fected by the coreids shrank, either that section of stem sank inwards or, in older, more substantial tissue, the necrotic cells pulled away from those immediately surrounding so that after several days the lesion was marked by a cavity, within which a darkened 'stroma', representing the original tissue, was sometimes distinguishable.

Mirids are known to possess a salivary endopolygalacturonase ('pectinase')[9]. As a simple microviscosimetric test of pectinase activity, the rate of flow (under a standard pressure difference) of microliter quantities of substrate was timed along a horizontal capillary. The substrate used was 3% citrus pectin in phosphate buffer containing salivary gland homogenate (one gland per 30 µl substrate). The extracts from *Helopeltis* reduced the viscosity of the substrate until it was indistinguishable from that of water within an hour at all pH values tested (5–8), but no activity could be found in *Amblypelta*. No cellulase (using a similar technique with methyl cellulose as substrate) was discovered in either mirid or coreid.

As a simple test for invertase, unbuffered 1% sucrose containing salivary gland homogenate was incubated for 2 h at room temperature (about 30°C), and a drop on paper tested with silver nitrate reagent[10]. Homogenate in distilled water and substrate alone were the controls. Invertase was clearly demonstrated in the salivary glands of *Amblypelta* but none was indicated in those of *Helopeltis*.

Amylase was tested by incubating 1% 'soluble starch' in 1% sodium chloride with gland homogenate, and testing the solution with iodine solution, followed by hydrogen peroxide (because of the strongly reducing properties of the salivary glands of some Heteroptera[5], a falsely positive result may be given unless the oxidizing agent is added.) Neither insect was shown to possess a salivary amylase; minute quantities of human saliva provided a positive test of method.

The injury caused by mirids to their food plants, including nonmechanical spread of symptoms, has been ascribed to their salivary pectinase[11], and perhaps this enzyme contributes to any histological differences of the lesions of *Helopeltis* from those of *Amblypelta*, but for both insects there remains the problem of how they remove the contents of unbroken cells, over 3 mm away from the opening of the stylet bundle, within tens of minutes.

In this, the method of feeding of *Helopeltis* and *Amblypelta* clearly differs from that of e.g. the pentatomid investigated by Hori[4], the lesions of which are formed by an irregular 'assemblage of radial, branch-like stripes' of cells individually pierced by the stylets, the dimensions of the lesion being determined by the maximum reach of the stylet bundle.

Zweigelt[12], considered that the 'salivary secretion' (at that time identified as the stylet sheath) of gall-forming aphids was osmotically active and created a flow of nutrients towards the insects' stylets; he also mentioned release by the insects of amylase and a toxic 'aphidolysin'. Kunkel[13] describing the feeding of Homoptera on parenchyma thought that they might continue to suck from the vacuole of a single cell thereby drawing on an inflow of nutrients from surrounding tissues. The phenomena described in the present work appear to be either far more rapid or on a much larger scale than those alluded to by either author, however; certainly the results of feeding of *Helopeltis* and *Amblypelta* would be difficult to ascribe simply to diffusion of solutes to a single cell or locus, unaugmented by some more pervasive stimulus.

The 'water-soaked' region that is initially formed presumably indicates that some component of the saliva of the insects is injected, rapidly infiltrating the intercellular region. In the mirid, this may well be aided by action of the salivary pectinase on the cell walls, but such an explanation will not do for the coreid. Moreover, the main barriers to diffusion of solutes in plants are cell membranes rather than cell walls, and it seems likely that the diffusion of solutes from physically unbroken cells that takes place within minutes in the lesions of either insect is due to more than impairment of the integrity of the walls alone.

Perhaps, for both mirid and coreid, release of hydrolytic salivary enzyme results in the liberation of osmotically active substances into the intercellular space, causing an outflow of liquid (containing nutrients) that is sucked back from the intercellular space from time to time by the insect. The swollen intercellular spaces in the *Amblypelta* lesions is consistent with such a possibility and its biochemical and biophysical aspects are at present under investigation in this laboratory.

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Acid rain affects egg-laying behavior of apple maggot flies

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Summary. The tephritid fruit fly, *Rhagoletis pomonella,* is less likely to attempt oviposition in host fruit that have been exposed to acid rain or to a simulated acid rain solution (pH < 3.8). Electrophysiological data suggest that acid rain residue on the fruit surface may interfere with the sensory mechanisms that the fly uses during recognition and acceptance of host fruit.

Key words. Acid rain; apple maggot fly; host plant selection; oviposition; pollution; *Rhagoletis pomonella.*

How acid precipitation affects insects and insect-plant interactions is poorly understood, although an increasing number of reports cite complex and varied impacts of acids on plant[2], water, and soil systems[3]. During field tests, we observed per-

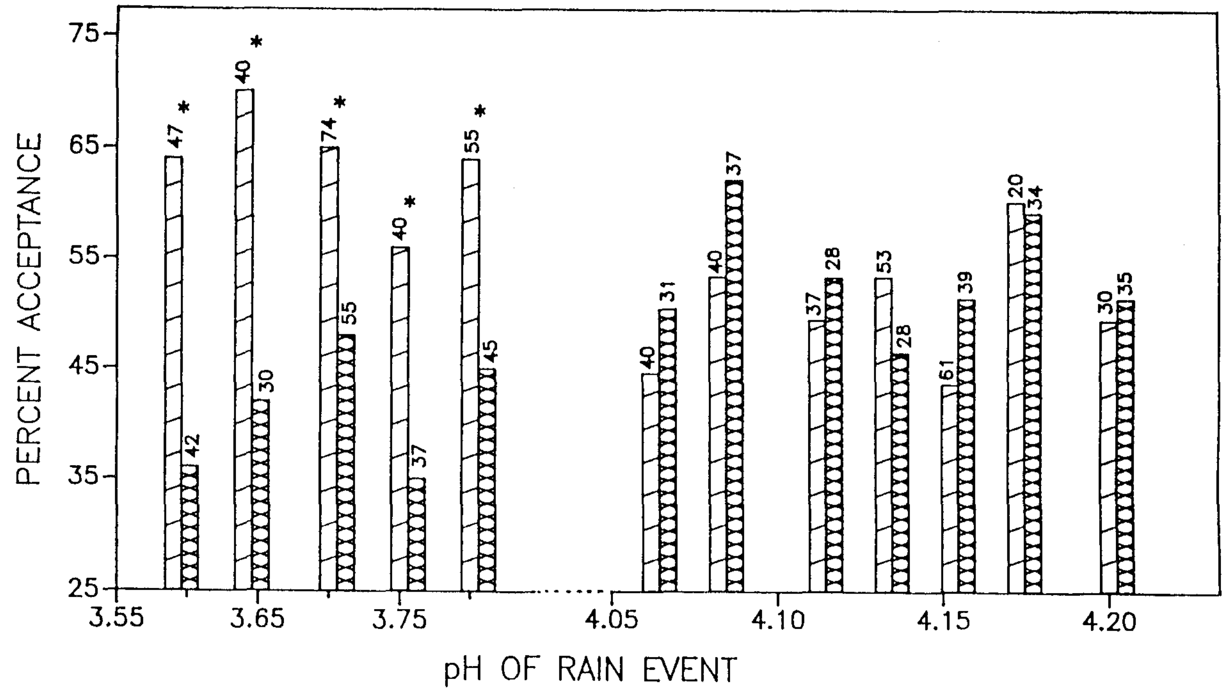

Figure 1. Effect of pH of rain event on *Rhagoletis pomonella* acceptance for egg-laying of rain-washed (xxxxx) versus protected (/////) *Crataegus* hawthorn fruit. Each pair of bars represents a bioassay of fruit following a single rain event and a star above the bars indicates that there is a significant difference ($p < 0.05$, G-test) between the 2 treatments. Values above bars represent the number of female arrivals on each fruit treatment. For illustrative purposes, beginning at pH 4.05, we expanded the x-axis scale.

plexing variability in egg-laying behavior of the apple maggot fly, *Rhagoletis pomonella* (W.) (Diptera: Tephritidae), when presented with fruit that had been exposed to different rainfall events. In a series of experiments, we first tested the hypothesis that these observations could be explained by differences in the pH of natural rains. Prior to rainfall initiation, picked hawthorn fruit were attached to wires and hung among naturally growing clusters of fruit in hawthorn trees located on the University of MA-Amherst campus. Half of the fruit was hung under plastic hoods with mesh sides and bottom, and thereby protected from rainfall. At the completion of each of 12 precipitation events that ranged in pH from 3.60–4.20, all fruit were brought into the lab. Determination of the pH of a rain event was made using samples taken from a collection apparatus stationed 0.2 km from the trees used in the experiments. Because the pH scale is logarithmic, at pH levels as low as these, a change in even 0.1 of a pH unit represents a substantial difference (approximately a 25% increase in hydrogen ion concentration) in acidity. The fruit were allowed to dry and were bioassayed in the laboratory by hanging 6 of the fruit 6–8 cm apart from the ceiling of a plexiglas-screen observation cage (30 × 30 × 30 cm). A single mature female, which had just begun egg-laying in a clean fruit, was introduced into the assay cage and allowed to fly to fruit overhead. A fly was observed for up to 2 h or until it flew to the cage wall. Acceptance (attempting egg-laying before leaving a fruit) or rejection (leaving a fruit without attempting egg-laying) was recorded for each visit. Three rain-washed and 3 protected fruit were bioassayed per cage, 4 cages were observed simultaneously, and a minimum of 12 flies was bioassayed per rain event. The bioassay results showed that females rejected fruit that were exposed to lower pH rains (3.60–3.80) significantly more often than fruit that had been protected from rain. In contrast, there was no significant difference between acceptance of protected fruit and fruit exposed to higher pH rains (4.06–4.20) (fig. 1).

In a second experiment, we evaluated the effect of simulated rain by 'acidifying' distilled water using a 3:2 molar ratio of sulfuric and nitric acids. Although acid precipitation is composed of a constellation of substances, these 2 acids are the primary components and this is the approximate ratio of these acids occurring in the Amherst area rainfall (O. T. Zajicek, Dept. of Chemistry, University of MA-Amherst, pers. comm.). For treated fruit, 0.5 ml of distilled water or an acidified solution was sprayed onto a hawthorn fruit and allowed to dry for circa 20 min before bioassay began. One fruit of each treatment was bioassayed per cage and 2 cages were observed simultaneously. The bioassay procedure was similar to that described previously. Females rejected fruit treated with the pH 3.6 solution significantly more often than untreated fruit or fruit treated with a pH 5.6 or 4.6 solution or distilled water (table, experiment 1).

One possible explanation that could account for a fly's decreased propensity to attempt oviposition in fruit treated with an acidic solution is acid-induced change in the fruit. It has been suggested that acid may affect surface characteristics of plant structures as a result of changes in the submicroscopic structure of epicuticular wax layer(s)[2]. Following exposure to highly acidic simulated or ambient rainfall, visible injury to fruit and leaves has been observed[2,4]. Such changes of the surface properties of fruit may alter important host recognition factors for *R. pomonella*, e.g. change in the 3-dimensional structure of the surface wax layer or altered volatilization of compounds produced by the epidermis. As one way to test this possibility, fruit were first treated with a pH 3.6 or 4.6 solution, allowed to dry for 2 h, then either not washed or washed with distilled water for 15 s, and then bioassayed as in the above experiments. Washing of the acid-treated fruit eliminated the acid treatment's effect on the behavior of *R. pomonella*: females accepted acid-treated, washed fruit just as frequently as fruit treated with a pH 4.6 solution (table, experiment 2). This suggests that irreversible

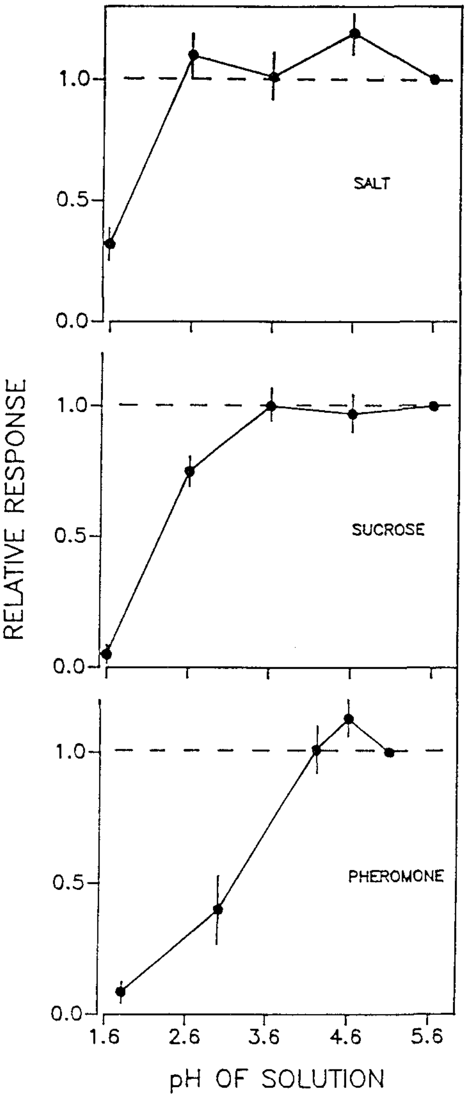

Figure 2. Effect of pH on the number of impulses generated by *R. pomonella* tarsal chemosensory cells following stimulation with salt (N = 17), sucrose (N = 12), or host-marking pheromone (N = 15). Bars represent SE. Results are expressed relative to response at pH 5.6 for salt and sucrose and at pH 5.4 for pheromone.

alterations to the fruit surface caused by the acid treatment are not responsible for our observations. Considerable additional study is necessary to evaluate the effects of acid on fruit.

A second explantation for our results is acid-induced changes in the fly. Positive and negative chemical and physical fruit stimuli are known to influence steps in fruit acceptance by *R. pomonella*[5–7]. Some of these stimuli are normally perceived by contact chemo- and mechanoreceptors. Acidity may affect these receptors, e.g. by altering the membrane proteins of the acceptor site or by interfering with the interaction of the stimulus molecule and the receptor, and hence, affect fly behavior.

As one avenue of testing this latter possibility, we conducted an electrophysiological study on a subgroup of contact chemosensory sensilla (Type D hairs) located on the distal segments of the fly's prothoracic leg that provide critical information for the fly[8]. Each D hair contains several sensory cells and three of these cells have been identified as sensitive to salt, sucrose, or host-marking pheromone[8]. The marking pheromone is deposited by a *R. pomonella* female on the fruit surface after she has laid an egg and deters subsequent females from further oviposition[9]. The standard hair-tip recording technique[10] was used to test the responses of the cells to 0.5 M NaCl, 0.5 M sucrose, and a high concentration of pheromone. Pheromone was collected by rinsing hawthorn fruit that had been used for oviposition in a known volume of distilled water. Amount of pheromone was estimated by counting the number of egg-laying punctures in each washed fruit: 1 puncture = 1 pheromone deposition event (DE). In this test, a concentration of 100 DE/ml was used. The stimulants were adjusted to a range of pH values (salt and sucrose: pH 1.6–5.6; marking pheromone: pH 1.8–5.4) using a 3:2 ratio of sulfuric and nitric acids. The highly acidic solutions were included because flies in nature may encounter concentrated acidic residues on a fruit surface following evaporation of rain droplets[11]. For example, we found in evaporation tests (on a glass surface) that simulated acid rain solutions which initially were at pH 4.6 dropped to pH 4.1 following 99% evaporation. Solutions initially at pH 3.6 and 2.6 dropped to pH 2.1 and 1.4, respectively. The responses of the cells were measured by counting the number of impulses elicited during a 1-s period of stimulation. At least 3 min elapsed between stimulations.

As the pH of the stimulating solution decreased, the number of impulses generated by each cell was reduced (fig. 2). Each cell appeared to show a different sensitivity to pH: inhibition was evident at pH 1.6 for the salt, at pH 2.6 for the sucrose, and at pH 3.1 for the pheromone (apparently the most pH sensitive) cell. Responses to sucrose at pH 1.6 and to pheromone at 1.8 were almost completely inhibited, whereas some response to salt was still evident at pH 1.6. For each cell, this inhibitory effect was reversible, which suggests that acid inhibition may not be due to membrane damage alone. An additional study we conducted demonstrated that when the concentration of a stimulating solution (sucrose) was decreased to a threshold level a fly may encounter in nature (i.e. to 0.01 M) (J. G. Stoffolano, unpublished data), the pH inhibition curve was shifted approximately 0.5 units towards higher pH (fig. 3). Thus, if a female's response to host fruit in nature was affected by low concentrations of host metabolites (e.g. sugars or acids), such responses could be influenced

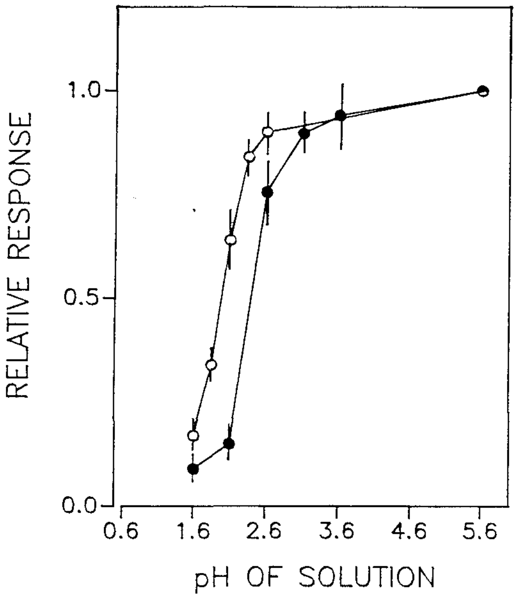

Figure 3. Effect of concentration on inhibition of the sucrose receptor by pH: ○—○ = 0.5 M sucrose (N = 11) and ●—● = 0.01 M sucrose (N = 20). Results for each concentration are expressed relative to the response at pH 5.6. Bars represent SE.

Percentage of *Rhagoletis pomonella* females attempting egg-laying in (Experiment 1) untreated *Crataegus* hawthorn fruit and fruit that had been treated with various pH solutions, and (Experiment 2) fruit that had been treated with a pH 4.6 or 3.6 solution, allowed to dry for 2 h, and then either not washed or washed with distilled water (DW)

Treatment solution pH	SO_4 µ M/l	NO_3	Female arrivals on fruit (No)	Females attempting egg-laying (%)
Experiment 1				
no treatment			100	69 a
> 6.8 (= DW)			92	79 a
5.6	13.2	12.0	99	67 a
4.6	23.8	21.2	70	76 a
3.6	99.3	92.6	96	49 b
Experiment 2				
4.6	25.1	22.8	102	74 a
4.6 followed by DW wash			88	75 a
3.6	105.3	98.7	94	44 b
3.6 followed by DW wash			74	79 a

Values in the last column followed by a different letter are significantly different at the 5% level (G-test).

by acid rain throughout the fly's peak oviposition period (July-August) when average rainfall pH in the Amherst area is well below 4 (O. T. Zajicek, pers. commun.).

R. pomonella females do in part assess the suitability of a potential host fruit on the basis of contact chemosensory information. However, these stimuli have not been identified, and further, it has not been established that tarsal D-sensilla respond to such compounds of the fruit surface. As a result, the actual significance of our electrophysiological recordings remains to be determined and additional avenues of study may be necessary to fully explain our behavioral observations, e.g. the possibility that perception of the acids alone by contact receptors is deterrent or that other sensilla (in addition to D-hairs) are involved in the perception of oviposition stimulants.

Visual characteristics of the fruit (including size, shape, and color) are known to be critical in selection of an egg-laying site[11] and these stimuli probably are not affected by the acid treatment. It is therefore not surprising that the observed reductions in fruit acceptance were no greater than 20–30% following a real or simulated event of acid precipitation. Further, it should be taken into account that our tests were conducted with picked fruits. Had we run tests on living plants, possible compounding effects of repeated acid deposition on growing tissue may have resulted in a more pronounced decline in fruit acceptance by these flies.

To our knowledge, this is the first demonstration that acid rain in a terrestrial system could influence the behavioral response of an animal, although in other insects, air pollutants, such as SO_2, dusts, and ozone, are known to cause change in feeding or oviposition behaviors[13, 14]. Regarding aquatic systems, a recent laboratory study demonstrated that decrease in pH modified the behavioral response of salmon to certain odors and suggests that acid rain could potentially impair recognition of olfactory stimuli during the spawning migration of these fish[15]. However, the degree to which acid rain (or any pollutants for that matter) influences animal behavior under natural conditions is unknown, and in most cases, the mechanisms responsible for pollution-induced changes in behavior are unclear. Indeed, whether we have established the sole mechanism responsible for change in *R. pomonella* egg-laying behavior on acid-rain-exposed fruit is moot. Nonetheless, several other electrophysiological studies have documented pH-dependent effects, e.g. on leg and mouthpart chemosensory hair cells of blow flies and flesh flies[16, 17], taste bud cells of rats[18], and chemoreceptors of puffer fish[19] and trout[20]. It is likely that a pH-dependent effect could be demonstrated for many, if not all, contact mechano- and chemosensory cells. Perhaps future studies will demonstrate that acid rain could interfere with the sensory mechanisms utilized by many insects, as well as other animals, during feeding or reproduction.

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