Effect of Simulated Insect Damage on Growth and Survival of Northern Red Oak (*Quercus rubra* L.) Seedlings

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ABSTRACT Effects of simulated insect damage—artificial defoliation and root damage in combination with two levels of watering—were studied to determine the potential effect on northern red oak seedlings (*Quercus rubra* L.). Treatments and treatment combinations caused significant differences in stem diameter, percentage of stem dieback, and mortality. Defoliation and a regime of decreased watering seemed to have the greatest effect on seedling growth and mortality. Root injury had no consistent direct effect, but interacted significantly with other factors. Insect damage to foliage and roots, together with water stress, may be a factor in poor survival of oak seedlings under field conditions.

KEY WORDS Insecta, *Quercus*, defoliation, roots

THE FAILURE of natural oak regeneration in eastern hardwood forests has been attributed to several causes, including insect damage (Marquis et al. 1976). Although the role of insects is not fully understood, we do know that insects affect all aspects of oak regeneration, from flowering to seedling establishment and growth.

Insect defoliation can have a positive, negative, or neutral effect on tree growth (Mattson & Addy 1975). Houston (1984) reports that defoliation by insects is a significant stress factor for many tree species. Significant loss of nutrients or metabolites, or both, and disruption of plant growth can result from defoliation of trees by insects (Schowalter et al. 1986). Heichel & Turner (1984) reported a reduction in branch growth and leaf production following 3 yr of simulated insect defoliation on northern red oak (*Quercus rubra* L.). Parker & Patton (1975) found that defoliation of black oak (*Quercus velutina* Lambert) seedlings results in a depletion of the starch reserves in the roots, an effect that is augmented by drought. A study by Larson (1975) revealed that defoliation of northern red oak seedlings in the fall reduces spring growth the following year.

Although the extent of defoliation can be detected easily on seedlings in the field, damage to the roots is nearly impossible to survey in a nondestructive manner. Consequently, little information is available about insect pests that cause root damage.

Very little is known about the effect of insects on oak seedling establishment and survival. Linit et al. (1986) found 25 families of insects associated with seedlings of northern red oak. Many species of defoliators and twig girdlers were identified. The Asiatic oak weevil, *Cyrtepistomus castaneus* (Roelofs), an insect pest that has been reported to be a defoliator and a root feeder of oaks (Triplehorn 1955, Roling 1979), was responsible for significant leaf area losses. Leaf area losses averaged about 22% of total leaf area, however the effect of this defoliation on subsequent growth and survival of the experimental seedlings was not determined.

In an ongoing study that is being done in southern Ohio to determine what factors influence seedling mortality in the field, Galford et al. (1988) have found that several insects are responsible for defoliation and root injury on oak seedlings. The majority of defoliation occurs before mid-July, and generally ranges from 1 to 50%, but can be as high as 100%. There was no attempt to quantify root injury in this study, because of the destructive nature of the necessary techniques and the long-term nature of this study.

Insect damage to seedlings may limit growth and competitive ability. Furthermore, this damage may be enhanced by abiotic factors such as inadequate moisture and light. These factors, acting singly or in combination, could significantly reduce seedling growth and survival.

In this study, insect damage was simulated through artificial defoliation and root damage of northern red oak seedlings. We assessed the effect of defoliation and root damage on seedling survival and growth, alone and in combination with inadequate moisture, another seedling stress factor that was simulated with a regime of reduced watering.

Materials and Methods

Collection and Handling of Plant Material. Acorns of northern red oak were collected under a single tree in the Mt. Gilead State Park, Morrow

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County, Ohio, in October 1984. Acorns were examined for insect damage, and all of the apparently uninfested acorns were stored in bags of moist sand at 5°C until May 1985. Following cold storage, the acorns were placed in a mixture of moist sand and peat (2:1) and allowed to germinate at 22°C.

Acorns were planted on 20 May 1985 when radicle development was evident and there was no sign of insect infestation. Acorns (640) were planted singly in a water-saturated commercial soil mixture (Terra-Lite, W. R. Grace & Company, Cambridge, Mass.) at a depth of 1–1.5 cm in 575-ml Styrofoam cups. Following planting, the 640 cups were randomly assigned to 16 groups and moved to a greenhouse maintained at 20–25°C; only natural lighting was used during the study.

Beginning 24 May, seedlings were watered twice weekly (see experimental treatments). Acorns were excised from all seedlings on 2 July to ensure that the seedlings were using the nutrients and water available from the pot, and not the remaining reserves in the cotyledon of the acorn. On 16 July, the seedlings were subjected to the appropriate defoliation and root excision (see experimental treatments). All seedlings were fertilized on 29 July with a 20-20-20 (NPK) soluble fertilizer. From 13 September until 3 October, seedlings were watered only once a week. After 3 October, the plants were moved temporarily to an unheated greenhouse and watering was discontinued. All seedlings were moved to an outdoor lathhouse (overwintering facility) on 20 November, where they were watered to saturation and mulched with sawdust. The seedlings were returned to the greenhouse on 15 March 1986 and watered semiweekly.

Experimental Treatments. Sixteen treatment combinations were tested; treatments included defoliation of seedlings (four levels), watering regimes (two levels), and root excision (two levels). The treatments were performed as follows:

Defoliation. Seedlings were subjected to 0, 50, 75, or 100% defoliation in a two-stage process. On 16 July, half of the leaves on each seedling received the appropriate amount of defoliation by excising with a scalpel, none, one-half, three-fourths, or all of each leaf (except the midrib, which was always left intact). One week later, the remaining leaves were subjected to defoliation using the same procedure. This technique is similar to one described by Poston et al. (1976), in which they found that cork borers, paper punches, and along-the-midrib bisections adequately simulate insect defoliation based on net photosynthesis of soybean leaves. Also, Hammond & Pedigo (1981) found that, based on water loss of soybean leaves, defoliation by a paper punch more accurately simulates insect defoliation than picking off leaflets. We performed our defoliation in two stages rather than one because typically in the field insect defoliation on oaks occurs over a period of days or weeks, rather than at one time (J. R. Galford, J.W.P. & S.L.W., unpublished data).

Simulated Water Stress. Beginning on 24 May, half of the seedlings received 50 ml tap water twice per week (hereafter referred to as low-watered seedlings), and the other half received 150 ml tap water twice per week (high-watered seedlings).

Root Damage. A portion of the roots from half of the seedlings was removed on 16 July. This was accomplished by cutting completely through the Styrofoam cup with a sharp knife, removing a portion of the roots and soil in the process. An effort was made to keep the uncut roots and attached soil intact during the removal process. The seedling (with the remaining soil) was then replanted in a new cup with fresh soil added to fill the cup to its original level.

To estimate the amount of root material removed in the excision process, a separate group of 48 seedlings (24 low-watered and 24 high-watered) were subjected to the excision procedure. After we removed the bottom portion of roots (as above), the remaining root tissue was excised from the seedling at the root collar. The soil was then washed from the bottom and top sections. Top and bottom portions were placed separately in paper bags, dried for 72 h in an oven at 105°C, and weighed. Based on weights of the dried root tissue, we estimate that 30.5 ± 1.9% ($\bar{x}$ ± SEM) of the root was removed in the excision process.

Recording and Analyzing Data. Five criteria were used to measure treatment effects: seedling height, seedling diameter, percentage of stem dieback, cumulative mortality, and overwinter mortality.

Seedling height was measured on 20 November by measuring the distance from the root collar to the end of the terminal bud. At the same time, the diameter of each seedling was measured at a point midway between the root collar and tip of the terminal bud.

Following the overwinter period in the lathhouse and after a 6-wk growing period in the greenhouse, tip dieback and overwinter mortality were ascertained. Dieback was recorded as the percentage of dead tissue (no living buds or leaves) from the tip of the main stem to the beginning of living tissue on that stem. All seedlings with no evidence of living buds or leaves were considered dead at the time of final readings. Cumulative mortality was determined from the total amount of mortality that occurred throughout the experiment, including overwinter mortality.

Data on seedling height, diameter, and dieback were analyzed by a three-factor analysis of variance. When significant differences occurred, means were separated by Student-Newman-Keuls (SNK) multiple comparison test at the 5% level. Seedling mortality data were analyzed using the FUNCAT procedure of SAS, a nonparametric test for frequency data (FUNCAT, SAS Institute 1982, 257–285). Significant differences in mortality among defoliation treatments were detected by 2×2 χ^2 contingency tables ($\alpha = 0.05$).

Table 1. Height, diameter, dieback, and mortality of red oak seedlings subjected to four levels of defoliation, two levels of watering, and two root injury treatments

Variable and treatment	% Defoliation							
	0		50		75		100	
	n	x̄ ± SEM[a]	n	x̄ ± SEM	n	x̄ ± SEM	n	x̄ ± SEM
Seedling height (mm)								
High water, no root injury	23	127.4 ± 5.9	27	133.0 ± 5.4	26	136.0 ± 7.4	26	137.7 ± 6.5
High water, root injury	29	129.2 ± 6.6	28	129.6 ± 6.0	29	137.9 ± 6.3	25	158.3 ± 8.5
Low water, no root injury	27	134.1 ± 6.9	28	136.2 ± 6.1	28	127.8 ± 5.6	28	133.7 ± 4.6
Low water, root injury	28	142.3 ± 6.3	26	128.8 ± 5.9	28	135.5 ± 4.3	24	138.5 ± 6.7
Seedling diameter (mm)								
High water, no root injury	23	2.22 ± 0.04	27	2.15 ± 0.05	26	1.98 ± 0.07	26	1.96 ± 0.05
High water, root injury	29	2.31 ± 0.06	28	2.18 ± 0.05	29	2.13 ± 0.05	25	2.07 ± 0.04
Low water, no root injury	27	2.17 ± 0.06	28	2.05 ± 0.05	28	1.92 ± 0.06	28	1.94 ± 0.03
Low water, root injury	28	2.13 ± 0.03	26	2.09 ± 0.05	28	1.93 ± 0.45	24	1.63 ± 0.05
% Dieback								
High water, no root injury	23	3.65 ± 2.0	27	11.7 ± 4.8	26	10.7 ± 5.5	26	17.1 ± 6.5
High water, root injury	29	16.24 ± 4.2	28	5.8 ± 3.0	29	7.5 ± 3.7	25	25.4 ± 7.3
Low water, no root injury	27	39.33 ± 7.2	28	22.5 ± 6.1	28	11.0 ± 4.1	28	27.9 ± 7.1
Low water, root injury	28	49.29 ± 6.3	26	27.3 ± 5.1	28	30.2 ± 5.9	24	58.4 ± 9.7
% Cumulative mortality								
High water, no root injury	27	14.8	27	3.7	27	11.1	27	14.8
High water, root injury	29	0	29	3.4	29	3.4	29	24.1
Low water, no root injury	28	21.4	28	7.1	28	3.6	29	20.7
Low water, root injury	29	20.7	28	7.1	28	10.7	26	57.7
% Overwintering mortality								
High water, no root injury	23	0	27	3.7	26	7.7	26	11.5
High water, root injury	29	0	28	0	29	3.4	25	12
Low water, no root injury	27	18.5	28	7.1	28	3.6	28	17.9
Low water, root injury	28	17.9	26	0	28	10.7	24	54.2

[a] SEM is not applicable to mortality data.

Results and Discussion

Height, diameter, dieback, and mortality data for the treatment combinations are given in Table 1. There were no significant differences in seedling height as a result of treatment combinations (Table 2). However, there were significant differences in seedling diameter, percentage of dieback, and mortality (cumulative and overwinter) caused by treatments and interactions (Table 2).

Over all treatments, mean stem diameters (±SEM) of seedlings subjected to 0, 50, 75, and 100% defoliation were 2.21 ± 0.03, 2.12 ± 0.03, 1.99 ± 0.03, and 1.91 ± 0.03 mm. All means were significantly different from one another according to SNK. Over all treatments, stem diameters on low-watered seedlings were significantly smaller than on high-watered seedlings (1.99 ± 0.02 versus 2.12 ± 0.02 mm); diameters on root-injured seedlings were not significantly different from uninjured seedlings (2.07 ± 0.02 versus 2.05 ± 0.02 mm).

Mean dieback over all treatments on seedlings subjected to 0, 50, 75, and 100% defoliation was 28.0 ± 3.2, 16.7 ± 2.5, 14.9 ± 2.5, and 31.7% ± 4.1. SNK showed significant differences in defoliation at 0 versus 50%, 0 versus 75%, 0 versus 100%, 50 versus 100%, and 75 versus 100%. Over all treatments, low-watered seedlings had significantly more dieback than seedlings that received high water (32.8 ± 2.2 versus 12.3% ± 1.8), and root-injured seedlings had significantly more dieback than uninjured seedlings (26.8 ± 2.3 versus 18.3% ± 1.3). Within most treatment combinations, percentage of dieback on low-watered seedlings was greater than on high-watered seedlings. Dieback was greatest on low-watered, root-injured seedlings (Table 1). However, lower levels of dieback at 50 and 75% defoliation within three of the four treatment combinations suggest that treatments interact to influence dieback; the nature of these interactions is unclear.

Cumulative mortality on seedlings subjected to 0, 50, 75, and 100% defoliation was 14.2, 5.3, 7.1, and 28.8%. Significant differences in mortality occurred in the following treatments: 0 versus 50%, 0 versus 100%, 50 versus 100%, and 75 versus 100%. The decline in mortality between 0 versus 50% and 0 versus 75% defoliation suggests that, as with dieback, there are interactions among factors; the end result is decreased mortality of seedlings at moderate levels of defoliation. Mortality was significantly higher on low-watered seedlings than on high-watered seedlings (18.3 versus 9.4%). Over all of the treatments, there was no significant difference between mortality on root-injured seedlings and uninjured seedlings (15.4 versus 12.2%). Root injury did interact with defoliation and with low water to affect cumulative mortality (Table 2). By far the highest cumulative mortality for any treat-

Table 2. Results of ANOVA and FUNCAT on seedling height, diameter, dieback, and mortality of red oak seedlings subjected to four levels of defoliation, two levels of watering, and two root injury treatments

Variable and source	df	Mean square	Test statistic[a]
Seedling height			
Defoliation (D)	3	2,009.6	1.9
Water (W)	1	218.7	0.2
Root injury (R)	1	1,829	1.8
D × W	3	2,311.6	2.2
D × R	3	1,444.8	1.4
W × R	1	78.8	0.1
D × W × R	3	710.9	0.7
Residual	414	1,066.7	
Seedling diameter			
Defoliation (D)	3	1.8	24.5**
Water (W)	1	2.1	26.8**
Root injury (R)	1	0.01	0.1
D × W	3	0.2	0.9
D × R	3	0.2	2.1
W × R	1	0.7	9.5**
D × W × R	3	0.2	2.7*
Residual	414	0.076	
% Dieback			
Defoliation (D)	3	7,361.6	8.3**
Water (W)	1	44,914.4	50.4**
Root injury (R)	1	9,447.3	10.6**
D × W	3	2,464.6	2.8*
D × R	3	1,817.7	2.0
W × R	1	4,685.6	5.3*
D × W × R	3	944.3	1.1
Residual	414	892.1	
% Cumulative mortality			
Defoliation (D)	3		30.8**
Water (W)	1		9.2**
Root injury (R)	1		1.5
D × W	3		6.2
D × R	3		9.6*
W × R	1		5.4*
D × W × R	3		2.9
% Overwintering mortality			
Defoliation (D)	3		27.6**
Water (W)	1		17.9**
Root injury (R)	1		1.7
D × W	3		14.0**
D × R	3		8.8**
W × R	1		4.0*
D × W × R	3		6.6

[a] The test statistic reported for seedling height, diameter, and dieback is F. The test statistic for mortality data is χ^2. Values followed by * are significant at the $P < 0.05$ level; values followed by ** are significant at the $P < 0.01$ level.

ment combination occurred on low-watered, root-injured seedlings subjected to 100% defoliation (Table 1). Seedling mortality is the ultimate measure of treatment effects. Overall, cumulative mortality was greatest in seedlings receiving defoliation or low water; the interaction of these two treatments resulted in the highest cumulative mortality.

A significant number of planted oak seedlings fail to survive the winter in experimental plots in the field (Galford et al. 1988). Because of these findings, we evaluated overwinter mortality as one parameter in this study. Defoliation of 0, 50, 75, and 100% resulted in overwinter mortality of 9.3, 2.8, 6.3, and 23.3%. Significant differences in mortality occurred between the following levels of defoliation: 0 versus 50%, 0 versus 75%, 50 versus 100%, and 75 versus 100%. Over all other treatments, low-watered seedlings had significantly higher levels of overwinter mortality than did high-watered seedlings (16.4 versus 6.1%). Root injury alone did not affect overwinter survival (Table 2). Over all the treatments, there were no significant differences in overwinter mortality between root-injured and uninjured seedlings (13.1 versus 9.3%); however, as with cumulative mortality, root injury did significantly interact with defoliation and the watering regimes to affect overwinter survival (Table 2). The greatest overwinter mortality occurred in those seedlings that received 100% defoliation, low water, and root damage (Table 1).

This study has demonstrated that artificial defoliation and root damage, designed to simulate insect damage, significantly affect certain aspects of growth and survival of red oak seedlings. Seedling height was unaffected by the treatments, probably because red oak has a determinant growth habit, and growth had ceased when the defoliation and root excision treatments were imposed. Seedling diameter was significantly reduced by defoliation, by low water, by root injury, and by the interaction of low water and root injury. Defoliation, low water, and root injury all significantly affected dieback, as did the interactions between these treatments.

In this experiment, we observed less dieback and mortality on low-watered seedlings that underwent moderate amounts (50 and 75%) of defoliation than on low-watered seedlings that received no defoliation (Table 1). One explanation for this is that although defoliation by itself may impose significant stress on a seedling, it may reduce transpiration, thus reducing the overall moisture deficit in a plant under water stress. The rate of transpiration is more important in controlling water stress of plants than the soil moisture content (Kramer 1983). Ostlie & Pedigo (1984) found that simulating insect defoliation by cutting leaves increased water loss initially in soybeans, but this effect was transitory because of the healing that occurred along the cut leaf edges. In a separate experiment, they also showed that defoliation of soybean plants actually reduced water loss over a 48-h period. Thus, moderate levels of defoliation in our test may have reduced the effect on seedlings of water stress or root damage, or both.

Findings by Galford (1986) and Linit et al. (1986) clearly show that many insect species are associated with oak seedlings in the field. Other species are probably also involved (Galford et al. 1988). These species include sucking insects, defoliators, stem borers and root feeders; the significance of the damage caused by these insects has yet to be determined. However, we suggest that processes similar to those recorded in our laboratory study occur in oak seedlings growing under natural conditions. To verify this assumption, these laboratory results must be validated in the field.

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