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PLANT ANIMAL INTERACTIONS

Rebecca C. Mueller · Brian D. Wade
Catherine A. Gehring · Thomas G. Whitham

Chronic herbivory negatively impacts cone and seed production, seed quality and seedling growth of susceptible pinyon pines

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Abstract Although herbivory often reduces the reproduction of attacked trees, few studies have examined how naturally occurring insect-resistant and susceptible trees differ in their reproduction, nor have these effects been experimentally examined through long-term herbivore removals. In addition, few studies have examined the effects of herbivory on the quality of seeds produced and the implications of reduced seed quality on seedling establishment. We evaluated the impact of chronic herbivory by the stem-boring moth, *Dioryctria albovittella*, on cone and seed production of the pinyon pine (*Pinus edulis*) during two mast years. Three patterns emerged. First, moth herbivory was associated with reductions in cone production, viable seed production and seed mass. Specifically, pinyons susceptible to moth attack had 93–95% lower cone production, and surviving cones produced 31–37% fewer viable seeds, resulting in a 96–97% reduction in whole tree viable seed production. In addition, surviving seeds from susceptible trees had 18% lower mass than resistant trees. Second, long-term experimental removal of the herbivore resulted in increased rates of cone and seed production and quality, indicating that moth herbivory was the driver of these reductions. Third, seed size was positively associated with seed germination and seedling biomass and height, suggesting that trees suffering chronic herbivory produce poorer quality offspring. Thus, the resistance traits of pinyons can affect the quality of offspring, which in turn may affect subsequent seedling establishment and population dynamics.

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R. C. Mueller (✉) · B. D. Wade · C. A. Gehring · T. G. Whitham
Department of Biological Sciences and Merriam Powell
Center for Environmental Research,
Northern Arizona University, Flagstaff,
AZ, 86011-5640 USA
E-mail: rcm5@dana.ucc.nau.edu
Tel.: +1-928-5239138
Fax: +1-928-5237500

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Introduction

High levels of herbivore damage often have negative impacts on plant reproduction, particularly in woody species (Obeso 1993). Decreased flower and/or cone and seed production of woody plants in association with or in response to insect herbivory have been documented for numerous herbivore–host plant combinations (Strauss 1991; Louda and Potvin 1995; Maron 1998). However, these studies have examined the impacts of outbreak herbivore species, as opposed to the impacts of chronic herbivory on host reproduction. In addition, although herbivory has been shown to result in decreased seed mass of mountain birch and horsechestnut (Kaitaniemi et al. 1999; Salleo et al. 2003; Thalmann et al. 2003), the potential effects of herbivory on the quality of surviving seeds are not fully understood for woody species. Although even fewer studies have examined the impact of herbivory on the vigor of progeny from attacked trees, recent studies suggest that these effects may be important. For example, Agrawal et al. (1999) found transgenerational induction in which seedlings from parents that suffered herbivore attack were more heavily defended than seedlings from non-attacked parents.

This study focused on pinyon pine (*Pinus edulis* Engelm.), a long-lived tree that is chronically attacked by the stem-boring moth, *Dioryctria albovittella* (Hulst) on cinder soils of northern Arizona (Whitham and Mopper 1985; Cobb et al. 1997). Within these sites, 70% of adult pinyons were susceptible to moth attack (Cobb et al. 1997). These herbivores preferentially attack vigorously growing terminal stems, resulting in high levels of mortality of the terminal cone-bearing shoots (Whitham and Mopper 1985), decreased radial growth (Ruel

and Whitham 2002), increased cone and conelet mortality (Cobb et al. 2002) and increased male function (Cobb et al. 2002). Pinyons that are resistant and susceptible to moth herbivory could be found growing adjacent to each other, which allowed us to contrast the reproductive traits of moth resistant and susceptible trees under the same environmental conditions. In addition, differences in resistance to moth attack were shown to be genetically driven (Mopper et al. 1991), so that variation in tree performance arising from moth herbivory was the result of inherent genetic traits. Most importantly, the long-term experimental removal of moths from susceptible trees provided the opportunity to demonstrate a cause and effect relationship between moth herbivory and changes in pinyon reproduction.

We examined the impacts of chronic herbivory by *D. albovittella* on pinyon cone and seed production and seed quality, and the impacts of seed quality on seedling growth. We addressed three questions. First, what are the effects of chronic herbivory on cone production during two mast years? Previous studies found that herbivory by *D. albovittella* resulted in decreased cone production (Whitham and Mopper 1985; Christensen et al. 1991; Christensen and Whitham 1991), but each study was limited to a single year and changes in the magnitude of these reductions may vary over time. Second, what are the impacts of moth herbivory on the quantity and quality of seeds produced? Specifically, does chronic moth herbivory decrease viable seed production and seed weight? Although the negative impacts of moth herbivory on cone production have been demonstrated previously, the impact of herbivory on seed quality has not been examined. Third, what are the effects of decreased seed weight on seedling vigor? The impact of seed size on seedling vigor has been observed for numerous woody species (Crawley 1983; Armstrong and Westoby 1993; Hutchings 1997; Bonfil 1998; Castro 1999; Turnbull et al. 2000; Khan and Shankar 2001; Beon and Bartsch 2003), but only recently have studies begun to examine the extended impacts of herbivory on the vigor of progeny (e.g., Bonfil et al. 1998; Agrawal et al. 1999).

Methods

Effects of moth herbivory on pinyon cones and seeds

To estimate the effects of chronic herbivory on cone and seed production during two mast years, cone counts and measures of seed viability and seed mass were performed on 17 moth-resistant and 13-moth susceptible mature pinyons during the mast years (years of synchronous reproduction) of 1992 and 1994. These trees were selected because levels of moth herbivory (number of moth-killed shoots) have been quantified on them since 1982. Mean age of resistant and susceptible trees was (mean ± 1 SE) 164.8 ± 8.5 and 155 ± 7.4, respectively ($t = 0.86$, $P = 0.36$). Mean number of moth-killed shoots for these groups was 24.5 ± 8.5 and 174.0 ± 40 in 1992 ($t = 14.8$. $P < 0.001$) and 75.0 ± 12.5 and 839.8 ± 216.1 in 1994 ($t = 13.6$, $P = 0.001$), respectively.

Ten to fifty cones per tree were collected in August and September when the majority of cones where closed, but a few were beginning to open, indicating seed maturity. Cones were placed in a greenhouse to dry and open naturally. After the cones opened, the number of seeds in each cone was counted, and potential seed viability was quantified. Because pinyons often produce hollow seeds, seed viability was tested using pentane. Seeds that sank in the pentane were classified as potentially viable (filled), and those that floated were classified as non-viable (hollow) (McLemore 1965). For simplicity, throughout the remaining text, potentially viable seeds are referred to as viable. Viable seeds were removed from the pentane, allowed to dry, and weighed to the nearest 0.01 g. The total number of cones per tree and the number of viable seeds per cone were used to calculate whole tree estimates of viable seed production.

Experimental moth removal

To determine if observed differences in cone and seed traits were due to chronic herbivory or innate differences between resistant and susceptible pinyons, we utilized a long-term moth removal experiment. This experiment was initiated in 1983 on pinyons approximately 60-years old (Whitham and Mopper 1985), and consisted of three classes: moth resistant, moth susceptible, and susceptible trees that had their moths removed (moth-removed). Moths were removed from the latter group of susceptible trees with an annual application of the systemic insecticide Cygon for a minimum of 6 years and maximum of 9 years (additional trees were added to the study in 1986) prior to the initiation of our cone census work. Smaller trees were selected for this experiment to increase the likelihood that insecticide was effectively applied to the entire tree. After 4 years of herbivore removal, the moth-removed trees and resistant trees had

Table 1 Shoot mortality due to herbivore attack and age of resistant, susceptible and moth-removed pinyons

		Resistant	Susceptible	Removed	*P*
No. of moth-killed shoots	1992	8.8 (2.2)[a]	115.5 (15.4)[b]	4.0 (0.7)[a]	< 0.001
	1994	12.5 (3.8)[a]	110.9 (13.1)[b]	9.76 (2.3)[a]	< 0.001
Tree age (years)		55.1 (3.5)	58.8 (3.8)	60.3 (2.2)	0.474

Data presented are means ± 1 SE. Different letters indicate significant differences ($\alpha \leq 0.05$)

similar levels of herbivore damage and both groups had less shoot damage than susceptible trees. Previous studies indicate that the application of this insecticide did not affect pinyon growth (Whitham and Mopper 1985). Total cones per tree were counted on 25 resistant, 25 susceptible and 26 moth-removed trees during the mast years of 1992 and 1994. Tree age and levels of shoot mortality due to moth herbivory for these trees can be found in Table 1.

To determine the impacts of moth herbivory on pinyon seeds, five to thirty cones per tree were collected from each study tree. Because moth herbivory often results in cone crop failure of susceptible trees (Cobb et al. 2002), insufficient cones were available from susceptible trees to accurately measure seed parameters. The older and larger susceptible trees described above produced sufficient seeds for accurate measurements, but the smaller and younger trees used in the experiment did not, and as a result, could not be included in measures of seed quality. Cones were allowed to dry and naturally open in a greenhouse, seeds were collected, and percent viable seeds and viable seed weight were measured. Of the trees included in cone counts, 21 resistant and 20 moth-removed pinyons produced seeds and were included in the study in 1992, and 22 resistant and 28 moth-removed pinyons were analyzed in 1994.

Seed size, germination and seedling effects

To examine the impacts of seed size on germination, viable seeds from resistant and moth-removed pinyons collected during the 1992 mast year were weighed to the nearest 100th of a gram and planted 1 cm deep in steam-sterilized field soil (120°C for 10 h) in 6.5-cm diameter by 24-cm deep plastic pots. Pots were maintained in a greenhouse with a 16:8-h light:dark photoperiod and temperatures of 24–27°C during the day and 13–16°C at night. Pots were watered with tap water and checked for germination daily. A seed was considered to have germinated when a shoot emerged above the surface of the soil. Germination was monitored for 3 months, and the seed size of germinants versus non-germinants was compared.

In order to examine the relationship between seed size and seedling vigor of pinyons, we collected two sets of viable seeds from 60-year-old resistant and moth-removed trees during the 1992 mast year. These seeds were then planted in field soil in plastic plots as described above. Seedlings were allowed to develop for 3 months and 6 months under the greenhouse conditions mentioned previously. Forty-eight seedlings from seeds ranging in weight from 0.12 g to 0.63 g were sacrificed after 3 months of development. Seedlings were washed to remove soil, and total seedling (shoot and root) biomass was measured. Sixty seedlings from seeds ranging in weight from 0.18 g to 0.51 g were allowed to develop for 6 months and then measured for height to the nearest centimeter. This group of seedlings was not sacrificed for biomass measurements so that they could be used in future studies.

Statistical analysis

Differences between resistant and susceptible trees in cone crop size were analyzed using a *t*-test. Differences in the number of viable seeds/cone and viable seed weight from 1992 and 1994 were analyzed using a single-factor MANOVA. If the MANOVA was significant at the 0.05 level, univariate *F*-tests were used to determine which of the variables contributed to the overall significant difference between resistant and susceptible trees. Cone production among resistant, susceptible and moth-removed trees was compared with an ANOVA, and seed parameters of resistant and moth-removed trees were compared with a MANOVA followed by univariate ANOVAS if the MANOVA was significant. Linear regression analyses were performed to compare seed weight to seedling biomass at 3 months and seedling height at 6 months. The biomasses of seedlings grown from seeds from resistant and susceptible trees, and the biomasses of seeds that germinated versus seeds that did not, were compared using *t*-tests. All data presented are means ± 1 SE.

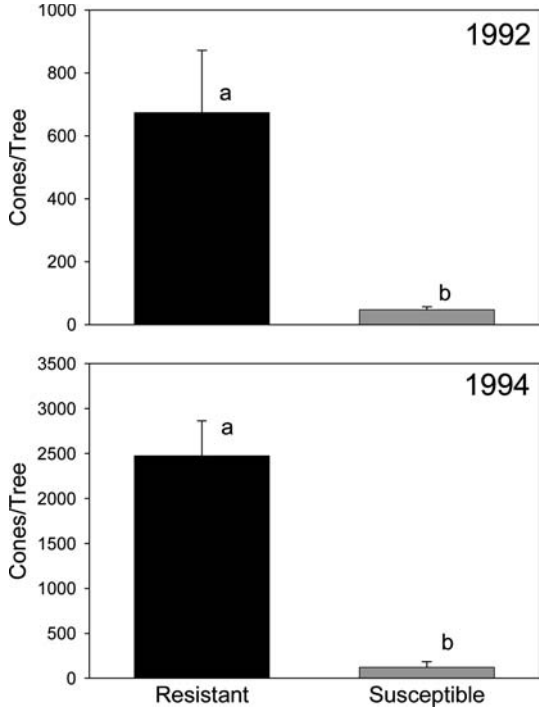

Fig. 1 Cone production of moth-susceptible trees was lower than resistant trees during the mast years of 1992 and 1994. *Different letters* indicate significant differences at $\alpha \leq 0.05$. *1992* Cone production of moth-susceptible pinyons was reduced by 90% in 1992. *1994* Similarly, cone production of susceptible trees was reduced by 93% in 1994

Results

Cone and seed production of moth resistant and susceptible pinyons

Susceptible trees that suffered chronic moth herbivory consistently produced significantly fewer cones than resistant trees. In the 1992 mast year, cone production of susceptible trees was 95% lower than resistant trees ($F_{1,31} = 8.6$, $P = 0.006$). Similarly, in 1994 susceptible trees produced 93% fewer cones than resistant trees ($F_{1,31} = 36.2$, $P < 0.001$) (Fig. 1).

Consistent with the above observations of the negative effects of moth herbivory on cone production, susceptible trees produced fewer viable seeds per surviving cone and seeds with lower mass than resistant trees in both 1992 (Wilk's Lambda = 0.617, $P < 0.001$) and 1994 (Wilk's Lambda = 0.342, $P < 0.001$). In 1992, susceptible trees produced 30% fewer viable seeds per cone ($F_{1,31} = 5.4$, $P = 0.007$), and in 1994, susceptible trees produced 37% fewer viable seeds per cone ($F_{1,31} = 11.94$, $P < 0.001$) than resistant pinyons. As a result of both the decrease in cone production and reduced numbers of viable seeds per cone of moth-susceptible pinyons, the estimated whole-tree viable seed production of susceptible pinyons was 97% lower than resistant trees in 1992 and 96% lower than resistant trees in 1994 (Fig. 2).

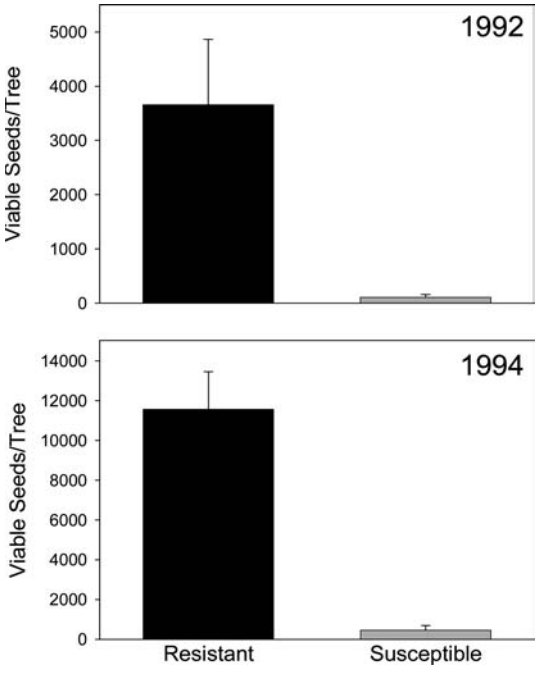

Fig. 2 Whole-tree viable seed production was reduced as a result of the combined effects of decreased cone production and decreased number of viable seeds per cone. *Different letters* indicate significant differences at $\alpha \leq 0.05$. *1992* Whole-tree viable seed production of moth-susceptible pinyons was lower than resistant pinyons in 1992. *1994* Similarly, whole-tree viable seed production of susceptible trees was lower than resistant trees in 1994

Because there is often a trade-off between seed number and seed size (Leishman 2001), susceptible trees with reduced seed production would be predicted to produce seeds of equal or higher mass than resistant trees. However, this hypothesis was not supported; we found the opposite pattern in which seeds from moth-susceptible trees were significantly smaller than resistant trees. In both 1992 and 1994, susceptible trees produced viable seeds that were 18% smaller than resistant trees (1992: $F_{1,31} = 5.53$. $P = 0.007$; 1994: $F_{1,31} = 6.76$, $P < 0.001$).

The impact of herbivory on cones and seeds

Cone production was significantly different among susceptible, resistant and moth-removed trees in both 1992 ($F = 7.68$, $P < 0.001$) and 1994 ($F = 6.7$, $P = 0.002$) (Fig. 3). In 1992, cone production of moth-removed trees was 82% greater than susceptible trees, and resistant trees produced an intermediate number of cones. In 1994, moth-removed and resistant trees produced 89% and 88% more cones than susceptible trees.

Because susceptible trees produced so few cones, we were unable to experimentally assess the effects of her-

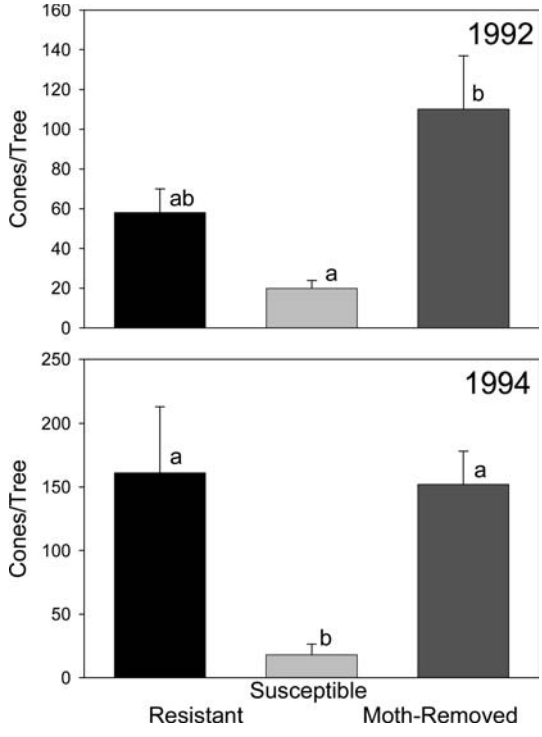

Fig. 3 Cone production of moth-susceptible and moth-removed pinyons during two mast years. *Different letters* indicate significant differences at $\alpha \leq 0.05$. *1992* The cone production of moth-susceptible pinyons was significantly lower than moth-removed pinyons, while the cone production of resistant pinyons was intermediate. *1994* The cone production of both resistant and moth-removed pinyons was significantly greater than susceptible pinyons. The cone production of resistant and moth-removed pinyons did not differ significantly

bivore removal on other relative fitness traits such as seed viability and seed mass. However, when moths were experimentally removed from susceptible trees, we found that the number of viable seeds per surviving cone and seed mass were similar between resistant and moth-removed trees in 1992 (Wilk's Lambda = 0.888, $F = 2.34$, $P = 0.11$), and 1994 (Wilk's Lambda = 0.959, $F = 1.01$, $P = 0.37$). The number of seeds per surviving cone for resistant and moth-removed trees was 4.36 ± 0.57 and 3.97 ± 0.53 in 1992 ($F = 0.49$, $P = 0.49$) and 3.65 ± 0.42 and 3.21 ± 0.45 in 1994 ($F = 0.354$, $P = 0.556$). Despite the cone crop failure of this group of susceptible trees, these data support our findings with larger trees and suggest that the moth is responsible for differences in seed traits between moth resistant and susceptible trees.

With the removal of moths, the mass of seeds produced by susceptible trees appeared to increase relative to moth-resistant trees. Seed mass of moth-removed trees trended to be greater than resistant trees in 1992, and significant differences were found in 1994. In 1992 mean mass of seeds from resistant and moth-removed trees was 0.298 ± 0.012 and $0.334\ \text{g} \pm 0.017$, respectively ($F = 3.11$, $P = 0.084$). In 1994, seed mass of these groups of trees were 0.283 ± 0.013 and $0.326\ \text{g} \pm 0.016$ ($F = 4.70$, $P = 0.037$), respectively.

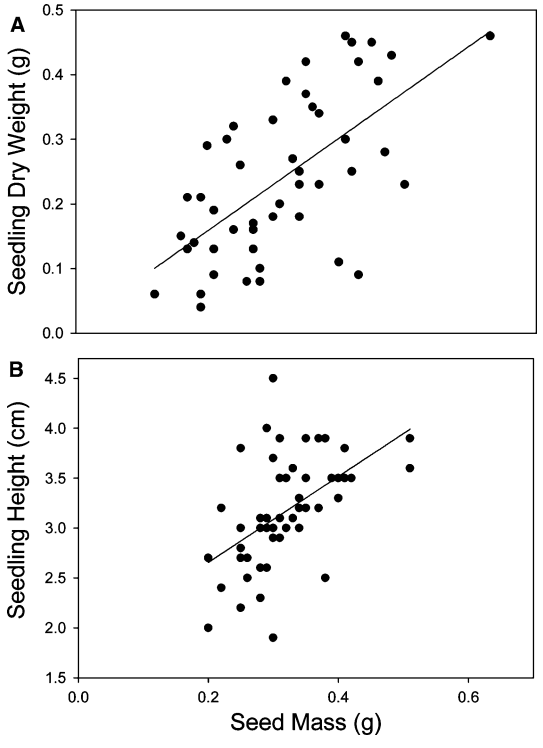

Fig. 4 **a** Seed mass was positively correlated with seedling biomass at 3 months. **b** Seed mass was also positively correlated with seedling height at 6 months. These relationships between seed size and seedling growth suggest that the negative effects of moth herbivory extend to the progeny of moth-susceptible pinyons

Seed size effects on seedlings

Seed mass was positively associated with seedling germination and growth. The mean mass of viable seeds that successfully germinated was $0.302\ \text{g} \pm 0.014$, while the mean mass of seeds that did not produce seedlings was $0.271\ \text{g} \pm 0.016$ ($t = 2.049$, $P = 0.027$).

Seed mass impacted seedling growth in terms of both biomass and height (Fig. 4). Greenhouse experiments showed a significant positive linear relationship between initial viable seed mass and seedling biomass that explained approximately 40% of the variation in seedling biomass ($r^2 = 0.40$, $F = 30.9$, $P < 0.001$, $y = 0.014 + 0.72x$). Furthermore, seedling height after 6 months was significantly positively correlated with initial seed weight ($r^2 = 0.285$, $F = 20.7$, $P < 0.001$, $n = 60$, $y = 1.79 + 4.31x$). These data argue that differences in seedling growth persist over time, even under less resource-limited conditions.

Discussion

Long-term observations and experiments demonstrate that chronic herbivory by the stem-boring moth was the mechanism driving observed patterns of decreased cone and seed production and reduced seed quality (i.e., seed mass) of susceptible trees. We also found that the mass of seeds produced by moth-removed trees appeared to be greater than resistant trees, suggesting a trade-off in resistance and plant performance (Obeso 2002). Determining if resistance to moth herbivory is associated with additional costs requires further study, such as quantifying cone and seed production in additional mast years and measuring other parameters such as shoot and trunk growth.

Although the seed parameters of the younger group of susceptible trees could not be accurately assessed due to a high percentage of trees with cone crop failure, the rebound in cone and seed production and seed mass observed in response to moth removal argues that moth herbivory caused the decline in cone and seed production and seed mass. Reductions in the number of viable seeds per surviving cone and seed mass also suggest that moth herbivory has indirect negative impacts on female reproduction in addition to the direct effects of increased rates of cone mortality found by Cobb et al. (2002).

Because moth attack results in the mortality of cone-bearing shoots (Mopper and Whitham 1989; Cobb et al. 2002), it is not surprising that moth-susceptible trees produced fewer cones than moth-resistant trees. The reductions in seed mass and seed viability that also resulted from moth herbivory were not clearly predicted by the foraging behavior of the moth, but may be due to carbon limitation induced by chronic loss of photosynthetic tissue. Previous studies have shown that susceptible pinyons suffer a variety of negative effects as a result of chronic moth herbivory, including reduced stem (Whitham and Mopper 1985) and radial growth

(Ruel and Whitham 2002) and reduced colonization by ectomycorrhizal fungi (Gehring and Whitham 1991), findings that are consistent with carbon limitation. Severe carbon limitation could result in lower energy allocation to reproduction as well, which would translate into fewer seeds per cone and/or lower seed mass, the patterns we observed in this study.

Insect herbivory has been previously shown to negatively impact seed quantity and quality of woody species. For example, Kaiteniemi et al. (1999) found that repeated simulated defoliation of *Betula pubescens* resulted in a 25% reduction in seed mass and viability. Similarly, Thalmann et al. (2003) found that defoliation of chestnuts by leafminers did not impact the number of seeds per fruit, the number of fruits per inflorescence, or the number of inflorescences per tree, but it did reduce seed and fruit weight by one half. Comparisons between our findings and these studies suggest that impacts of chronic herbivory may be more severe than those resulting from a single or small number of severe defoliation events. In addition, the effects of shoot borers like *D. albovittella*, that damage the tissues that produce fruits and seeds may be more detrimental than the effects of insects that attack tissues less associated with reproduction.

Herbivory and the probability of seedling establishment

The dispersal and survival of seeds from susceptible trees will likely be negatively impacted by moth-induced reductions in cone production, seed production and seed biomass. Because avian seed dispersers avoided pinyon stands and individual trees with low cone crops (Christensen and Whitham 1991; Christensen et al. 1991), they may no longer forage on susceptible trees or in stands dominated by susceptible trees. In the absence of birds, mammals were more likely to utilize the seed crop (Christensen and Whitham 1993). Because seed-dispersing mammals may act as both seed dispersers and seed predators (Vander Wall 1997), their behavior will have an important impact on pinyon-seed fate. Also, because pinyon jays tend to be long-distance dispersal agents, whereas rodents tend to be local dispersal agents, insect herbivory may shift the mean dispersal distance and alter the genetic structure of the pinyon population (Christensen and Whitham 1991; Brown et al. 2001).

Seed mass also affects the probability of successful seedling establishment. Seed mass has been shown to be positively correlated with numerous seedling fitness parameters, such as increased germination rate, accelerated germination timing, and enhanced seedling survival and growth (Gomez 2004). In addition, the number of seeds placed in a single cache by rodents is negatively correlated with seed mass (Vander Wall 2003), which should increase effective seedling recruitment because emergent seedlings are likely to experience less competition from conspecifics (Howe 1989).

In addition to the negative impacts of herbivory on patterns of pinyon recruitment, herbivore-mediated reductions in seed mass result in decreased seedling growth, indicating that the impacts of moth herbivory extend to the progeny of attacked trees. The mass of seeds that germinated was 10% greater than seeds that did not germinate. In addition, we found a positive correlation between seed size and seedling size (Fig. 3). Because differences in seedling size have been shown to affect survivorship, fecundity, and the outcome of biotic interactions, particularly competition (Weiner 1990), the smaller progeny of susceptible trees are likely less vigorous than the progeny of resistant trees. For example, seedlings grown from larger seeds were better able to tolerate herbivory (Armstrong and Westoby 1993), and were also more likely to survive stressful conditions such as drought (Leishman and Westoby 1994). Larger seeds may be particularly important to plants in light of the predicted increased frequency and severity of drought in the Southwest US (IPCC 2001) and associated increases in insect outbreaks (Logan et al. 2003). Although we suspect that differences in seedling performance resulting from variation in seed biomass may be even more pronounced in the field where drought stress and herbivores are present, this hypothesis remains to be tested.

Reduced vigor of seedlings derived from small seeds may in part be associated with their ectomycorrhizal mutualists. Gehring and Whitham (1994) found a strong positive association between seedling size and ectomycorrhizal colonization, although the driver of this relationship was not known. Other studies have also shown that plant species with larger seeds are better able to form mycorrhizal associations than species with small seeds (Muthumukar and Udaiyan 2000; Green and Newberry 2001). Pinyon seedlings derived from small seeds may be less successful in establishing this important mutualism, which in turn negatively affects their subsequent growth and survival. In addition, recent studies showed that insect-resistant pinyons supported different mycorrhizal communities than susceptible trees (Gehring and Whitham 2002; Whitham et al. 2003). Whether these differences were determined by the mycorrhizal associations they obtained as seedlings, or were derived through subsequent interactions with herbivores (Gehring and Whitham 1991), remains to be determined.

Herbivory and the persistence of moth susceptible pinyons

Dioryctria albovittella reduced female function of susceptible trees directly through increased rates of cone mortality on attacked shoots (Whitham and Mopper 1985; Cobb et al. 2002), and indirectly via increased conelet abortion of unattacked shoots (Cobb et al. 2002). These combined mechanisms have resulted in complete lack of female reproduction in a large percentage of susceptible trees. We found that 39% and

60% of the older susceptible trees produced no cones in 1992 and 1994, respectively. Similarly, from 1996 to 1999, Cobb et al. (2002) found that 55% of susceptible trees suffered total cone crop failure.

Although moth herbivory severely limits female reproduction, male function in susceptible trees was often increased. Cobb et al. (2002) found no difference in the male function of younger resistant and susceptible trees, but with increasing age susceptible trees produced 1.5 times more pollen than resistant trees. The elevated rates of pollen production (Cobb et al. 2002) and the higher abundance of susceptible trees within cinder sites (Mopper et al. 1991; Gehring and Whitham 1994; Cobb et al. 1997) may counteract selection against susceptible genotypes (Cobb et al. 2002), and help explain the persistence of this genotype within pinyon populations even under extreme herbivore pressure.

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