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Can *Rhagoletis pomonella* flies (Diptera: Tephritidae) learn to associate presence of food on foliage with foliage color?

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Abstract. *Rhagoletis pomonella* (Diptera: Tephritidae) flies were exposed for 3 days in laboratory cages to yellowish, green or white surrogate leaves with or without food (sucrose) on the leaf surface. When tested in an arena minutes after fly removal from an exposure cage, yellowish surrogate leaves were more attractive to tested flies than green surrogate leaves irrespective of the nature of surrogate leaves to which flies had been exposed. However, flies exposed to green surrogate leaves having food exhibited greater propensity to alight on green surrogate leaves than flies exposed to yellowish or white surrogate leaves having food. This propensity disappeared when flies were tested 24 h after termination of exposure to green surrogate leaves having food. There was no evidence of enhanced propensity of flies exposed to yellowish surrogate leaves having food to alight on yellowish surrogate leaves when tested minutes after removal from an exposure cage. We discuss the potential ecological significance of the evidence presented here that *R. pomonella* flies are capable of learning to associate the presence of food with green color of leaf surface on which food could be found.

INTRODUCTION

Increasingly, there is evidence that insects of a variety of species occupying a broad range of habitats are capable of learning and remembering information associated with habitat resources (Papaj & Prokopy, 1989; Szentesi & Jermy, 1990; Papaj & Lewis, 1993). Information learned includes elements of chemical as well as visual stimuli of resources. Learning of visual stimuli, such as color, is known to occur in a diversity of insects such as bees (e.g., Gould, 1993), wasps (e.g., Shafir, 1996), saprophagous and coprophagous flies (e.g., Folkers & Spatz, 1981; Fukushi, 1989), hymenopterous parasitoids (e.g., Wardle, 1990; Wackers & Lewis, 1994) and phytophagous lepidopterans (e.g., Traynier, 1986; Lewis & Lipani, 1990; Goulson & Cory, 1993; Weiss, 1995; Kandori & Ohsaki, 1996), grasshoppers (e.g., Bernays & Wrubel, 1985; Holliday & Holliday, 1995), locusts (e.g., Raubenheimer & Blackshaw, 1994), and tephritid fruit flies (e.g., Prokopy et al., 1994). In most of these studies on color learning, the insect under investigation has been shown to associate the presence of a rewarding stimulus (e.g., food) with the color of surface on or adjacent to which the stimulus is offered.

The apple maggot fly, *Rhagoletis pomonella* (Walsh), like other frugivorous tephritids, emerges from a puparium in the soil beneath a host tree and commences foraging for food shortly thereafter. Principal types of food include foliar leachate and insect honeydew (important sources of carbohydrate) and bird feces (a source of protein) (Hendrichs & Prokopy, 1994), all of which occur predominantly or exclusively on surfaces of host or non-host foliage. Carbohydrate as food is required on a daily basis by apple maggot flies

to ensure survival, whereas protein is not required for survival (but is necessary for egg development) and is consumed less often (Hendrichs & Prokopy, 1994). The color of host or non-host foliage frequented by food-foraging apple maggot flies may vary from yellowish (characteristic of young or nutrient stressed leaves) to dark green (characteristic of non-stressed more mature leaves). We are unaware of information showing a possible association between leaf color and availability of foliar leachate or bird feces; but aphids are more likely to be found in association with yellowish than green leaves (Kring, 1970).

To date, apple maggot flies have been shown to be capable of learning to find green fruit against a background of green foliage, thereby enhancing the probability of locating inconspicuous but potentially quality sites for oviposition (Prokopy et al., 1994). Apparently the act of oviposition is associated with the color of fruit that has been found. Here, we asked whether apple maggot flies are able to learn to associate the presence of carbohydrate as a source of food with the color of foliage (in the form of colored surrogate leaves) on which carbohydrate is present, and thereby enhance the probability of finding acceptable sites for feeding.

MATERIAL AND METHODS

All flies originated from larvae that infested field-collected apples near Amherst, Massachusetts (USA). Upon eclosion, both sexes were held together for 3 days in 30 × 30 × 30 cm cages constructed of wire screen and Plexiglas at 25°C, 60% RH and 18L : 6D. The light source consisted of tubular fluorescent lamps, every other one of which was either a daylight fluorescent tube (Sylvania F40 CW) or grow-light fluorescent tube (Sylvania FYO GRO), intended as a unit to approximate the quality of outdoor light (Shields, 1989). The top of each cage was covered with a sheet of white filterpaper to diffuse incoming light. Walls about 20 cm to the rear and front of each cage were covered with white paper to enhance entry of diffuse light into the cage, where light intensity on the cage floor (covered with white paper) averaged 1,600 lux. Water and carbohydrate (granular sucrose) were provided on the cage floor. On Day 4, groups of about 50 females were transferred into adjacent separate cages and remained there for 3 days, during which females could gain experience with surrogate leaves on which food was or was not present.

Surrogate leaves were constructed of white filter paper cut into the shape of apple leaves, each 10 cm long × 6 cm wide. They were colored by dipping into a solution of food dye (Durkee, Wayne, New Jersey) and water: 50% green dye and 50% water for green leaves; 47% yellow dye, 3% red dye and 50% water for yellowish leaves. Spectral reflectance curves of surrogate leaves and fresh-picked green and yellowish apple foliage are shown in Fig. 1. Surrogate leaves having food were dipped in a solution of dye containing 15% granulated sugar, an amount sufficient to provide needed carbohydrate throughout the fly exposure period. After surrogate leaves were dry, we used double-sided-sticky tape to attach four leaves to a 28 × 28 cm piece of white filter paper (Fig. 2) and then attached the filter paper to the rear wall of a cage. Except where stated otherwise, the only source of food in a cage during the 3-day fly exposure period was sucrose on surrogate leaves.

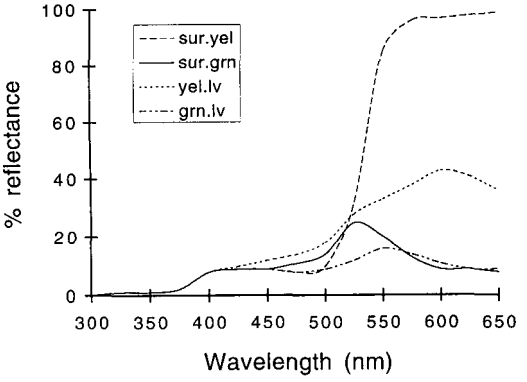

Fig. 1. Reflectance patterns of real and surrogate green and yellowish leaves within the visual spectrum of *R. pomonella.*

Assays of female response to color of surrogate leaves were carried out on Day 7 under above temperature, humidity and light conditions. The assay arena was

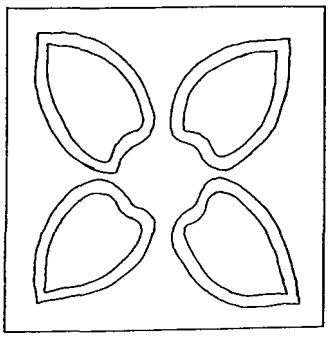

Fig. 2. Arrangement of surrogate leaves in exposure cages and test arena. Where two colors were present simultaneously, leaves of same color were diagonally opposite one another.

cubical (50 × 50 × 50 cm) and enclosed entirely in white paper except for the front, which was open to permit entry. A 28 × 28 cm piece of white paper having four surrogate leaves (Fig. 2) without food was centered on the back wall. Two of the leaves were green (diagonal to one another) and two were yellowish. Assay females were released singly from a horizontal 2 × 2 cm platform of white paper (dipped in 15% sucrose solution and dried before use) 25 cm above the arena floor and 25 cm to the front and center of the surrogate leaves. An assay female was selected randomly from an exposure cage, excluding those females present on surrogate leaves at the time of selection. It was transferred to the release platform by allowing it to walk on a small piece of moist white filter paper attached to a probe and then gently nudging it from the filter paper onto the platform. One fly from each exposure treatment was tested and removed from the arena before the next replicate of testing was begun.

For data analysis, we excluded all flies that did not leave the platform within the 10-min observation period (less than 2% of those tested), all flies that left the platform by crawling rather than by flying (less than 3% of those tested), and all flies that flew from the platform but did not alight directly upon or within 1 cm of a surrogate test leaf. We are uncertain as to why the latter flies did not respond positively to a surrogate test leaf (some may have engaged in escape-type behavior as a reaction to being transferred from an exposure cage to the release platform), but the proportion that did not respond to a test leaf (generally about two-thirds) was quite consistent among treatments within an experiment and among experiments. Perhaps if we had deprived flies of opportunity to ingest sucrose for a brief period (e.g. a few hours) between exposure in treatment cages and testing or if we had used a release platform devoid of sucrose, the proportion of non-responding flies would have been less. By so doing, however, we could have risked flies forgetting what they had experienced in exposure cages or risked even more flies exhibiting escape behavior after release on the platform. We included flies landing within 1 cm of a surrogate test leaf as being responders to the leaf because many kinds of insects showing positive response to a visual stimulus land at points where contrast between stimulus and background is greatest (i.e., at or nearby the edge of a stimulus) (Prokopy & Owens, 1983). For flies within an experiment that did respond to surrogate test leaves, we used a G-test for goodness of fit (Sokal & Rohlf, 1981) to determine whether differences in response to treatments were significant.

RESULTS

Our first question asked whether fly exposure to surrogate leaves lacking sucrose affected fly response to surrogate test leaves. When flies were exposed to four yellowish, four green or four white surrogate leaves in the absence of sucrose on the leaves (sucrose was on the cage floor), there was no significant difference among exposure treatments in the pattern of fly response to two surrogate yellowish leaves and two surrogate green leaves in the test arena ($P < 0.823$) (Table 1, Exp. 1). Irrespective of exposure treatment, 30–31% of responders alighted on yellowish leaves and 2–3% on green leaves.

Our second question asked whether fly exposure to surrogate leaves having sucrose affected fly response to surrogate test leaves. With sucrose now present on all four leaves (but not on the floor) in the exposure cages, there was a significant difference among exposure treatments in pattern of fly response to surrogate leaves in the test arena ($P < 0.001$) (Table 1, Exp. 2). Among flies exposed to four yellowish or four white leaves, 31–32% alighted on yellowish leaves and 1–2% on green leaves. Among flies exposed to four green leaves, 26% alighted on yellowish leaves but 7% on green leaves. Results from

the first and second experiments indicate that fly responses were similar following exposure to different colors of surrogate leaves lacking food, but that response to green leaves was enhanced by prior exposure to green leaves having food.

TABLE 1. Proportion of *R. pomonella* females exposed for 3 days to sucrose (on white paper on the floor or on yellowish, green or white surrogate leaves on a wall) in laboratory cages that alighted on yellowish or green surrogate leaves when tested in a laboratory arena.

Exp.	Location of sucrose	No. surrogate leaves in exposure cage			Hours tested after exposure	No. females tested	% tested females alighting on leaves		Results of statistical analysis		
		Yellowish	Green	White			Yellowish	Green	d.f.	χ^2 value	P value
1	Floor	4	0	0	0	148	31	2	2	0.39	0.823
		0	4	0	0	154	31	3			
		0	0	4	0	145	30	3			
2	Leaves	4	0	0	0	215	32	2	2	14.32	0.001
		0	4	0	0	214	26	7			
		0	0	4	0	215	31	1			
3	Leaves	4	0	0	0	203	38	3	1	16.43	0.001
		0	4	0	0	209	26	13			
4	Leaves	4	0	0	0	260	38	2	1	0.13	0.719
		0	4	0	24	258	42	3			
5	Leaves[1]	2(–)	(2+)	0	0	147	25	11	1	9.17	0.003
		0	0	2(+),2(–)	0	142	31	2			

[1](–) refers to leaves without sucrose; (+) refers to leaves with sucrose.

Our third and fourth questions asked whether the pattern of results of the second experiment could be repeated using a slightly altered protocol (omission of the treatment of fly exposure to white leaves having sucrose) and whether flies could remember for 24 h experience gained during exposure to surrogate leaves having sucrose. As in the second experiment, there was a significant difference between exposure treatments in pattern of fly response to surrogate leaves in the test arena ($P < 0.001$) (Table 1, Exp. 3). Again, fly response to green leaves was enhanced by prior exposure to four green leaves having food. When flies were exposed to the same treatments as in Exp. 3 but allowed to remain an extra 24 h in the exposure cages in the absence of any sucrose on surrogate leaves (which were removed at the end of Day 6), there was no significant difference between exposure treatments in fly response to yellowish or green leaves in the test arena ($P < 0.719$) (Table 1, Exp. 4). This result suggests that after a day without exposure to food or color, flies may have lost memory of their experience of finding food in association with a particular color of surrogate leaf.

Our final question asked whether the pattern of results obtained in the second and third experiments would hold true if flies were given a choice among leaves in exposure cages rather than being exposed to only a single kind of leaf. We thus exposed flies in cages to two green surrogate leaves with sucrose and two yellowish surrogate leaves without sucrose, or to two white surrogate leaves with sucrose and two white surrogate leaves without sucrose. There was a significant difference between exposure treatments in pattern of fly response to two surrogate yellowish leaves and two surrogate green leaves in the test arena ($P < 0.03$) (Table 1, Exp. 5). Flies exposed to two white leaves with sucrose and two

white leaves without sucrose responded similarly to flies exposed to four white or four yellowish leaves with sucrose in Exps 2 and 3 (31% alighted on yellowish leaves, 2% on green leaves). Flies exposed to two green leaves with sucrose responded similarly to flies exposed to four or two green leaves with sucrose in Exps 2 and 3 (25% alighted on yellowish leaves, 11% on green leaves). These results confirm that the pronounced tendency of color-naive apple maggot flies to alight on yellowish compared with green leaves can be significantly modified by the experience of acquiring food on green leaves.

DISCUSSION

Together, our findings show that female apple maggot flies are capable of learning to associate the presence of carbohydrate food (sucrose) with color of leaf surface on which food could be found. The effect of learning was expressed as an enhanced tendency to alight on green surrogate leaves following 3 days of exposure to green leaves having food. There was no evidence of an enhanced tendency to alight on yellowish surrogate leaves following 3 days of exposure to yellowish leaves having food. Our findings are similar to those of Bernays & Wrubel (1985), who showed an enhanced response of *Melanoplus* grasshopper nymphs to green color following exposure to food presented in association with green color but lack of enhanced response to yellow color following exposure to food presented in association with yellow color.

Among females tested here that had not been exposed to food in association with surrogate leaves present in the cage (i.e. females in Exp. 1), about 11 times more alighted on yellowish than on green surrogate leaves under dual-choice test conditions. Owens (1982) found that slightly more than a two-fold greater number of apple maggot flies alighted on real yellowish compared with real green apple foliage under dual-choice test conditions in a white-walled test chamber. Although the surrogate green leaves used here approximated real green leaves in intensity at wavelengths of peak reflectance for each (500–550 nm) within the insect-visible spectrum of 300–650 nm (Fig. 1), the surrogate yellowish leaves reflected light much more intensively than real yellowish leaves at wavelengths of peak reflectance for each (550–650 nm) (Fig. 1). The greater the intensity of reflectance of green and yellow hues between 500–650 nm, the more attractive such hues are to apple maggot flies (Owens, 1982).

Our finding that exposure of apple maggot flies for 3 days to food in association with yellowish leaf surrogates did not result in a detectable increase in attraction to yellowish leaf surrogates (beyond that shown by flies exposed for 3 days to food in association with white leaf surrogates) suggests that attraction to or discrimination of real or surrogate yellowish leaves may be so strong that, relatively speaking, it is unmodifiable by experience. Similarly, attraction of apple maggot flies to red colored fruit proved so strong that it too could not be enhanced through 3 days of exposure to red fruit for oviposition (Prokopy et al., 1994). On the other hand, fly attraction to green foliar surrogates, which was less than to yellowish ones, could be enhanced by 3 days of exposure to food in association with green surrogates. Similarly, apple maggot fly attraction to green-colored fruit, which was less than to red-colored fruit, could be enhanced through 3 days of exposure to green fruit for oviposition (Prokopy et al., 1994). Together, these findings are consistent with hypotheses of Vet et al. (1990), who predicted that innately strong responses to resource stimuli are less modifiable by experience that are innately weaker responses. If we had

used artist pigments (Owens & Prokopy, 1986) rather than food dyes to more precisely mimic the hue and intensity of yellowish leaves, perhaps fly response to yellowish leaf surrogates would have been weaker and thus more amenable to modification by experience.

Prokopy et al. (1994) showed that apple maggot fly detection of inconspicuous green fruit against a background of green host tree foliage improved as a consequence of prior experience finding and ovipositing in green fruit. Even though apple maggot fly attraction to green surrogate leaves increased as a consequence of prior experience finding and feeding on green leaf surrogates, the amount of increase was consistently slight (albeit statistically significant). Indeed, the increase involved only a relatively small proportion of responding individuals (no more than 25% in any experiment) that were attracted to green rather than to yellow leaf surrogates as a consequence of prior exposure to green surrogates having sucrose. Perhaps over evolutionary time, carbohydrate as food for apple maggot flies has been so consistently or sufficiently more abundant or of higher quality on yellowish than on green leaves (owing to greater probability of aphids being present on yellowish leaves) that apple maggot flies have undergone selection to respond to yellowish leaves to such an extent that response to green leaves is always considerably less, regardless of the amount or quality of carbohydrate that might be found there on occasion and regardless of the amount of experience obtained in finding high quality carbohydrate in association with green rather than yellowish leaves. The difference in value between green fruit and red fruit as potential egglaying sites may be consistently less than the difference in value between green foliage and yellowish foliage as potential feeding sites, thereby possibly accounting for the seemingly greater ability of apple maggot flies to learn to find green fruit as opposed to green leaves. Alternatively, because the vast majority of foliage in habitats encountered by apple maggot flies is green (not yellowish), there may be little value in learning to associate a possibly infrequent presence of high quality carbohydrate with such a frequently encountered visual stimulus.

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