Potato Leafhopper (Homoptera: Cicadellidae) Utilization of Alfalfa as a Host: The Role of Non-Host Stimuli[1]

Lane M. Smith II[2], William O. Lamp[3], and Edward J. Armbrust

Illinois Natural History Survey and Illinois Agricultural Experiment Station
607 East Peabody
Champaign, IL 61820

J. Entomol. Sci. 27(1): 56-64 (January 1992)

ABSTRACT The effect of non-host crabgrass, *Digitaria sanguinalis* (L.) Scop., on potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), utilization of alfalfa, *Medicago sativa* L., as host was investigated in binary choice experiments that measured oviposition and residency preference of adults. Leafhoppers preferred to oviposit and reside on pure alfalfa over alfalfa mixed with crabgrass. Successive experiments in which crabgrass was presented to leafhoppers with fewer sensory cures (i.e. tactile, gustatory, visual, olfactory) demonstrated that olfactory cues alone from crabgrass may explain the lower preference equally as well as any combination of other cues. Thus, volatile chemicals from crabgrass reduced acceptability of alfalfa for the potato leafhopper, although visual stimuli are not completely excluded. Additional experiments showed that leafhopper density, lighting, and alfalfa density all may influence leafhopper host utilization in laboratory experiments.

KEY WORDS host plant utilization, oviposition, preference, non-host stimuli, volatile chemicals, potato leafhopper, *Empoasca fabae*

Host plant utilization by herbivores has been described as a catenary process that requires attraction or acceptance involving several sensory modalities (Thorsteinson 1960, Schoonhoven 1968). This process has been especially studied among monophagous and oliphagous herbivores, which require specific host stimuli that operate over long distances. In contrast, polyphagous herbivores often have abundant and easily-located hosts and thus mechanisms of host acceptance rather than those of host finding may be of particular importance in host plant selection (Lance 1983, Jermy 1971).

The role of background weedy grasses on the population ecology of potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), a serious pest of alfalfa and other crops in the eastern half of the United States, has been the focus of recent research. These studies were prompted by the observation that weed control resulted in an increase in potato leafhopper abundance (Peterson 1979). In subsequent studies, this observation was verified by associating a reduction in adult leafhopper abundance (number per area) as well as a reduction in intensity (number per alfalfa stem) with the presence of weedy grasses in field plots (Lamp et al. 1984a, Oloumi-Sadeghi et al. 1987). These grasses, which included large crabgrass, *Digitaria sanguinalis* (Scop.), were unsuitable for reproduction and

[1] Accepted for publication 15 November 1991.
[2] Current address: Department of Entomology, Auburn University, Auburn, AL 36849.
[3] Current address: Department of Entomology, University of Maryland, College Park, MD 20742.

development of nymphs under laboratory conditions, although a small percentage of adults could survive on grass for several days (Lamp et al. 1984b). This research suggests that leafhopper-induced crop losses in alfalfa may be moderated by the manipulation of non-host grasses in alfalfa production systems.

This reduced abundance of potato leafhopper in the presence of grasses could be explained by a variety of mechanisms (Lamp et al. 1984a). Non-host grasses may alter leafhopper behavior, resulting in a net migration away from grass-infested alfalfa. Altieri et al. (1977) came to this conclusion with a similar species, *Empoasca kraemeri* Ross and Moore, in bean fields. They found that grass macerate sprays (i.e. blended mixtures of plant foliage and water) prevented leafhoppers from colonizing beans in the field. In laboratory experiments, they found that grass apparently hindered the leafhopper from finding bean plants.

Other workers have found that non-hosts can affect the behavior of other pest insects. Latheef and Ortiz (1983) demonstrated that many common herbs reduced oviposition by imported cabbageworm. Saxena and Khan (1985, 1986) showed reduced feeding in a leafhopper mediated by olfaction with extracts of neem plant. Koul (1986), using a noctuid moth, and Saxena and Basit (1982) using a leafhopper, also demonstrated reduction in oviposition due to limonene and several other plant constituents mediated by olfaction and gustation.

In the present study, we investigated the effects of crabgrass on potato leafhopper residency and oviposition preference using choice experiments. In addition, we investigated various stimuli that might influence this preference.

Materials and Methods

Leafhoppers were obtained from a colony started two years earlier from field-collected adults and reared at 26°C on fava beans, *Vicia faba* L. Adults from cages in which oviposition had occurred four weeks earlier were used in experiments. Greenhouse alfalfa (vegetative stage, cultivar 'WL304') and crabgrass (vegetative) were presented as 25 cm stems with four stems in 3% sucrose solution per 60 ml glass vial. Using cut stems reduced variance in plant quality and amount of plant material, and avoided moisture differences from potting soil.

For paired-choice experiments, wood frame cages were employed that had two adjacent sliding glass sides, two adjacent organdy covered sides, a wood panel bottom and mylar covered top (30 × 30 × 50 cm high). Velcro strips at the edges of the glass sides allowed cages to be paired. Paired cages containing test and control materials were separated by glass sides, which, when raised, allowed leafhoppers to move freely from one side to the other. In each experiment, adults, without regard to sex, were aspirated in equal proportions to both of the paired cages before the start of each experiment. During these investigations, the initial percentage of females was 50.5% (± 0.06%, n = 33). The actual number used depended on the number available from the colony each week and ranged from 40 to 150 adults. At the end of the experiment, the glass was lowered (Fig. 1).

Leafhopper response was measured by egg deposition for oviposition preference and adult location for residency preference between paired control and test cages. The control cage contained four vials of alfalfa separated on the corners of a 15 cm square, while the test cage material varied with each experiment. Eggs deposited in the plant tissues in the control and test cages were counted using a lactophenol-fuchsin clearing/staining technique (Lamp and Smith 1989). Preference

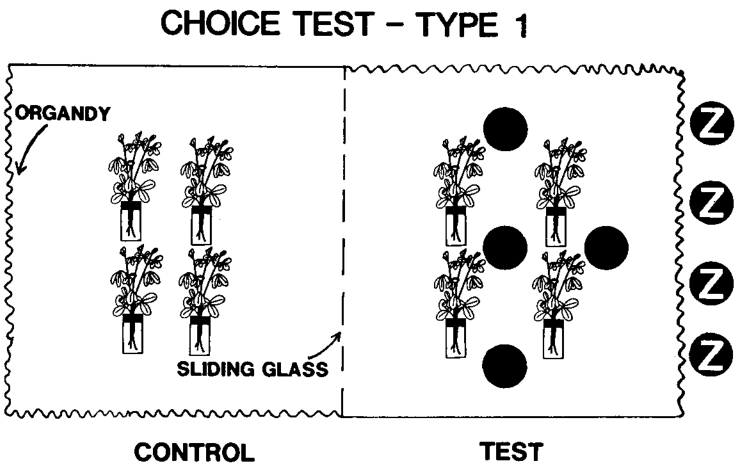

Fig. 1. Schematic diagram of choice experiments. Filled circles and Z in a circle represent possible positioning of test material in and out of cages.

trials for oviposition lasted for six days, except as noted. Adult residency was measured by counting males and females in the control and test cages at the end of the 1 and 6 day residency trials. In host preference tests of various leafhoppers, Nuorteva (1952) observed that at least 24 hours were required to observe definitive leafhopper reaction to test material.

Effect of light and alfalfa density. Initial experiments combined measurements of both oviposition and adult residency preference. In the first experiment, the effect of varying alfalfa distribution in the cage on leafhoppers was considered using the paired cages. In one cage, the four vials of alfalfa were separated on the corners of a 15 cm square (control cage, labelled "sparse"), while in the other cage the vials were contiguous (test cage, labelled "dense"). Those trials were combined with the effect of light on leafhoppers in a two-way ANOVA. One side of the cage was oriented toward a west-facing window and the other away from this light source. In this experiment all trials were two days in duration. In a second experiment, a much larger wood frame cage covered with organdy (120 × 80 × 110 cm tall) was also employed to test the effects of light and alfalfa density. On six occasions, three hundred leafhoppers were aspirated into the cage in which treatments were arranged in four groups. Light, measured with a hand-held photographic light meter (Gossen Model 1266-175), and temperature, measured with a thermograph (Weather measure Corp., Model T306), were recorded in each of the corners of the cage for one week. The effect of light on oviposition was analyzed using Friedman's randomized block test. The effect of plant density (sparse or dense) on oviposition was examined by placing dense and sparse alfalfa in paired positions, and analyzed by a paired t-test.

Effect of crabgrass. Paired-choice experiments were conducted to examine oviposition preference with four vials of alfalfa in both the control and test cages. In both cages (control and test), the alfalfa vials were separated on the corners of a 15 cm square (Fig. 1). First, the general effect of crabgrass was investigated by placing four vials of crabgrass in the test cage. Second, the visual and olfactory stimuli of crabgrass were removed, leaving gustatory and tactile stimuli, by placing four vials of crabgrass outside the test cage. Third, the olfactory stimuli of grass were differentiated from visual stimuli by placing four vials of alfalfa coated with a crabgrass macerate outside the test cage. The macerate was prepared by grinding 100 g of crabgrass blades from greenhouse plants in 200 ml of water. Solids were filtered off, and the alfalfa stems were dipped in the liquid until wetted. The macerate was allowed to air-dry before each trial. Fourth, as a control, alfalfa was placed outside the test cage. Fifth, the additional effects of crabgrass macerate in physical contact with leafhoppers were tested by placing four vials of alfalfa coated with crabgrass macerate inside the test cage. A similar series of paired-choice experiments was conducted to examine the residency preference of adults. The number of replications for each experiment is listed in Table 1.

Table 1. Choice-values for potato leafhopper oviposition and adult residency preference.

Experiment	Choice value* + SD	n	Probability for t-test c-value=1
Oviposition preference			
grass inside @ 6 days	0.77 ± 0.04	7	0.001
grass outside @ 6 days	0.86 ± 0.12	5	0.01
grass macerate out @ 6 days	0.83 ± 0.13	4	0.05
alfalfa outside @ 6 days	1.32 ± 0.40	9	0.05
grass macerate in @ 6 days	0.82 ± 0.11	4	0.03
Adult residency preference			
grass inside @ 6 days	0.81 ± 0.20/0.92 ± 0.12†	5	NS
grass outside @ 6 days	0.77 ± 0.23/0.94 ± 0.13	5	NS
grass outside @ 1 day	0.74 ± 0.23/0.83 ± 0.16	6	0.05/0.05‡
grass macerate out @ 6 days	0.81 ± 0.10/0.74 ± 0.21	6	0.01/0.03
alfalfa outside @ 6 days	1.44 ± 0.15/1.45 ± 0.10	4	0.001/0.001
grass macerate in @ 6 days	1.01 ± 0.18/1.01 ± 0.13	6	NS/NS

* Choice value ranges from 0 (preference for control) to 2 preference for test) with 1 signifying no preference.

† Means for females and males, respectively.

‡ Probabilities for females and males, respectively.

An index of preference was used to calculate oviposition or residency preference for all paired choice experiments: c = 2(test response)/[(test response) + (control response), [where c is the choice value Kogan and Goeden 1970). This formula produced a scale of values from 0 to 2, with 0 indicating complete preference for the control cage, and 2 complete preference for the test cage. Except for the initial ANOVA, choice experiments were analyzed with Student's t-test (null hypothesis, c=1). Regression analysis was also used to examine the effect of leafhopper density on choice values.

Results

Effect of light and alfalfa density. Alfalfa density and light intensity (experiment 1) had no significant effect on leafhopper oviposition or adult residency preference in the paired-choice experiments (ANOVA; df 3, 13; $p > 0.05$). In the second, large cage experiment, eggs were deposited most in the corner receiving the greatest amount of light and least in the darkest corner (chi-square; df = 3; $p = 0.025$). Temperature was the same in all corners and therefore did not influence results (temperature ranged from 20.5 to 26.0° C simultaneously in each corner). Light levels were 6.7 ± 1.0, 5.3 ± 0.5, 4.1 ± 0.4, and 3.8 ± 0.2 foot-candles for the four corners, while the sums of ranks for eggs oviposited were 20, 17, 13, and 10 respectively for each corner (Friedman's randomized block and analysis). The oviposition preference of potato leafhopper was significantly affected by alfalfa density in a large cage experiment; more eggs were found in the sparse alfalfa (172 ± 33) than in dense alfalfa (67 ± 17; $n = 4$, $p = 0.05$).

Even though light and density were not significant factors affecting oviposition or residency preference in the paired-choice experiments, these effects were controlled in all subsequent experiments to reduce variation that may occur as demonstrated in large cage experiments.

Effect of crabgrass on oviposition preference. Oviposition preference for the test alfalfa was reduced when crabgrass was inside the test cage (t-test, $p = 0.001$, Table 1). Crabgrass outside the test cage also lowered oviposition preference for the test alfalfa ($p = 0.01$) showing that tactile and gustatory stimuli do not play a major role in preference for the control. When crabgrass macerate was applied to alfalfa outside the test cage, reduced preference for the test alfalfa was observed again ($p = 0.05$). Alfalfa outside the test cage significantly increased oviposition preference for the test alfalfa ($p = 0.05$), corresponding to approximately 66% of eggs oviposited in alfalfa next to alfalfa outside the cage. When crabgrass macerate was applied directly to alfalfa in the test cage, reduced preference for the test alfalfa was observed again ($p = 0.03$). The c-value for this experiment was also approximately equal to the c-value for the experiment in which crabgrass was inside the cage, indicating no additional response to stimuli from direct contact with crabgrass macerate.

There were no systematic effects of leafhopper density on oviposition preference (pooled regression $R^2 = 0.02$, NS, see Table 2) except for the experiment with grass inside the test cage. In this case, there was a negative relationship (slope = -0.54, $p = 0.01$); increasing density increased preference for the control.

Effect of crabgrass on adult residency preference. When crabgrass was in the test cage, greater numbers of males and females were observed in the control cage, but preference for the control cage was not significantly demonstrated with a t-test. However, there was a singificant positive regression of choice values with increasing leafhopper density ($p = 0.01$, Table 2); as leafhopper density increased, grass had less influence on adult location. When crabgrass was outside the test cage, males and females showed the same trend in favor of the control (Table 1), though not significant with a t-test. This result is explained by the positive regression with increasing leafhopper density ($p = 0.02$, Table 2).

When this last experiment was performed for only one day, the choice values for both male and females were similar to the 6-day experiment, although this time statistical significane was obtained with a t-test ($p = 0.05$ for both male and

Table 2. Potato leafhopper density in laboratory cages correlated and regressed with oviposition and adult residency preference (c-values).

	Regression*		
Experiment	R^2	slope	P
Oviposition preference:			
grass inside @ 6 days	0.77	-0.54	0.01
grass outside @ 6 days	0.29	1.12	NS
grass macerate out @ 6 days	0.40	1.07	NS
alfalfa outside @ 6 days	0.04	-0.01	NS
grass macerate in @ 6 days	0.08	-0.32	NS
pooled data†	0.02	-	-
Adult residency preference:			
grass inside @ 6 days	0.83	2.04	0.01
grass outside @ 6 days	0.85	0.47	0.02
grass outside @ 1 day	0.61	0.88	NS
grass macerate out @ 1 day	0.01	0.23	NS
alfalfa outside @ 6 days	0.12	0.00	NS
grass macerate in @ 1 day	0.05	0.54	NS
pooled data	0.27	-	-

* For sample size see Table 1.

† Only experiments containing crabgrass were used in calculation of regression.

females, Table 1), indicating, as for oviposition preference, gustatory and tactile stimuli do not effect residency preference. Unlike the six-day observation periods, 1 day observation periods did not give significant regressions. In all further experiments of adult residency preference, only one-day experiments were used. When alfalfa treated with crabgrass macerate was placed outside the test cage, eliminating any visual stimuli specific to grass, adults still preferred the control cage ($p = 0.01$ and $p = 0.03$ for females and males, respectively). Having more alfalfa outside the test cage significantly increased preference for the test cage for both males and females ($p = 0.02$ and $p = 0.01$ respectively, see Table 1). When crabgrass macerate on alfalfa was placed inside the test cage, adults showed no preference.

Discussion

Light intensity had a significant effect on potato leafhopper oviposition preference as shown in the large cage experiment. Cherry et al. (1977) and others have used the attractiveness of light to catch potato leafhoppers in the field. However, the preference for light by ovipositing females in this study may have been an indirect effect by changing the quality of the alfalfa in the vials. Different levels of light on cut stems may have produced differences that females reacted to over six days. In either case, light may affect the outcome of trials, and it must be controlled or accounted for in experiments.

Vegetation density (independent of quantity) also affected oviposition preference. Although significant oviposition preference was not observed in the paired-cage tests, oviposition preference for sparse alfalfa was demonstrated in the large cage experiment. This phenomenon may be related to flight behavior. Because flight was the major means of locomotion between stems, as observed in this study, spacing between bunches of alfalfa may have allowed easier access to each stem. This observation is supported by field data which shows leafhoppers prefer sparse alfalfa over dense alfalfa (Smith 1987).

As might be expected, adult density moderates the effect of oviposition and residency preference. The greater the number of leafhoppers, the less other stimuli affect preference, because of competition between individuals for resting and oviposition sites. Sex ratio was not observed as a factor in these experiments. Though all experiments were initiated with nearly 50% females, mortality during the experiment was variable, averaging 14% (±5%, n = 33) with more males dying than females. Correlations of sex ratio with results were not significant.

The series of experiments on oviposition and adult residency preference with different forms and presentations of crabgrass, demonstrated how leafhoppers reacted to crabgrass. Crabgrass inside the test cage reduced preference for alfalfa in the test cage. After demonstration of this, removing crabgrass to the outside of the cage, and then using a crabgrass macerate on alfalfa, we successively removed tactile, gustatory and then visual stimuli specific to crabgrass. When alfalfa was placed outside the test cage, leafhoppers were attracted to it, indicating that the presence of the crabgrass macerate reduced oviposition and residency preference through olfaction only because alfalfa remained the same color. When crabgrass macerate was applied directly to test alfalfa, there was no increase in oviposition preference for the control due to enhanced gustatory or tactile stimuli. Residency preference was not affected and possible sugars in the macerate may have attracted adults, while still providing the compounds to reduce oviposition preference.

Results for adult residency preference were not as significant as those for oviposition preference, even though in many cases the value of the CI were similar for each preference for a given test. One reason for this difference may lie in the specificity of the behaviors we observed. Oviposition preference is related to a very specific daily behavior of mature females that is very important in the leafhoppers life history, while residency preference can be related to any number of behaviors, such as resting, feeding, escape response (hiding from investigators), mating, preening, etc. Therefore, we might hypothesize that the deterrent properties of crabgrass may affect the measurement of specific behaviors more than a spectrum of behaviors.

By comparison with Saxena et al. (1974), Saxena and Saxena (1974, 1985a, 1975b) and Saxena (1979), we can suggest that alfalfa is attractive in this experiment to potato leafhoppers because of the increased humidity associated with it, though it may have an odor attractive to potato leafhopper. Like *Amrasca devastans,* the potato leafhopper is a polyphagous species and probably relies on general cues during host-finding from a distance.

Clearly, crabgrass macerate can not be equated directly with the olfactory properties of whole crabcrass. Many internal compounds are released by maceration and may have an effect as well. The effect of crabgrass macerate observed in these experiments is similar to Altieri et al. (1977), and it is suggestive of the olfactory properties of whole crabgrass. Altieri et al. (1977) used the macerates for successful control of *E. kraemeri* in field plots. Their results may rest on the same non-preference observed for the potato leafhopper in this study. The results of the present study also may explain results observed by Peterson (1979), Lamp et al. (1984a), and Oloumi-Sadeghi et al. (1987) on the reduction of potato leafhopper density in alfalfa fields in the presence of grass weeds, including crabgrass. Crabgrass may have visual stimuli that influences leafhopper behavior, because we did not directly exclude visual cues in the experiments. Nevertheless, we suggest that olfactory stimuli, i.e., a volatile compound from the non-host crabgrass, can reduce potato leafhopper oviposition and adult residency in alfalfa.

Acknowledgments

We would like to thank Renee Sherman, Anne Kirts, and Todd Elmore for their technical assistance. This publication is a contribution of the Illinois Natural History Survey and the Illinois Agricultural Experiment Station (College of Agriculture, University of Illinois at Urbana-Champaign) and was supported in part by Project 12-339 (NC-149) and the United States Department of Agriculture under agreement No. 71-59-2481-1-2-039-1.

Reference Cited

Altieri, M. A., A. van Schoonhoven, and J. Doll. 1977. The ecological role of weeds in insect pest management systems: review illustrated by bean (*Phaseolus vulgaris*) cropping systems. PANS 23: 195-205.

Cherry, R. H., K. A. Wood, and W. G. Ruesink. 1977. Emergence trap and sweep net sampling for adults of the potato leafhopper from alfalfa. J. Econ. Entomol. 70: 279-282.

Jermy, T. 1971. Biological background and outlook of the antifeedant approach to insect control. Acta Phytopath. Acad. Sci. Hung. 6: 253-260.

Kogan, M., and R. D. Goeden. 1970. The host-plant range of *Lema trilineata daturaphila* (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 63: 1175-1180.

Koul, O. 1986. Feeding deterrence induced by plant limonoids in the larvae of *Spodoptera literatura* (F.) (Lepidoptera, Noctuidae). Z. angewandte Entomol. 95: 166-171.

Lamp, W. O., R. J. Barney, E. J. Armbrust, and G. Kapusta. 1984a. Selective weed control in spring-planted alfalfa: Effect on leafhoppers and planthoppers (Homoptera: Auchenorrhyncha), with emphaisis on potato leafhopper. Environ. Entomol. 13: 207-213.

Lamp, W. O., M. J. Morris, and E. J. Armbrust. 1984b. Suitability of common weed species as host plants for the potato leafhopper, *Empoasca fabae.* Entomol. Exp. Appl. 36: 125-131.

Lamp, W. O. and L. M. Smith. 1989. Sampling objectives and problems. Misc. Publ. Entomol. Soc. Am. 72: 3-9.

Lance, D. R. 1983. Host-seeking behavior of the gypsy moth: The influence of polypyhagy and highly apparent host plants. *In* Herbivorous Insects, S. Ahmad (ed.). Academic Press, New York. p. 201-224.

Latheef, M. A., and J. H. Ortiz. 1983. The influence of companion herbs on egg distribution of the imported cabbageworm, *Pieris rapae,* on collard plants. Can. Entomol. 115: 1031-1038.

Nuorteva, P. 1952. Die Nahrungespflanzenwahl der Insketen im Lichte von Untersuchungen an Zikaden. Ann. Acad. Scien. Fennicae. Series A. IV. Biologica 19: 5-90.

Oloumi-Sadeghi, H., L. R. Zavaleta, W. O. Lamp, E. J. Armbrust, and G. Kapusta. 1987. Interactions of the potato leafhopper (Homoptera: Cicadellidae) with weeds in an alfalfa ecosystem. Environ. Entomol. 16: 1175-1180.

Peterson, K. 1979. The influence of weed control on alfalfa stand, production, and the incidence of other pests. M. S. thesis, Southern Illinois University, Carbondale. 58 pp.

Saxena, K. N. 1979. Physiology of leafhoppers: their behavior and nutrition. Final Tech. Report, USDA P. L. 480 Proj. No. A 7-ENT-109. 95 pp.

Saxena, K. N., and A. Basit. 1982. Inhibition of oviposition by volatiles of certain plants and chemicals in the leafhopper, *Amrasca devastens* (Distant). J. Chem. Ecology. 8: 329-338.

Saxena, K. N., J. R. Ghandi, and R. C. Saxena. 1974. Patterns of relationship between certain leafhoppers and plants. I. Responses to plants. Entomol. Exp. Appl. 17: 303-318.

Saxena, R. C., and Z. R. Khan. 1985. Electonically recorded disturbances in feeding behavior of *Nephotettix virescens* (Homoptera: Cicadellidae) on neem oil-treated rice plants. J. Econ. Entomol. 78: 222-226.

1986. Aberrations caused by neem oil odour in green leafhopper feeding on rice plants. Entomol. Exp. Appl. 42: 279-284.

Saxena, K. N., and R. C. Saxena. 1974. Patterns of relationships between certain leafhoppers and plants, part IV. Role of sensory stimuli in orientation and feeding. Entomol. Exp. Appl. 17: 493-503.

1975a. Patterns of relationships between certain leafhoppers and plants, part IV. Sequence of stimuli determining arrival on a plant. Entomol. Exp. Appl. 18: 207-212.

1975b. Patterns of relationships between certain lefhoppers and plants, part III. Range and interaction of sensory stimuli. Entomol. Exp. Appl. 18: 194-206.

Schoonhoven, L. M. 1968. Chemosensory of host-selection. Annu. Rev. Entomol. 13: 115-136.

Smith, L. M. II. 1987. Determination of mechanisms by which weed grasses affect the potato leafhopper (*Empoasca fabae* (Homoptera: Cicadellidae)) in alfalfa. Ph.D. thesis, Univ. of Illinois, Urbana.

Thorsteinson, A. J. 1960. Host selection in phytophagous insects. Annu. Rev. Entomol. 5: 193-218.