L.) and *Barypeithes pellucidus* (Boh.) (Coleoptera: Curculionidae) are serious pests of the horticultural industry (Brandt 1992), causing extensive damage to ornamental conifers and reducing wholesale tree value from $30–72 per metre of height to almost zero (pers. comm. John Somerville, Somerville Nurseries Inc., Alliston, Ontario). Root weevils cause damage in two ways: first, larvae feed on tree roots, limiting the tree's ability to absorb water and nutrients;

and second, adults feed on needles and twigs (Gambrell 1938; Stocks 1938). Both activities discolour tree foliage and reduce the tree value because of its poor quality and form.

The behaviour, biology, and habitat of *O. ovatus* and *B. pellucidus* are similar and both are difficult to sample. As adults during mid-to-late summer, they are nocturnal feeders and move to the soil litter or other sheltered places during the day (Treherne 1912; Campbell et al. 1989). Immature weevils are found in the soil where their seasonal distribution in the root-zone of the host plant is unknown. Eggs and first instars are less than 1 mm in size, and later instars are generally only a few millimetres long (Treherne 1914; Borg 1981).

Populations of immature stages of *O. ovatus* and *O. sulcatus* (Fab.) have been monitored by destroying the host plant, sifting, or sifting and washing relatively large volumes of soil (Emenegger and Berry 1978; Garth and Shanks 1978). Montgomery et al. (1979) developed a modified washing-flotation technique that reduced the time and labour involved in such sampling while increasing the likelihood of recovering the immature stages. Mellors et al. (1982) and Harcourt and Binns (1989) developed techniques for sampling larvae of the alfalfa snout beetle, *O. ligustici* (L.), feeding on alfalfa roots. These techniques are not suitable for ornamental conifers because tree roots extend less than 30 cm into the soil (they are usually root-pruned at least once a year) compared with more than 60 cm for alfalfa. All these approaches damage the tree and are labour-intensive because of the large volumes of soil necessary for processing. Such destructive sampling is unacceptable in ornamental tree nurseries because of the high value of individual trees.

An effective sampling method for root weevils is necessary to determine the presence and density as well as the timing and location of various life stages in the soil. Previous methods for sampling immature *O. ovatus* and *O. sulcatus* have been used solely to detect the various stages during development. No attempts have been made to test the effectiveness of these methods at predicting population density, which must be estimated to make appropriate decisions for managing root weevils. Managers require sampling techniques that are easy to implement and accurate in their predictions.

Our study had two objectives: (*i*) to examine the seasonal distribution of root weevil larvae in the root-zone of young ornamental conifer trees to determine the best location and timing for sampling; (*ii*) to identify the most appropriate sample unit for estimating the density of larval populations under individual trees without destructively sampling the whole tree. Such information will allow nursery pest managers to improve the timing and location of control measures.

Materials and Methods

A Colorado spruce (*Picea pungens* Engelm.) plantation infested with the root weevils *O. ovatus* and *B. pellucidus* was selected near Hockley, Ontario (44°01' N, 79°58' E). The plantation, which was planted in 1987, contained 8-year-old trees spaced at 1.8 by 0.9 m. Trees were 50–80 cm tall. An area containing 90 trees in the northwest corner of the plantation was selected as the study area. The soil at the study area was a sandy loam (65% sand, 22% silt, 13% clay) containing 2.3% (of the oven-dried weight) organic matter; soil pH was 7.8. Immature root weevils are active in the soil during late summer and early spring (Campbell et al. 1989); consequently sampling took place from 27 July to 24 October 1990 and from 29 April to 4 June 1991. Root weevils overwinter in the soil during the third or fourth instar (Wilcox et al. 1934). Samples of larvae were selected at random and sent to D.M. Anderson at the U.S. National Museum of Natural History for identification because identification of larvae requires the aid of a taxonomist.

Distribution. One tree was selected at random from the study area about every 2 days. The soil beneath each tree was excavated in two layers. A 60-cm square of surficial soil was removed to a depth of 15 cm on the 1st day of each excavation. A bottom layer (15–30 cm) was removed on the 2nd day. Before each excavation, soil samples were taken from beneath

each tree with a 40-cm length of 9-cm-diameter copper pipe. Using the stem of the sample tree as the centre, the surface area of the soil was divided into four compass quadrants. Soil samples were collected at 10 (1 core), 20 (3 cores), and 30 cm (1 or 2 cores) from the tree stem in each quadrant. The samples were placed in plastic bags and their position recorded. The remaining soil for each 15-cm layer was removed and placed in plastic bags.

Individual soil samples were sifted with two moveable screens (top mesh = 3.1 mm; bottom mesh = 1.6 mm) fastened one above the other on a table to allow soil to fall through sequentially. The first screen eliminated coarse soil material, and the second trapped large root weevil larvae. Soil passing through the bottom screen was funnelled into a collecting box underneath the sifting table where it was transferred to a washing table. The material on both screens was examined for root weevils before any material was discarded from either screen. The number of weevils collected at this point was recorded and all specimens were placed in alcohol.

Sifted soil was washed using the washing table developed by Montgomery et al. (1979) but modified to increase water supply. Samples were washed for about 15 min or until the water ran clear. Samples were placed in the table's washing funnel where water flowed up through a bottom opening. By controlling the flow rate of the water, the fine and light materials in the soil (including root weevil larvae) were washed to the top of the funnel and out through a spout where they flowed through two Tyler^{R} soil sieve screens (top 425 μm; bottom 300 μm). The top sieve collected mostly organic matter such as fine roots, plant seeds, and large root weevil larvae (probably third, fourth, and fifth instars); the bottom sieve collected fine sand and small root weevil larvae (probably first and second instars). The remaining sand in the funnel was discarded after washing. Material in the top sieve was examined for root weevils and also discarded. The material in the bottom sieve was transferred to a glass bowl containing a solution of magnesium sulphate (specific gravity 1.15, 20°C) in which small larvae floated to the surface. Specimens were collected and their number recorded.

Histograms of the number of larvae in soil samples (L) were inspected to determine if these data were normally distributed with common variance. The distributions suggested that a logarithmic transformation was required, and data were transformed to natural logarithm ($x + 1$). The general linear models procedure (SAS Institute Inc. 1989) was used to assess variation in L based on several effects. A mixed-effect model was used in the form

$$L_{ijklm} = \mu + P_i + T_{ji} + R_k + D_l + Q_m + PR_{ik} + PD_{il} + PQ_{im} + RD_{kl} + RQ_{km} + DQ_{lm} + PRD_{ikl} + e_{ijklm}$$

where L_{ijklm} is the observation at the i^{th} sampling period, the j^{th} tree nested within the i^{th} sampling period, the k^{th} distance from the stem, the l^{th} soil depth, and the m^{th} quadrant; μ is the overall mean; P_i is a fixed effect resulting from the i^{th} sampling period; T_{ji} is a random effect resulting from the j^{th} tree nested within the sampling period; R_k is a fixed effect resulting from the k^{th} distance from the stem; D_l is a fixed effect resulting from the l^{th} soil depth; Q_m is a fixed effect resulting from the m^{th} quadrant; PR_{ik} is a fixed interaction effect resulting from the i^{th} sampling period and the k^{th} distance; PD_{il} is a fixed interaction effect resulting from the i^{th} sampling period and the l^{th} soil depth; PQ_{im} is a fixed interaction effect resulting from the i^{th} sampling period and the m^{th} quadrant; RD_{kl} is a fixed interaction effect resulting from the k^{th} distance and the l^{th} soil depth; RQ_{km} is a fixed interaction effect resulting from the k^{th} distance and the m^{th} quadrant; DQ_{lm} is a fixed interaction effect resulting from the l^{th} soil depth and the m^{th} quadrant; PRD_{ikl} is a fixed interaction effect resulting from the i^{th} sampling period, the k^{th} distance, and the l^{th} soil depth; and e_{ijklm} is the random error. The type III sum of squares (sometimes referred to as the partial sum of squares, SAS Institute Inc. 1989) was used from the analysis of variance. Least-square means were used to make pairwise comparisons.

Sampling. The number of larvae collected in soil samples was compared with the total number of larvae found under individual conifers to determine whether or not soil samples could reliably replace whole tree sampling (i.e. sifting all soil under a tree to a 30-cm depth and 60-cm diameter to count all larvae). The total number of larvae consisted of all larvae within the soil samples as well as the remaining soil between samples in both 15-cm layers.

Regression analysis was used to test the linear relationship between the number of larvae in the soil samples and the total number of larvae found to a 30-cm depth of soil beneath an individual tree (Systat^R 5.0, Stats; Wilkinson 1990). The linear model used was of the form

$$T = cM + e$$

where T is the total number of larvae beneath the tree, M is the mean number of larvae in the soil samples, c is the slope of the line, and e is the random error. The constant was omitted from the model forcing the estimated regression line through the origin.

Results and Discussion

Based on larval samples sent to D.M. Anderson, 44% of the weevils collected were *O. ovatus* and the remainder were *B. pellucidus*. The information collected in our study is applicable to both root weevils because both weevils have similar life cycles (Browne 1968; Campbell et al. 1989; Brandt 1992). Twenty-nine trees were excavated to determine the larval distribution of root weevils: 22 between 27 July and 24 October 1990, and the remainder between 29 April and 4 June 1991. To develop and test a non-destructive sampling technique, 34 trees were excavated: 29 from the distribution portion of the study plus another five trees.

Distribution. Sampling period, tree nested within sampling period, and distance from the tree stem all significantly affected the number of larvae in the soil samples (Table 1). The significant effect of sampling period was expected because of changes in root weevil population density within a generation. The significant effect of trees within sampling period was also expected because populations varied considerably between trees. Tree roots were not distributed evenly beneath trees; consequently there was a significant effect of distance from the stem on the number of larvae in soil samples. The number of larvae decreased significantly in samples collected at 10, 20, and 30 cm from the stem ($P = 0.0001$). Tree roots were highly concentrated directly beneath the tree and decreased with increased distance from the stem. Quadrant or its interactions with any of the other effects did not significantly affect the number of larvae. Larval distribution beneath the tree was not significantly different for compass direction. Soil depth did not significantly affect the number of larvae in soil samples nor did the interaction between sampling period, soil depth, and distance from the stem.

Interactions between sampling period and distance from the tree stem, sampling period and soil depth, and distance from the tree stem and soil depth were significant (Table 1). The horizontal distribution of larvae can be deduced by examining the distribution of larvae at varying distances from the tree stem over time (Fig. 1). The number of larvae in samples was significantly higher ($P < 0.05$) at 10 cm than at 20 and 30 cm, and significantly higher at 20 cm than at 30 cm for the first six sampling periods from 27 July to 24 October 1990. In the final two periods in the spring of 1991, larvae were distributed evenly beneath the tree with no significant differences occurring between the three distances ($P > 0.05$). This change in distribution suggests that larvae moved out from the stem as they grew, perhaps in search of fine roots as they exhausted sources close to the stem. The number of larvae at 10 and 20 cm immediately prior to winter was significantly higher than that at any distance in the spring. Perhaps mortality occurred during the winter of 1990–1991 but this could not be confirmed because dead larvae were not found in the spring during excavations.

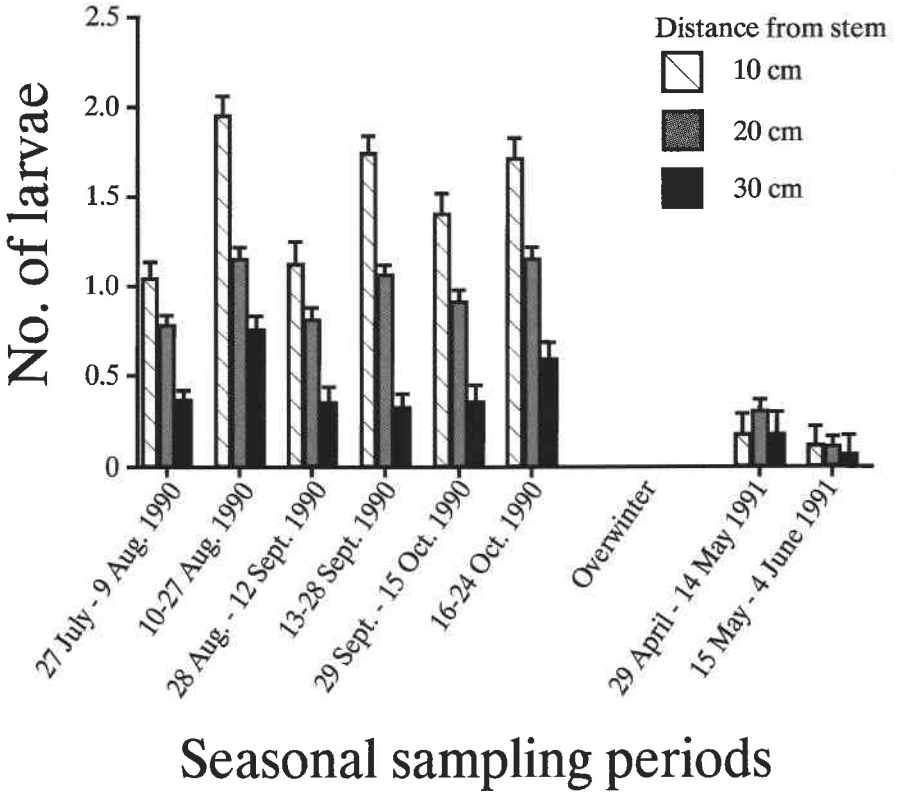

FIG. 1. Least-square means of the number of root weevil larvae found in soil samples collected beneath Colorado spruce trees at various distances from the tree stem. All sampling took place near Hockley, Ontario, in 1990 and 1991. The numbers of root weevil larvae in soil samples were transformed using the natural logarithm.

TABLE 1. Analysis of variance for number of root weevil larvae* in soil samples taken from beneath individual Colorado spruce trees (*Picea pungens* Engelm.) near Hockley, Ontario, in in 1990 and 1991

Source	df	MS	*F*	*P* >*F*
Sampling period (P)	7	20.89	56.80	0.0001
Tree (within P)	21	4.58	12.45	0.0001
Distance from stem (R)	2	41.19	112.01	0.0001
Soil depth (D)	1	0.77	2.10	0.1475
Quadrant (Q)	3	0.66	1.80	0.1462
Interaction (P × R)	14	2.28	6.20	0.0001
Interaction (P × D)	7	0.89	2.41	0.0188
Interaction (P × Q)	21	0.49	1.33	0.1471
Interaction (R × D)	2	1.84	4.99	0.0069
Interaction (R × Q)	6	0.11	0.30	0.9352
Interaction (D × Q)	3	0.03	0.09	0.9666
Interaction (P × D × R)	14	0.52	1.40	0.1430
Error df		1217		

*The numbers of root weevil larvae in soil samples were transformed using the natural logarithm.

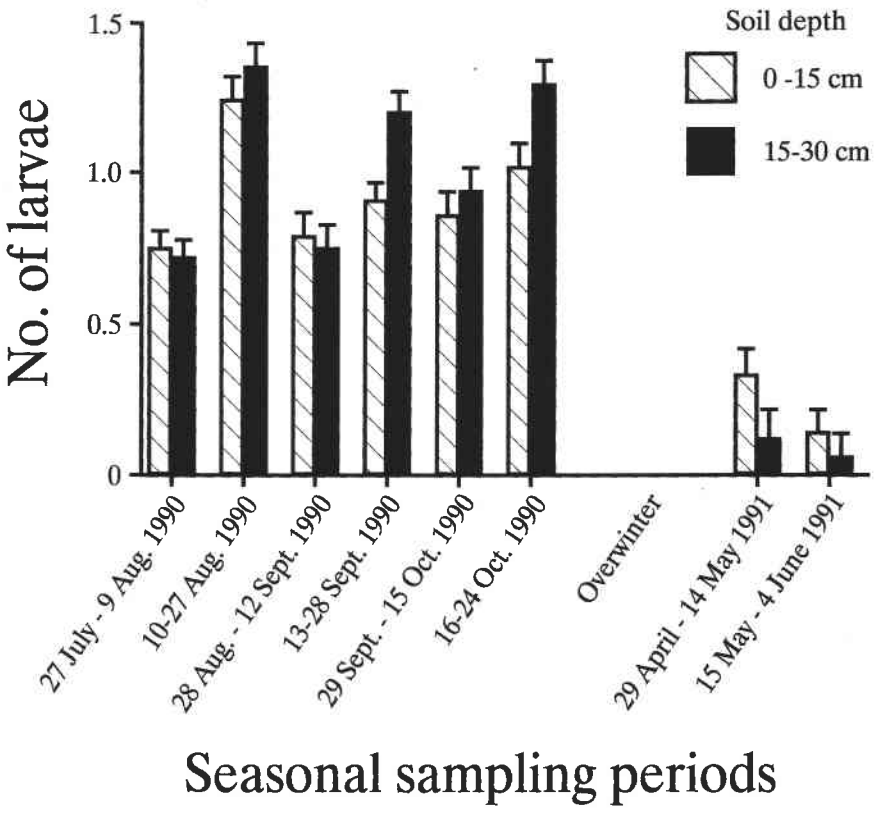

FIG. 2. Least-square means of the number of root weevil larvae found in soil samples collected beneath Colorado spruce trees at soil depths of 0–15 and 15–30 cm. All sampling took place near Hockley, Ontario, in 1990 and 1991. The numbers of root weevil larvae in soil samples were transformed using the natural logarithm.

The vertical distribution of larvae beneath the tree is illustrated in Figure 2. In the first three sampling periods from 27 July to 12 September, the numbers of larvae found in the upper and lower 15 cm of soil were not significantly different ($P > 0.05$). During the fourth and sixth periods, the number of larvae in the upper 15 cm of soil was significantly less ($P < 0.05$) than that found in the lower 15 cm. In the fifth period, the number of immature weevils in the upper 15 cm of soil was less than that in the lower 15 cm but the difference was not significant ($P = 0.51$). It was apparent that there was a shift over time with more larvae found deeper in the soil as winter approached, with the majority reaching a depth of 15–30 cm. In the spring, the distribution of larvae was more even and the number of larvae was not significantly higher in the upper 15 cm. As with the horizontal distribution, this change in distribution from skewed at depth in the fall to an even distribution in the spring indicates that larvae moved up in the soil in the spring, probably in preparation for pupation. In addition, the number of larvae in the spring was significantly less than that in the fall ($P < 0.05$), suggesting winter mortality.

Combining the results of the horizontal and vertical distribution of immature weevils suggests that root weevil larvae moved to feed on the roots of the host tree. As young larvae consumed the food supply and winter approached, larvae moved further from the tree and

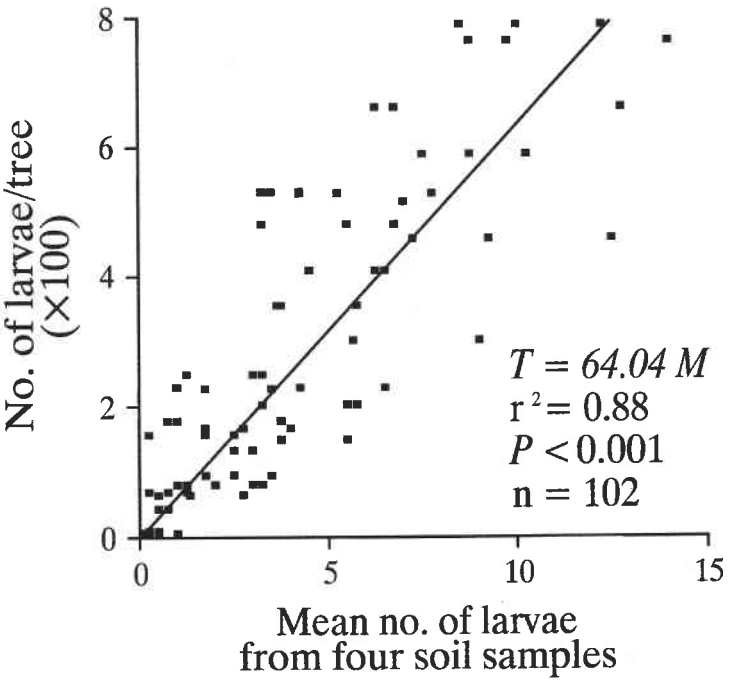

FIG. 3. Mean number of root weevil larvae in four soil samples taken 20 cm from the stem to a depth of 30 cm and the total number of larvae in the soil beneath each Colorado spruce tree ($n = 102$). Samples were collected near Hockley, Ontario, in 1990 and 1991.

deeper into the soil (>15 cm). By the spring of the next season, larvae moved up in the soil to the upper 15-cm horizon, probably to pupate.

The distribution of larvae beneath the tree has important implications for pest management. Any control strategy for root weevil larvae must target where they are active. The application of a soil insecticide or entomopathogenic nematodes requires that applications be timed and located correctly. Movement of soil insecticides is restricted by soil texture, structure, moisture, and organic matter (Harris 1964, 1966; Nielsen and Boggs 1985). Entomopathogenic nematodes are affected by soil moisture and texture (Moyle and Kaya 1981; Georgis and Poinar 1983*a*, 1983*b*, 1983*c*). Therefore, any application of control agents requires that either (*i*) the material is incorporated or injected into the soil near larvae or (*ii*) the material is applied when larvae (or pupae) are relatively close to the soil surface. The information developed here allows the pest manager to optimize the efficacy of current control strategies through better timing and accurate site application of control agents.

Sampling. Numbers of larvae collected in soil samples separated by distance from the stem, quadrant, soil depth, and number of samples per tree were compared with the total number found under the tree (Table 2). The best correlation was achieved with soil samples taken 10 cm from the tree stem. This sample represented a mean of four soil samples (0.0076 m^3) taken 90° from one another on four sides of the tree to a depth of 30 cm ($r^2 = 0.885$, $P < 0.001$). The second best correlation was with a mean from four samples taken 90° from one another on four sides of the tree at 20 cm to a depth of 30 cm ($r^2 = 0.875$, $P < 0.001$). A similar combination at 30 cm from the tree gave an r^2 value of only 0.559 ($P < 0.001$).

TABLE 2. Regression analysis relating the number of root weevil larvae in soil samples taken at various distances from the stems of Colorado spruce trees to the total number of larvae beneath the tree based on soil depth, quadrant, and number of samples per tree. Samples were collected near Hockley, Ontario, during 1990 and 1991

Distance (cm)	Soil depth (cm)	Quadrant	Samples per tree (no.)	r^2	Coefficient	SE of coefficient*
10	0–30	All	4	0.885	33.38	2.10
20	0–30	All	4	0.875	64.04	2.40
30	0–30	All	4	0.559	100.65	15.56

*Probabilities for all SE of coefficients were $P < 0.001$.

These findings are consistent with the distribution of larvae discussed earlier. The best correlation was obtained from samples collected at 10 cm, which is where most larvae were found. The second and third best correlations were obtained from samples that had the second and third highest number of larvae, respectively. Other combinations of distance from the stem, quadrant, depth, and number of soil samples per tree yielded r^2 values ranging from 0.354 to 0.816. Analyses at various sampling periods during the year did not improve the r^2 values (e.g. September versus April).

Numbers of immature weevils collected in soil samples in each of the four quadrants were not significantly different (Table 1) indicating that unbiased samples could be taken on any side of a tree. The relationship between number of larvae in soil samples at 20 cm from the stem and the whole population under the tree was linear and described as $T = 64.04M$ (Fig. 3). The independent and dependent variables were highly correlated ($r^2 = 0.875$). Regression analysis indicated the presence of 10 outliers in the data as well as 12 data points with large leverage ($0.030 >$ leverage value < 0.076). Examination of these data points failed to show errors in data collection or entry. Outliers and data points with large leverage were probably indicative of the inherent variation in populations of root weevil larvae beneath the host tree.

This is a practical sampling procedure for the pest manager. To estimate the population of larvae in the soil at any time of the year, the procedure would require only four soil samples around each tree, 20 cm from the stem, and 30 cm deep. We found that large larvae, which predominate in September or early October, were easy to find. For pest management programs, sampling in the fall would be preferable to the spring because this would allow sufficient time to plan control options for the following spring when immature weevils are closer to the soil surface.

It is important for pest managers to know where root weevil larvae are distributed around the host tree, especially when implementing control strategies. Larvae are the most suitable stage for implementing control strategies because they are the most damaging stage to ornamental conifers and the least mobile. If larvae are adequately controlled, tree mortality can be reduced and damage lessened from later adult attack. For ornamental tree growers the ability to control these serious pests is vital to their competitiveness in domestic and export markets.

Soil sampling is an efficient tool for estimating populations of root weevil larvae under young conifers. Our sampling procedure provides specific recommendations for timing, location, and sample numbers for estimating weevil populations. Further work is needed to correlate spatial distribution of root weevils with damage. Similar relationships have been established for the alfalfa snout beetle (Harcourt and Binns 1989). If damage indices could be developed, pest managers would be able to establish an action threshold for root weevil larvae attacking conifers.

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