CROWDING AND A PLANT'S ABILITY TO DEFEND ITSELF AGAINST HERBIVORES AND DISEASES

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Competition is accepted as an important selective factor for many plant populations. Herbivores and plant diseases can strongly affect the competitive ability of plants (e.g., Chadwick 1960; Nicholson et al. 1970) and can even reverse the outcome of competition between species (e.g., Bentley and Whittaker 1979; Dirzo and Harper 1980). Mechanisms of plant competition include preemption of nutrients, light, water, and other resources; monopolization of limited pollinators and agents of seed dispersal; and interference through allelopathy. The competitive hierarchy of seedlings becomes more pronounced as they grow, resulting in a skewed distribution of fitness (Harper 1977).

Plant crowding affects plant-insect interactions in a variety of ways. Pimentel (1961) presented evidence that increased plant density was associated with reduced diversity and density of herbivorous insects. Kareiva (1983) reviewed many subsequent studies that found a similar negative relationship between plant density and herbivore abundance, but he urged caution in drawing conclusions about mechanisms from these observations.

Several studies have found that herbivores have a more detrimental effect on their host plant when the plants are crowded. Defoliation had a more negative effect on the seed production of Abutilon theophrasti (Malvaceae) when plants were grown at high density (Lee and Bazzaz 1980). Similarly, Ipomopsis arizonica (Polemoniaceae) was less able to compensate for damage caused by herbivores when it was grown with competitors (Maschinski and Whitham 1989). Tobacco seedlings (Nicotiana sylvestris, Solanaceae) responded to damage by increasing foliar alkaloid content (Baldwin 1988). However, "pot-bound" plants did not show this induced increase following damage.

In recent years, the notion that plants are unresponsive victims of herbivores and diseases has been replaced by observations that plants may change following such attacks. Furthermore, some of these induced plant responses make the plant a less suitable host for subsequent infestations by viruses, bacteria, fungi, insects, mites, and vertebrate herbivores (reviews in Horsfall and Cowling 1980; Rhoades 1983; Fowler and Lawton 1985; Raupp and Tallamy 1990). Examples of the reverse also abound; damage to a plant can make it a more suitable host for...
parasites (see, e.g., Hare and Dodds 1978; Williams and Myers 1984; Danell et al. 1985). Although the mechanisms of induced resistance are poorly understood at present, we present evidence that crowded cotton seedlings are less able to defend themselves by induced resistance against leaf-feeding spider mites and against a vascular fungus disease than are less crowded seedlings. Furthermore, the evidence we present suggests that the induced responses involved in this system are active processes rather than properties resulting from the passive deterioration of plant tissue following herbivore or pathogen damage.

INDUCED RESISTANCE IN COTTON

Cotton seedlings show induced resistance to a variety of parasites that challenge it. Seedlings that were root-inoculated with conidia of Verticillium dahliae (the fungus that causes vascular wilt disease) recovered and then became systematically resistant to subsequent symptomatic attacks of this disease (Schnathorst and Mathre 1966; Schnathorst 1981). Resistance can be induced in cotton plants against spider mites and herbivorous insects. The population growth of two-spotted spider mites, Tetranychus urticae, was reduced on plants that had been briefly damaged as cotyledons by feeding mites (Karban and Carey 1984) or by mechanical abrasion (Karban 1985). In the field, populations of T. pacificus were less likely to build up on cotton plants that had been damaged as cotyledons (Karban 1986). Induced resistance in cotton was systemic throughout the plant and was not specific to the herbivorous species. Plants whose cotyledons had been damaged by spider mites were more resistant to beet armyworm caterpillars (Karban 1988) and to symptoms of Verticillium wilt (Karban et al. 1987), in addition to mites, as mentioned above. Conversely, plants that had been exposed to Verticillium fungus supported fewer spider mites than did uninoculated controls (Karban et al. 1987).

The basic experiment showing induced resistance against spider mites was performed 28 times, and the strength of resistance varied considerably (Karban 1987). In this experiment, seedlings were randomly assigned to one of two treatments: a "damaged" treatment and an undamaged "control" treatment. Cotyledons assigned to the damaged treatment hosted T. turkestani mites for 5 days. Plants of the control treatment received no mites. Methods are summarized in figure 1 and described in detail elsewhere (Karban and Carey 1984; Karban 1985). In repeating the basic procedure, many environmental factors have varied among the different experiments (Karban 1987). A post hoc analysis of these experiments suggested that (among many other things) the strength of induced resistance varied in proportion to pot size.

POT SIZE AND THE STRENGTH OF INDUCED RESISTANCE AGAINST MITES

We conducted an experiment to examine the relationship between the strength of induced resistance against spider mites and the size of the pot in which the plants were grown. We used three pot sizes, which held the following soil vol-
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![Diagram of plant experiment](image)

**Fig. 1.—** Schematic diagram of the basic experiment, which showed induced resistance against spider mites. The shaded leaves show the sites of mite releases. (From Karban 1987.)

In the two categories of smaller pot size, plants that had been damaged previously had mite populations no different from those of undamaged controls (fig. 2). In larger pots, however, mite population growth was significantly reduced on plants that had been previously damaged. These results were consistent with our observations from prior experiments. Plants in small pots were small; they may have supported few mites because they provided few resources, lower-quality resources, or both. Since spider mites are leaf feeders, the results were also considered in terms of the number of mites per gram of leaf biomass (table 1). The results were qualitatively similar to those presented in figure 2 in that the two damage treatments were different only for the large pots. In other words, the interaction between pot size and damage treatment was large, although it missed statistical significance (table 1). The results of this experiment suggested that plants in small pots, with access to fewer resources, were less able to show an
induced response sufficient to affect mite population growth. If resources available to the seedlings limit their ability to respond, then we expected that results would be similar if plant density rather than pot size was varied.

PLANT DENSITY AND THE STRENGTH OF INDUCED RESISTANCE AGAINST MITES

To examine the effect of plant crowding on the strength of induced resistance against spider mites, we varied plant density and damage to the cotyledons. Cotton seeds were sown into pots containing 500 ml of soil at the following densities: 1, 2, 4, and 8 individuals per pot. Half of the pots were assigned to be damaged with 8 Tetranychus turkestanii females per plant; the other pots held undamaged controls. The experiment was otherwise identical to the one described above and illustrated in figure 1. Each treatment was performed eight times. Mite population data were analyzed using a two-way analysis of variance (ANOVA), with
CROWDING AND PLANT DEFENSE

**TABLE 2**

**ANALYSIS OF VARIANCE OF NUMBER OF MITES PER PLANT**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage treatment*</td>
<td>15,181</td>
<td>1</td>
<td>15,181</td>
<td>6.9</td>
<td>0.011</td>
</tr>
<tr>
<td>Plant density†</td>
<td>66,600</td>
<td>1</td>
<td>66,600</td>
<td>30.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment × Density‡</td>
<td>9,376</td>
<td>1</td>
<td>9,376</td>
<td>4.3</td>
<td>0.043</td>
</tr>
<tr>
<td>Error</td>
<td>131,962</td>
<td>60</td>
<td>2,199</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Damaged by mites versus undamaged (controls).
† Log$_2$-transformed.
‡ Best-fit linear models: for controls, number of mites $= -39.7(\log_2$ plant density $) + 161.9$; for damaged plants, number of mites $= -18.0(\log_2$ plant density $) + 110.4$.

...damage treatment and plant density as main effects. Plant densities were converted to logarithms (base 2) in all analyses.

For both damaged and undamaged plants, numbers of mites per plant decreased as plant density increased (fig. 3). Not only was plant density significant in the ANOVA of mite numbers, but the damage treatment also explained a significant portion of the variation (table 2). Furthermore, the interaction between damage treatment and plant density was significant (table 2). The difference in mite population growth between damaged and control plants was relatively great when plant density was low, and it disappeared at higher plant densities (fig. 3). These results are similar to those in the pot-size experiment in that induced resistance was apparent only when the seedlings were grown in uncrowded conditions.

Crowded plants did not grow as large as less crowded individuals (data not provided; $F = 87.7, \text{df} = 1,60, P < 0.01$). This effect was not different for the two damage treatments ($F = 0.2, \text{df} = 1,60, \text{NS}$), and damage had no effect on plant biomass ($F = 0.08, \text{df} = 1,60, \text{NS}$). Because crowded plants were smaller, it is...
interesting to determine how mite numbers varied in response to leaf quality and to the amount of foliage available. One way to factor out the difference in leaf quality is to consider the number of mites per gram of leaf biomass (fig. 4; table 3). The number of mites per gram of leaf decreased as plant density increased. Of most interest, the interaction between damage treatment and plant density was significant. This was caused by a large effect of damage when plants were not crowded and a lack of effect when they were.

PLANT DENSITY AND INDUCED RESISTANCE AGAINST *VERTICILLIUM* WILT

An experiment was conducted to determine if induced resistance against the vascular fungus that causes *Verticillium* wilt would be similarly affected by plant density. As in the preceding experiment, we established four plant-density treatments: 1, 2, 4, and 8 plants per pot (500 ml of soil). We randomly assigned plants in each density treatment either to be inoculated with *Verticillium* conidia or to serve as uninoculated controls (10 replicates of each treatment). All plants were removed briefly from the soil at the cotyledon stage. The main root of each plant

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**Fig. 4.**—Plant density versus spider mite population size per gram of leaf biomass.
TABLE 3
ANALYSIS OF VARIANCE OF NUMBER OF MITES PER GRAM OF LEAF BIOMASS WHEN PLANT CROWDING AND DAMAGE TREATMENT WERE VARIED

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage treatment*</td>
<td>331,169</td>
<td>1</td>
<td>331,169</td>
<td>7.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Plant density</td>
<td>3,746,317</td>
<td>1</td>
<td>3,746,317</td>
<td>82.30</td>
<td>0.01</td>
</tr>
<tr>
<td>Treatment × Density</td>
<td>243,432</td>
<td>1</td>
<td>243,432</td>
<td>5.39</td>
<td>0.02</td>
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<tr>
<td>Error</td>
<td>2,731,073</td>
<td>60</td>
<td>45,518</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Damaged by mites versus undamaged (controls).

slated to be damaged was clipped approximately 5 cm below the soil surface, and the remaining root was dipped in a suspension of fungal conidia (approximately 5 × 10^5 conidia per milliliter, mild strain SS-4; for detailed methods, see Schnathorst and Mathre 1966). Roots of controls were clipped and dipped in distilled water. Plants of both treatments were repotted at the assigned density. Root inoculation with conidia caused the foliage of infested plants to appear wilted and to develop necrosis. After 45 days, plants had recovered from the root inoculation. At this time, all plants were challenged by three injections in the upper stem with a suspension of conidia of V. dahliae (strain SS-4; 0.1 optical density, or approximately 10^7 viable conidia per ml). Plants were checked weekly over the next 4 wk, and the percentage of chlorotic or necrotic foliage at peak infection was recorded for each plant. Since the plants in a pot are not independent sampling units, analyses were conducted on the mean for each pot. Data were analyzed using a two-way ANOVA, with percentages normalized by arc-sine transformation and plant densities transformed into logarithms (base 2).

Control plants, which had not been exposed to the root inoculation but were stem-injected, developed disease symptoms on approximately half of the leaf area. This proportion was unaffected by plant density (fig. 5). Other experiments indicate that plant density can affect “noninduced” susceptibility to Verticillium wilt (Brody, Karban, and Schnathorst, MS), but no such effect was found here. Plants previously damaged by root inoculation developed less chlorotic or necrotic leaf tissue when challenged with stem injections of conidia. Furthermore, the level of induced resistance exhibited by these damaged plants was influenced by plant density (i.e., the interaction between damage treatment and plant density was significant in table 4). At low plant density, the strength of induced resistance was greater than at high plant density (fig. 5).

MECHANISMS OF INDUCED RESISTANCE

Induced resistance against both spider mites and symptoms of Verticillium wilt was greater when the seedlings were less crowded. We posit that the plants were less able to show an induced response as they became more crowded. However, this leaves unresolved the question of whether the induced resistance was an active plant response to damage or the direct result of passive removal of plant
**FIG. 5.**—Plant density versus the strength of induced resistance against symptomatic *Verticillium* wilt disease. Error bars show one standard error.

### TABLE 4

**ANALYSIS OF VARIANCE OF THE PERCENTAGE OF LEAF AREA SHOWING DISEASE SYMPTOMS**

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage treatment*</td>
<td>5,714</td>
<td>1</td>
<td>5,714</td>
<td>37.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Plant density†</td>
<td>1,714</td>
<td>1</td>
<td>1,714</td>
<td>11.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Treatment × Density‡</td>
<td>1,340</td>
<td>1</td>
<td>1,340</td>
<td>8.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Error</td>
<td>11,751</td>
<td>76</td>
<td>155</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.**—Percentage of leaf area showing chlorosis or necrosis was arc-sine-transformed.
* Roots inoculated with a conidial suspension or with distilled water.
† Log$_2$-transformed.
‡ Best-fit linear models: for controls, area with symptoms = $0.48(\log_2$ plant density) + 46.34; for damaged plants, area with symptoms = $7.80(\log_2$ plant density) + 18.06.

tissue. Fortunately, the following two models of induced plant responses lead to different predictions.

*Passive Removal*

According to a passive-removal model of induced resistance, the initial damage (caused by either mites or root inoculation of *Verticillium*) results in a smaller or less productive seedling than the control. When this "deteriorated" plant is challenged by a second plant parasite, the parasite does less well than on the control plant. The causal agent is not any chemical or physical change that benefits the plant; rather, the plant is physiologically impaired and, hence, of reduced nutritional quality for the parasite. It helps to envision the host plant as a limited resource. A damaged plant provides less of this limited resource to its
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<table>
<thead>
<tr>
<th>PLANT QUALITY FOR PARASITE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. PASSIVE DETERIORATION</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Damaged</td>
</tr>
<tr>
<td><strong>B. ACTIVE RESPONSE</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Damaged</td>
</tr>
<tr>
<td>Induced Resistance</td>
</tr>
</tbody>
</table>

**PLANT DENSITY**

Fig. 6.—Prediction of models of mechanisms of plant response to damage and to plant crowding. The model of passive deterioration predicts that damage and crowding are additive, and hence, the curves diverge. The active-response model predicts that induced resistance is possible only at low densities, and hence, the curves converge.

Parasites than does an undamaged plant. Crowding also reduces the quantity or quality of the plant as a resource to parasites. The negative effects of crowding and damage act additively or synergistically, making the damaged, crowded plants a lower-quality resource than damaged, uncrowded plants. Thus, this model predicts that the curves representing the quality of damaged and undamaged plants for a parasite should run parallel or diverge as plant density increases (fig. 6A).

**Active Response**

If induced resistance involves an active plant response, then uncrowded plants will have more resources to utilize in this response than will crowded plants. At low plant density, such a response to previous damage reduces plant quality for parasites relative to control plants. However, as plant density increases, plants are less able to respond, and plant quality for parasites becomes similar for damaged and control plants. A model of active plant response predicts that the greatest damage-induced difference in plant quality occurs at low density and that, as density increases, the difference in quality diminishes (fig. 6B). The difference between the two curves at any density is caused by the active plant response.

The important prediction of the model is that, at low plant density, the quality of the plant for the parasite is different between damaged and control plants, whereas, at high plant densities, this difference is reduced and the curves converge. Convergence versus divergence of the curves as a function of plant crowding is more informative than the slopes of either of the curves considered alone. For example, plant quality may increase for both damaged and control plants as plants become more crowded. Even under these conditions, prior damage might
reduce the plant's ability to accumulate induced defenses, and crowding might exacerbate this problem. This scenario produces curves for both damaged and control plants that increase but diverge as a function of crowding. The curves do not have to be linear, as depicted in figure 6B, for this model to apply.

Empirical Evidence

Results from experiments involving both spider mites and *Verticillium* fungus support the model that cotton seedlings are responding actively to damage (figs. 2–5). In both cases, an induced response was apparent when plants were not crowded and diminished as plant crowding increased. This was true even when differences in leaf biomass were accounted for (table 3; figs. 4, 5). Thus, it does not appear that the amount of foliage available to the herbivore is responsible for the effect we observed. Other evidence also suggests that induced resistance in cotton is more than a process of passive removal. Quantity of leaf tissue and other morphological measures did not differ between controls and plants that had been damaged (Karban and Carey 1984; Karban 1987).

There is some evidence in other plant systems for both passive deterioration and active induced responses. Tuomi et al. (1984) argued that induced resistance could be caused by damage that produced nutrient imbalances in the plant. If this mechanism were operating, they reasoned, fertilizing the plant when it was damaged should negate the effects of the damage. They found that birch trees increased foliar concentrations of phenolics following damage to their roots. However, this effect was alleviated when the trees were fertilized, supporting their hypothesis. They did not assess the significance of increased phenolics on birch parasites. In a later set of experiments, Haukioja and Neuvonen (1985) found that fertilization at the time of defoliation did not lessen the induced resistance of birch foliage to caterpillars. Similarly, Bryant (1987, p. 1324, fig. 3) found that fertilizing willows did not reduce the induced response, measured in terms of biomass consumed by hares. Obviously, more work is required before an understanding of the mechanisms of induced resistance is achieved. Because the phenomena currently categorized as "induced resistance" are so diverse, there is no reason to assume that one mechanism applies in every case.

SUMMARY

Populations of spider mites were reduced on cotton seedlings that had been damaged as cotyledons by a brief bout of feeding by mites. However, this induced resistance was found only for seedlings grown in relatively uncrowded conditions. The strength of induced resistance against both spider mites and *Verticillium* wilt diminished as plant density increased. Results were qualitatively similar when differences in foliage quantity were factored out. This suggests that induced resistance in cotton does not result from a passive removal of plant tissue but is caused by an active response. Crowded plants may be unable to express the response to damage. If this result is general, then preventing induced resistance against herbivores and diseases may represent an important and overlooked consequence and mechanism of plant competition.
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LITERATURE CITED


