Induced Resistance and Interspecific Competition Between Spider Mites and a Vascular Wilt Fungus

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The fungal pathogen *Verticillium dahliae* was less likely to cause symptoms of verticillium wilt on cotton seedlings that had been previously exposed to spider mites than on unexposed cotton seedlings. Conversely, populations of the spider mite *Tetranychus urticae* grew less rapidly on seedlings that had been inoculated with *V. dahliae* than on uninoculated controls. Changes caused by pathogen or herbivore attack reduced the suitability of the host plant to a diversity of organisms. This result suggests that highly unrelated organisms that share a host plant may interact strongly.

ALTHOUGH IMMUNIZATION IS THE primary means of disease control in animals, induced resistance in plants has received relatively little attention. Recently plant pathologists have found that restricted inoculation of bacteria, viruses, and fungi can induce resistance in plants against subsequent disease caused by these pathogens (*1*). Restricted infection with fungi has been shown to provide protection against later fungal attacks (*2*). Cotton (*Gossypium hirsutum*) seedlings that had been inoculated at the roots with the fungal pathogen *Verticillium dahliae* (SS-4 strain) developed few symptoms of verticillium wilt disease when they were reinoculated with a conidial suspension of the same strain. By contrast, seedlings that had not been inoculated at the roots showed no induced resistance and developed severe wilt symptoms (*3*). Resistance against a severe strain (T-1) of *V. dahliae* in cotton can be induced by inoculating the roots or infesting soil with a mild strain (SS-4) of the fungus (*4*).

Attacks by herbivorous insects and mites induce chemical and physical changes in many host plants (*5*). In several systems host changes caused by herbivore damage have had deleterious effects on subsequent herbivores. Herbivores fed on damaged plant tissue had lower survival rates (*6–9*), reduced individual growth rates (*7, 9–11*), and reduced adult weight or fecundity, or both (*8, 9, 11, 12*). However, Fowler and Lawton (*13*) have argued that most of these effects are weak and may not exert a strong influence on populations of herbivores.

Populations of spider mites grew more rapidly on new growth of cotton seedlings whose cotyledons were undamaged than on new growth whose cotyledons had been damaged previously by spider mites (*14*). The induced resistance was systemic, protecting parts of the seedling that had not been damaged or had not even been present at the time of damage (*14*). Induced resistance can last for at least 60 days and has been demonstrated in the field as well as in the laboratory (*15*).

Induced resistance can have strong interspecific effects. Resistance against *Tetranychus urticae* was induced by previous feeding of *T. urticae* or *T. turkestani* (*14*) or even by mechanical damage to the cotyledons (*16*). Survival to pupation by *Spodoptera exigua* (Lepidoptera: Noctuidae) was reduced on cotton plants that had previously been exposed to spider mites compared with survival on unexposed controls (*17*). Results of several studies suggest that plant pathogens and herbivorous arthropods may interact by altering the quality of their shared host plant (*18*).

Here we report that changes in cotton seedlings caused by previous exposure to spider mites reduced the probability of infection and severity of symptoms caused by the vascular wilt fungus *V. dahliae*. The interaction was symmetrical, so that populations of spider mites grew less rapidly on cotton seedlings that were infected with *V. dahliae* than on uninoculated controls.

In these experiments, the cotton cultivar Acala SJ-2, which is tolerant to the SS-4 strain of *V. dahliae*, was used. One cotton seed was sown into each of eighty, 3-inch (7.5-cm) plastic pots in a greenhouse. When the cotyledons had expanded, the pots were divided into an experimental group that was infested with mites and an uninfested control group. Each plant in the experimental treatment received eight *T. turkestani* adult female spider mites. Covers made of plastic or acetate were placed over the pots to prevent movement of mites between pots. All plants of both treatments were moved into a growth chamber maintained at 28° ± 2°C during the time that mites were present (*19*).

Fourteen days after mites had been placed on the cotyledons, all plants of both treatments were dipped in a solution of Kelthane (100 ppm dicofol), which killed all mites (*20*). After removal of mites, all plants were moved back to the greenhouse, and temperature was maintained at or below 25°C (*19*). After an additional 14 days, all of the plants were inoculated by hypodermic injection with a conidial suspension of *V. dahliae* (*21*). Over the next 30 days, plants were checked for symptoms of verticillium wilt. No plant measurements were taken for the two treatments because previous studies have shown that exposure of cotyledons to mites at the density used in this experiment does not affect growth or morphology of the seedlings (*14, 15, 22*). The entire experiment was replicated three more times and the development of chlorosis, necrosis, and wilting of leaf tissue was recorded (*23*). Plants were inoculated with conidia 3 days, rather than 14 days, after mites had been removed in the second experimental replicate.

Population growth of *T. urticae* was compared on seedlings that were inoculated with *V. dahliae* and on uninoculated controls (*24*). One seed was sown into each of forty 3-inch pots in a greenhouse. Plants with one true leaf were divided into two groups, those that were inoculated with *V. dahliae* and controls (*21, 25*). Seven days after inoculation with fungus, three adult female *T. urticae* were placed on each plant. Plants

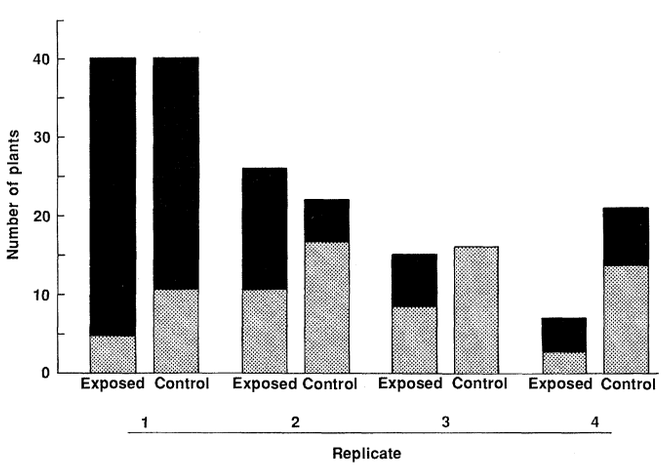

Fig. 1. Effects of mite damage on susceptibility to verticillium wilt. Within each replicate, the number of plants that developed foliar wilting due to verticillium (stippled bars) is less after being exposed to mites than for controls that were not exposed to mites. Shaded bars, no wilting.

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Table 1. Percentage of leaf tissue of each plant that developed chlorosis or necrosis caused by *V. dahliae*.

Mite treatment	Leaf tissue that developed chlorosis or necrosis (%)				
	0	1–25	26–50	51–100	Total
Exposed	15	15	14	5	49
Unexposed control	3	15	14	30	62
Total	18	30	28	35	111

were covered with acetate cylinders and placed in growth chambers at 28° ± 2°C (*19*). Fourteen days later, leaves were removed one at a time to facilitate counting of mites (*26*). This experiment was repeated and growth measurements of leaves and stems were taken (*27*).

Plants that had previously been exposed to mites were less likely than controls to develop foliar wilting caused by *V. dahliae* (Fig. 1) (*28*). Similarly, a smaller percentage of leaf tissue developed chlorosis or necrosis due to verticillium inoculation on plants that had been exposed to mites (Table 1) (*29*). Resistance to verticillium wilt was apparently systemic since plant parts not present during the exposure to mites were protected.

Plants that had been inoculated with *V. dahliae* supported smaller populations of spider mites than controls without the fungus (Fig. 2) (*30*). Plants that had been inoculated with fungus were shorter and had less leaf area than controls although the number of leaves and mean internode length were not different in this (*31*) and other studies (*32*). Some of the negative effect of verticillium wilt on mite populations may be due to the reduction in quantity of leaf tissue caused by fungal infection (*33*).

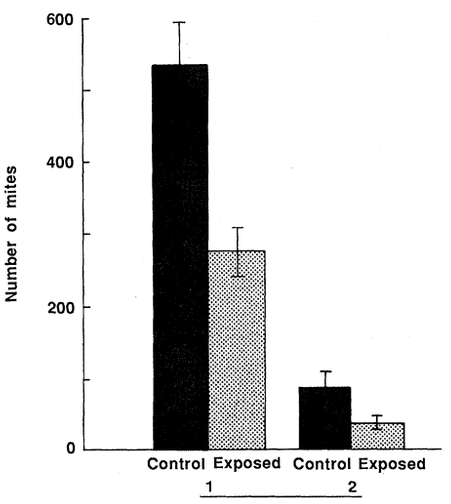

Fig. 2. Mite population growth affected by fungal infection. Mean number of *T. urticae* mites on plants that had been inoculated with *V. dahliae* (stippled bars) was less than the mean number of mites on uninoculated controls (shaded bars). Error bars show 1 SE. Sample sizes were 20 controls and 19 inoculated plants in replicate 1 and 19 controls and 20 inoculated plants in replicate 2.

These results show that *V. dahliae* and tetranychid spider mites can strongly and negatively affect one another. Since mites were no longer present in the first experiment when seedlings were inoculated with conidia, mite feeding must have induced changes in the plant that reduced its susceptibility to the fungus (Fig. 1 and Table 1). The second experiment (Fig. 2) did not indicate whether the reduction of mite populations on seedlings infected with *V. dahliae* was caused by an induced plant response or by some other form of interspecific competition because both fungus and mites were present concurrently.

This demonstration of negative interspecific effects between a fungus and an herbivorous arthropod is unlike others that have been reported (*18*). The interaction is repeatable, it is symmetrical, and it causes statistically significant effects. Experimental inoculations eliminate problems of causality associated with using "natural experiments," unmanipulated by the investigator, which lack adequate controls.

These results suggest that responses of cotton plants, unlike most vertebrate immune responses, lack specificity. They can be triggered by a wide diversity of biological or physical perturbations to the cotton seedling. They also have deleterious effects on a wide variety of organisms that subsequently challenge the plant.

Several possible mechanisms could produce a general resistance pattern. Resistance may result from a physiological change in the plant, which requires no mechanism of recognition or response to a unique component of the attacking pathogen or herbivore (*34*). Such a physiological change need not be adaptive for the plant but may reflect wounding or deterioration of metabolic functions. The first species attacking the plant may deplete the most desirable nutrients or plant parts. Alternatively, resistance may be caused by metabolites that undergo activation or de novo synthesis and accumulate in the plant after attack. Chemicals that accumulate after damage may have activity against a diversity of organisms. For example, phytoalexin isoflavonoids reduce fungal growth and also deter feeding by herbivorous insects (*35*). There is some evidence that cotton tannins provide resistance against both *V. dahliae* (*36*) and herbivorous insects (*37*).

Ecologists have traditionally looked for negative interspecific interactions between species that have close taxonomic relatedness. This was based on the intuition that closely related species should have similar requirements and utilize similar resources (*38*). Recently many ecologists have concluded that interspecific interactions, particularly competition, between terrestrial plant-feeding species are rare and unimportant relative to other factors that affect populations and organize communities (*39*). This study describes a negative interaction between two species that are highly unrelated and that utilize their shared host plant in very different ways. However, both fungus and mites are dependent on the host and both affect it by their presence. Perhaps dependence of herbivores and pathogens on their host and, conversely, the effects of these parasites on the plant provide for better intuition about the importance of competitive interactions than does taxonomic relatedness. If competitive interactions between highly unrelated species, which have received little attention in the past, prove to be common, then ecologists must revise their thinking about the importance of these interspecific effects on the community structure of organisms that utilize plants as hosts.

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19. Mite dispersal was reduced and mite population growth enhanced by placing the plants in growth chambers. However, conditions are more favorable for infection and symptom expression caused by *V. dahliae* in the greenhouse than in a growth chamber.
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21. Three injections were made into the upper stem of each plant with a suspension of strain SS-4 (0.1 optical density or approximately 10^7 viable conidia per milliliter).
22. R. Karban, in preparation.
23. Sample sizes for the second replicate were 26 plants exposed to mites, 21 controls; for the third replicate, 15 exposed, 16 controls; and for the fourth replicate, 7 exposed, 21 controls.
24. *Tetranychus turkestani* females were used to damage the cotyledons because they are more destructive than *T. urticae* [J. N. Simons, *J. Econ. Entomol.* **57**, 145 (1964)]. *Tetranychus urticae* females were used for the bioassay of the effect of wilt because they are more sensitive than *T. turkestani*. It was not possible to remove the fungus once seedlings had been inoculated; hence, this procedure does not distinguish competition from resistance against mites induced by the fungus.
25. Controls were either not injected or received three injections of distilled water into the upper stem. No differences in growth were found between these two types of controls.
26. At 28°C, *T. urticae* complete a full generation in 12 days; thus 14 days allowed at least one generation [J. R. Carey and J. W. Bradley, *Acarologia* **23**, 333 (1982)].
27. We measured stem height from the soil to the top of the plant, stem height above the cotyledon node, number of expanded true leaves, area of the true leaves, and mean internode length.
28. Mite treatment and wilting were not independent in a three-way test: $G = 13.636$, df = 1, $P < 0.005$. The frequency of wilt was not independent of replicate ($G = 44.784$, df = 3, $P < 0.005$) although the interaction between replicates, mite treatment, and wilt was not significant ($G = 6.842$, df = 3, $P > 0.05$). Even under controlled conditions, with cotton of the same variety, mites from the same laboratory cultures, and fungi of the same strain, incidence of mites and wilt varied greatly between replicates in response to subtle environmental changes [see (*22*)].
29. Mite treatment and number of plants with symptoms of chlorosis and necrosis were not independent in a three-way test: $G = 27.022$, df = 3, $P < 0.005$. Leaf symptoms were not independent of replicate but the interaction between replicates, percentage of leaf tissue with symptoms, and mite treatment was not significant.
30. For the first replicate: $t = 3.59$, df = 37, $P < 0.01$; for the second replicate: $t = 1.97$, df = 37, $P < 0.06$. One plant that had been inoculated with fungus in the first replicate and one control plant in the second replicate had no mites and were excluded from the analyses.
31. Mean stem height in centimeters above soil ± 1 SE for controls was 16.75 ± 0.48, for plants with fungus was 12.48 ± 0.44, $t = 6.52$, df = 38, $P < 0.001$. Height in centimeters above cotyledons for controls was 7.70 ± 0.46, for plants with fungus was 4.53 ± 0.38, $t = 5.35$, df = 38, $P < 0.001$. Mean internode length in centimeters for controls was 1.76 ± 0.11, for plants with fungus was 1.45 ± 0.50, $t = 0.61$, df = 38, not significant (NS). Number of leaves for controls was 4.45 ± 0.13, for plants with fungus was 4.50 ± 0.15, $t = 0.25$, df = 38, NS. Leaf area in square millimeters for controls was 936.85 ± 34.21, for plants with fungus was 681.80 ± 56.49, $t = 3.86$, df = 38, $P < 0.001$.
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33. Although plants exposed to fungi were smaller than controls, on average, it seems unlikely that size alone is responsible for the difference in mite populations. Leaf area and mite population do not appear to be positively correlated (for this study $r^2 = 0.075$, df = 37, NS).
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Development of Two Types of Calcium Channels in Cultured Mammalian Hippocampal Neurons

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Calcium influx through voltage-gated membrane channels plays a crucial role in a variety of neuronal processes, including long-term potentiation and epileptogenesis in the mammalian cortex. Recent studies indicate that calcium channels in some cell types are heterogeneous. This heterogeneity has now been shown for calcium channels in mammalian cortical neurons. When dissociated embryonic hippocampal neurons from rat were grown in culture they first had only low voltage–activated, fully inactivating somatic calcium channels. These channels were metabolically stable and conducted calcium better than barium. Appearing later in conjunction with neurite outgrowth and eventually predominating in the dendrites, were high voltage–activated, slowly inactivating calcium channels. These were metabolically labile and more selective to barium than to calcium. Both types of calcium currents were reduced by classical calcium channel antagonists, but the low voltage–activated channels were more strongly blocked by the anticonvulsant drug phenytoin. These findings demonstrate the development and coexistence of two distinct types of calcium channels in mammalian cortical neurons.

THE ENTRY OF CALCIUM THROUGH voltage-gated membrane channels is essential for many neuronal functions (*1*). In the mammalian brain Ca^{2+} entry participates in the generation of various forms of electrical activity, such as dendritic spikes (*2*), rhythmic firing (*3*), normal and epileptiform burst discharges (*4*), as well as in the secretion of neurotransmitters and neuromodulators (*5*). The influx of Ca^{2+} may also couple neuronal activity to metabolic processes and induce long-term changes in neuronal and synaptic activity (*6*). Studies in brainstem slices (*3*) and cultured sensory neurons (*7*) have demonstrated the coexistence of two types of Ca^{2+} channels in some mammalian neurons. We have employed patch-clamp techniques (*8*) and cultured rat hippocampal neurons (*9*) to investigate whether the Ca^{2+} channels in differentiated neurons from a mammalian cortical structure are also heterogeneous (*10*), and to characterize the development and distribution of these channels during neuronal growth.

Hippocampal neurons were dissociated from 18- to 19-day-old rat embryos and maintained in culture for 4 to 6 weeks (*11*). Whole-cell membrane currents were recorded from the somatic region of the neurons (*12*). Calcium currents (I_{Ca}'s) were isolated from other voltage-dependent membrane currents by ionic substitution and addition of sodium and potassium channel blockers

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