Induced Resistance of Cotton Seedlings to Mites

Abstract. *Mite populations grew more rapidly on new growth of cotton seedlings that had never been exposed to mites than on new growth of plants whose cotyledons had been previously exposed to them. Experiments in which a second mite introduction on the exposed plants involved a different mite species produced this same result. The substance or substances responsible for the response are transported systemically among leaves of cotton seedlings.*

Restricted inoculation of bacteria, viruses, and fungi can induce resistance in plants against subsequent diseases that are caused by these pathogens (*1*). Phytoalexins, which are responsible for resistance, have been shown to deter feeding by herbivorous insects (*2*), although the role of these chemicals in plant-herbivore interactions is not well understood. Attacks by herbivores have been shown to induce chemical and physical changes in many host plants (*3, 4*). For several of these systems, there is evidence suggesting that the induced plant changes are associated with lower rates of subsequent herbivory.

Defoliation of larch by the bud moth may produce higher bud moth larval mortality and lower fecundity (*5*). Sitka spruce weevils may exhibit decreased larval success and ovipositional avoidance of *Picea sitchensis* trees that had been attacked previously (*6*). Beet fly larvae that develop on plants that had previously hosted beet flies may suffer greater mortality than flies growing on beets that are attacked for the first time (*7*). Insects reared on foliage of plants that had been artificially defoliated have been reported to feed less (*8*), have prolonged development (*9, 10*), reduced survival (*10, 11*), and reduced weights at pupation (*10*) compared to insects reared on undefoliated foliage. However, results from experiments with simulated herbivory and artificial defoliation may be quite different than results from actual herbivory (*12*). Rhoades experimentally defoliated alder and willows with tent caterpillars and webworms (*13*). Insects fed leaves from trees that had been defoliated grew more slowly than those fed leaves from unattacked control trees. These findings suggest that herbivores may induce plant changes that control subsequent insect populations.

We report that changes in cotton seedlings, induced by prior experimental herbivory, reduced the population growth of phytophagous mites. Cotton plants that were exposed briefly to mites supported much smaller numbers of mites than unexposed controls when mites were reintroduced. The induced plant changes not only affect components of the life table but act transgenerationally to reduce mite populations.

Three species of the spider mite complex (*Tetranychus urticae*, *T. turkestani*, and *T. pacificus*) are major pests of cotton in California (*14, 15*). All three species may occur on cotton, although *T. turkestani* predominates early in the growing season and is replaced by *T. urticae* and *T. pacificus* in the middle to late season in the San Joaquin Valley (*15, 16*).

Three cotton seedlings (Acala SJ-2) were grown in each of 24 4-inch plastic pots maintained at 28° ± 2°C in growth chambers. The plants were randomly assigned to an experimental group which was infested with mites or to a control group which was not. Each plant in the experimental treatment received 16 *T. urticae* adult females as soon as the cotyledons had unfolded. Plastic acetate cylinders were placed on top of each experimental and control pot to prevent emigration or immigration of mites. Five days after the mites had been placed on the seedlings, both experimental and control plants were dipped in a Kelthane solution (100 ppm), which killed all mites (*17*). Twelve days later, three *T. urticae* females were placed on the most recently expanded new leaf of each plant of both treatments and cylinders were again placed on top of each pot. By this time, the cotyledons had dried and many had fallen off. As true leaves mature, mites move up the plant (*18*) and no mites were found on the cotyledons. After 14 more days, each leaf was removed, and mite populations were counted (*19*). The entire experiment was repeated with eight *T. turkestani* females for the first introduction, followed by three *T. urticae* females for the second introduction, as described above (*20*).

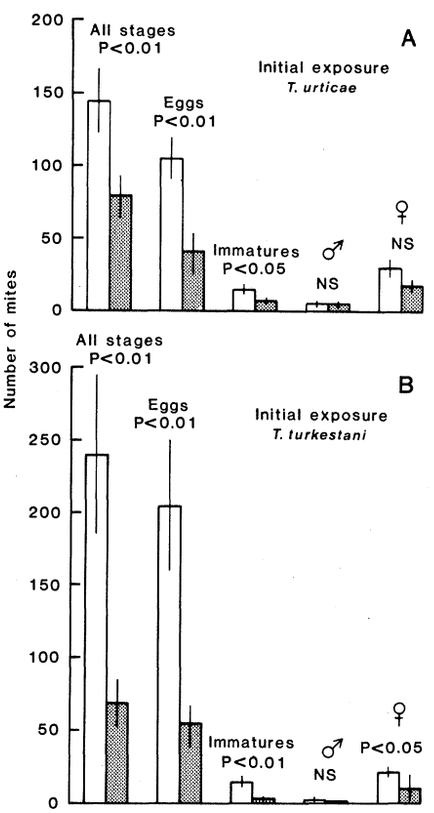

Fig. 1. Mean number of *T. urticae* on plants that had been previously exposed to mites (shaded bars, $n = 12$) and controls with no previous exposure (open bars, $n = 12$). Standard errors of the mean are shown. Results are shown for (A) *T. urticae* as the first species and (B) *T. turkestani* as the first species (*22–24*).

Plants that had been exposed to mites during the cotyledon stage showed no essential differences from the unexposed controls in appearance, size, or number of leaves produced (*21*). Fewer mites, of all stages, were found on plants that had previously been exposed than on unexposed controls (Fig. 1) (*22*). This was true when the first and second exposures were of the same mite species (Fig. 1A) and of different species (Fig. 1B). Much of this difference between the previously exposed seedlings and unexposed controls is due to greater numbers of eggs on the latter (*23*). Differences in the numbers of immatures and adults were smaller, although in some cases significant (*24*). It is not clear from this experiment whether differences in survival or fecundity (or both) are responsible for these results.

The induced factors that are responsible for reduced mite population growth are not known. They must be transported through the plant since the initial group of mites fed only on the cotyledons and the second group fed on the true leaves. These experiments show that the response is not species specific; an initial exposure to *T. turkestani* inhibited subsequent population growth of *T. urticae*.

This system is unlike others that have been reported (*4–13*). Resistance can be induced experimentally and repeatedly by briefly exposing the plants to herbivores under controlled conditions. The basic experiment has now been repeated eight times with similar and statistically significant results (*25*). Experimental inoculations eliminate problems of causality associated with using uncontrolled "natural experiments." The use of her-

bivores rather than artificially induced damage is also preferable. The induction that we describe produced large reductions in the populations of herbivorous mites. For the most part, other investigators considered only plant changes or, at most, the impact of induced changes on bioassays measuring insect performance, rather than the impact on herbivore populations. The induced changes that we describe did not dissipate for at least 12 days.

The finding that mites can induce resistance to subsequent herbivory is potentially of great importance in pest and disease control. It may be possible to inoculate a plant against various herbivores and pathogens. A more thorough understanding of the mechanism and specificity of the induced resistance should allow facilitation of a practical inoculation.

RICHARD KARBAN
JAMES R. CAREY
Department of Entomology, University of California, Davis 95616

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19. At 28°C, tetranychid mites 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)].
20. Eight *T. turkestani* females were placed on each seedling rather than 16 *T. urticae*; *T. turkestani* are more damaging to cotton cotyledons than *T. urticae* [J. N. Simons, *J. Econ. Entomol.* **57**, 145 (1964)]. It was not possible to repeat the experiment with *T. turkestani* as the species for the second mite introduction because *T. turkestani* individuals are more sensitive to the miticide than are *T. urticae*. The procedure follows the phenology in the field where *T. turkestani* often precedes but rarely follows *T. urticae*.
21. No statistically significant differences were found between plants of the two treatments for six plant characteristics (R. Karban, in preparation): total stem length, stem growth during the experimental period, mean internode distance, total leaf area (including cotyledons), mean area of true leaves, and number of leaves.
22. For *T. urticae* as the first species: $F(1, 22) = 9.80$, $P < 0.01$. For *T. turkestani* as the first species: $F(1, 22) = 9.32$, $P < 0.01$.
23. For *T. urticae* as the first species: $F(1, 22) = 11.32$, $P < 0.01$. For *T. turkestani* as the first species: $F(1, 22) = 9.01$, $P < 0.01$.
24. For *T. urticae* as the first species: immatures, $F(1, 22) = 4.32$, $P < 0.05$; males, $F(1, 22) = 0.50$, not significant; and females, $F(1, 22) = 2.45$, not significant. For *T. turkestani* as the first species: immatures, $F(1, 22) = 8.40$, $P < 0.01$; males, $F(1, 22) = 1.58$, not significant; and females, $F(1, 22) = 5.17$, $P < 0.05$.
25. At the mite densities reported for the initial inoculation, control plants built up populations of mites that were approximately twice the size of mite populations on unexposed controls. The size of the final mite population buildup is inversely correlated with the size of the initial mite exposure (R. Karban, in preparation).
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Simian Sarcoma Virus–Transformed Cells Secrete a Mitogen Identical to Platelet-Derived Growth Factor

Abstract. *Normal rat kidney (NRK) cells transformed by simian sarcoma virus (SSV) release into the culture medium a biologically active mitogen with properties identical to those of human platelet–derived growth factor (PDGF). Like PDGF, the growth factor derived from SSV-NRK cells was shown to be stable to heat and sensitive to reducing agents. It was capable of inhibiting binding of labeled PDGF to the receptor on human fibroblasts. It also stimulated the phosphorylation of the same membrane protein (185 kilodaltons) in isolated plasma membranes from human fibroblasts. Immunoprecipitation of metabolically labeled proteins released by SSV-NRK cells showed that a 34-kilodalton protein was specifically precipitated by antiserum to PDGF. Upon reduction, this protein had a molecular size of 17 kilodaltons. PDGF has been shown to consist of two 14- to 18-kilodalton proteins linked by disulfide bonds.*

Investigations of human platelet–derived growth factor (PDGF), a potent mitogen for mesenchymal-derived cells (*1*), have provided insight into the mechanism by which the v-*sis* oncogene of the simian sarcoma virus brings about cell transformation. PDGF represents the major growth factor activity in human serum and normally circulates stored in the α-granules of platelets (*2*). PDGF has been isolated and characterized from serum (*3*), platelets (*4, 5*), and platelet-rich plasma (*6*). Elucidation of the amino-terminal amino acid sequence of PDGF has shown that it consists of two homologous polypeptide chains (PDGF-1 and PDGF-2) linked by disulfide bonds (*7*). Computer analysis of the partial sequence of PDGF revealed a homology with the transforming gene product $p28^{sis}$ of the simian sarcoma virus (SSV) (*8*), an acute transforming retrovirus of primate origin (*9, 10*), implying that the two proteins arose from the same or closely related genes. This relation has been confirmed by others (*11*). More recent studies (*12*) have shown that $p28^{sis}$ and PDGF show antigenic similarities and a common structural configuration.

These studies provided a basis for understanding the process by which the v-*sis* gene induces cell transformation, apparently involving the constitutive expression of a PDGF-like protein. However, it is not yet known whether the action of the v-*sis* product is mediated through an extra- or intracellular signal. The extracellular signal would require the release of the gene product into the extracellular space with subsequent binding to specific cell membrane receptors in a manner similar to that established for PDGF. We now report that SSV-transformed cells release a biologically active mitogen that has properties similar to those of human PDGF.

The conditioned medium obtained from SSV-NRK (normal rat kidney) cells stimulates the incorporation of [^{3}H]thymidine into quiescent BALB/3T3 cells (clone A31) (*4*) (Table 1). When approximately 50 μl of conditioned medium was concentrated to one-fifth of its original volume, the activity of the concentrate was similar to that of 5 ng of pure PDGF. Conditioned medium obtained from untransformed NRK cells did not exhibit significant activity. The heat stability and sensitivity to reducing agents of the SSV-NRK mitogen and PDGF were compared (Table 1). Both the SSV-NRK mitogen and PDGF retained 90 percent of their activity after being boiled for 10