not be necessary since exclusive anti-isotypic immunization does not abrogate the immunosuppressive capacity of OKT3, as is illustrated by the patient described above (Fig. 3) (*12–14*).

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Methylcyclopentanoid Monoterpenes Mediate Interactions Among Insect Herbivores

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Beetles secrete an array of chemicals generally believed to mitigate attack by predators. Methylcyclopentanoid monoterpenes secreted by larvae of the willow leaf beetle, *Plagiodera versicolora*, deter feeding by conspecific adults. Furthermore, the secretion elicits a strong repugnancy response in larvae of another willow herbivore, *Nymphalis antiopa*. Leaves bearing beetle larvae are less likely than leaves not bearing beetles to be frequented and consumed by *Nymphalis* larvae. Predator defense may not be the sole function of glandular secretions produced by herbivorous insects; secretions may also mediate interactions among herbivores that use a common resource.

MANY SPECIES OF LEAF BEETLES belonging to the family Chrysomelidae have evolved exocrine glands that produce an array of chemicals (*1, 2*). Typically, nine pairs of glands line the dorsal margins of the thorax and abdomen of larvae. When disturbed, larvae evert these glands and expose drops of secretion (*3, 4*). The chemical composition of these secretions varies within and among species and is influenced by host plant chemistry in some species (*1, 2, 4*). The major classes of chemical compounds secreted by the Chryosomelinae include six types of methylcyclopentanoid monoterpenes, salicylaldehyde, benzaldehyde, juglone, and phenyl esters (*2, 4*).

The ecological role of exocrine secretions in the Chrysomelidae is generally believed to be one of defense against vertebrate and invertebrate predators (*5*). However, of the many trophic associations of leaf beetles, predators pose only one threat. Because of their habit of feeding on leaf surfaces, many Chrysomelidae larvae share their food with a variety of vertebrate and invertebrate herbivores. In addition to competing for resources, small herbivores risk the danger of being consumed by larger ones as they graze (*6*). One solution to this problem is to feed selectively on plant parts unlikely to be eaten by large consumers (*6*). An alternative is to defend the occupied resource chemically and make it unattractive to other consumers. While this latter possibility has received little attention, it is the focus of this paper.

The leaf beetle *Plagiodera versicolora* (Laich.) is an oligophagous herbivore whose larvae and adults consume the foliage of willows and poplars (*7*). The chemical compositions of glandular secretions produced by *Plagiodera* larvae are mixtures of methylcyclopentanoid monoterpenes. Compounds reported in the secretion include plagiodial, epiplagiodial, chrysomelidial, epichrysomelidial, and plagiolactone (*1, 2, 8, 9*). Analysis of the glandular secretions of beetles used in this study confirmed the presence of two methylcyclopentanoid monoterpenes similar to those found in other American beetle populations (*10*).

The genus *Salix* includes one of the largest faunas of invertebrate herbivores (*11*). In view of this rich diversity, the potential for interactions between species is great. For larvae of *Plagiodera* this situation is further complicated by adults feeding on the same plant. We tested the hypothesis that *Plagiodera* larvae can influence the feeding behavior of other herbivores and that this effect is mediated by the glandular secretion. A series of bioassays examined intraspecific interactions between *Plagiodera* larvae and adults (*12*). We first investigated the ability of beetle larvae to exclude adults from a resource. In each petri plate, five first-instar *Plagiodera* larvae were placed on one of two randomly chosen leaf disks (0.32 cm^2) punched from a single leaf. Two adult leaf beetles were added to each plate and allowed to feed. At the end of 7 hours, leaf disks bearing *Plagiodera* larvae were eaten less than disks without larvae ($T = 34$, $n = 18$, $P < 0.024$, Wilcoxon matched-pairs signed-ranks test) (Fig. 1).

We were concerned that the mere presence of larvae rather than the defensive secretion might prevent adult beetles from consuming a leaf. To clarify the role of the defensive secretion in the interaction, we conducted two bioassays. In the first, larvae were frozen to make them incapable of secreting chemicals and placed on leaf disks at a density of five larvae per disk; the remainder of the assay was conducted and evaluated as the previous one had been. The second set of assays used defensive secretions only. As before, leaf disks were placed in petri plates. One disk was treated with the secretion of two *Plagiodera* larvae expressed by pinching the caudae of the larvae and wiping the secretion on the leaf surface. Two *Plagiodera* adults were added to the plates and allowed to feed for 50 minutes. Leaf disks protected by frozen larvae or the secretion alone were consumed less than unprotected disks (dead larvae, $T = 12$, $n = 20$, $P < 0.005$); larval secretion, $T = 1$, $n = 6$, $P < 0.031$) (Fig. 1).

Plagiodera larvae were able to exclude adults of their own species from a common food resource. Furthermore, this exclusion was mediated by the glandular secretion. Leaf disks with dead beetle larvae were consumed less than those without larvae. The mode by which adult leaf beetles detect and avoid incapacitated larvae is unknown. However, defensive secretions adhere to the cuticle of Chrysomelidae (*13*). Perhaps the cuticle of dead larvae was contaminated with traces of secretion that were detected and avoided by adults.

We investigated interspecific interactions

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of *Nymphalis antiopa* larvae with *Plagiodera* larvae. One of the more dramatic responses of *Nymphalis* larvae to adverse stimuli is the voiding of the gut contents. We quantified the strength of this emetic response by collecting the vomit on a filter-paper disk and measuring the area of filter paper stained green. To test the repugnancy response of *Nymphalis* to *Plagiodera*, beetle larvae were placed on willow leaves in petri plates at densities of 0, 1, 5, and 10 per leaf; an additional treatment of ten frozen larvae was also used. Individual *Nymphalis* larvae exhibited strong repugnancy responses to the leaf beetle larvae after 1 hour. The strength of this response depended on density, with more *Plagiodera* larvae eliciting a greater response [analysis of variance, $F(4, 45) = 20.042$, $P < 0.001$] (Fig. 2). This relation was probably due to increased encounters between *Nymphalis* and *Plagiodera* larvae. In addition, *Plagiodera* larvae incapable of secreting elicited no repugnancy response. A second bioassay further implicated the secretion as an interspecific feeding deterrent. Leaf disks treated with *Plagiodera* secretion were less eaten by *Nymphalis* larvae after 30 minutes ($T = 0$, $n = 8$, $P < 0.004$). The amount of treated leaf disk eaten in half an hour was 3 ± 2.2 mm^2 ($\bar{X} \pm$ SEM) compared with 22 ± 3.8 mm^2 for untreated disks.

We conducted the final series of tests to determine whether *Plagiodera* larvae excluded *Nymphalis* larvae from a common resource. The experimental system consisted of two willow shoots removed from the same branch, each bearing two leaves of equal age (Fig. 3). The cut ends of the shoots were placed in water-filled vessels capped with a rubber septum. The septum was coated with petroleum jelly to prevent egress of larvae during the assay. Ten first-instar larvae were placed on one leaf. After approximately 45 minutes, when beetle larvae were well aggregated and feeding, four *Nymphalis* larvae were introduced into the system. Two were placed on the leaf holding the willow beetles, and the other two were placed on the corresponding leaf on the shoot lacking *Plagiodera* larvae. At 10-minute intervals for a period of 2 hours, the locations of all *Nymphalis* larvae were recorded. At the end of 2 hours, each leaf was measured to determine how much had been consumed by the caterpillars.

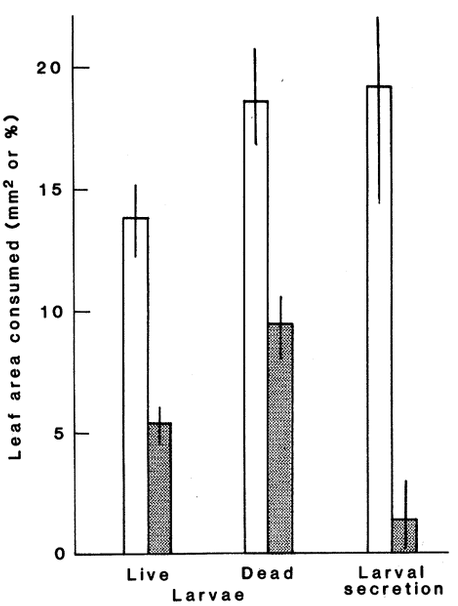

Fig. 1. Leaf area consumed by *P. versicolora* adults offered leaf disks with (shaded bars) or without (open bars) live ($n = 18$) or dead ($n = 20$) *P. versicolora* larvae or their glandular secretion ($n = 6$). Consumption was measured in square millimeters for trials with larvae and as the percentage of the disk consumed for trials with secretion. Bars represent means and vertical lines, standard errors.

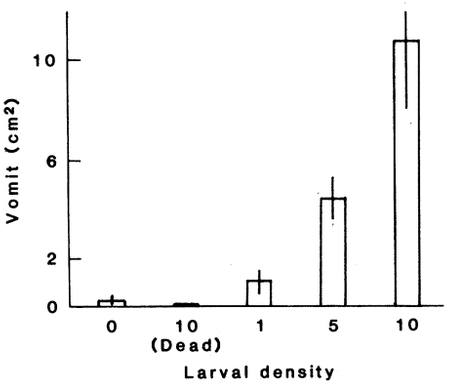

Fig. 2. Vomit produced by *N. antiopa* larvae ($n = 10$) in response to various densities of *P. versicolora* larvae. The legend is as for Fig. 1.

Nymphalis larvae were observed about twice as frequently on the shoot lacking *Plagiodera* larvae ($T = 37.5$, $n = 18$, $P < 0.024$) (Fig. 3). An analysis of the within-shoot location revealed that *Nymphalis* larvae were uniformly distributed over leaves and stem on the shoot without *Plagiodera* [$\chi^2(2) = 1.361$, $P < 0.1$, Friedman's method for randomized blocks] (Fig. 3). On the shoot with *Plagiodera*, however, *Nymphalis* larvae were not uniformly distributed [$\chi^2(2) = 7.513$, $P < 0.025$]. The position least frequently occupied was the leaf with *Plagiodera* larvae, and the most frequented position was the stem. The pattern of *Nymphalis* feeding corroborates the pattern observed in the previous assay. The amount of leaf area consumed on the shoot with *Plagiodera* was significantly less than that on the shoot lacking larvae ($T = 37$, $n = 18$, $P < 0.024$) (Fig. 3). For the shoot lacking *Plagiodera*, no differences between leaves were found in leaf area eaten ($T = 53$, $n = 18$, $P < 0.1$). However, for the shoot with *Plagiodera*, the leaf bearing willow larvae was less eaten than the one lacking larvae ($T = 36$, $n = 18$, $P < 0.024$).

Although we have demonstrated that a chemical produced by larvae deters feeding by adults of the same species, this possibility has been suggested before (*14*). That *Plagiodera* adults avoid larvae and their secretion is consistent with distributions of beetles observed in the field (*15*). In more than 1000 observations of *Plagiodera* adults and larvae feeding synchronically on the same tree, the two stages have never been observed feeding on the same leaf simultaneously (*12*).

The repugnancy response of *Nymphalis* larvae to *Plagiodera* larvae resembles enteric discharges of other insects to adverse biotic stimuli (*16*). *Nymphalis* larvae failed to respond to nonsecreting beetle larvae; therefore, the defensive secretion alone seems to mediate emesis in *Nymphalis*. Enteric discharges such as those of *Nymphalis* larvae are thought to be defensive in function (*16*). Close encounters between the immature stages of these willow herbivores initiate reciprocal, antagonistic chemical exchanges.

It has been suggested that small herbivores should select feeding sites on plants unlikely to be grazed by larger herbivores (*6*). An alternative solution exists if the small herbivore can defend itself and its immediate food resource. Methylcyclopentanoid monoterpenes secreted from eversible glands enable *Plagiodera* larvae to repel larger herbivores chemically and reduce their feeding at

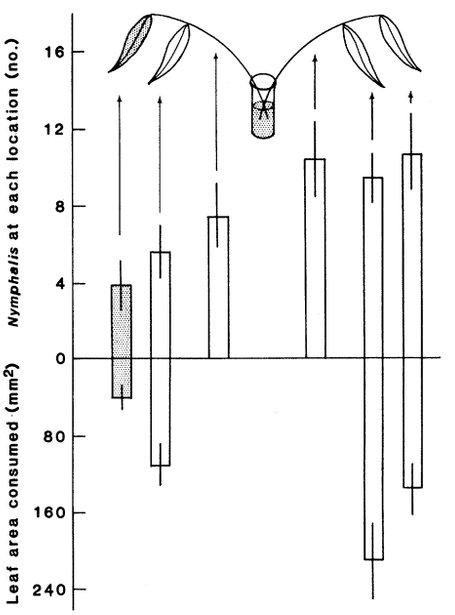

Fig. 3. Location and leaf area consumed by *N. antiopa* larvae on willow shoots with and without *P. versicolora* larvae during a 2-hour interval ($n = 18$). Shading represents the leaf with *Plagiodera* larvae. The legend is as for Fig. 1.

a common location. Although the role of glandular secretions in the Chrysomelidae as antipredator defenses is firmly established (*1, 2, 5, 16*), these chemicals may also mediate interactions within the second trophic level. Insect defensive secretions may play multiple ecological roles.

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Binding of the Sp1 Transcription Factor by the Human Harvey *ras*1 Proto-oncogene Promoter

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Members of the *ras* gene family encode proteins that when overproduced or mutated can transform immortalized mammalian cells. It is therefore important to understand the mechanisms by which the *ras* genes are regulated. The promoter region of the human Harvey *ras* proto-oncogene c-Ha-*ras*1 initiates RNA transcription at multiple sites and contains repeated copies of the hexanucleotide GGGCGG and its inverted complement CCGCCC, referred to as GC boxes. These GC boxes consist of sequences identical to those found in the SV40 early promoter, where the human cellular transcriptional factor Sp1 binds. Footprinting analysis with deoxyribonuclease I was used to show that Sp1 binds to six GC box sequences within the c-Ha-*ras*1 promoter. An in vivo transfection assay showed competition between the 21–base pair repeats of the SV40 promoter and the c-Ha-*ras*1 promoter for common regulatory factors. In this system the presence of Sp1 is apparently required for c-Ha-*ras*1 transcription. Analysis of deletions of the c-Ha-*ras*1 promoter region by means of a transient expression assay revealed that the three Sp1 binding sites closest to the RNA start sites were sufficient for full transcriptional activity.

THE *ras* GENES HAVE ATTRACTED A great deal of attention because of their possible role in the development of human cancers. There are three functional *ras* genes in the human genome, c-Ha-*ras*1 (cellular Harvey *ras*) (*1, 2*), c-Ki-*ras*2 (cellular Kirsten *ras*) (*1, 3*), and N-*ras* (*4, 5*); two other *ras* genes, c-Ha-*ras*2 and c-Ki-*ras*1, are pseudogenes (*6, 7*). The three functional genes code for proteins (p21) that are very closely related to each other. Because p21 proteins have guanosine triphosphatase (GTPase) activity (*8*) it has been suggested that their normal function may be as G-like regulatory proteins involved in the normal growth control of cells (*9*). Several groups of investigators have shown that a normal c-*ras* gene can be activated by a single point mutation that changes the glycine residue at position 12 to a valine (*10–12*). If the expression of a normal *ras* gene is enhanced by placing it under the control of a strong promoter, it can transform NIH 3T3 cells (*13*); however, there are as yet no examples of human tumors expressing very high levels of normal *ras* products. We examined the regulation of transcription of the c-Ha-*ras*1 gene to help define its role in controlling cellular growth.

We had earlier identified the promoter region of the human c-Ha-*ras*1 gene (*14*) and found that it contains multiple RNA start sites and ten repeats of a GGGCGG sequence and its inverted complement CCGCCC (called GC boxes). However, neither a TATA box nor a CAAT box were found in their characteristic positions upstream from the transcriptional start sites (*14*). These specific features of the promoter region are very similar to those found in the promoter region of the human epidermal growth factor (EGF) receptor gene (*15*). The GC box sequence was originally found in the 21–base pair (bp) repeats of the SV40 early promoter (*16*). This sequence binds the human HeLa transcription factor Sp1 (*17–20*). It was reported that five Sp1 molecules can bind to the tandemly repeated GC boxes of the SV40 early promoter and stimulate transcription in vitro (*17–20*). The GC boxes of some other viral genes [for example, herpes "immediate-early" [IE]3 and herpes *tk* genes (*21, 22*)] and cellular genes [mouse dihydrofolate reductase gene and the monkey β region (*23, 24*)] are also recognized by Sp1 protein. Recently the consensus sequence $^{G}_{T}$GGGCGG$^{GGC}_{AAT}$ for Sp1 recognition was deduced by the comparison of the sequences of 19 Sp1 strong binding sites (*25*). We found that the promoter region of the c-Ha-*ras*1 gene contains eight 10-bp GC boxes resembling this consensus sequence (Fig. 1).

To determine whether the transcription of this proto-oncogene is regulated by sequence-specific interactions of Sp1 to the promoter, as has been demonstrated for SV40, we tested the c-Ha-*ras*1 promoter for its ability to bind the Sp1 transcription factor in a deoxyribonuclease I (DNase I) footprint assay. An isolated DNA fragment end-labeled on either DNA strand was incubated with various amounts of partially purified Sp1, subjected to partial DNase I digestion, and analyzed as described in the

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