

Suppression of Leafminer (Coleoptera: Buprestidae) Populations on Turkey Oak (Fagaceae) Using Implants of Acephate

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ABSTRACT Herbivory by leafmining insects is notoriously difficult to manipulate for ecological studies because eggs and larvae generally cannot be moved easily among plants without damaging plant tissue and killing larvae. We report on the effectiveness of stem implants of acephate (*O,S* dimethyl acetylphosphoramidothioate) for protecting turkey oak, *Quercus laevis* Walter, foliage from leafminer, *Brachys tesselatus* F., herbivory. Implanting acephate capsules into the stem of turkey oak trees before budbreak significantly reduced overall leafminer damage (both adult foliage feeding and larval mining) without any evident negative effects on tree acorn production (number and weight of acorns). This technique is currently being used to examine long-term costs of leafminer herbivory on growth and reproduction of turkey oak. It is likely that a similar approach can be used to manipulate leafminer densities for ecological studies on other tree species, as has been shown for herbivores on conifers and numerous crop species.

KEY WORDS *Quercus*, Buprestidae, acephate, herbivory, leafminer, pesticide

THE EFFECTS THAT herbivores have on their food plants is the source of considerable controversy. Although studies indicate that herbivores can have negative effects on plants (they can limit plant distribution [Harper 1977], reduce plant reproductive potential [Whitham and Mopper 1985], and modify plant competitive ability [Dirzo and Harper 1982]), other studies have demonstrated either positive effects (Belsky 1986) or no effects of herbivores on plants (Lee and Bazzaz 1980). However, because plant responses to herbivory are often plastic, being influenced by plant nutritional status, water status, and history of defoliation, results from these numerous studies are not necessarily contradictory. Instead, variable responses to herbivory likely reflect complex interactions between the herbivore, plant, and environment (Maschinski and Whitham 1989). Long-term empirical studies, especially in natural systems, are needed to demonstrate how these multiple factors interact to affect plant life history responses to herbivory (Maschinski and Whitham 1989).

Leafmining insects provide ideal systems for ecological studies of plant responses to herbivory because they leave a conspicuous record of their presence that allows accurate estimation of their densities and the amount of tissue damage they inflict on plants (Hespenheide 1991, Opler 1973).

Also, leafminer densities are often very high in both natural and agricultural systems and thus result in substantial loss of photosynthetic tissue (Condrashoff 1964, Turnbow and Franklin 1981, Fujiie 1982) and early leaf abscission (Faeth et al. 1981, Maier 1989). Unfortunately, leafminer populations are notoriously difficult to manipulate for ecological studies because their eggs are generally inserted into the leaf tissue or glued to the leaf surface (Hespenheide 1991) and thus cannot be moved among plants. Furthermore, larvae typically complete development inside of a single leaf, and thus, larval densities cannot be manipulated without damaging plant tissue and exposing larvae to desiccation and predation. Current techniques for manipulating leafminers (for example, exclusion/inclusion cages and bags, Faeth 1991, Heinz and Parrella 1992) typically are work intensive and often have inadvertent negative effects on plant growth (for example, they can shade photosynthetic tissue and increase humidity). However, systemic pesticides may allow low-maintenance manipulation of leafminer populations for ecological studies, without negative effects on plant growth and reproduction (Whitham and Mopper 1985).

Topically applied acephate (*O,S* dimethyl acetylphosphoramidothioate), and stem implants of acephate, provide broad spectrum control of insects on several conifers (Sandquist and Erickson 1991, Fatzinger et al. 1992, West and Sundram

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1992) and many crop species (Bull 1979, Frank et al. 1984, Sato et al. 1992, Russell et al. 1993, Antonious and Snyder 1994). Stem implants require minimal insecticide use and thus minimize environmental effects; only target trees are affected (West and Sundram 1992). They also allow targeting of selected trees in a natural population of otherwise untreated trees, permitting controlled, randomized designs. Finally, because implants are light-weight and portable, they are practical for use in ecological experiments in remote areas.

As part of a long-term study examining the effect of leafminer herbivory on turkey oak, *Quercus laevis* Walter, reproduction, we have manipulated larval and adult densities of *Brachys tessellatus* (F.) using stem implants of acephate. Here we report on the effectiveness of these implants for protecting turkey oak foliage from *B. tessellatus* herbivory, and discuss the implications of these results for the manipulation of leafminer densities in ecological experiments.

Materials and Methods

Natural History of the *Q. laevis*—*B. tessellatus* System. Turkey oak is a small, scrubby tree, abundantly distributed throughout the coastal plain sandhill communities of the southeastern United States. *B. tessellatus* is a leaf-mining beetle abundant on, and largely restricted to, turkey oak (Turnbow and Franklin 1981). Although numerous herbivores attack *Q. laevis* (for example, leaf galls, stem galls, and leaf chewers), *B. tessellatus* is the most conspicuous and abundant herbivore on this oak in South Carolina, resulting in approximately an order of magnitude more tissue loss than all other foliar herbivores combined (K.J.W., unpublished data).

Beetles overwinter in the leaf litter (inside mined leaves) and, in a typical year, emerge as adults in March–April, coincident with (or slightly before) *Q. laevis* budbreak. Adults feed on expanding leaves through April and begin oviposition on fully expanded leaves in late April. Eggs are glued to the upper surface of leaves. Larvae enter leaves under their eggs, where they undergo 5 instars, and finally pupate in November–February.

Beetle Manipulations. This study was conducted during an ≈ 9 mo period during 1994, at 2 sites in South Carolina; Sesquicentennial State Park in Richland County, South Carolina, and a plot of unmarked county owned property along Clemson Road, ≈ 3 km south of the Clemson University Sandhills Research and Education Center in Richland County, South Carolina (subsequently referred to as the Clemson Road site). Both sites are located in the sandhills dominated by longleaf pines, *Pinus palustris* Miller, and turkey oak.

Acephate implants consisted of a gelatin capsule containing 200 mg acephate powder, enclosed inside a plastic cartridge to facilitate insertion into the tree (MINI ACECAPS 97, Creative Sales, Fre-

mont, NE). Implants were inserted into the trunk between 50 cm and 1 m above ground, ≈ 7 d before budbreak. Because the date of budbreak could only be predicted (based on the size of the buds) at the time of implant insertion, some trees were implanted as much as 15 d before budbreak, and others as little as 1 d before budbreak. One implant was inserted for each 2.5 cm of tree diameter. In total, 71 trees were implanted at Sesquicentennial State Park (with 146 controls) and 28 trees were implanted at Clemson Road (with 50 controls).

Treatment trees were haphazardly chosen from a pool of previously marked trees. The remaining trees were used as controls. Treatment trees were spatially intermixed with controls, and did not differ from controls in either size (diameter at 20 cm, which correlates well with total leaf number; C.W.F., unpublished data) or phenology (Mann–Whitney *U* tests, $P > 0.05$).

Date of budbreak and tree size were collected for all trees. Bud break was defined as the 1st date on which half of the buds on the tree had broken. Following bud break on the earliest tree all marked trees were examined daily for bud break. Tree size was estimated as the circumference of the trunk at 20 cm above ground level. This diameter was positively correlated with circumference of the tree at 1 m above ground, and, in a subsample of 47 trees at the Sesquicentennial site, positively correlated with the log of the total number of leaves on the tree ($r^2 = 0.59$, $P < 0.001$; C.W.F., unpublished data). Neither tree height nor tree diameter at 1 m correlated better with the total number of leaves ($r^2 = 0.59$ and $r^2 = 0.45$, respectively).

Assessment of Adult Feeding Damage. Early in the season (late March to late April) adult *B. tessellatus* congregate and feed on recently expanded foliage. Because leaves are attacked while still expanding, adult feeding has a large effect on the resulting size of these leaves (reducing total leaf area by as much as 20%). We estimated the amount of leaf area lost to adults on both treated and control trees on 1 May (after leaves had fully expanded) and 13 August (after most adults had died). Leaf area loss was estimated independently by 2 observers, 1 of whom was blind to the experimental design. Each tree was subjectively classified as having approximately <5, 5, 10, 15, or >15% leaf area loss. Although these estimates are subjective, they are relatively precise; both observers classified almost all trees identically (Pearson moment correlation >0.945 at each site; all trees sampled).

Assessment of Egg and Larval Densities. The number of eggs laid on leaves of treated and control trees was estimated 1st in late April (26–27 April, Sesquicentennial State Park only) and again in late May (at both sites, 26–31 May). Egg densities were estimated by haphazardly selecting 20 leaves from each tree (10 leaves from the west side

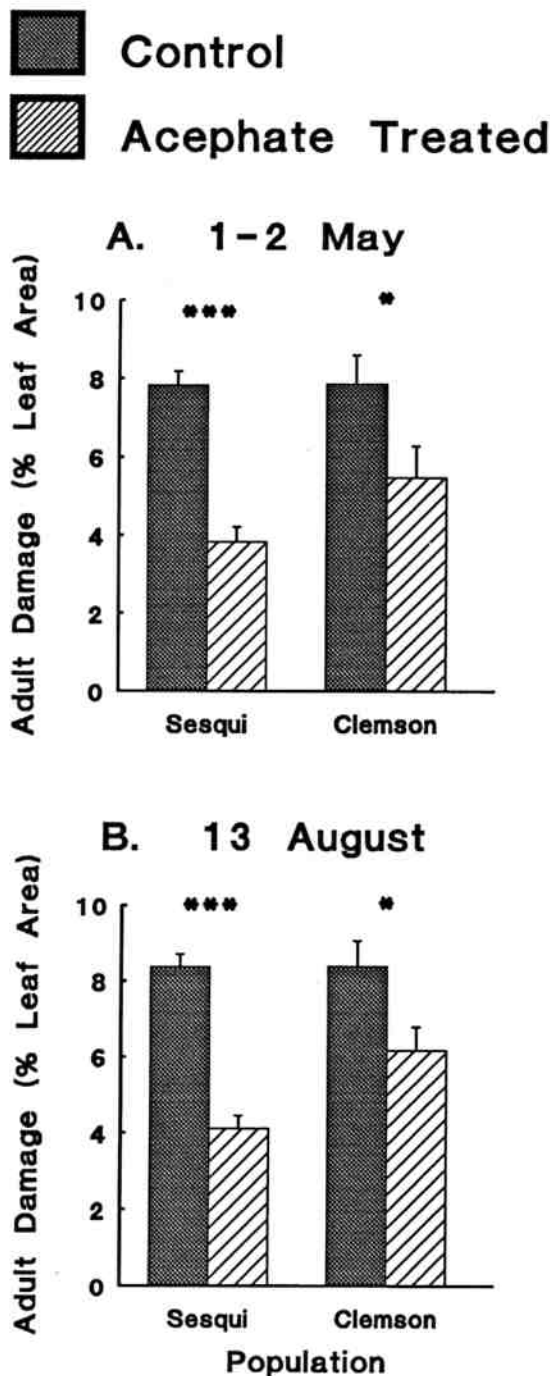


Fig. 1. Effect of acephate implants on the mean percentage (± 1 SEM) of turkey oak leaf area lost to adult *B. tessellatus* feeding. (A) 1–2 May 1994, after leaves had fully expanded, and (B) 13 August after most adults had died. Sesquicentennial State Park (Sesqui), control $n = 146$, acephate $n = 71$; Clemson Road (Clemson), control $n = 50$, acephate $n = 28$. *, $P < 0.05$; ***, $P < 0.001$ (Mann–Whitney U test; Wilkinson 1990).

of the tree, and 10 from the east). The total number of eggs, number of parasitized eggs, and number of newly initiated mines was recorded for each leaf.

At the end of the season (9–13 September), just before leaf abscission, 100 leaves were haphazardly selected on each tree ($\approx 1/2$ from each the east and west sides of the tree) and censused without removing them from the tree. The number of small (< 1 cm²), medium (1–5 cm²), large (> 5 cm²), and successful mines was recorded for each leaf, where small, medium, and large reflects the size of the mine when the larvae died, and successful mines are any mine with a living larva in it on the date of census (note that all successful mines were > 5 cm²). In a previous experiment, $> 90\%$ of these larvae successfully pupated (K.J.W., unpublished data). Numbers of leaf rollers, *Archips* sp., leaf tiers, *Croesia semipurpurana* (Kearfott), and leaf galls were also recorded. Because ants remove eggs from leaves during the summer, egg densities were not counted again at the end of the season.

Acephate Effects on Endophytic Leaf Fungi.

Five leaves were haphazardly selected on each of 18 control and 10 treatment trees at the Sesquicentennial State Park site. To remove surface microorganisms, leaves were washed in 95% ethanol, then soaked for 1-min in 70% ethanol, and transferred to 33% chlorine bleach for 5 min. Leaves were sampled by punching six 1-cm holes along the midvein and 6 others away from the midvein (12 holes per leaf). Leaf samples were placed on half-strength sterilized corn meal media and allowed to grow at room temperature for 8 wk.

Assessment of Tree Reproductive Success.

The number of acorns produced, and the average size of these acorns, was estimated for every tree at Sesquicentennial State Park. Like many oaks, *Q. laevis* is a 2-yr oak; flowers are fertilized in their 1st yr (March–April of year 1), and overwinter as developing ovaries. These ovaries are matured into acorns the following year, and eventually fall from the tree in September–November of year 2. Thus, the maximum number of acorns a tree can produce in a year is dependent on the number of ovaries matured in the previous year. These ovaries (immature acorns) were counted on every tree before budbreak (February and March). The number of acorns produced by a tree at the end of the summer was then scaled by the number of ovaries on the tree before budbreak. Our analysis of tree reproductive success was subsequently based on this proportion of ovaries successfully matured into acorns.

The size of acorns was estimated by collecting > 15 acorns from each tree (except for trees that produced < 15 acorns, in which case all acorns were collected). Unfortunately, the phenology of acorn maturation varies substantially among trees, and the weight of any individual acorn varies substantially with phenology. To insure that all acorns were collected at approximately the same stage (af-

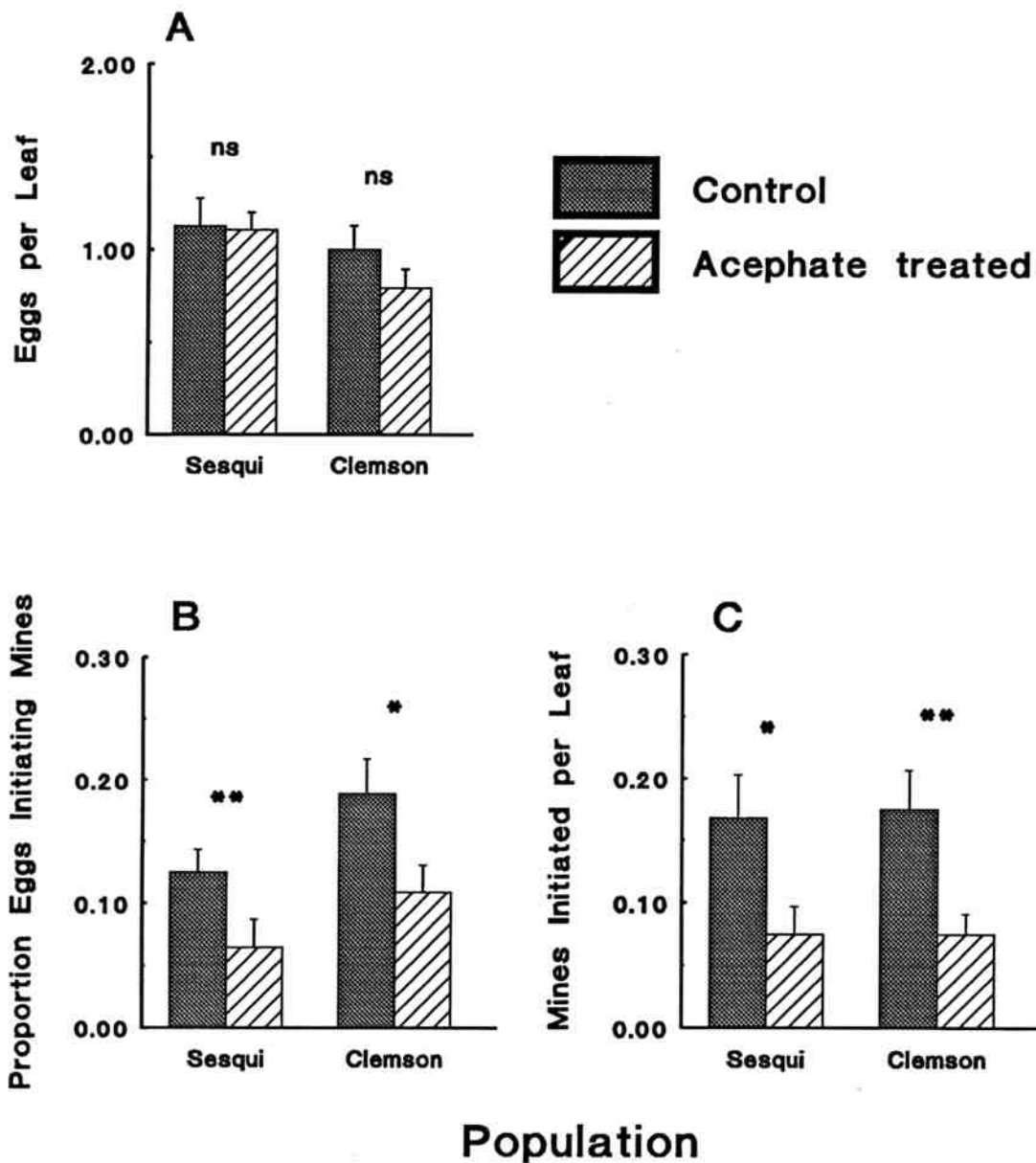


Fig. 2. Effect of acephate implants on (A) the number of *B. tessellatus* eggs laid per turkey oak leaf, (B) the proportion of these eggs that successfully initiated mines, and (C) the number of mines initiated per leaf. All bars are means \pm 1 SEM. Sesquicentennial State Park (Sesqui), control $n = 36$, acephate $n = 29$; Clemson Road (Clemson), control $n = 27$, acephate $n = 28$. ns, Not significant; *, $P < 0.05$; **, $P < 0.01$ (Mann-Whitney U test; Wilkinson 1990).

ter maturation, but before dropping from the tree), all trees were examined for acorn maturation beginning the end of September; acorns were gently twisted in their caps, and if they easily came free, they were harvested from the tree. Otherwise, the acorns were tested again in 2–3 d.

Harvested acorns were stored over winter in plastic bags at 4°C. In February 1995, these acorns were transferred to wet sand and allowed to ger-

minate. Germinating acorns (4 acorns per family) were subsequently transferred to \approx 3-liter pots containing wet sand (collected from the field sites) and grown in a greenhouse at 22–23°C at a photoperiod of 15:9 (L:D) h. To estimate potential effects of acephate on early growth of seedlings, the height of each seedling was measured, and the number of leaves present counted, 8 wk after germination. For analysis of germination and growth

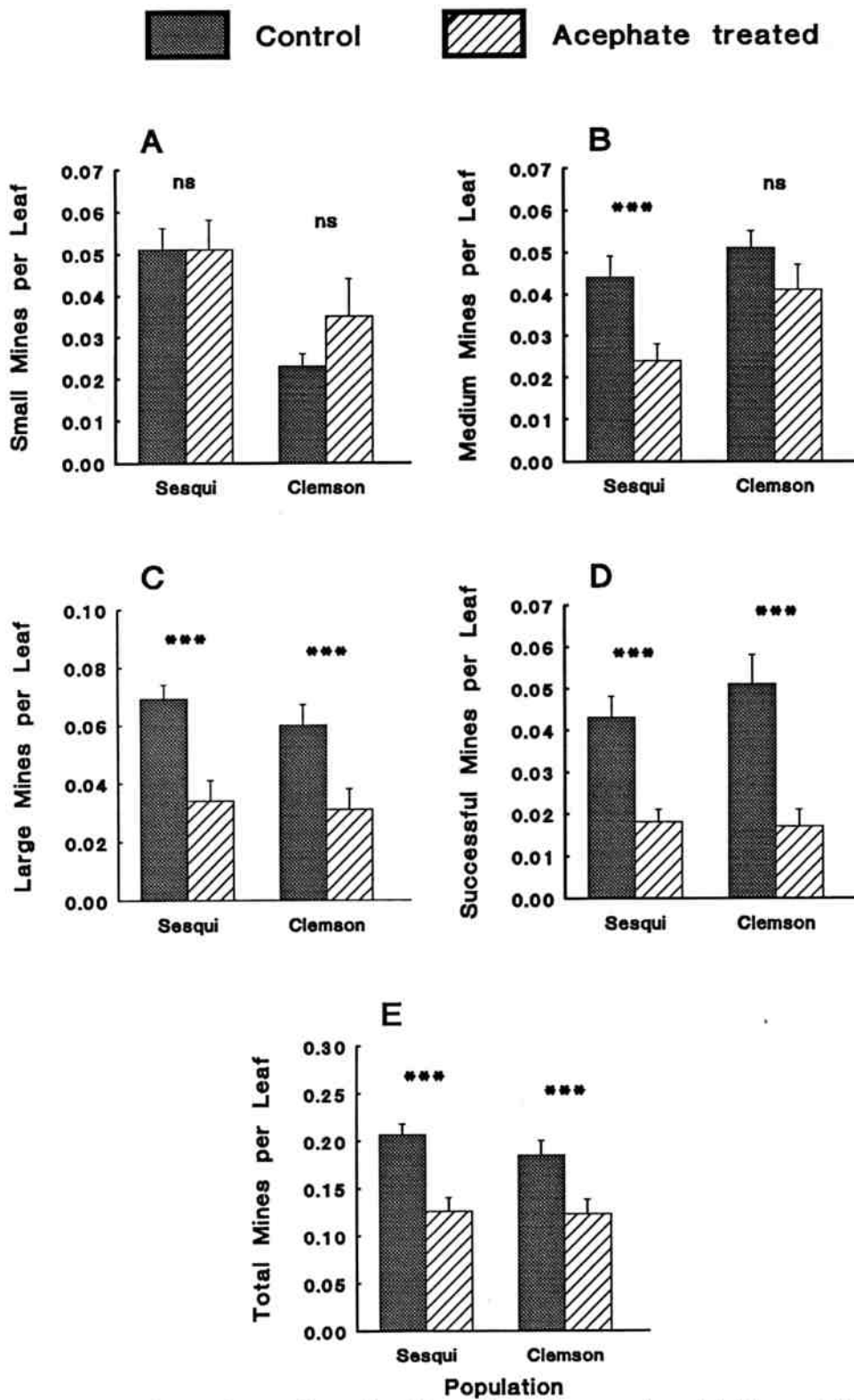


Fig. 3. Effect of acephate implants on the number of *B. tessellatus* mines per turkey oak leaf (mean \pm 1 SEM). (A) small mines, <1 cm²; (B) medium mines, 1 to 5 cm²; (C) large mines, >5 cm²; (D) successful mines, containing surviving larvae at the time of census; and (E) the total number of mines. Populations examined and sample sizes as in Fig. 1. ns, not significant; ***, $P < 0.001$ (Mann-Whitney *U* test; Wilkinson 1990).

data, seedlings from the same family were pooled, with each family average treated as an individual data point.

Laboratory Preference Experiment. To confirm that adult beetles actively avoid feeding on acephate treated leaves, adult females collected from the field were confined in 800-cm³ plastic boxes with 2 turkey oak leaves of approximately the same size; 1 leaf was harvested from a tree treated with acephate (harvested 4–8 wk after the tree was pegged), the other from a control tree. Beetles were allowed to feed on these leaves for 2 d, after which females were scored as preferring either treated or control leaves. Because the beetles ate almost exclusively control leaves, it was not found necessary to quantify the amount of leaf area lost for control versus treatment leaves.

Results and Discussion

Manipulation of Adult and Larval Herbivory on Turkey Oak. At both Sesquicentennial State Park and Clemson Road, acephate treated trees lost $\approx 1/3$ – $1/2$ as much leaf area to adult *B. tessellatus* feeding as control trees (Fig. 1). This observation was consistent with our laboratory experiment on adult feeding; when presented with leaves from both treated and control trees, female beetles fed largely on untreated leaves (22 of 26 beetles, sign test, $P < 0.01$). The laboratory experiment also indicated that, in addition to an adult feeding deterrent, treated leaves were toxic to beetles; most females presented with treated leaves in closed plastic boxes died within 4 d (29 of 40 beetles), whereas very few females died when presented with only control leaves for 4 d (4 of 84 beetles). It is unclear, however, whether females died from feeding on treated leaves or from volatiles trapped in the unventilated plastic boxes.

Although adults avoided feeding on acephate treated foliage, there was no evidence that they avoided laying eggs on these trees; the number of eggs laid on treated and control trees did not differ significantly in either our April or May censuses (Fig. 2A). However, eggs laid on treated trees were less likely to initiate mines than eggs laid on control trees (Fig. 2 B and C). This resulted in fewer medium (1–5 cm²), large (>5 cm²), and successful mines per leaf on treated trees at the end of the season (Fig. 3), although there was no difference between treatments in the number of small mines (<1 cm²) per leaf (Fig. 3A). Of larvae that were reared successfully to the end of the season (collected just before leaf abscission), there was no difference in weight of larvae on control and treatment trees (mean \pm SE: control = 19.9 \pm 0.5 mg [24 trees; 83 larvae], treated = 19.4 \pm 0.8 mg [27 trees; 116 larvae]; Mann–Whitney *U* test, $P = 0.85$).

Although acephate implants were effective against *B. tessellatus*, there was no evidence of acephate treatment effects on the densities of any

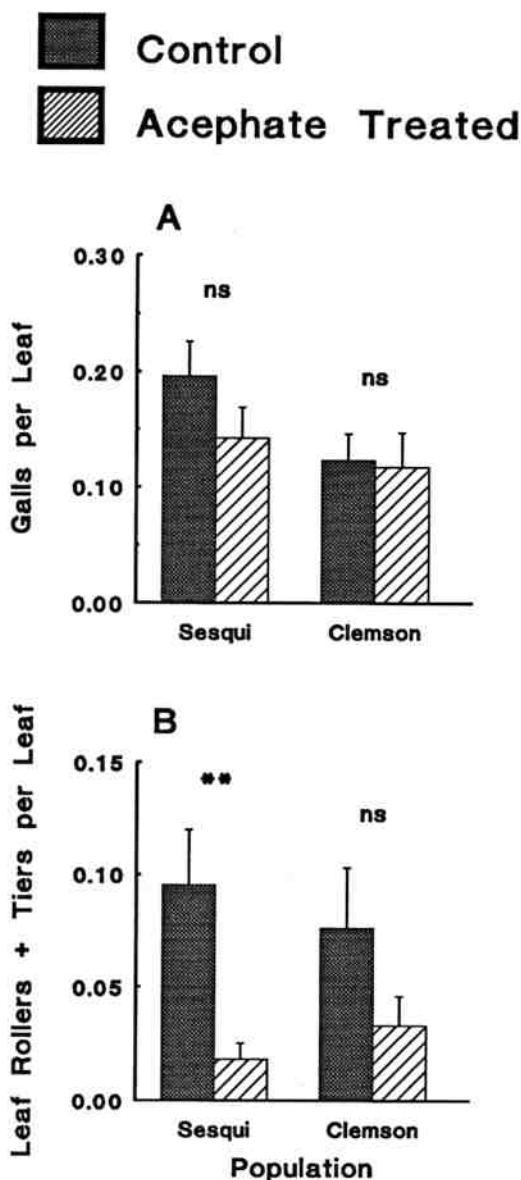
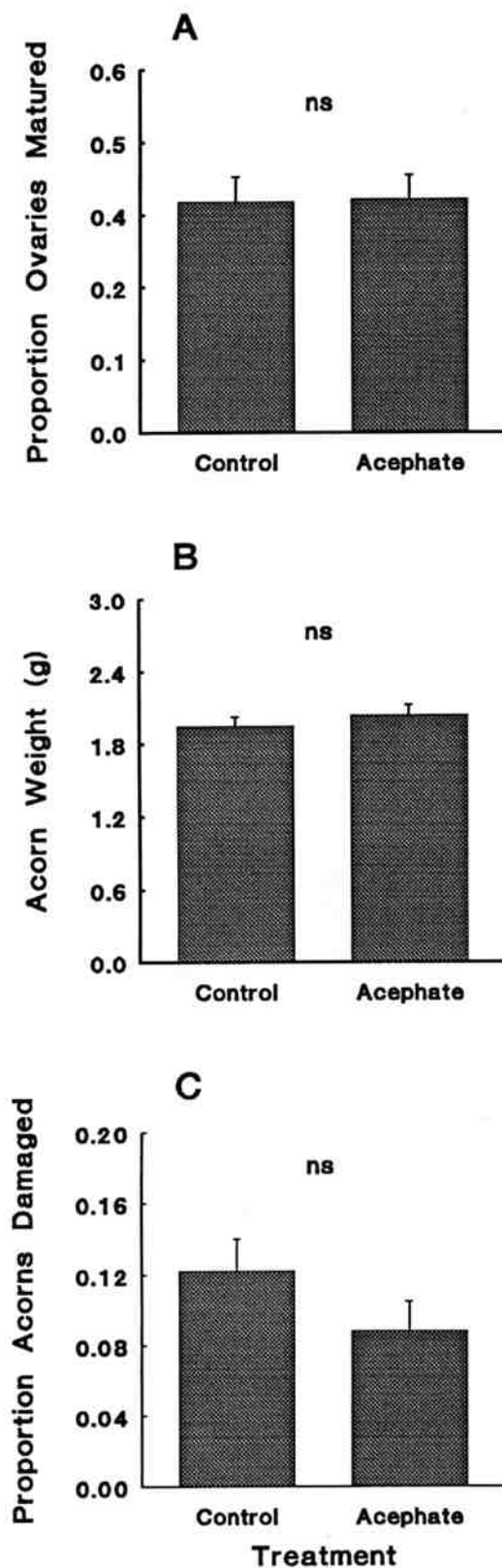


Fig. 4. Effect of acephate implants on leaf herbivores other than *B. tessellatus* (mean \pm 1 SEM). (A) leaf galls (all species combined), (B) leaf rollers + leaf tiers (combined). Populations examined and sample sizes as in Fig. 1. ns, Not significant; **, $P < 0.01$ (Mann–Whitney *U* test; Wilkinson 1990).

leaf-galling insect (Mann–Whitney *U* tests, $P > 0.05$ for each test; sample sizes as in Fig. 1), nor for the overall density of leaf-galls (all leaf-galls lumped together, Mann–Whitney *U* test, $P > 0.05$; Fig. 4A). Acephate did, however, reduce the number of leaf rollers, *Archips* sp., and leaf tiers, *C. semipurpurana*, per leaf at Sesquicentennial State Park (not significant for Clemson Road site; because of the small number of insects, both species were combined for the analysis; Fig. 4B). Because



leaf rollers are generally uncommon, and are responsible for <1% leaf area loss (C.W.F. and K.J.W., unpublished data), the small observed reduction in densities are likely insignificant to the trees. Thus, our acephate treatment appears to be largely specific to *B. tessellatus* among significant foliage damaging insects (gallers and miners). We did not, however, examine effects of the acephate treatment on stem-galling or wood-boring insects.

In the subsample of trees for which endophytic fungi were cultured from leaves, there was no evidence of an effect of acephate treatment on either the presence/absence of hyphomycete or coelomycete fungi, nor on the number of hyphomycete or coelomycete species per leaf (Mann-Whitney *U* tests, $P > 0.05$ for each) (control, $n = 18$ trees; treatment, $n = 10$ trees; Sesquicentennial site only).

Effects of Acephate on Tree Reproduction. There was no evidence of negative effects of acephate implants on oak tree reproductive success. Treated trees successfully matured the same proportion of their ovaries as control trees (Fig. 5A), and produced acorns that were as large as those produced by control trees (Fig. 5B). Acorns of acephate treated trees also germinated as well as acorns of control trees (proportion germinated, mean \pm SEM, acephate, 0.912 ± 0.033 , $n = 34$ families; control, 0.905 ± 0.028 , $n = 55$ families; Mann-Whitney *U* test, $P > 0.05$), and grew as rapidly as control trees (seedling height, mean \pm SEM, acephate, 11.31 ± 0.46 cm, $n = 27$ families; control, 12.51 ± 0.38 cm, $n = 43$ families; number of leaves, mean \pm SEM, acephate, 6.89 ± 0.32 , $n = 27$ families; control, 7.16 ± 0.21 cm, $n = 43$ families; Mann-Whitney *U* tests, $P > 0.05$ for each). This also indicates that there is no short-term cost of herbivory of beetle damage on tree reproduction; treatment trees, which suffered less damage than acephate treated trees, did not produce more or larger acorns than treated trees (Fig. 5). However, this does not imply the absence of long-term costs of leafminer herbivory, nor the absence of long-term negative effects of pesticide treatment on tree growth and reproduction. Like many trees (Maschinski and Whitham 1989), turkey oak has an extensive root system and likely retains large carbohydrate reserves in their roots and may not be sensitive to short-term changes in

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Fig. 5. Effect of acephate implants on turkey oak reproductive success. (A) The proportion of ovaries successfully matured into acorns, (B) the weight of these acorns, and (C) the proportion of acorns attacked by an acorn herbivore (*Curculio* species). These data were collected at Sesquicentennial State Park only. ns, Not significant (Mann-Whitney *U* test; Wilkinson 1990). Acorn maturation, $n = 75$ (control) and 52 (acephate); acorn weight, $n = 79$ (control) and 41 (acephate); acorn damage, $n = 101$ (control) and 50 (acephate).

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