epinephrine per kilogram once or on succeeding days. In rats without ligatures, that dose caused no adverse effects.

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Cucumber Beetle Resistance and Mite Susceptibility Controlled by the Bitter Gene in Cucumis sativus L.

Abstract. *Antibiotic and nonpreference mechanisms are related in cucumber through the action of the* bi *gene and the absence of cucurbitacins. Cucurbitacins attract cucumber beetles and cause feeding whereas they have an antibiotic effect on two-spotted mites.*

Insects cause tremendous economic losses to agriculture, both directly and indirectly from the cost of control measures. Chemical insecticides cause additional concern because they contribute to environmental pollution. The development of cultivars that are resistant to insect attack would seem to be an ideal method of control. Three mechanisms of insect resistance are tolerance, antibiosis, and nonpreference (*1*).

Nonpreference is due to the absence of an attractant or the presence of a repellant. Antibiosis refers to adverse effects on the biology of the insect. These mechanisms are generally assumed to be independent, but we find they are related and that resistance to specialized insect pests (cucumber beetles) is related to susceptibility to general pests (two-spotted mites) in cucumber, *Cucumis sativus* L.

A class of tetracyclic triterpenoids called cucurbitacins found in the Cucurbitaceae are specific feeding attractants for cucumber beetles of the Crysolimadeae (*2*), and a quantitative relationship exists between these substances and damage by these insects. Cucumbers of the *bibi* genotype completely lack cucurbitacins (*3*) and would seem to be useful as types resistant to cucumber beetles. However, we wondered what was the role of cucurbitacins in the economy of the plant (*4*).

In order to study the effects of cucurbitacins in vivo on the biology of nonspecific pests of cucurbits, "isogenic populations" (*5*) were created by crossing 'Marketer' and 'Eversweet' cultivars of *Cucumis sativus* L. and backcrossing the F_1 hybrid to 'Eversweet.' The resulting populations segregated one bitter plant (*Bibi*) for each nonbitter plant (*bibi*). The two phenotypes were identified by tasting the cotyledons or by a chemical test (*3*) and were transplanted to the field or to pots in the greenhouse.

Cucumber field plots of 50 bitter (*Bibi*) and 50 nonbitter (*bibi*) plants were artificially infested with two-spotted mites, *Tetranychus urticae* Koch. After 1 month, the nonbitter plants were nearly dead and showed signs of much mite feeding whereas the bitter plants were relatively free of mites. It was obvious that nonbitter plants had many more mites and had suffered much more damage from mite feeding than the bitter plants. Differential oviposition does not seem to be a factor in these results since equal numbers of eggs were laid on each phenotype.

Many more mites of all ages were able to develop on nonbitter than on bitter cucumber plants in greenhouse experiments (Table 1). The largest differences, however, were between larvae and young adult mites or the nymphal stages. Feeding on bitter plants seemed to have a deleterious effect on the development and growth of the early larval stages.

The length of feeding period had an effect on the mortality of mite larvae. Larvae were reciprocally transferred after various feeding periods on either bitter or nonbitter hosts. Those designated "long feeding period" were larger and darker due to ingested plant pigments as compared to the smaller, lighter colored "short feeding period" mites. These larval classes differed in age by about 2 days, depending on the temperature. The handling of the young

Table 1. Larval mortality of two-spotted mites on three genotypes of cucumber. Data pooled from oviposition of five females during 2 days; three replications of five plants per replication.

Host	Genotype	Larvae (No.)	Nymphs (No.)	Larval mortality (%)
Bitter	*BiBi*	1072	13	98.79
Bitter	*Bibi*	896	34	96.21
Nonbitter	*bibi*	1592	1512	5.03

Table 2. The influence of feeding duration on bitter or nonbitter plants on larval mortality of two-spotted mites. In the first experiment below, the larvae fed on primary and secondary hosts of the same genotype. In the second and third experiments below, the larvae fed on primary and secondary hosts that differed in genotype. In addition, the duration of feeding was varied before the mites were transferred from one host to the other in these two experiments.

Secondary larval feeding host	Primary larval feeding host	Larvae* (No.)	Nymphs (No.)	Larval mortality (%)
Nonbitter	Nonbitter	150	127	15
Bean	Bean	100	76	24
Bitter	Bitter	150	34	77
	Long feeding period			
Bitter	Nonbitter	150	113	25
Nonbitter	Bitter	200	32	84
Bean	Bitter	350	92	74
	Short feeding period			
Bitter	Nonbitter	100	14	86
Nonbitter	Bitter	150	100	33
Bean	Bitter	100	78	22

* Each host plant received 50 larvae.

mites seemed to have some harmful effects, but the mites which had fed for a long period on the bitter plants had a mortality rate more than three times that of those which had either a short feeding period on bitter plants or which had fed on beans or nonbitter cucumber plants (Table 2).

Attractants evolved as protectants against herbivore feeding and their role as attractants came later as certain groups such as the cucumber beetles overcame this resistance (*4*). It became an advantage to these adapted insects to have access to a food source on which they alone could feed without ill effects. Thus, cucumber beetles evolved the ability to respond positively toward plants containing cucurbitacins. However, cucurbits retain their chemical protection against mites and presumably against other pests which have not evolved detoxifying mechanisms.

Thus, we feel that cultivars resistant to insects through the nonpreference mechanism, where the selected genotypes lack an attractive substance, may be susceptible to attack by other insects. The nonpreference and the antibiotic mechanisms of insect resistance are not independent in the Cucurbitaceae and this is probably also true of many other plant-insect relationships. Insect attractants are commonplace (*4*) and their evolution from protectants to attractive substances through insect coevolution seems the best explanation of these paradoxical chemicals (*2*).

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Antiviral Resistance by the Polyinosinic Acid–Poly(1-vinylcytosine) Complex

Abstract. *The antiviral activities of analogs of the double-stranded complex of polyinosinic and polycytidylic acids [poly(I)·poly(C)], which is a potent interferon inducer, have been studied. Structural changes that modify the polymer backbone substantially, such as loops or 2′ → 5′ phosphodiester bonds, lead to decreased antiviral activity. Unexpectedly, however, the complex of polyinosinic acid and poly(1-vinylcytosine), which is only a much more distantly related analog of poly(I) • poly(C), shows high activity. It is postulated that the high activity is related to the reduction of the charge/mass ratio and to the existence of this complex in an aggregated state; these are two factors that generally enhance the uptake of compounds by cells.*

Several polyanions are able to protect mammalian cells against viruses, the principal mechanism being the induction of formation of an antiviral protein, interferon. The double-stranded complex of polyinosinic and polycytidylic acids [poly(I) • poly(C)] is more effective than other polymeric inducers —for instance, by a factor of 10^5 compared with the synthetic polycarboxylates, which, furthermore, are inactive in vitro (*1, 2*). With a view to improving the stability of the complex to enzymatic hydrolysis and decreasing the level of toxic side effects, a number of poly(I) • poly(C) analogs were prepared and studied. Earlier studies suggested that the poly(I) • poly(C) system is very sensitive to structural alteration. If the ribose moieties of either chain are replaced by deoxyribose, the complex has a similar double helical structure, but its activity is lost (*2*). Thus, a certain type of backbone is required to obtain a very active inducer. Several compounds structurally very close to poly(I) • poly(C) have been prepared and studied. Replacement of the phosphate oxygen by sulfur slows down the enzymatic hydrolysis, and the corresponding polymer is a good interferon inducer (*3*). We studied poly(I) • poly(C) analogs that contained a drastically altered poly(C) component and, surprisingly, found one which is highly active.

The compounds tested and the experimental findings are collected in Table 1. Assays for antiviral activity were carried out with cultures of human skin fibroblasts at 24°C, a temperature below the dissociation temperature (T_m) of the least stable complex. Bovine vesicular stomatitis virus (VSV) was used as a challenge virus. Induction of cellular resistance to the viral infection was measured colorimetrically (*4*) and by virus titer reduction (*2*). The production of extracellular interferon was also measured indirectly by colorimetry (*4*). The amount of extracellular interferon produced by the cell line used was very low [poly(I) • poly(C) inducing about ten times more interferon than poly(I) • poly(VC)]. (The meaning of VC is explained in the next paragraph.) Interferon was characterized by its sensitivity to trypsin and resistance to ribonuclease (*5*). Thus interferon was definitely present; on the other hand, not all the antiviral action of the compounds tested need necessarily stem from interferon induction. Components of complexes, when tested separately at the same concentrations, did not show any antiviral protection ability.

Poly(I) • poly(C) was tested as the standard inducer. The results in Table 1 are in agreement with data described previously (*1, 2*). Among the compounds tested a considerable activity was also shown by poly(I) • poly(VC). In this analog poly(I) is complexed with high-molecular-weight poly(1-vinylcytosine), $[-CH(1\text{-cytosyl})-CH_2-]_n$, containing about 7 percent of uracil residues (*6*). There are three major differences, compared to poly(I) • poly(C). First, the cytosine strand lacks the regular sugar phosphate backbone and does not carry any electric charge. Second, the complex contains four cytosine residues for each inosine residue, and thus must display some looping in the poly(VC) strand. Third, the complex has a tendency to form large aggregates which can be easily sedimented (1 hour at 10,000 rev/min) when the aqueous propylene glycol in which it was prepared (*6*) is replaced by aqueous buffer ($10^{-2}M$ phosphate buffer, *p*H 7.0; $10^{-3}M$ $MgCl_2$; $0.15M$ NaCl) through extensive dialysis.

To elucidate reasons why, in spite of these differences, the activity was conserved, three other analogs were studied. The first analog, poly(I) • poly(CU), has the helical structure of the poly(I) • poly(C) complex, but inter-