PHYTOCHEMICAL BASIS OF LEARNING IN *Rhagoletis pomonella* AND OTHER HERBIVOROUS INSECTS

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Abstract—Examples of phytochemically-based learning of host preference in herbivorous insects are reviewed in the context of traditionally important issues: the number and kinds of chemicals involved; which sensory modalities are affected; whether peripheral or central nervous processing is altered; and whether learning is associative or not. A fifth issue addressed here— whether experience enhances a feeding or ovipositing insect's propensity to accept familiar chemical stimuli or to reject novel chemical stimuli—has been ignored in previous studies. Following the review, evidence is presented indicating that female apple maggot flies (*Ragoletis pomonella*) learn to reject both novel physical and novel chemical stimuli.

Key Words—Learning, phytochemistry, host preference, herbivorous insects, *Rhagoletis pomonella*, Diptera, Tephritidae.

INTRODUCTION

The location and selection of host plants by herbivorous insects has been portrayed historically as a simple, invariant behavioral sequence (Thorsteinson, 1960). In particular, responses to the secondary and nutrient chemical constituents of plants have been viewed generally as consequences of innate releasing mechanisms (*sensu* Tinbergen, 1969), stereotyped and relatively resistant to environmental factors. The continuing accumulation of evidence for learning of host preference in herbivorous insects (Jermy et al., 1968; Dethier, 1980; Prokopy et al., 1982; Rauscher, 1983; Papaj and Rauscher, 1983; Stanton, 1984; Papaj, 1986a, b) and saprophagous insects (Jaenike, 1982, 1983, 1986), as well as recent theoretical discussions of learning as a factor in the evolution of host discrimination (Rauscher, 1983; Papaj and Rauscher, 1983; Stanton, 1984), de-

mand a critical examination of the thesis that an insect's responses to phyto-
chemicals are static and independent of prior feeding or egg-laying history. In
this paper, we first review the evidence for learning involving plant chemicals
and then introduce our own work on learning to chemical cues in fruit accep-
tance behavior by the apple maggot fly, *Rhagoletis pomonella*. We define
"learning" as a reversible change in behavior with experience; we use "in-
duction" synonymously with learning, but apply that term particularly to effects
of diet on the feeding preference of lepidopterous larvae.

GENERAL REVIEW

Induction of Larval Feeding Preference. Yamamoto and Fraenkel (1960)
and Jermy et al. (1968) can be credited with first showing convincingly that
host preference depends on prior feeding history. Since then, feeding or ovi-
position preference has been altered by feeding or oviposition experience almost
whenever the issue has been examined, predominantly in lepidopterous larvae
(see reviews by Hanson, 1983; De Boer and Hanson, 1984), but also in adult
Lepidoptera (Stanton 1984; Traynier 1984; Papaj 1986a–c), Diptera (Jaenike,
1982, 1983, 1985; Prokopy et al., 1982, 1986; Cooley et al., 1986) Orthoptera
(Cassidy, 1978) and Coleoptera (Phillips, 1977; Rausher, 1983).

Most studies of so-called induction of feeding preference adopted the assay
developed by Jermy et al. (1968) in which individuals reared on a particular
host plant or artificial medium are presented simultaneously with an array of
hosts, nonhosts, or artificial media. Preference was usually defined by the rel-
ative proportion of material of each type consumed after a certain time has
elapsed or after a certain absolute amount of material is ingested. In the original
work by Jermy et al. (1968), for example, larvae of the tobacco hornworm,
Manduca sexta, were reared on tobacco (*Nicotianea* spp.), Jerusalem cherry
(*Solanum* spp.), and tomato (*Lycopersicon* spp.); larvae were subsequently of-
fered plant disks of all three species simultaneously. The assay was terminated
when a larva had consumed approximately half of one disk. The results were
unambiguous: larvae always consumed more leaf material of the plant species
on which they were reared.

In other words, the order in which host species were preferred was altered
by prior experience—in the sense of Papaj and Rausher (1983), a modification
of the rank order of preference. In other cases the rank order of preference
remained the same, but the degree to which different species were consumed
still depended on feeding history—in the sense of Papaj and Rausher (1983), a
modification of the specificity of preference. For example, when *Manduca* lar-
vae were reared on certain nonhost species, those species were consumed more
frequently than if the larvae were reared on host plants, but never more fre-

quently than the host plants themselves (De Boer and Hanson, 1984). In general, the outcome of induction, i.e., whether rank order or specificity is altered by experience, may depend upon the taxonomic relatedness of the test plant species (Wasserman, 1982; De Boer and Hanson, 1984).

The notion that host experience causes changes in an herbivorous insect's responses to host chemical stimuli was implicit in an important hypothesis about the processing of host-selection information advanced by Jermy et al. (1968). Presuming that induction of preference in lepidopterous larvae was mediated by changes in responses to phytochemicals, Jermy and colleagues argued that discrimination among plant species must involve, at least in part, assessment of qualitative differences in the profiles of phagostimulants and deterrents of different plant species. If quantitative differences only were important, e.g., if larvae discriminated among plants merely by measuring the level of a compound shared by those plants, then induction to one host should have enhanced the acceptability of all host species more or less equally. In fact, induction always resulted in increased consumption of the inducing plant relative to alternative plants. Qualitative differences among plants could reflect, for example, differences among plants in the kinds of compounds perceived by the insect or differences in the relative concentrations of more than one compound shared by those plants.

Evidence for Phytochemical Basis of Larval Induction. The assumption of a phytochemical basis of larval induction of feeding preference has been supported by a number of studies. In a study by Schoonhoven (1969), for example, the gustatory organs of *Manduca* larvae reared on tomato plants exhibited electrophysiological responses to saps from an array of host and nonhost species that differed from those of larvae reared on an artificial medium. Impulse rates from a gustatory organ, the medial maxillary sensilla styloconica, were generally higher for larvae fed on artificial diet than for those fed on tomato leaves. Similarly, the impulse rates generated by contact with a variety of pure chemicals (sodium chloride, salicin, solanin, and inositol) were greater after feeding on artificial diet lacking these compounds than after feeding on tomato.

The responses of larvae to single compounds can also be modified by rearing on media containing those compounds. Electrophysiological responses to the secondary compound, salicin, by *Manduca* larvae were altered by prior feeding on diets containing salicin (Schoonhoven, 1969). When salicin was incorporated into artificial medium or tomato leaves, the medial maxillary sensilla styloconica of larvae exposed to the salicin-laced diet fired less rapidly than the sensilla of larvae exposed to diet alone.

Induction of preference for diets containing particular compounds is not confined to secondary plant compounds only. The medial maxillary sensilla styloconica of *Manduca* larvae fed on artificial diet spiked with 0.01 M inositol (a weakly stimulatory sugar) fired at a lower rate upon contact with inositol than

did those of larvae fed on artificial diet alone (Schoonhoven, 1969). Similarly, Städler and Hanson (1978) induced successfully *Manduca* larvae to an artificial diet containing the nutrient linoleic acid. Larvae fed on a standard wheat germ diet and larvae fed on a similar diet lacking lineoleic acid and other nutrient compounds consumed proportionately more of their respective diets in choice tests. Städler and Hanson used choice tests featuring a variety of extracts of the diets as well as tests with pure lipid components to confirm that experience altered feeding responses to linoleic acid.

Components of Behavior Altered by Induction. Like most induction studies using natural foliage for testing, preference for a host chemical or host test extract has often been deduced from the relative amounts of chemically treated and untreated material consumed by larvae in choice assays. These so-called "cafeteria tests" facilitate a large number of replicates and experiments (e.g., Städler and Hanson, 1978; De Boer and Hanson, 1984), but compromise efforts to define which components of host-selection behavior are altered by prior experience. Saxena and Schoonhoven (1978, 1982), in sharp contrast, used behavioral assays in which individual insects were observed directly to elucidate the behavioral mechanisms underlying induction of larval preference.

Saxena and Schoonhoven (1978) reared a group of *Manduca* larvae on a wheat germ diet containing the terpene, citral, and another group on a plain wheat germ diet. Results of a tightrope choice test in which larvae walked along a wire or Plexiglas edge between two experimental disks revealed that citral-exposed larvae turned more frequently towards a citral-medium disk than a plain-medium disk. Moreover, induction to diet was symmetrical. Larvae reared on plain medium turned more frequently towards a plain-medium disk than a citral-medium disk. Larvae reared on citral medium consumed more citral medium than plain medium in both choice and no-choice presentations; larvae fed plain medium consumed more plain medium in both choice and no-choice presentations. Although differences in consumption between citral-experienced and citral-naive larvae presumably represent some unknown composite of differences in orientation, biting and feeding responses, and/or arrestment, Saxena and Schoonhoven contended that both orientational and contact feeding responses were altered by prior experience with citral medium or plain medium.

Saxena and Schoonhoven (1982) extended these assays using *Manduca* larvae reared and tested on natural foliage. As commonly found in such studies, prior experience with a plant species tended to increase the extent to which that species was consumed relative to other plants. In addition, larvae tended to turn more frequently towards the plant on which they were fed, in both choice and no-choice tests. Interestingly, the rank order of orientational preferences of larvae reared on different diets did not always correspond to the rank order of feeding preferences. This result suggests that some other behavior besides orientation is also altered by prior feeding history. Furthermore, the alteration in orientational preference was apparently olfactorily-based, at least in part. Lar-

vae reared on tomato, for example, chose tomato leaves over blank controls in the turning assay much more often than did larvae reared on an artificial diet. This effect of experience on orientation behavior disappeared entirely when test tomato leaves were presented behind a glass barrier.

Sensory Modalities and Multicomponent Chemical Basis of Induction. The contention that both orientation and contact behavior are affected by prior feeding history suggests that more than one sensory modality is involved in induction of larval preference. More direct and detailed information about the sensory modalities affected by induction was supplied by the ablation experiments of Hanson and Dethier (1973). After raising *Manduca* larvae on either tomato or Jerusalem cherry, they extirpated systematically the olfactory and gustatory organs of fourth- and fifth-instar larvae. Using standard leaf-disk choice tests, the relative consumption of tomato and Jerusalem cherry was measured for normal and operated larvae. Loss of either the gustatory organ (maxillary sensilla styloconica) or both olfactory organs (antennae and maxillary palps) reduced markedly the strength of induction, relative to unoperated larvae. A lesser but significant degradation of induction followed the removal of the antennae alone or the palps alone. The residual effect of prior experience on relative consumption of tomato and Jerusalem cherry after ablation of either the olfactory or gustatory systems suggested strongly that both systems were involved independently in induction of host preference.

This systemic redundancy in induction of preference was paralleled in Hanson and Dethier's studies by a redundancy in the number of discriminatory organs within each system: both discrimination and induction of discrimination persisted after the unilateral ablation of the maxillary sensilla styloconica, antennae, and palps. The unilateral extirpation procedure not only indicated that more organs of each type were present than were required to discriminate or to be induced to discriminate among food plants, but confirmed that the effects of the bilateral gustatory and olfactory ablations on induction were not merely the result of surgical trauma.

The activity of both olfactory and gustatory systems in induction of preference in lepidopterous larvae implies that responses to more than one class of chemical compound are modified by prior feeding history. This inference was supported by Städler and Hanson (1976, 1978), who recorded the behavioral and electrophysiological responses of larvae reared on tomato and Jerusalem cherry to host extracts prepared with a variety of solvents. Using a modification of the standard cafeteria test, larval preference was measured as the proportion of glass-fiber disks impregnated with a particular solvent extract that was consumed by a larva. While larvae discriminated among a number of extracts, only the nonpolar hexane and polar water extract carried the inducing activity. Thus, experience with a host plant apparently altered responses to more than one but less than all of the phytochemicals used in discrimination. The fact that responses to only a subset of the phytochemicals used to select hosts were appar-

ently altered by prior experience may help to explain the common observation (De Boer and Hanson, 1984) that the extent to which preference among particular plant species is induced in late-instar larvae is not necessarily coincident with the extent to which early-instar larvae discriminate among those species.

Adult as well as immature insects may be conditioned to more than one chemical in plant or plant-derived resources. A multicomponent chemical basis of learning of adult oviposition preference in *Drosophila* spp. was postulated by Jaenike (1983). After exposing larval and adult *D. melanogaster, D. pseudoobscura,* and *D. immigrans* to artificial media containing one of various substances (sodium chloride, ethanol, ethyl acetate, lactic acid, piperidine, and peppermint oil), Jaenike (1982) presented adults simultaneously with media with and without these substances. Rearing larvae on these media had no effect on the number of eggs laid by adults in various substrates, adding to the tremendous body of evidence that adult preference is rarely susceptible to larval conditioning (Papaj and Rausher, 1983). Exposure of adult flies to peppermint-containing food, however, significantly reduced the aversion of adults of all three species towards laying eggs in this medium. While this result may be a bonafide example of habituation to a deterrent substance (Manning, 1967; McGuire, 1984), Jaenike (1983) nevertheless questioned the extent to which learning of acceptance of individual deterrent compounds (particularly ones that were not present in natural food resources) reflected the kind of learning possible in natural breeding substrates.

Jaenike (1983) therefore exposed larval and adult *Drosophila melanogaster* to more natural breeding substrates derived from apple, tomato, squash, and banana. As before, adult preference did not depend on the type of food on which larvae developed. In two-choice tests, however, individuals laid a greater proportion of eggs in the particular substrate on which they fed as adults than did individuals that fed on the other substrate. In a second experiment, adult flies were exposed to either apple, tomato, orange, grape, or onion food and tested simultaneously on all five media. Adult exposure to a certain medium sometimes, but not always, induced a preference for ovipositing on that medium. Adults that fed on grape or tomato, for example, laid proportionately more eggs in grape or tomato, respectively, than did adults in the other treatments. Feeding on apple, orange and onion, however, did not increase the proportion of eggs laid on those substrates, relative to feeding on alternative food sources. In addition, exposure to one food source sometimes increased the relative acceptability of other foods. Flies kept on apple, for instance, laid proportionately more eggs on grape than flies kept on tomato, orange, or onion. This so-called "cross-induction" phenomenon was not reciprocal: flies maintained on grape medium did not show an enhanced propensity to accept apple medium.

A mechanism proposed by Jaenike (1983) to explain cross-induction assumed that prior experience with a given food resource influences responses to an array of repellent and attractive compounds. While details of this scheme

were entirely speculative, cross-induction itself is probably genuine. Enhancement of relative acceptibility of one host species upon experience with another is evident in a great number of studies on induction of preference (even though it was not usually acknowledged; e.g., Jermy et al., 1968; Yamamoto, 1974; Saxena and Schoonhoven, 1982; De Boer and Hanson, 1984). Indeed, its generality may be very much understated simply because induction experiments typically assay discrimination between only two plant species, conditions under which cross-induction cannot be deduced.

Effects of Experience on Peripheral and Central Processing. Presuming a multicomponent chemical basis of induction of lepidopterous larvae, Schoonhoven (1977) proposed alternative schemes in which experience with a food resource was accompanied by changes in either the central or the peripheral nervous system. Both models embrace the notion that discrimination is the outcome of comparison of the across-fiber firing pattern of sensory receptors with a centrally located template. The first model supposes that prior experience affects feeding behavior by changing the internal template of the phytochemical spectrum against which the across-fiber firing pattern of the sensory receptors is matched. The second model supposes that prior experience alters the across-fiber firing pattern of the sensory receptors itself.

Both Schoonhoven (1969) and Städler and Hanson (1976) reported evidence that changes in the sensitivity of the gustatory maxillary sensilla styloconica to particular chemicals accompany induction of host preference. While these studies clearly corroborate a hypothesis of peripheral control of induction, they do not exclude the possibility of correlative changes in the central nervous system.

A central nervous system component in conditioning of feeding behavior was emphasized in Szentesi and Bernays' (1984) study of the physiological basis of habituation to nicotine hydrogen tartrate (NHT) by grasshopper nymphs (*Schistocerca gregaria*). Habituation, defined as the waning of response to a stimulus with repeated exposure to that stimulus, is widely presumed to involve central nervous processes. While a number of investigators have defined effects of host experience on feeding (e.g. Schoonhoven, 1969; Gill, 1972, cited in Szentesi and Bernays, 1984) or oviposition (Jaenike, 1982, 1983) preference that could potentially be attributed to habituation (McGuire, 1984), few studies controlled for effects of resource deprivation on preference. In most procedures, food with and without deterrent was offered to groups of insects ad libitum for a specified period of time, after which responses to the stimulus were measured. No attempt was made to control for the effect of differences in consumption rate of the alternative diets on subsequent preference. Thus, a difference in preference among treatments could be due to the specific effect of repeated exposure to the deterrent or to the general effect of resource deprivation or to both. By ensuring that all locusts consumed food at the same average rate, regardless of the presence or absence of a deterrent in their diet, Jermy et al.

(1982) and Szentesi and Bernays (1984) verified that a depression of deterrency was due to repeated exposure to the deterrent and not to food deprivation.

Periods of sensory stimulation even without ingestion of the deterrent substance was sufficient to cause habituation to NHT by locust nymphs. Grasshoppers whose maxillary palp sensilla had been encapsulated with nicotine hydrogen tartrate (NHT) solutions ate more NHT-treated diet than control grasshoppers whose palps were encapsulated with water, even though both groups were reared on an NHT-free diet of fresh wheat (Szentesi and Bernays, 1984). An additional result demonstrated that habituation via sensory stimulation was associated with changes in the central nervous system. Before testing, the maxillary palps of groups of NHT-exposed and NHT-naive individuals were covered with a nylon sleeve and rendered inaccessible to sensory stimulation. Despite concealment of the palps, individuals whose palps were previously immersed in NHT solutions ate more NHT-impregnated food in the test period than did individuals whose palps were previously immersed in water.

Chemical Cues Involved in Associative Learning by Herbivorous Insects. Associative learning, defined as the association of a neutral stimulus with a nonneutral stimulus through the temporal pairing of those stimuli, is usually considered to entail changes in the central nervous system. Two instances in which a nonneutral host-selection chemical cue is associated with a neutral visual cue are known, both in adult papilionoid Lepidoptera.

Searching behavior of ovipositing cabbage butterflies, *Pieris rapae*, was altered by prior experience with particular varieties of their cabbage hosts (Traynier, 1984). Exposure of adult females to either Earlyball or Wongbok cabbage varieties for as short as 30 min predisposed individuals to land more frequently on those types in the first 5 min of a two-choice test. Efforts to reverse the effects of training in this experiment were unsuccessful, suggesting that short training periods induced relatively long-term changes in oviposition preference.

Taking advantage of the fact that paper disks wetted with sinigrin (a common component of cabbage and other crucifers) and water solutions elicited oviposition, Traynier (1984) next attempted to train female butterflies to disks of two shades of green, Shoalhaven Green and Great Green. Although females tended to land first on Shoalhaven Green disks regardless of prior experience, this preference in two-choice tests was greater for females preexposed to sinigrin-treated Shoalhaven Green disks than for females preexposed to sinigrin-treated Great Green disks. A single contact with a sinigrin-treated Shoalhaven Green disk—even without oviposition—altered significantly a butterfly's choice of colored disks. Thus, the visual cue (color or monochromatic hue) was apparently associated with the perception of sinigrin solution directly and not indirectly via the events corresponding to egg-laying.

Association of a visual cue with chemical releasers of oviposition behavior also occurs in pipevine swallowtail butterflies (Papaj, 1986a, b). In a large

outdoor screened enclosure, adult female *Battus philenor* butterflies were trained to search selectively in an array of host and nonhost plants for leaves of a shape (defined by maximum leaf length–maximum width ratio) similar to that of the *Aristolochia* host species to which they were exposed. Unlike cabbage butterflies, the effects of learning were reversed readily upon exposure to an alternative host species with a significantly different leaf shape. As in cabbage butterflies, contact with a host plant without oviposition was sufficient to induce a change in alighting responses.

Methanolic extracts of *Aristolochia macrophylla* applied to artificial substrates or to nonhost plants elicited oviposition by gravid females. When placed on nonhost plants sprayed with these extracts, butterflies alighted predominantly on leaves of a shape similar to that of the treated nonhost species. Contact with treated nonhost plants even without oviposition was sufficient to increase significantly a female's propensity to alight on leaves of a shape similar to that of the treated nonhost plant.

Many studies of associative learning (e.g., Traynier, 1984) neglect to control for the nonassociative effects of sensitization and pseudoconditioning. Sensitization is defined as a change in response coincident with repeated presentations of a neutral, conditioned stimulus whether or not that stimulus is paired with a nonneutral, unconditioned stimulus (McGuire, 1984; Tully, 1984). Cabbage butterflies, for example, may have come to prefer to land on green disks even if green disks without sinigrin treatment had been presented repeatedly. The fact that experienced *Pieris* butterflies often laid eggs on untreated disks in subsequent tests may be evidence of this nonassociative process.

The searching behavior of pipevine swallowtail butterflies, by contrast, provided an internal control for sensitization. *Battus* butterflies searching for a host plant on which to deposit eggs land periodically on leaves. Upon alighting, they drum their tarsi and presumably stimulate tarsal chemoreceptors (Feeny et al., 1983). If the leaf belongs to a nonhost species, the female immediately resumes search. If the leaf belongs to a host, the female often lays a small egg cluster and resumes search. The great majority of leaves on which a female alights are nonhost plants and, in fact, the standard assay for leaf-shape response (Rausher, 1978) records only the kinds of nonhost leaves on which an individual lands.

Whereas a single contact with a treated nonhost plant induced a search mode for leaves of a shape similar to that of that plant, repeated contacts with untreated nonhost plants in the enclosure array did not. Moreover, both in the enclosure (Papaj, 1986a) and in the field (Papaj, 1986b), a single contact with a host plant whose leaf shape differed from that for which an individual was searching originally often induced a new search mode for the leaf shape of the newly discovered host plant. Such abrupt switches in nonhost alighting behavior never occurred after "mistaken" contacts with a nonhost plant with the "wrong" leaf shape. The most parsimonious explanation for these host-re-

leased switches is that female butterflies associated the neutral shape stimuli of the host leaves on which they landed with positive host phytochemical stimuli.

Pseudoconditioning is defined as a change in response coincident with repeated presentations of the nonneutral, unconditioned stimulus whether or not that stimulus is paired with the neutral, conditioned stimulus (McGuire, 1984; Tully, 1984). Pseudoconditioning of leaf-shape response could have been possible if repeated contact with host extract (i.e., the unconditioned stimulus) resulted in search modes for particular leaf-shapes. Specifically, repeated contact with extracts prepared from the broad-leaved host species, *A. macrophylla*, should have induced preferences for broad leaves. In fact, search modes depended only on the leaf shape of the nonhost species (i.e., the conditioned stimulus) to which the extract was applied.

LEARNING IN APPLE MAGGOT FLY

Repeated oviposition in a particular fruit species alters the degree to which female apple maggot flies, *Rhagoletis pomonella* (Walsh), attempt to oviposit into various fruit species in future encounters (Prokopy et al., 1982, 1986). Females ovipositing successively in hawthorn fruits, for example, accepted subsequent hawthorn fruits more frequently than did females ovipositing successively in apples. The training effect was reciprocal: females exposed to apples accepted apples more frequently than females exposed to hawthorn fruits. The effect of experience on responses to alternative fruit species could be reversed by repeated exposure to a different fruit species. Although the effect of experience on apple maggot fly oviposition-site selection was previously described as associative learning (Prokopy et al., 1982), this assignation was premature. In fact, almost nothing was known at the time about the stimuli involved in learning. Hence, it was not possible to show that one cue was associated with another through prior experience.

Prokopy et al. (1986) established an alternative dimension along which to classify effects of experience on host preference. They pointed out that experience with a host type could alter the difference in acceptance of two host types in either of two ways. First, prior experience with a particular host type might act to enhance the likelihood of acceptance of that host type in subsequent encounters. Alternatively, experience with one host type might depress the tendency to accept alternative host types in subsequent encounters. Although previous studies of learning of preference failed to distinguish between "learning to accept" and "learning to refuse" a host type, most investigators have assumed that learning enhances the propensity to accept the familiar resource type. In fact, Prokopy et al. (1986) and Cooley et al. (1986) discovered that

both the apple maggot fly and another tephritid fly, the Mediterranean fruit fly (*Ceratitis capitata* Weidemann), learn to reject the novel fruit type.

This conclusion was reached by comparing the fruit acceptance of flies exposed to a single fruit species with that of flies that had never been exposed to fruit (i.e., naive flies) or with that of flies exposed alternately to more than one fruit species (i.e., untrained flies). A female apple maggot fly ovipositing successively into hawthorn fruit, for example, had no greater propensity to accept a test hawthorn fruit than did a female ovipositing alternately into hawthorn and apple. Instead, the female trained on hawthorn was simply less prone than the untrained female to accept an apple. Similarly, females exposed to apples were less prone than the naive or untrained females to accept a test hawthorn fruit. In short, females exposed to a particular fruit species were always more likely to reject the alternative fruit species than were naive or untrained females.

Whether learning to reject the novel fruit type is mediated by responses to chemical stimuli was not known previously. In the following study, we investigated two cues—fruit surface chemistry and fruit size—that could be involved in learning of fruit acceptance by ovipositing female *R. pomonella*. These stimuli were chosen because both are known to be important in discrimination of oviposition sites by naive females (Diehl and Prokopy, 1986). We asked the following questions: (1) Do females exposed to particular fruit species discriminate among models differing in size or in chemistry? (2) If females learn based on fruit size or chemistry, do they learn to accept familiar fruit stimuli or to reject the novel fruit stimuli? (3) Is an association between fruit size and chemistry formed through prior experience with host fruit?

METHODS AND MATERIALS

Adult female *R. pomonella* flies originated from puparia collected from infested apple fruit on Orchard Hill at the University of Massachusetts at Amherst. Adult flies were kept at 25°C, 16:8 light–dark photoperiod, and about 60% relative humidity. On day 1, 25 females and five males were placed in each of three 30 × 30 × 30-cm Plexiglas and aluminum screen cages. On day 7, two Red Delicious apples (ca. 60 mm diam) were hung with wire from the ceiling of cage 1. On the same day, 10 *Crataegus mollis* fruits (ca. 15 mm diam) were hung with wire from the ceiling of cage 2. Cage 3 received no fruit. Every two days, fruits were replaced with fresh fruit. This training regime was replicated three times. The number of eggs deposited in a single sample of used apples and hawthorn fruits was estimated by counting the number of oviposition punctures into the fruit surface.

On day 14, we began testing the propensity of experienced and naive flies to attempt to oviposit in a variety of fruit models. The models were prepared

by coating plastic and clay spheres of apple (65 mm in diameter) and hawthorn (15 mm in diameter) sizes, respectively, with ceresin wax dyed red. Ceresin wax in the form of hollow hemispheres is known to elicit relatively high rates of oviposition by apple maggot flies (Prokopy and Bush, 1973).

Models of each size were treated with surface fruit chemicals of either apple or hawthorn fruits, using the following procedure. Parafilm (American Can Co.) was stretched and wrapped around individual apples or groups of between six and eight hawthorn fruits. The parafilm was left on the fruits for between three and seven days at room temperature. The parafilm wrap was then removed and applied to wax models of each size category. The surface originally in contact with the surface of the fruit was always placed in contact with the wax surface of the model. The parafilm wrap was removed from the models after 2 hr, and the models were used immediately in testing. Each day, freshly treated models were used in testing. A preliminary study (Papaj, unpublished) revealed that ceresin wax domes pretreated with parafilm wrapped in host fruit received significantly more oviposition punctures and eggs than domes pretreated with blank parafilm.

Assay flies were selected by introducing, in alternating fashion, a large or small red ceresin wax sphere into each treatment cage. When a female flew or walked onto the model, she was placed in an empty test cage and allowed to rest for 5 min. Virtually all flies were eventually tested using these criteria. The fly was then allowed to walk onto the first model. An acceptance of the model was recorded if the female attempted to oviposit (i.e., bore into) in the model. A rejection was recorded if the female flew or walked from the model or if the female remained on the fruit 5 min without boring. Following acceptance (which was interrupted before egg deposition could occur) or rejection, the fly was permitted to rest 5 min before presentation of the next model.

The order of presentation of the models was randomized, changing each time a series of flies from each treatment was tested. Approximately 85 flies from each training regime were tested on each of the following four models: (1) apple size treated with apple-exposed parafilm, (2) apple size treated with hawthorn-exposed parafilm, (3) hawthorn size treated with apple-exposed parafilm, and (4) hawthorn size treated with hawthorn-exposed parafilm.

About 25 flies were tested on two additional models: one apple-sized and one hawthorn-sized model, each exposed to blank parafilm only.

RESULTS

Although flies in each fruit-exposure group were given equal opportunity to lay eggs in fresh fruit, apple-exposed flies laid many fewer eggs than hawthorn-exposed flies. In one sample three-day period, flies laid 234 eggs into one

set of 10 hawthorn fruit. Flies laid only 34 eggs into two apples in the same period (this difference is highly significant; $G = 167.35$, $P < 0.0001$). Casual observations suggested that flies had difficulty penetrating the apple skin with their ovipositor.

For all groups of females tested on models treated with blank parafilm, large, apple-sized models were accepted less frequently than small, hawthorn-sized models; this difference was significant for hawthorn-exposed and naive flies (Table 1). In addition, females exposed previously to hawthorn fruit exhibited an even greater difference in their propensity to bore into large vs. small models (0.12 vs. 0.36, respectively) than did naive females (0.27 vs. 0.54, respectively), but this trend was not significant. Previous experience with apple, by comparison, had no detectable effect on a female's propensity to attempt to oviposit in apple- vs. hawthorn-sized models.

Likewise, there was no effect of apple exposure on acceptance of models pretreated with parafilm wrapped previously around fruit. Females exposed to apples attempted to oviposit into apple- and hawthorn-treated spheres of either size as frequently as naive females (Table 2). Both apple-exposed and naive flies attempted to oviposit less frequently in large models compared to small models. Neither group of flies, however, exhibited a difference in propensity to bore into an apple-treated model or a hawthorn-treated model of a given size.

Females exposed to hawthorn fruits, by contrast, differed markedly from naive females in their acceptance of particular models pretreated with parafilm wrapped previously around fruit (Table 2). First, although each group attempted to bore more often into small models than large models of a given parafilm treatment, hawthorn-exposed females attempted to oviposit proportionately less frequently into apple-sized models of a given parafilm treatment than did naive females. Second, the training reponse to fruit model size was paralleled by a

TABLE 1. PROPORTION OF ACCEPTANCES BY FEMALES EXPOSED TO APPLE OR HAWTHORN FRUITS AND BY NAIVE FEMALES ON MODELS DIFFERING IN SIZE AND PRETREATED WITH BLANK PARAFILM

		Model type	
Exposure	N	Apple-sized[a]	Hawthorn-sized[a]
Apple	26	0.19 a1	0.38 a1
Hawthorn	25	0.12 a1	0.36 a2
Naive	26	0.27 a1	0.54 a2

[a] Proportions within a column that are followed by the same letter are not significantly different by a two-way G-test at $P = 0.05$. Proportions within a row that are followed by the same number are not significantly different by a two-way G-test at $P = 0.05$.

TABLE 2. PROPORTION OF ACCEPTANCES BY FEMALES EXPOSED TO APPLE OR
HAWTHORN FRUITS AND BY NAIVE FEMALES ON MODELS DIFFERING IN SIZE
AND PARAFILM PRETREATMENT

Exposure	N	Apple-sized[a]	Hawthorn-sized[a]
A. Apple-treated model			
Apple	81	0.35 a1	0.63 a2
Hawthorn	84	0.14 b1	0.48 b2
Naive	86	0.43 a1	0.72 a2
B. Hawthorn-treated model			
Apple	81	0.39 a1	0.62 a2
Hawthorn	84	0.28 b1	0.69 a2
Naive	86	0.49 a1	0.69 a2

[a] Proportions within a column that are followed by the same letter are not significantly different by a two-way G-test at $P = 0.05$. Proportions within a row that are followed by the same number are not significantly different by a two-way G-test at $P = 0.05$.

training response to fruit model parafilm treatment. Naive females exhibited no difference in acceptance of hawthorn-treated and apple-treated models within a size category. Hawthorn-exposed females, by contrast, attempted to bore considerably less often into an apple-treated model than into a hawthorn-treated model of a given size category.

In summary, responses to both size and surface chemistry were altered by prior experience with hawthorn fruits. The hawthorn-exposed flies accepted least often an apple-treated and apple-sized model and most often a hawthorn-treated and hawthorn-sized model. Training was associated with the rejection of novel fruit stimuli: the propensity of hawthorn-exposed females to attempt to oviposit into the hawthorn-treated and hawthorn-sized models was no greater than that of naive females. Rather, the propensity of hawthorn-exposed females to attempt to oviposit into apple-sized and/or apple-treated models was consistently less than that of naive females.

Finally, a log-linear chi-square model (Bishop et al., 1975) was used to assess the interaction of learned responses to model size and chemistry. The analysis tested the independence of the following factors: response (accept vs. reject), exposure (hawthorn-experienced vs. naive), size (apple-sized vs. hawthorn-sized), and parafilm treatment (apple-treated vs. hawthorn-treated). The significant response × exposure × size effect ($G = 4.32$, $P < 0.05$) confirms statistically that flies exposed to hawthorn fruits differed from naive flies in propensity to bore into large and small fruits. The significant response × exposure × parafilm treatment effect ($G = 6.39$, $P < 0.01$) confirms that flies

exposed to hawthorn fruits differed from naive flies in propensity to bore into apple-treated and hawthorn-treated fruit. Most importantly, the lack of a four-way interaction (response × exposure × size × parafilm treatment effect, $G = 0.44$, $P > 0.05$) indicates that the effect of training on size discrimination did not depend on the chemical features of the fruit model. Conversely, the effect of training on discrimination of fruit chemistry did not depend on the size of the fruit model.

DISCUSSION

A fundamental dichotomy in learned responses to novel vs. familiar chemical stimuli has been ignored previously. In many investigations of induction of feeding preference, the proper controls by which to identify this kind of conditioning were not executed. ''Naive'' insects were typically first-instar larvae that had not yet fed on plants or older larvae that had fed on a ''neutral'' artificial medium. When naive insects are much younger than experienced insects, however, level of experience is necessarily confounded with developmental stage. Moreover, insects feeding on artificial diets are not ''naive'' because such diets are not ''neutral'': lepidopterous larvae can be induced to prefer particular artificial diets over others and even over natural foliage (Städler and Hanson, 1978; Saxena and Schoonhoven, 1982). Using ''untrained'' individuals that have been exposed to a mixture of host species or a mixture of artificial media is a viable, but rarely considered, alternative. Of course, feeding insects can be ''untrained'' only to the extent that they will consume alternative foods.

An additional methodological complication has afflicted most studies of induction of larval feeding preference. Although cafeteria tests have assayed adequately the relative consumption of alternative foods, the *absolute* consumption rate of alternative food items across control and experimental groups has rarely been measured. Without measuring absolute consumption rates, however, it is almost impossible to deduce whether experience with a particular host species causes an absolute increase in the tendency to consume that host or an absolute decrease in the tendency to consume novel hosts, or both.

Despite these difficulties, many investigators have assumed that prior host experience enhances the acceptability of familiar food. Schoonhoven (1969), for example, presumed that induction in *Manduca* larvae was associated with decreased deterrence of the familiar diet. Similarly, Jaenike (1982, 1983) and McGuire (1984) contended that exposing adult *Drosophila* flies to peppermint-oil medium reduced the flies' aversion to peppermint oil. Städler and Hanson (1978) stated that induction of *Manduca* larvae to particular artificial diets both increased consumption of the rearing media and decreased consumption of alternative media.

Our present results indicate that alteration of a female apple maggot fly's responses to both physical (i.e., model size) and chemical (i.e., parafilm treatment) cues took the form of learning to reject the novel model type, paralleling the results of previous experiments (Prokopy et al., 1986; Cooley et al., 1986) with tephritid flies tested on natural fruit.

Interestingly, the propensity of hawthorn-exposed flies to bore into the hawthorn-treated, hawthorn-sized model was identical to that of naive flies. This result could be interpreted in one of two ways. First, fruit size and the fruit chemical constituents extracted and transferred with the parafilm treatment may be the only characters which females learned. Hawthorn-experienced flies might have accepted the hawthorn-sized, hawthorn-treated model as frequently as did naive flies because all of the relevant stimuli with which they were familiar were present. If so, experienced flies may be attending either to the absence of familiar stimuli or to the presence of novel stimuli when they reject novel fruit models. Such a fortuitous result seems unlikely. Fruit size and parafilm-extracted chemicals probably represent only a subset of the stimuli.

Alternatively, experienced flies may not be attending to the absence of familiar stimuli at all when they reject novel fruit or fruit models. Rather, they may reject novel fruit or novel fruit models because of the presence of novel stimuli. Thus, even though the hawthorn-sized, hawthorn-treated model does not bear all of the stimuli learned by flies, it would still be accepted by the experienced flies as vigorously as by the naive flies because of the absence of any novel stimuli.

In this study, apple maggot flies clearly learned to reject models with novel chemical stimuli, but only when exposed to hawthorn fruit. Exposure to apples failed to alter the females' fruit acceptance pattern, relative to that of naive females. In fact, apples always receive many fewer oviposition attempts than do hawthorn fruit in laboratory assays (Prokopy et al., 1985) and have consistently yielded a weaker training effct than hawthorn with real test fruit in previous experiments (Prokopy et al., 1986). Nevertheless, we do not yet know if females trained successfully to apple learn to accept familiar chemical stimuli or to reject novel chemical stimuli.

We also do not know if learning in apple maggot flies constitutes associative learning. The lack of an interaction in training to models of different sizes and parafilm treatments implies that size and chemistry cues were not associated during learning. If size and chemistry became associated through experience, the hawthorn-exposed flies should have been proportionately less inclined to bore into an apple-sized, apple-treated model than was expected based on their responses to the models with mixed size and chemistry characteristics (i.e., the apple-sized, hawthorn-treated and hawthorn-sized, apple-treated models). This negative result does not, however, exclude an association between size and/or chemistry and some other unknown stimuli.

By using fruit models to train as well as to test female apple maggot flies, we hope to address these questions. We hope also to shed light on the principal issues outlined in our review: whether learning based on host chemistry involves one or more than one chemical constituent; which sensory modalities are involved in learning of preference; and whether changes in the central or peripheral nervous system accompany experience with particular host species.

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