# Insect and Disease Impacts on Blue Oak Acorns and Seedlings<sup>1</sup>

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Abstract: We studied the impacts of diseases and arthropods on acorns and naturally occurring seedlings of blue oak, Quercus douglasii, in northern California. Damage levels were always significantly higher in acorns collected from the ground than those picked from the tree. Accurate assessments of damage levels could only be made by dissecting acorns, since external symptoms were frequently absent from acorns with severe damage to the embryo. Levels of insect and disease damage to acorns varied significantly between locations and between trees. Foliar feeding by several types of insects had no apparent effect on the survival of natural blue oak seedlings over 3 years. Water stress appeared to be the primary factor affecting seedling survival. Although many seedlings survived the complete loss of their shoots and resprouted, repeated resprouting was associated with increased mortality. Overall seedling mortality after 3 years was 25 percent, but ranged from 5 percent to 65 percent at different plots. We suggest that blue oak seedlings are adapted to persist for a number of years beneath mature trees and may constitute an important source of advance regeneration.

As part of a research project sponsored by the California Department of Forestry and Fire Protection we have been studying the effects of diseases and arthropods on different life stages of blue oak. Due to their small size and limited energy reserves, acorns and seedlings are potentially quite susceptible to destruction by insects and diseases. While losses of acorns due to insect and disease damage has been noted by various authors (Griffin 1971, Knudsen 1987, Borchert 1990), methods used to assess damage levels have not been standardized. A number of damage and mortality factors have been implicated in the failure of blue oak seedlings to develop into saplings. However, the impacts of arthropods and diseases on the survival of natural blue oak seedlings have not been studied previously.

We developed and applied methods to assess the impacts of diseases, arthropods, and other damage factors on acorns and naturally-occurring seedlings of blue oak throughout northern California. This paper summarizes some of the results of our studies conducted between the summer of 1988 and May 1990. Results from these and related studies are reported in Swiecki and others (1990).

#### **METHODS**

#### Acorns

Insect and disease impacts on blue oak acorns were assessed by visually rating internal and external symptoms of collected acorns. We rated 26 blue oak acorn samples in 1988 and 14 in 1989. Acorns were collected either from the ground beneath trees or from the lower branches of tree canopies. Over the 2 years, we also collected paired canopy and ground samples from 10 trees. Sampled trees were in blue oak and mixed oak woodlands in the northern California counties of Amador, Butte, Glenn, Napa, Sacramento, Solano, Sonoma, Tehama, and Yuba. Most of the sampled trees were in the vicinity of the seedling study plots described below. Acorns were placed in plastic bags and held at room temperature (20-25 C) until rated. From each sample, 25 acorns were drawn at random for rating.

External symptoms rated included the degree of external color change, the presence of insect oviposition injury and exit holes, and presence of mycelium and/or sporulation. Sporulating fungi were identified to genus where possible. After external symptoms were rated, each acorn was cut longitudinally into quarters, and the sections of the embryo were separated from the seed coat to allow inspection of all faces. The embryo sections were further sliced or broken as necessary to determine the extent of symptoms and inspect for insects. The percentage of the acorn embryo affected by discoloration, decay, and insect feeding was estimated using a 0 to 6 pretransformed scale, where 0 = no damage and 6 = >97.5 percent (Swiecki and others, theseproceedings). The percentage intervals in this scale are pretransformed using the arcsin transformation, which permits analysis by parametric statistical techniques without further transformation (Little and Hills 1978). In addition, we noted the presence and type of visible fungal signs, type of insect injury, and the number and type of insect larvae within each acorn.

## Seedlings

In the summer of 1988, we established nine plots to study naturally occurring seedlings in Amador, Butte, Glenn, Napa, Solano, and Sonoma counties. The locations of the plots are shown in Swiecki and others (these proceedings). Plots were selected to represent a variety of different site conditions (Swiecki and others 1990). At each location, 50 blue oak seedlings located along a belt transect were selected at random and marked by placing a small wooden stake or wire stake flag 10 cm due

<sup>&</sup>lt;sup>1</sup>Presented at the Symposium on Oak Woodlands and Hardwood Rangeland Management, October 31 - November 2, 1990, Davis, Calif.

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north of the seedling. Sites subject to cattle grazing were also marked prior to the start of seasonal grazing by anchoring an aluminum tag at ground level with a heavy gauge galvanized nail 10 cm due south of the seedling. A portable metal detector was subsequently used to relocate seedlings when the stakes or flags were destroyed or dislodged by cattle or rodents. With very few exceptions, seedlings selected for the study were no taller than 10 to 15 cm, and had no more than 20 leaves at the start of the study.

We rated the plots at irregular time intervals in the latter half of 1988 and throughout the growing season in 1989. Final ratings were made in the spring of 1990. During each rating, we collected data on seedling morphology and damage symptoms. Numbers of live and dead leaves were counted, and the percentage of leaf area affected by necrosis, chlorosis and other discoloration, chewing (free feeding through the entire leaf), window feeding (feeding on only one leaf surface), and stippling (fine chlorotic or necrotic spotting caused by arthropods with piercing-sucking mouthparts) was estimated using the 0 to 6 pretransformed scale previously described . We also coded the types of insects observed, disease symptoms, and other types of damage seen during each rating. We estimated the degree of shading each seedling would receive, based on its topographic position, position relative to the canopy, and canopy density. Three levels of shading were assigned: 0 =full sun, 1 = shaded part of the day, 2 = shaded most of the day, except at low solar angles.

At the start of the study in 1988, we examined each seedling carefully to determine whether shoots had been produced in previous years. If no evidence of stems formed in previous seasons was found, seedlings were assigned to the "new" seedling age class. Seedlings continuing growth on 1-year old or older wood and those with evidence of previous shoots were assigned to the "existing" seedling age class. In subsequent years, we rated seedlings for the age of the oldest live shoot only. If the oldest live shoot arose from ground level, seedlings were assigned to the "current" shoot age class. If new growth was produced on a preexisting stem above ground level, seedlings were assigned to the "previous" shoot age class.

# Data Analysis

The statistical significance of mean differences was determined using fixed or mixed model analysis of variance. Correlations between continuous variables were evaluated with standard linear regression analysis or stepwise multiple regression. Nonparametric rank correlation analysis was used for ratings. Proportional data were subjected to contingency table analysis, and the significance of differences between proportions was determined by either Fisher's exact test or Chi square as appropriate. Tests or comparisons reported as significant are significant at the P < 0.05 level unless otherwise noted.

# RESULTS

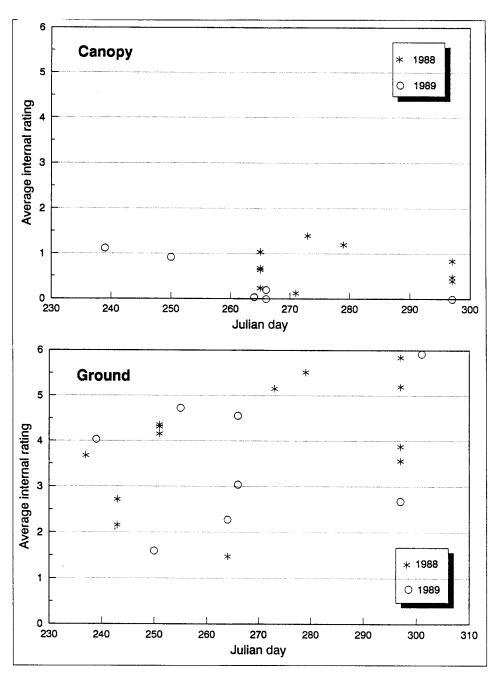
# Acorns

Substantial numbers of acorns, many of which were diseased and/or insect infested, began to drop by early August in some of our study areas. The average internal damage rating for acorns collected from the ground  $(3.8 \pm 2.3)$  was significantly higher (P<.001) than for acorns picked from oak canopies (.6  $\pm$ 1.2). For the 10 paired canopy/ground samples, site of collection (canopy or ground) and the site by tree interaction were both highly significant (P<.001) in the analysis of variance. The incidence of internal fungal growth, internal insect boring, feeding/oviposition wounds, exit holes, and internal damage ratings > 3 (more than 20 percent of the embryo affected) were significantly greater in acorns collected from the ground than those collected from the canopy. Average internal damage ratings for canopy and ground samples remained relatively constant over the period from late August through late October (figure 1).

External discoloration, oviposition injury, and fungal growth were not significantly associated with substantial internal injury, i.e. internal ratings > 3. Only insect exit holes were generally indicative of substantial internal damage; 81 percent of the acorns with exit holes had internal damage ratings > 3. However, acorns lacking exit holes could not be assumed to be sound, since 82 percent of acorns with internal ratings > 3 did not have insect exit holes.

The larvae of a filbert weevil, *Curculio pardus* (Chittenden), and the filbertworm, *Cydia latiferreana* (Walshingham), accounted for almost all of the insect injury we observed in blue oak acorns. No more than a single filbertworm larva was found in each blue oak acorn infested with this species, but 1 to 8 filbert weevil larvae were found in each acorn (overall mean  $1.6 \pm 1.2$ ). Both insects were found in the same acorn 5.4 percent of the time. Up to 47 percent of the acorns in some samples contained weevil larvae whereas the incidence of filbertworm infestation ranged up to 60 percent. Average internal damage ratings in acorns infested by each species did not differ significantly (3.1  $\pm 1.6$  for filbert weevil and  $3.1 \pm 2.1$  for filbertworm).

Among the internal symptoms, internal fungal growth was most frequently associated with high damage ratings. Acorns were commonly colonized externally and/or internally by species of *Penicillium* and *Aspergillus*. In many cases, external fungal colonization was limited to the abscission scar at the cap end of the acorn, and did not progress into otherwise intact acorns. Extensive internal sporulation, particularly by *Penicillium*, was commonly seen in acorns with insect exit holes and moderate amounts of internal insect boring. Other fungi observed in or on acorns included *Fusarium oxysporum*, Schlechtend.:Fr. *Stemphylium* sp., *Trichothecium* sp., and an unidentified Coelomycete.

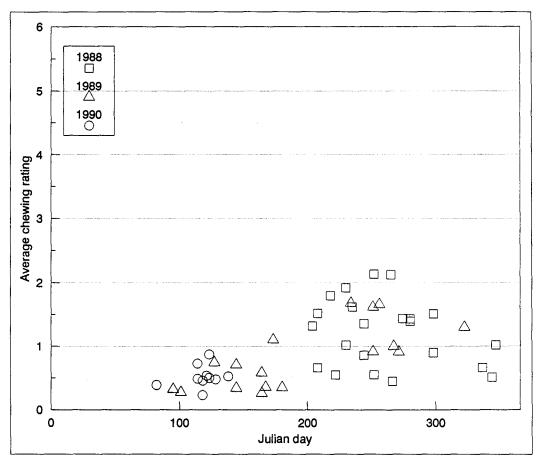


**Figure 1**—Average internal damage ratings for blue oak acorn embryos collected from the tree canopy or ground for August through November of 1988 and 1989 (Julian day 240 = 28 August, Julian day 300 = 28 October).

### Seedlings

Various insects and their feeding symptoms or products were observed on seedlings, but only chewing, window feeding, and stippling were common enough and of sufficient intensity to warrant severity ratings. Of these, chewing damage was the most important, although average levels of chewing damage seldom exceeded a rating of 2 (2.5 to 20 percent of foliage affected) (figure 2). Most window feeding and stippling damage occurred before May. Early-season chewing damage to young foliage was seen through mid-June, and a second period of chewing damage was seen in July and August. Significant positive correlations (P<.001) were observed between the maximum leaf count and maximum levels of chewing, window feeding, and stippling damage in 1989. Within each plot, the distribution of seedlings with insect damage was highly variable. Heavily-damaged seedlings were often observed within close proximity (10 to 30 cm) of seedlings with little or no insect damage.

Progressive foliar necrosis was the most significant foliar symptom in all plots, and increased steadily throughout the season (figure 3). Foliar chlorosis also tended to increase over



**Figure 2**—Average chewing damage ratings for blue oak seedlings for all plot ratings in 1988 through 1990 (Julian day 100 = 10 April, Julian day 200 = 19 July, Julian day 300 = 28 October).

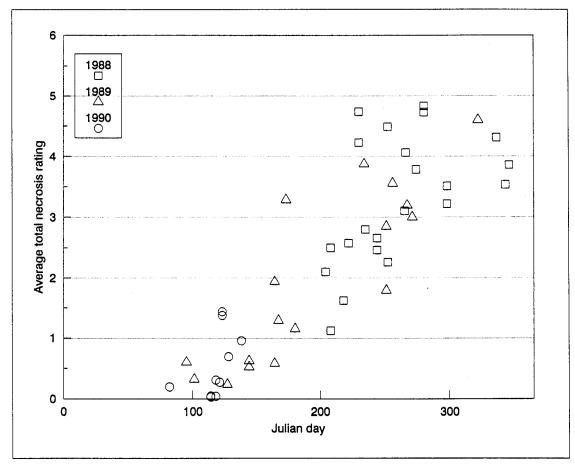
the season from the beginning of July onward. Overall, these progressive chlorotic and necrotic symptoms were consistent with those caused by water stress. Damage to seedling foliage and shoots by aerial plant pathogens was insignificant at all of our study sites.

Mortality was based on the last year that a live shoot was observed at any rating because many seedlings successfully resprouted following complete foliar or shoot necrosis. A total of 383 seedlings were tracked across all 3 years of the study. Of these, 34 (8.9 percent) died in 1988 and 59 (15.4 percent) died in 1989, leaving 290 survivors (75.7 percent) in the spring of 1990. Survival over the study period varied widely between plots, and ranged from 95 percent to 35 percent. The average maximum number of leaves per seedling in 1989 was significantly lower in seedlings that died that year ( $4.7 \pm 1.0$ ), than in seedlings that survived into 1990 ( $10.5 \pm 0.4$ ).

Regression analysis showed that among individual site environmental variables, reference evapotranspiration (ETo) was positively correlated ( $r^2$ =.461, P=.044) and precipitation was negatively correlated ( $r^2$ =.394, P=.070) with seedling mortality. In the model derived through stepwise multiple regression analysis, mortality increased with increasing ETo and decreasing soil depth ( $r^2$ =.683, P=0.032): (percent mortality)<sup>0.5</sup> = .443(ETo) - .049(Soil depth) - 44.2.

At the start of the study in 1988, 35 percent of the seedlings had no obvious evidence of shoots produced in previous years. However, many of these seedlings may still have been resprouts, since attached acorns were seldom observed. Of the seedlings that survived into 1989,26 percent resprouted from ground level after death of the previous year's shoot, whereas the remainder continued growth on stems that had been produced the previous year. In 1990, 31 percent of the surviving seedlings had resprouted from ground level due to death of the previous shoot. However, even though seedlings were able to resprout from ground level following death of the previous year's shoot, repeated resprouting was clearly associated with reduced survival. A comparison of seedlings that were alive in both 1988 and 1989 shows that subsequent mortality was highest in seedlings which died back to the ground and resprouted each year (37.5 percent) and lowest in plants whose shoots survived both years (92 percent). Seedlings rated as new in 1988 were more likely to die back to the ground in subsequent years, and had significantly lower rates of survival in 1989 and 1990 (79 and 56 percent, respectively) than those starting with existing shoots in 1988 (95 and 85 percent, respectively).

All of the major damage symptoms we rated varied significantly between plots. Among the damage variables, only chewing damage and total necrosis ratings were associated with



**Figure 3**—Average total foliar necrosis ratings for blue oak seedlings for all plot ratings in 1988 through 1990 (Julian day 100 = 10 April, Julian day 200 = 19 July, Julian day 300 = 28 October).

plant mortality. Seedlings that died in 1988 experienced significantly lower levels of chewing damage (P=.026) than those that survived. Although chewing damage was negatively correlated with mortality in 1988, no relationship was detected between chewing damage in 1988 or 1989 and mortality in 1989. Total necrosis ratings in 1988 and 1989 were positively correlated with mortality in 1989 in 2 by 2 contingency table analyses (P=.033 and .053, respectively). Seedlings that were alive in 1990 showed higher average chewing ratings and lower average necrosis ratings in 1988 and 1989 than seedlings that died in those years (table 1).

Although few seedlings (7 percent of total) were located in full sun locations, the relative level of shading had no significant effect on seedling and shoot survival over the course of the study. Maximum average leaf counts in 1989 and 1990 were significantly lower in seedlings under the highest level of shading than in the remaining seedlings. For 1989, least squares means for maximum leaf counts were  $10.1 \pm 1.5$ ,  $9.6 \pm .6$ , and  $7.5 \pm .6$  for the low, intermediate, and high shading levels, respectively. In 1989, chewing damage increased as shading level increased, although this effect was not observed in 1988.

### DISCUSSION

Considerable damage to the acorn embryo normally occurs in acorns that have exit holes. Substantial internal damage is also likely in extremely light-weight acorns that lack exit holes, since these acorns normally show severe decay and dehydration of the embryo. However, the results of our study indicate that acorns which do not meet these criteria may also have high levels of internal damage. Since internal damage cannot be reliably predicted from external symptoms, damage may have been under- or overestimated in previous studies where only external damage symptoms were recorded. We found that destructive sampling was the only reliable method for assessing absolute levels of internal damage in acorns. While this method precludes the use of rated acorns in germination tests, we expect that germination and seedling vigor are likely to be adversely affected at internal damage ratings of 3 or greater. Acorns with internal damage ratings of 5 or more are clearly nonviable.

Damaged acorns are prone to early abscission, contributing to the significant disparity between acorn damage levels found in samples collected from the ground and those collected from

 Table 1—Means for chewing ratings and total necrosis ratings by seedling survival class.

| Last year seedling           | Average chewing rating       |                | Average total necrosis rating |              |
|------------------------------|------------------------------|----------------|-------------------------------|--------------|
| was rated live               | <u>1988</u>                  | <u>1989</u>    | 1988                          | <u>1989</u>  |
| 1988<br>1989                 | 1.2 A <sup>1</sup><br>1.4 AB | 0.7 A          | 3.9 A<br>3.8 A                | 2.8          |
| 1990<br>Analysis of variance | 1.7 B                        | 1.3 B          | 2.6 B                         | 2.2          |
| F value<br>P level           | 3.97<br>.020                 | 11.70<br>>.001 | 13.89<br>>.001                | 3.46<br>.064 |

<sup>1</sup>Means followed by the same letter within a column are not significantly different at P=.05 (mean separation by LSD following significant F in the analysis of variance table).

tree canopies. Various authors (Griffin 1971, Schettler and Smith 1980, Menke and Fry 1980) have also noted that insectinfested acorns tend to drop before sound acorns. Therefore, the method of acorn collection will significantly affect the estimate of overall damage levels due to insects and disease. However, few authors have stated the method of collection when reporting acorns infestation levels.

Levels of damage in acorns collected from the ground did not decrease as the season progressed, even though the population of damaged acorns on the ground should have been diluted by the less-damaged population from the canopy. It is possible that the supply of sound acorns on the ground is preferentially depleted by vertebrate acorn consumers. Alternatively, insect and pathogen activity may be increasing over this interval, leading to the establishment of new infestations and infections and an increase in damage from established infestations and infections. Regardless of the mechanism, the net result is that a high proportion of the acorns available for recruitment have reduced viability due to insect damage and decay.

Although seed-boring insects are frequently cited as agents affecting acorns in California, the role of microorganisms has received relatively little attention. Our observations suggest that various fungi and possibly bacteria are important pathogens of blue oak acorns in northern California. Acorns are often extensively decayed by these agents even in the absence of significant insect damage. It appears that wounds produced by insects are probably the most common site of entry for the pathogens affecting blue oak acorns. A similar etiology is reported for the "drippy nut" disease of live oak acorns (Hildebrand and Schroth 1967). In addition to effects on acorn germination, various fungi, particularly *Aspergillus* and *Fusarium* spp., produce mycotoxins which may pose a hazard to livestock or wildlife species that consume large quantities of the mast crop.

Direct and indirect effects of water stress appeared to be the most significant factor influencing the growth and mortality of blue oak seedlings in our study. While damage caused by root pathogens, root-feeding insects, and vascular pathogens can be expressed as progressive water stress symptoms, we believe that low soil moisture is the most likely cause of the symptoms we observed. Symptoms consistent with severe water stress, including progressive foliar chlorosis and necrosis and shoot dieback, developed from early June through at least mid-September. Soil moisture levels and blue oak seedling water potentials decrease substantially over this interval (Welker and Menke 1987). The regression analysis of site characteristics also points to water stress as an important mortality factor at our study plots.

Although natural blue oak seedlings are fed upon by a variety of insects, insect herbivory did not significantly affect seedling survival in our study. This is not surprising, given the relatively low levels of damage seen in most seedlings. Furthermore, as indicated by Welker and Menke (in press), some loss of foliage may promote seedling survival by reducing the rate at which seedling water potentials decrease. In 1988, mortality was actually lower in seedlings with high levels of chewing damage. However, mortality rates were also low in seedlings with high leaf counts and low levels of foliar necrosis (Swiecki and others 1990). Therefore, we believe that the negative correlation between chewing and mortality simply reflects the preference of foliar feeding insects for larger seedlings and those with more live leaf tissue.

Although varying degrees of leaf loss due to insect herbivory did not adversely affect seedling survival, loss of the entire shoot did reduce survival. Repeated allocation of stored photosynthate to replace shoot tissues may reduce root growth (Welker and Menke in press), restricting the seedling's access to soil moisture and eventually leading to lethal water stress. However, even though they can be killed by water stress and repeated shoot loss, our data shows that small blue oak seedlings are anything but ephemeral. Overall mortality was only 25 percent over 3 years, and was as low as 5 percent in some plots. Over 60 percent of the seedlings that resprouted in both 1988 and 1989 still emerged in 1990, in spite of drought conditions during these 3 years. Similarly, Griffin (1971) observed 88 percent seedling survival in blue oak 3 years after planting, despite 60 percent shoot mortality in the first summer.

The low percentage of seedlings we located in full sun was the same as that reported by Muick and Bartolome (1987). There were no differences in seedling mortality observed over the course of our study that would account for the low numbers of seedlings away from tree canopies. Lack of dispersal and poor acorn germination in exposed sites (Griffin 1971) could account for the relative lack of seedlings in the open. Open and shaded sites may also differ in the depth of the litter layer, herbaceous plant cover, soil moisture levels, soil condition, soil nutrient levels, temperature, light intensity or other factors that may affect seedling establishment. However, even though the balance of factors under tree canopies is relatively favorable for seedling establishment and persistence, they apparently do not favor the transition to the sapling class. Muick and Bartolome (1987) report that although seedlings are abundant beneath existing blue oak tree canopies, saplings seldom develop in this position.

We believe that persistence in the seedling stage may be part of the reproductive strategy of blue oak, as suggested by Knudsen (1987) for *Q. lobata*. Blue oak seedlings are clearly capable of surviving for a number of years under blue oak canopies. These persistent seedlings may constitute a reserve of regeneration which is recruited into the sapling stage only when canopy gaps open or entire stands are killed by fire or removed by cutting. We have seen situations in various locations where a relatively evenaged stand of saplings appears to have developed from preexisting seedlings following removal of the canopy by cutting. An analogous situation, referred to as advance regeneration, has been observed for oaks in the eastern U.S. (Beck 1970, Sander and others 1976) where oaks may persist as seedlings or saplings for 6 to 15 years or more, and are still capable of developing into trees following removal of the overstory.

The lack of blue oak saplings in many stands has been cited as evidence of poor regeneration (Muick and Bartolome 1987, Bolsinger 1988). However, if persistent seedlings rather than saplings are the norm for advance regeneration in blue oak, the lack of saplings may be a poor indicator of potential regeneration. Under this scenario, the factors that would have the greatest impact on recruitment are those affecting the establishment and persistence of seedlings and the survival and growth of released seedlings. Although insects and plant pathogens apparently have little impact on the survival of established seedlings, their effects on seedling establishment and the performance of released seedlings remain to be studied.

#### ACKNOWLEDGMENTS

This study was supported by Contract 8CA74545 from the California Department of Forestry and Fire Protection, Forest and Rangeland Resources Assessment Program. We thank Dale M. Woods, Kevin Rice, and Tyson Holmes for providing helpful comments on earlier versions of the paper.

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