

AN ABSTRACT OF THE THESIS OF

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Title: EXAMINATION OF FACTORS AFFECTING LARVAL MORTALITY

OF THE BLACK VINE WEEVIL, *Otiorhynchus sulcatus* Fab., ON CONTAINER

GROWN RHODODENDRONS.

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Richard G. Clarke

Two types of exogenous mortality factors, density-independent and density-dependent, were identified for the black vine weevil, *Otiorhynchus sulcatus* Fab., developing on container grown rhododendrons.

The addition of the insecticide acephate, (O.S.-Dimethyl acetylphosphoramidothioate, Orthene[®] 75S) to the rhizosphere of rhododendrons in which larval *O. sulcatus* develop was categorized as a density-independent mortality factor. Acephate when applied as a drench, killed both early and late instar larvae. The mode of entry was primarily contact. Application of acephate drenches to control early instar (I-III) larvae prevents fatal injury (girdling) to container grown rhododendrons.

A life table was constructed for the black vine weevil on container grown rhododendrons at three densities (beginning with 25, 75, and 150 eggs per plant) in the process of identifying mortality factors. The greatest mortality occurred during the first instar as

the larvae become established on the root systems of host plants.

At high larval densities (> 4 larvae per plant, in a 23 x 22 cm plastic container) the amount of available stem exoxylary tissue became a density-dependent mortality factor. This is because the larvae require exoxylary tissue to successfully complete development on rhododendron, although they do not feed on it prior to the fourth instar. Therefore, mortality increased with an increase in insect density because of competition for the limited amount of exoxylary tissue. The percent of stem exoxylary tissue removed (consumed) by larvae was dependent on the volume of rhododendron roots. Plants with large (1622 ± 223 ml) root volumes had significantly less ($P < 0.01$) stem exoxylary tissue removed than plants with small (360 ± 265 ml) root volumes.

EXAMINATION OF FACTORS AFFECTING LARVAL MORTALITY
OF THE BLACK VINE WEEVIL, Otiorhynchus sulcatus Fab.,
ON CONTAINER GROWN RHODODENDRONS

by

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EXAMINATION OF FACTORS AFFECTING LARVAL MORTALITY
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ON CONTAINER GROWN RHODODENDRONS

I. INTRODUCTION

Several species of the genus Otiorhynchus (Coleoptera: Curculionidae) are important pests of cultivated plants in North America (Warner and Negley 1976). The larger member of the genus, the black vine weevil, Otiorhynchus sulcatus Fab., was first recorded as a pest of cultivated plants in North America by Riley (1871). Adults of this genus are flightless (Warner and Negley 1976). The legless larvae develop on host plant root systems.

The black vine weevil, named because of its occurrence on grape, is distributed over Northern and Middle Europe, France, Italy, Australia, New Zealand, and Tasmania (Smith 1932) where it attacks greenhouse and nursery crops, ornamental plantings, and food commodities such as grapes, strawberries, and raspberries (Cram 1971, Cone 1963, Essig 1933, Smith 1927 and Wilcox et al. 1934). It has been reported to be indigenous to North America (Hamilton 1889 and Schwarz 1890). Others contend that it was introduced via infested plant material from Europe (Sassler 1920 and Weiss 1916). The distribution map of Warner and Negley (1976) strongly supports the latter viewpoint (Figure 1). Regardless, O. sulcatus is widely distributed in the United States and is a major pest of Oregon's nursery and ornamental crops.

Until recently, chlorinated hydrocarbon insecticides provided control of black vine weevils in nursery stock (Gambell and Strickland 1950, Mason 1960 and Saunders 1970). With the banning of these

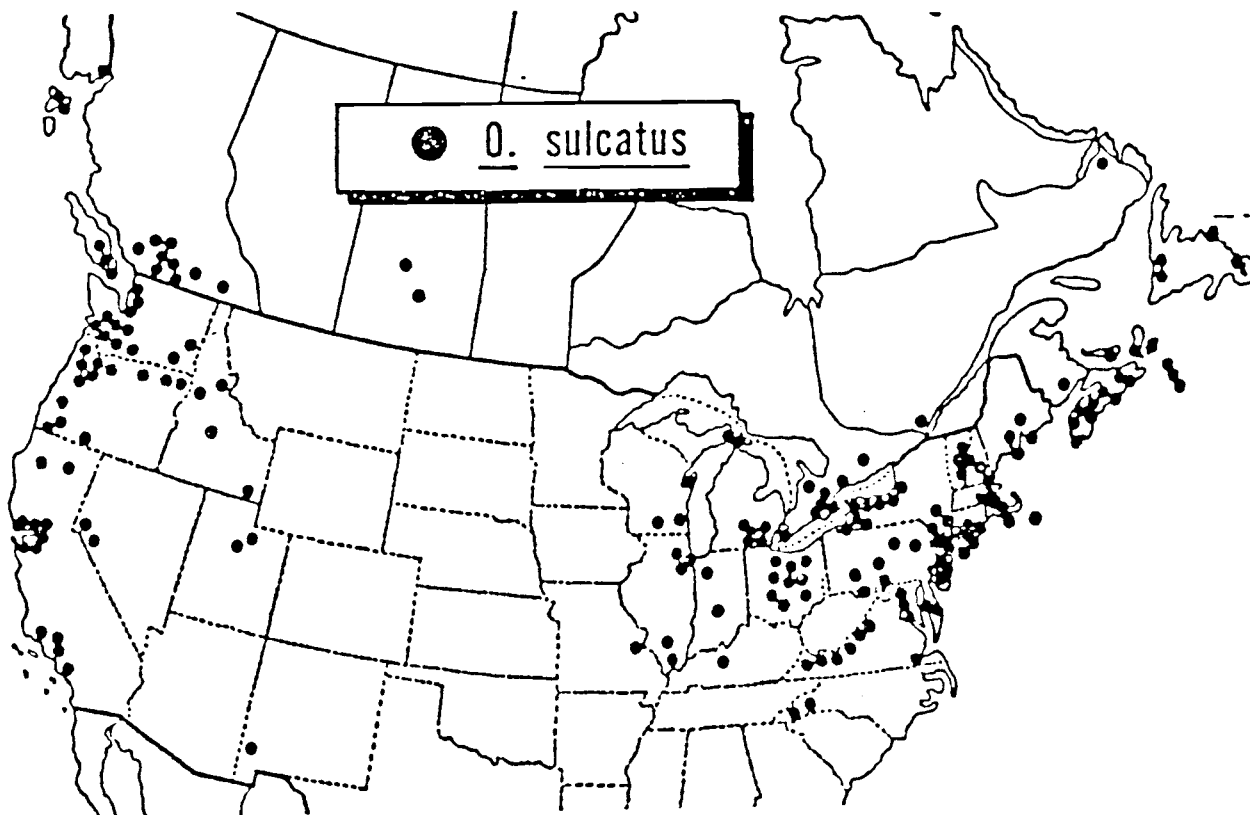


Figure 1. Distribution of *Otiorhynchus sulcatus* Fab. in the United States and Canada (from Warner and Negley 1976).

insecticides from the commercial market, root weevils have again reached economically damaging levels in Oregon nurseries.

In 1977, acephate (O, S-Dimethyl acetylphosphoramidothioate, Orthene[®] 75 S) was registered as a foliar spray for the control of adult root weevil species on rhododendrons. Acephate reduces foliar damage (leaf notching) caused by the nocturnally feeding adults. However, neither it nor other insecticides are registered as soil treatments to control larvae which cause plant death.

The major objectives of this research focused on O. sulcatus on container grown rhododendron were to: 1) test the efficacy of acephate as a larvicide in drench applications; 2) determine the proper timing of acephate treatments; 3) study larval instar development under outdoor and greenhouse conditions; 4) quantify larval mortality in the pre-girdling, girdling, and post-girdling larval development phases; 5) determine possible density-dependent mortality factors; and 6) determine the relationship of larval density and root volume to the amount (%) of exoxylary tissue consumed. This research was initiated in the fall of 1976 and completed in the spring of 1979.

II. LITERATURE REVIEW

The black vine weevil, Otiorhynchus sulcatus Fab., was described in 1792 as Curculio sulcatus (Smith, 1932). According to Smith (1932), Germar (1824) placed sulcatus in the genus Otiorhynchus after Latreille (1802-5) had placed sulcatus in Brachyrhinus. Schoenherr (1843) placed Brachyrhinus in synonymy with Otiorhynchus. In most European and American literature prior to 1920 sulcatus was placed in the genus Otiorhynchus. More recently, American authors have followed Leng (1920) and use Brachyrhinus (Smith 1932). The International Commission of Zoological Nomenclature (1972) in its Official List of Generic Names in Zoology place it under Otiorhynchus and this is the accepted practice.

Black vine weevil adults are oblong, black, with yellowish tufts of setae and coarsely punctured striae on fused elytra. The thorax is shorter than it is wide, subcylindrical, and densely covered with rounded tubercles bearing apical setae. The femora are club-shaped and have a single tooth located distally (Smith 1932). Keys to adult root weevil species of the Pacific Northwest include: Hatch (1971); Warner and Negley (1976), and Wilcox et al. (1934). The latter also presents keys to the eggs, larvae, and pupae.

Adults emerge from pupal cells in the soil in late May and June. The emerging adults dig their way to the soil surface at night using deciduous mandibular tips. These are lost during emergence, leaving characteristic mandibular scars. Adults begin feeding on foliage shortly after emergence (Smith 1932).

Adults have a variable preoviposition period that is both host

plant and temperature dependent (Cram 1958 and Smith 1932). It has been reported to be from 2 to 4 weeks (Shanks and Finnigan 1973, Mote and Wilcox 1927) and from 6 to 9 weeks long (Garth 1977 and Smith 1932). Ovariole dissection is a useful technique for determining the length of the preoviposition period and also the stage of ovariole development of field collected weevils (Bell 1978, Cram 1958 and Ritcher and Kamm 1972). Female O. sulcatus are parthenogenic, and no males have been reported (Smith 1927).

Oviposition begins in July and continues into early fall with peak intensity in August (Cram 1965a, Garth 1977, and Mote and Wilcox 1927). Overwintering adults initiate oviposition in April (Garth and Shanks 1978). A black vine weevil lays between 300-900 eggs per season. The number of eggs is affected by host plant (Smith 1932 and Penman and Scott 1976), moisture, and adult habitat (Smith 1932). The number of eggs laid varies in a cyclic pattern (Penman and Scott 1976).

Eggs are spherical (0.70 mm in diameter) and whitish in color when deposited. They are deposited randomly under host plants (Smith 1932 and Cram 1965a). In 1-3 days, the chorion darkens to chestnut brown with egg hatch occurring in 10-20 days (Smith 1932). Eggs deposited under plant debris and soil have a higher survival rate than those deposited on the soil surface (Shanks and Finnigan 1973). Soil must be porous for larval penetration and the degree of compaction affects larval mortality (Smith 1932).

There are usually six larval instars but seven are possible (Smith 1932). Larval development ranges from 74 to 230 days depending on temperature. Prior to each molt, larvae construct earthen cells.

Instars I-III feed on young roots while instars IV-VI feed on older roots and the stem. Exoxylary tissue appears to furnish nutritional requirements necessary for pupation (Smith 1932). Smith (1927) reports a lower fat accumulation in larvae reared on strawberry compared to dock, cyclamen, or yew.

The pupal period averages 2-3 weeks. Pupae are white at first followed by darkening of the eyes, mandibles, and appendages at the approach of adult transformation. There are prominent apical spines located on the pupae which apparently prevent direct contact with the cell wall (Smith 1932).

Adults are univoltine with overlapping generations (Smith 1932 and Garth 1977). In greenhouses they deposit 40% more eggs than do adults in the field. However, cessation of egg laying and the initiation of a quiescent period occur in synchrony in greenhouse and outdoor populations (Smith 1932).

Otiorhynchus sulcatus do not disperse far from pupal emergence sites. Dispersal averaged less than 10 m in release-recapture experiments, though the potential for traveling greater distances exists. No directional preference was exhibited (Garth 1977 and Maier 1978).

Control measures for black vine weevils have included bran baits, barriers, screens and traps, arsenates and chlorinated hydrocarbon insecticides (Gambell and Strickland 1950, Mason 1960, Neiswander 1953, Schread 1960, Weiss 1915 and Wilcox et al. 1934). All control tactics have been aimed at the adults. Weiss (1915) exemplified the frustration in black vine weevil control programs in his statement "it is considerably cheaper to sell the insects along with the plants."

III. DEFINITION OF TERMS

Girdle - a ring made by the removal of the bark and cambium around a plant stem.

Girdling - to cut a girdle around a plant, usually to kill by interrupting the circulation of water and nutrients.

Exoxylary tissue - all tissues external to the xylem.

Available stem exoxylary tissue - all exoxylary tissue present from the soil line to the root crown (Figure 2).

Percent stem exoxylary tissue removed (ETR) - this refers to the percentage of the total available exoxylary tissue removed by O. sulcatus larvae. This value is based on a visual estimation.

O. sulcatus Host Plant Relationships

Pre-girdling phase - the phase of O. sulcatus development in which instar I-III are present and feeding on small roots, not on stem exoxylary tissue.

Girdling phase - the phase of O. sulcatus development in which instar IV-VI are present and feeding on roots and available exoxylary tissue, causing fatal injury.

Post-girdling phase - the phase of O. sulcatus development in which prepupal (late VI instar) and pupal stages are present and not feeding on the host plant.

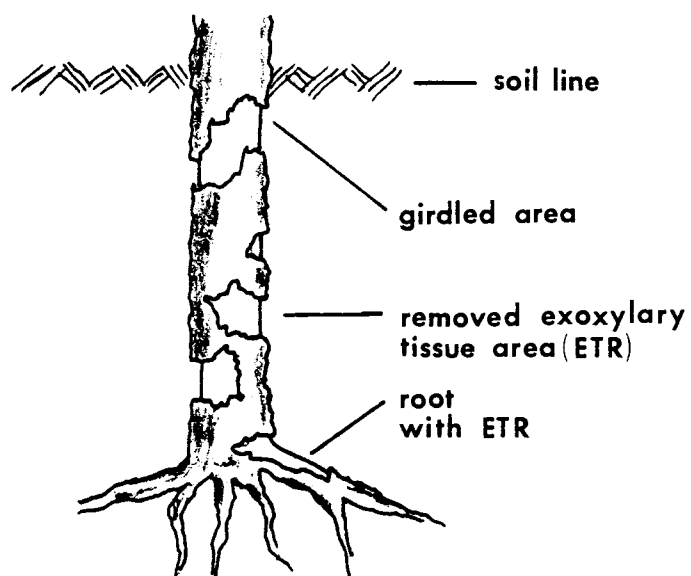


Figure 2. Illustration of a rhododendron stem and root crown showing typical *O. sulcatus* feeding injury. Area of stem below soil line is available for larval feeding.

IV. MATERIALS AND METHODS

Rhododendron Culture

Rhododendrons, ranging in age from six months to two years, were transplanted into one of two standard potting media (Table 1) and allowed 30 days to become established. Younger plants were potted in 18 x 17 cm plastic containers and older plants in 23 x 22 cm plastic containers. Varieties included: Alice (Rhododendron griffithianum x), Anna Rose Whitney (R. griersonianum x griffithianum x catawbiense x arboreum), 'Blue Ensign', 'Blue Peter', Jean Marie (R. griffithianum x), Mrs. Furnival (R. griffithianum hybrid x caucasium hybrid), Mrs. G.W. Leak (R. griffithianum x caucasium x hardy hybrid), Pink Pearl (R. griffithianum x catawbiense x arboreum), Purple Splendour (R. pontiam x), Roseum Elegans (R. catawbiense x), 'Sappho', Unique (R. camplyocarpum x), Vulcan (R. griffithianum x griersonianum).

A. *Otiorhynchus sulcatus* Development Outdoors

On September 22, 1976, 30 rhododendrons (var. Pink Pearl) were each inoculated with 50 O. sulcatus eggs. The eggs were placed around the base of each plant and covered with a thin layer (1 cm) of moist peat moss to prevent dessication¹. Egg viability was estimated by holding 100 eggs in a petri dish (9 x 1.5 cm) lined with moist filter paper and daily counting the number of larvae present. This was

¹This technique was utilized whenever eggs were used to infest plants. Black vine weevil eggs were furnished by Dr. Carl H. Shanks Jr. at the Southwestern Washington Research Unit (Washington State University), Vancouver, WA.

Table 1. Components of two potting media used for growing rhododendrons.

Medium A		Medium B	
Douglas Fir bark (quarter-minus) ^a	100%	Douglas Fir bark (not sifted)	60%
Fertilizer content ^b :		Peat moss	30%
Osmocote 18-5-11	747 g	Sand	10%
Super Phosphate 0-45-0	253 g	Fertilizer content as in Medium A	
Dolomite	202 g		
Gypsum	101 g		

^aBark sifted through a 0.25 in screen.

^bAmount/1.3 m³.

replicated four times. Head capsule measurements were recorded for each larvae recovered from the plant root systems in the biweekly sampling periods using an ocular micrometer on a binocular microscope.

B. Evaluations of Acephate as a Larvicide

Four tests were conducted to determine the larvicidal activity of acephate (Orthene[®]) applied as a drench treatment using the 75% soluble powder formulation.

Timing of drench applications - On October 6, 1976, 30 rhododendrons, potted in medium B (Table 1), were inoculated with 50 eggs. Four groups of six plants were randomly assigned one of four acephate treatment dates: Fall=November 5, Winter=January 8, Spring=March 11, and Fall/Spring. Acephate (75S formulation) was applied as a drench (500 ml/plant) at a rate of 6 g ai/3.8 liters (1190 ppm) to each rhododendron group on their respective treatment date. One group of six was left untreated. Plants were held before and after treatment in an unheated outdoor polyethylene shelter. Evaluations were made 14 days after treatment by hand searching each root system and recording the number of live larvae and the number of girdled plants.

Rate of application - Three rates of acephate applied as a drench treatment were evaluated for larval mortality. On September 26, 1977, 48 rhododendrons (var. Blue Peter), each inoculated with 50 eggs, were divided into two groups (A and B) with these subdivided into four lots of six. Drench treatments at rates of 6 g, 3 g, and 1.5 g ai per 3.8 liters were each applied (500 ml per plant) to one lot of six plants. Six plants were drenched with distilled water and set aside as a control. Group A was treated at 4.5 weeks and group B at

10.5 weeks post-inoculation. Evaluations were made 12 weeks after inoculation by hand searching each root system and recording the number of live larvae and the number of girdled plants.

Acephate activity on 1st instars - The effect of acephate on 1st instar larvae was evaluated by placing 25 newly hatched larvae on a 9 cm sheet of filter paper in a Buchner funnel and pouring 35 ml of acephate (6 g ai/3.8 liters) over them. The drench solution was filtered off and the larvae were transferred to another sheet of moist filter paper in a petri dish kept at 21.1°C. Counts were made after 1, 6, and 20 hours of the cumulative number of dead larvae per dish. Controls were treated similarly using distilled water. The experiment was replicated four times.

Acephate activity on late instars - The effect of acephate on 5th and 6th instar larvae was evaluated in two experiments. One experiment involved drenching larvae with 35 ml of acephate at 6 g ai/3.8 liters in petri dishes containing potting medium A (Table 1). Five insects were used per dish, with four replications. Counts were at 2, 3, and 4 days post-treatment of the cumulative number of dead larvae per dish.

In a similar experiment, to test the residual quality of acephate, larvae were placed in potting medium drenched with 35 ml of acephate at 6 g ai/3.8 liters 12 hours earlier. Larvae were removed after 24 hours and held in untreated medium.

C. Mortality During Development

Life table - This experiment quantified larval mortality during O. sulcatus development on container grown rhododendrons. A life

table at three densities was constructed to assist in identifying density-dependent and -independent mortality and the mortality factors involved.

Three groups of 70 rhododendrons, varieties Blue Peter, Jean Marie, Unique, and Vulcan, designated A, B, and C, were infested with 25, 75, and 150 eggs, respectively. Enough eggs to simultaneously inoculate all plants were not available. Therefore, half of the 70 plants in each group, numbered 1-35, were inoculated October 1-4, 1978 with the remainder (numbered 36-70) inoculated one week later. The infested rhododendrons were kept in a greenhouse at 18-20°C.

Mortality during the egg stage was estimated by placing 100 eggs in each of four 9 x 1.5 cm petri dishes and recording the number of eggs that hatched. The time for egg hatch was also used to estimate the beginning of larval activity on the rhododendrons.

Sampling began the week of November 11th and continued until adult emergence February 13, 1979. Larvae were collected by hand searching through the root system of each plant. Larval instars were determined by measuring head capsule widths. During the three month sampling period, 10 randomly selected plants per density were sampled every two weeks. The difference in the time of inoculation was compensated for by alternately sampling five plants per week from plants 1-35 and 36-70, respectively. Estimates of the amount (%) of stem exoxylary tissue removed from each plant were also recorded.

Development index - During most sampling periods, O. sulcatus specimens were distributed over one or more instars and/or life stages. To simplify data analysis, the average development stage (development index) of individuals recovered during a particular

sampling period was calculated using the method of Dyer et al. (1968) and McCambridge (1974).

Black vine weevil instars (I-VI) were assigned an index value of 1.0-6.0 with pre-pupae, pupae, and adults assigned values of 7.0, 8.0, and 9.0, respectively. Development index (DI) values for each sampling period was calculated by the following formula:

$$DI = \frac{\sum(S)(n)}{N}$$

where DI = development index

S = stage of insect development (e.g., 1.0, 2.0, etc.)

n = number of individuals in stage of development

N = total number of individuals in all stages of development

Percent mortality was calculated for each density per sampling period and each development phase. Duncan's new multiple range test was utilized to examine statistical differences in mortality between the density levels. Life tables were constructed estimating specific mortality rates during each sampling period (age interval). The mean percent stem exoxylary tissue removed was calculated for each density per sampling period. This was, in turn, correlated with mortality using correlation analysis.

D. Relationship of Exoxylary Tissue Removal to Root Volume

The objective of this experiment was to determine the relationship of larval density and root volume to the amount (%) of exoxylary tissue removed. The amount of exoxylary tissue removed was hypothesized to be dependent on both the size of the root system and the number of IV-VI instar larvae present.

The two factors, root volume and insect density, were fixed at two and three quantitative levels, respectively, in a completely randomized 2 x 3 factorial arrangement.

Levels of root volume:

1. Small = 360 ± 265 ml
2. Large = 1622 ± 223 ml

Levels of insect density:

1. 3
2. 6
3. 10

Rhododendron root volumes were estimated by water displacement. Roots covered with a plastic bag were submersed in a full bucket of water. The overflow collected in a tub was measured in a graduated cylinder. Plants with small root volumes were approximately 10 months old and with large root volumes about two years old.

Rhododendrons were infested with IV and V instar larvae reared on the roots of strawberry plants in the greenhouse. Larvae were allowed to develop to pupae on the test plants and the percent exoxylary tissue removed (ETR) per plant was recorded.

V. RESULTS

A. Otiorhynchus sulcatus Development Outdoors

Between October 1976 and May 1977, six instars were produced during O. sulcatus development on rhododendrons (var. Pink Pearl) under outdoor conditions. Mean head capsule widths and ranges are presented in Table 2. The larvae developed through instars I-III in less time (about two months) than through instars IV-VI (about five months) because of cooler temperatures during the latter period.

Larval feeding on stem exoxylary tissue was not noticed prior to February 1977 or until 4.5 months into their development. Stem feeding became very evident in subsequent samples with all plants being girdled. Damage by instars I-III was limited to young roots. Instars IV-VI produced stem injury and girdling by feeding on stem exoxylary tissue. A mean percent of 3.8 ± 0.77 SE pupae and 3.0 ± 0.77 SE adults were collected in May 1977 from the rhododendrons outdoors that were initially inoculated with eggs. Cumulative mortality was, therefore, 92% and 96% at pupation and adult emergence, respectively.

B. Evaluations of Acephate as a Larvicide

Timing of drench applications - Acephate drenches provided 100% control of black vine weevil larvae on container grown rhododendrons (Table 3). No larvae were found on root systems of treated plants. In contrast, a mean of 4.0 ± 1.95 SE larvae were recovered from untreated plants. Four of six plants treated in the Spring were girdled, indicating larval activity until drench application. There were no girdled plants in the Fall treatment group or in the Fall/Spring treatment

Table 2. Developmental period and head capsule measurements of *O. sulcatus* instars reared outdoors on container grown rhododendrons^a.

Instar	Number of Days ^b of Growth	Head Capsule Width (mm)		N
		\bar{X}	Range	
I	0- 15	0.29±0.02	0.27-0.32	20
II	20- 34	0.42±0.06	0.36-0.48	41
III	48-104	0.62±0.04	0.58-0.65	31
IV	62-118	0.91±0.08	0.83-0.99	77
V	118-155	1.18±0.05	1.13-1.23	34
VI	130-211	1.52±0.07	1.45-1.59	61

^aRhododendrons potted in 23 x 22 cm plastic containers and kept in an unheated outdoor polyethylene shelter.

^bThe number of days since inoculation that instar specimens had developed on rhododendron roots.

Table 3. Number of *O. sulcatus* larvae recovered from and number of girdled rhododendrons^a following acephate drench treatments.

Acephate Drench Treatment ^b	Number ^c of Larvae	Number of Plants Girdled	% of Plants Girdled
Fall November 5	0	0	0
Winter January 8	0	1	17
Spring March 11	0	4	67
Fall and Spring	0	0	0
Checks	24	4	67

^aRhododendrons potted in 23 x 22 cm plastic containers and kept in an unheated outdoor polyethylene shelter.

^b500 ml of acephate (Orthene[®] 75 S) at a rate of 6 g ai/3.8 liters of H₂O applied to six plants. Checks were drenched with 500 ml distilled H₂O.

^cThe number of live larvae recovered 14 days after treatment. Each plant was inoculated with 50 eggs on September 26, 1976.

group. In the latter treatment, all larvae had been killed by the Fall drench. A single girdled plant was found in the Winter treatment group. These results indicate that early and late instar larvae are susceptible to acephate.

Rate of Application - Acephate were effective at all rates tested (Table 4). Again, control of both early (group A) and late (group B) instar larvae was observed. Plant mortality, i.e. number of girdled plants, was substantially reduced by applying the acephate drench at 4.5 weeks (group A) into larval development to control instars I-III (Figure 3).

An average of 58.6 ± 8.0 SE percent of the available stem exoxylary tissue was removed by larvae from plants in group B prior to treatment. Sixty-one percent of these plants died even after drenching had eliminated the pest. On the other hand, an average of 13.3 ± 6.7 SE percent of the stem exoxylary tissue was removed from group A plants prior to treatment. Only 12% of these plants died because of larval feeding. Eighty percent of the untreated plants died (Figure 3).

Acephate activity on 1st instar larvae - The toxic effect of acephate on 1st instar *O. sulcatus* was demonstrated (Table 5). Within six hours, 52% of the larvae eventually killed by acephate had died. By 20 hours, 67% of the treated larvae had died.

Acephate activity on late instar larvae - Mortality was greater when 5th and 6th instars were held in the acephate treated media (Table 6) than when they were exposed then removed. Even then, there appeared to be enough residual activity after 12 hours to kill 60% of the larvae given a 24 hour exposure to treated media. In all cases, death was not immediate, indicating a delay in the pest's reaction to the insecticide.

Table 4. Number of *O. sulcatus* larvae recovered from and number of girdled rhododendrons^a after application of three rates of acephate applied as a drench treatment.

Treatment ^b Group	Rate ^c ai	Number of Larvae ^d	Number of Plants Girdled
A Checks	1.5	0	1
	3.0	0	0
	6.0	0	1
	-	30	5
B Checks	1.5	2	2
	3.0	0	5
	6.0	0	4
	-	29	5

^aRhododendrons potted in 23 x 22 cm plastic containers and kept in a greenhouse at 18-20°C.

^bGroup A: larvae allowed to develop 4.5 weeks prior to treatment.
Group B: larvae allowed to develop 10.5 weeks prior to treatment.
Each plant was inoculated with 50 eggs on September 26, 1977.

^cGrams of acephate (Orthene[®] 75 S) per 3.8 liters of H₂O. Each of six plants per rate were drenched with 500 ml. Checks drenched with 500 ml distilled H₂O.

^dThe number of live larvae recovered 12 weeks after inoculation.

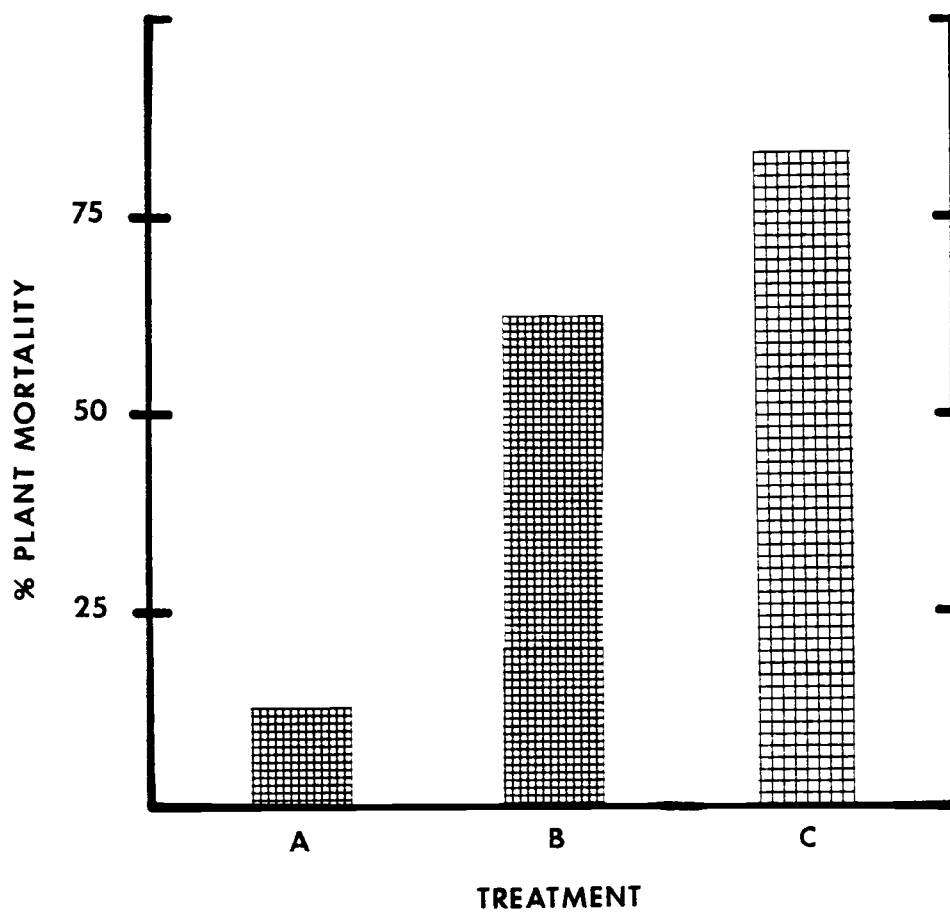


Figure 3. Percent mortality of rhododendrons after acephate drench treatment at (A) 4.5 weeks and (B) 10.5 weeks of larval feeding. Evaluation of treated and untreated (C) plants was made 12 weeks after inoculation.

Table 5. Acephate activity on 1st instar O. sulcatus larvae.

Treatment ^a	Hours After Treatment	Total ^b Number of Larvae		% Mortality	Adjusted ^c % Mortality
		Live	Dead		
Acephate Drench	1	108	17	14	14
	6	53	72	58	52
	20	19	106	85	67
Water Drench	1	125	0	0	-
	6	117	8	6	-
	20	103	22	18	-

^aAcephate (Orthene[®] 75 S) applied at a rate of 6 g ai/3.8 liters of H₂O; 35 ml filtered over larvae on filter paper.

^bTotal number of larvae in five replications.

^cAdjusted mortality = treatment - water drench mortality.

Table 6. Acephate activity on 5th and 6th instar O. sulcatus larvae.

Treatment	Days After Treatment	Total ^d Number of Larvae		% Mortality	Adjusted ^e % Mortality
		Live	Dead		
Acephate	2	15	5	25	20
Drenched	3	9	11	55	45
Medium ^a	4	4	16	80	60
Acephate	2	12	8	40	35
Drenched	3	6	14	70	60
Larvae ^b	4	1	19	95	75
Water	2	19	1	5	-
Drenched	3	18	2	10	-
Larvae ^c	4	16	4	20	-

^aLarvae placed in medium 12 hours after treatment and removed 24 hours later. 35 ml of acephate (Orthene[®] 75 S) at a rate of 6 g ai/3.8 liters of H₂O was used to drench medium in petri dishes.

^bLarvae in medium at time of treatment.

^cLarvae in medium drenched with distilled H₂O.

^dTotal number of larvae in four replications.

^eAdjusted mortality = treatment - water drench mortality.

C. Mortality During Development

Life table - A life table was constructed for O. sulcatus developing on container grown rhododendrons at three densities (Table 7). The initial mortality (26%) in the pre-girdling phase (age interval 0-2 weeks) was because of egg mortality. During the age interval of 2-4 weeks, mortality was high (77%) for all densities. Few additional larvae died in either density in the final age interval of the pre-girdling phase. No differences in O. sulcatus mortality were noted among rhododendron varieties. Apparently, density-independent factors were responsible for mortality in this development phase.

During the first age interval (6-8 weeks) of the girdling phase, the mortality rate observed in density A was lower than in density C. This trend continued throughout the girdling phase (6-12 weeks). A density-dependent factor or factors were present in this phase of development.

Mortality rates during the post-girdling phase (12-14 weeks) were higher for densities B and C than for A. At the beginning of the final age interval (14-16 weeks), 38 larvae were alive in the low (A) density group, while only three remained alive in the high (C) density group.

Density-dependent mortality - There was no significant difference ($P>0.05$) in larval mortality among densities at the end of the pre-girdling phase (Table 8). However, by the final age interval (10-12 weeks) of the girdling phase, percent mortality between the low (A) and the high (C) densities was significantly different ($P=0.05$). At the end of the post-girdling phase (14-16 weeks) the percent mortality between the low (A) and intermediate (B) densities were also

Table 7. Life table for O. sulcatus reared at three densities on container grown rhododendrons.^a

	x	d_x^d			l_x^f			q_x^g		
Development Phase ^b	Age Interval ^c (Weeks)	Density ^e			Density			Density		
		A	B	C	A	B	C	A	B	C
Pre-girdling	0- 2	65	195	390	250	750	1500	26	26	26
	2- 4	140	427	854	185	555	1100	76	77	77
	4- 6	3	13	19	45*	128	256	6	10	7
Girdling	6- 8	1	0	151	44*	115	237	2	0	64
	8-10	1	51	55	43*	116	86	2	44	64
	10-12	4	10	11	42	65	31	2	15	35
Post-girdling	12-14	0	38	17	38	55	20	10	69	85
	14-16	19	2	2	38	17	3	50	12	67

^aRhododendrons potted in 18 x 17 cm plastic containers and kept in a greenhouse at 18-20°C.

^bPhase of O. sulcatus development as it relates to host plant damage.

^cAge intervals correspond with sampling periods, e.g., 0-2 weeks = sampling period #1.

^dNumber dying in age interval out of total number of eggs per density.

^eTotal number of eggs placed on 10 plants per density: A=250, B=750, and C=1500.

^fNumber surviving at beginning of age interval out of total number of eggs per density.

^gMortality rate (%) that occurred within age interval.

* Estimation based on the average number of larvae recovered within each age interval of the girdling phase.

significantly different ($P=0.05$). Density-dependent factors appear to be responsible for mortality after the pre-girdling phase.

Exoxylary tissue removed - Stem exoxylary tissue (ETR) was not removed by larvae prior to the 4th instar (Figure 4). At this time (age interval 4-6 weeks), percent mortality was approximately equal at all densities (Table 8). Between the 4th and 6th instar (girdling phase), the percent ETR increased most at the high density and least at the low density. It was during this period that significant differences ($P=0.05$) in percent mortality between these two groups was noted (Table 8).

Correlation of mortality and ETR - There was a significant correlation ($P=0.01$) between the percent mortality in O. sulcatus at all densities and percent ETR (Figure 5). However, the higher the density, the stronger the correlation (Figure 5, B and C). The coefficient of determination (R^2) for the low (A) density was 0.44; R^2 for the higher (B and C) densities was 0.87 and 0.99, respectively. Apparently O. sulcatus mortality was more dependent on the percent ETR at high densities. The amount of available exoxylary tissue appears to be a key density-dependent mortality factor.

D. Relationship of Exoxylary Tissue Removal to Root Volume

The amount of available stem exoxylary tissue removed (ETR) by O. sulcatus larvae was dependent on root volume and independent of the larval densities tested (Figure 6). The mean percent ETR from plants with small and large root volumes was significantly different ($P<0.01$; Table B, Appendix II).

Although the percentage of tissue removed increased with an

Table 8. Percent mortality in the three development phases of O. sulcatus on container grown rhododendrons at three densities¹.

Development ² Phase	Life Stages	\bar{X} % Mortality ³ Density Level		
		A ⁴	B	C
Pre-girdling	Egg - IV	82 ^a	85 ^a	84 ^a
Girdling	IV - VI	88 ^b	93 ^b	99 ^c
Post-girdling	VI - Adult	92 ^b	98 ^c	100 ^c

¹Rhododendrons kept in a greenhouse at 18-20°C.

²Phase of O. sulcatus development as it relates to host plant damage.

³Average mortality occurring on 10 plants per density. Means followed by same letter were not significantly different at P=0.05 (Duncan's new multiple range test).

⁴Density levels: A=25, B=75, and C=150 eggs per plant.

Figure 4. Percent exoxylary tissue removed (ETR) by O. sulcatus during development on rhododendron at three densities. Development index values 3-6 correspond to instars III-VI; values 7-9 correspond to prepupae, pupae, and adults, respectively. Each data point averaged over 10 plants, with the number of eggs per plants: A=25, B=75, and C=150.

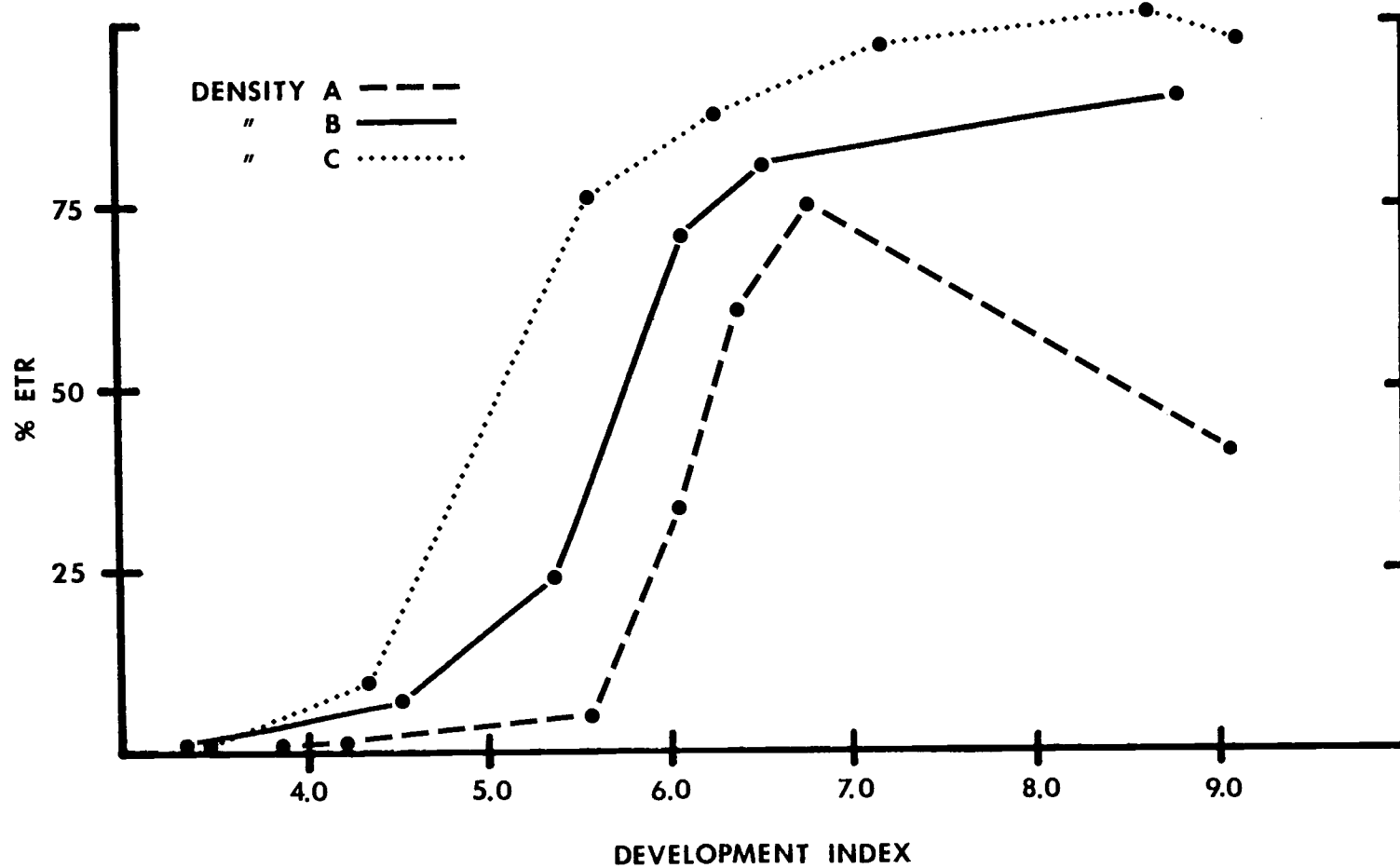


Figure 4.

Figure 5. Relationship between percent mortality of O. sulcatus populations and percent stem exoxylary tissue removed (ETR) by larval feeding on rhododendrons. Each coordinate is an average of 10 plants. O. sulcatus population densities: A=25, $\bar{B}=75$, and C=150 eggs per plant, respectively. Regressions significantly correlated at $P=0.01$, with $n=6$.

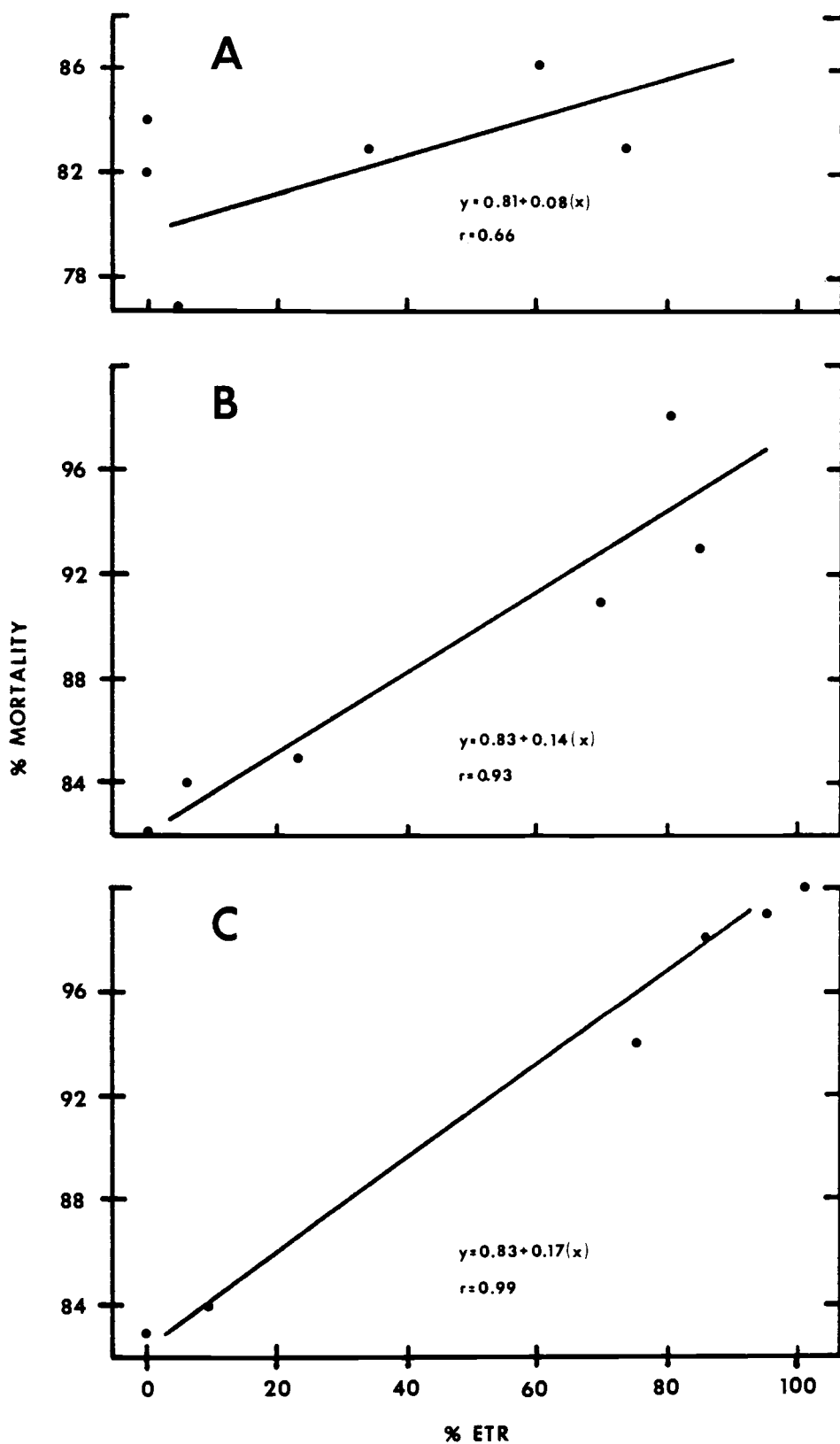


Figure 5.

increase in larval density, the difference was not significant ($P>0.05$) among plants having the same root volume (Figure 6). Twenty-four percent of the plants having large root volumes were killed by the larvae. In contrast, 76% of those plants with small root volumes were killed.

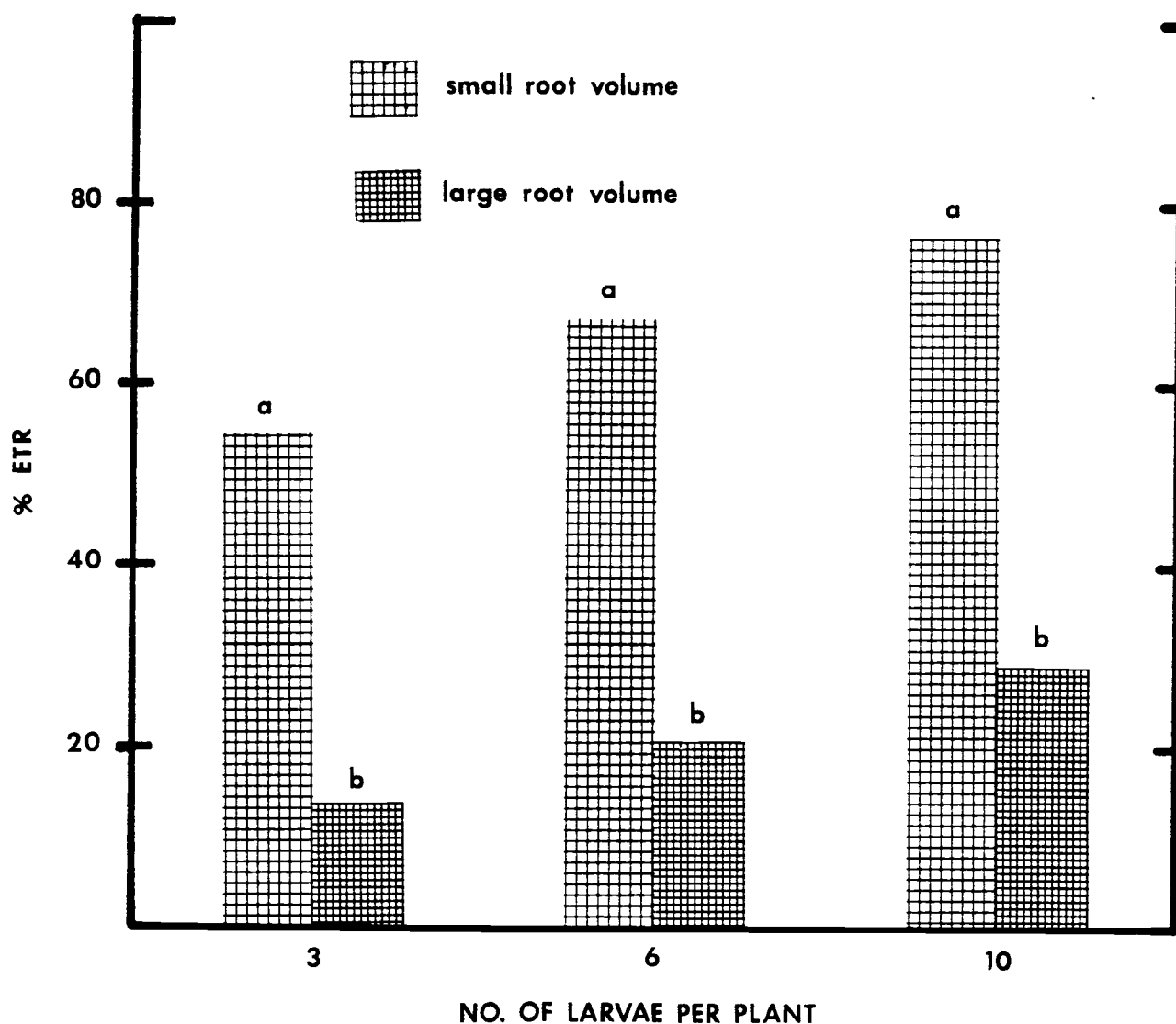


Figure 6. Average percent stem exoxylary tissue removed (ETR) from 10 plants by three densities (3, 6, and 10 larvae/plant) of *O. sulcatus* completing development on container grown rhododendrons. IV and V instar larvae were transferred at respective densities to plants with small (360 ± 265 ml) and large (1622 ± 223 ml) root volumes. Bars with the same letter are not significantly different (F-test) at the 5% level.

VI. DISCUSSION AND CONCLUSIONS

Two types of exogenous mortality factors, density-independent and density-dependent (Price 1975), were identified as affecting larval mortality of the black vine weevil on container grown rhododendrons.

The insecticide acephate was categorized as an exogenous density-independent factor (Price 1975) causing larval mortality when applied to the rhizosphere of infested rhododendrons. The 75% soluble powder formulation (Orthene[®] 75 S) applied at a rate of 6 g ai/3.8 liters as a drench treatment to black vine weevil infested container grown rhododendrons caused 100% mortality in nearly all cases to both early and late instar larvae.

Proper timing of the acephate drenches to control I-III instars substantially decreased plant mortality caused by O. sulcatus infestation. Drenches need to be applied in October or November in outdoor situations when larvae are in the pre-girdling phase of development. Proper timing in greenhouse environments will depend on close observation to determine the presence of adult weevils (by watching for foliar damage).

The registration of acephate (Orthene[®] 75 S) as a drench would be beneficial to the nursery industry. Drench applications can prevent economic losses in commercial nursery operations utilizing Douglas fir bark potting media and container systems for rhododendron and other woody ornamental cultivation.

Differences found in the mortality rates of O. sulcatus developing at three densities on container grown rhododendrons indicated that both density-independent and density-dependent factors were involved.

Comparison of these mortality rates during the three development phases (pre-girdling, girdling, and post-girdling) was a useful method to begin identifying and categorizing mortality factors.

The largest percent mortality occurred during the pre-girdling phase of development (instars I-III) and was approximately equal among all densities. This indicated that density-independent factors were acting on larvae during movement toward and establishment on the root system. These density-independent factors are probably physical in nature. Physical factors previously suggested include: soil or media compaction (Smith 1932), temperature (Mason 1960 and Shanks and Finnigan 1973), and moisture (Shanks and Finnigan 1973 and Smith 1932).

A density-dependent factor was evident in the girdling phase (instars IV-VI). Competition for a limited food resource, available stem exoxylary tissue, was the key mortality factor identified. During this period, mortality increased 15% in the high (C) density group compared to 6% in the low (A) density group. Since an equal amount of roots remained on all plants sampled, the differences in mortality rates can be attributed to intraspecific competition (Odum 1971) for the limited food resource--stem exoxylary tissue. This type of density-dependent competition was defined by Miller (1967) as interference competition. The activity of one individual limits another individual's access to a necessary resource.

Smith (1932) mentioned the possible need for O. sulcatus to obtain exoxylary tissue from woody plants to complete development. Results presented in this study prove that the observation was correct. Larvae apparently require a specific amount of exoxylary tissue

to successfully complete development on rhododendrons. At densities above 4-5 larvae (IV and V instar) per plant, few, if any, larvae obtain enough of the tissue to complete development. At the end of the girdling phase, about 25% of the stem exoxylary tissue remained on the plants in the low (A) density group while only 1% remained on plants in the high (C) density group.

The mortality rates observed on container grown rhododendrons in the greenhouse are comparable to those occurring outdoors. When rhododendrons were grown outdoors mortality was 92% at pupation (beginning with 50 eggs per plant) and 96% at adult emergence. This rate is between the 92 and 98% mortality rates observed for densities A (25 eggs) and B (75 eggs) on plants kept indoors.

The percentage of stem exoxylary tissue removed from container grown rhododendrons was dependent on root volume and independent of the larval densities tested. The amount (%) of exoxylary tissue removed from plants with large root volumes was significantly less ($P < 0.01$) than that removed from plants with small root volumes. Older rhododendrons with large root volumes have considerably more secondary growth on roots near the root crown in comparison to younger plants (Figure 2). This additional tissue, available for larval feeding, accounts for the lower percentage of stem exoxylary tissue removed.

Consequently, the chance of larvae girdling plant stems decreases when plants are older. A very low number (3) of larvae may cause severe injury to small rhododendron plants. This may explain why fatal injury to large rhododendrons is not common. Therefore, black vine weevil control programs should emphasize treatment of young plants.

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APPENDICES

APPENDIX I

Table A. Number of *O. sulcatus* larvae recovered, development index (DI), percent mortality, and percent stem exoxylary tissue removed (ETR) from container grown rhododendrons at three densities^a.

Density ^b	Sample ^c Number	DI	Number of Larvae			% ETR SE	Number of Plants Girdled
			Total	X SE	% Mortality		
A	1	3.8	40	4.0±0.83	84	0.5± 0.5	0
	2	4.2	45	4.5±0.99	82	1.0± 0.7	0
	3	5.5	58	5.8±1.21	77	5.0± 1.5	2
	4	6.0	38	3.8±1.07	85	34.5±14.4	7
	5	6.3	30	3.0±0.75	88	60.5±12.6	10
	6	6.7	38	3.8±1.05	85	74.5±10.9	9
	7	8.9	19	1.9±0.46	92	41.5±12.7	6
B	1	3.9	128	12.8±1.50	83	0.5± 0.5	0
	2	4.5	115	11.5±2.60	84	6.3± 1.6	8
	3	5.3	116	11.6±2.40	85	24.5±11.9	4
	4	6.0	65	6.5±0.58	91	71.5±11.1	10
	5	6.3	55	5.5±0.70	93	85.0± 6.2	10
	6	6.4	17	1.7±0.86	98	80.5±11.5	10
	7	8.7	15	1.5±0.45	98	88.5± 9.5	9
C	1	3.4	256	25.6±4.40	83	1.0± 0.7	0
	2	4.3	237	23.7±2.60	84	10.0± 5.4	6
	3	5.5	86	8.6±2.10	94	75.0± 9.5	9
	4	6.2	31	3.1±2.00	98	86.0± 6.2	10
	5	7.1	20	2.0±0.73	99	95.0± 3.4	10
	6	8.5	3	0.3±0.30	100	100.0± 0.0	10
	7	9.0	2	0.2±0.13	100	95.0± 4.8	9

^aRhododendrons potted in 18 x 17 cm plastic containers and kept in a greenhouse at 18-20°C.

^bNumber of eggs placed on each of 10 plants per sample: A=25, B=75, and C=150.

^cSamples taken at biweekly intervals, beginning 4 weeks after inoculation October 1-4 and 8-12, 1978.

APPENDIX II

Table B. Results of analysis of variance testing differences in the percent stem exoxylary tissue removed by *O. sulcatus* from container grown rhododendrons having small and large root volumes^a.

ANOVA TABLE			
Source of Variation	df	SS	MS ^b
Among treatments	5	34706.95	6941.39**
Root volume	1	31053.75	31053.75**
Density of larvae	2	3478.80	1739.40 ^{n.s.}
Within treatments (error term)	54	56866.70	1050.09

^aIV and V instar larvae placed on plant root systems at the following densities per plant: 3, 6, and 10. Evaluations made 2 months later. Mean root volumes: small=360±265 ml; large=1622±223 ml.

^b**Significantly different at $P < 0.01$.

^{n.s.}Not significantly different at $P > 0.05$.