

For submission to:
Journal of Agricultural
and Urban Entomology

Send correspondence to:
William Lamp
Department of Entomology
University of Maryland
College Park, MD 20742
301/405-3959 (voice)
301/314-9920 (fax)
lamp@umd.edu

**Feeding Site Preference of Potato Leafhopper (Homoptera: Cicadellidae)
on Alfalfa and its Effect on Photosynthesis¹**

William O. Lamp, Gary R. Nielsen, Clara B. Fuentes², and Bruno Quebedeaux³

Department of Entomology, University of Maryland, College Park, MD 20742

Footnotes

¹ Accepted for publication 3-August-2004

² Environmental Protection Agency, Biopesticides and Pollution Prevention Division,
Arlington, VA 22202

³ Department of Natural Resource Sciences and Landscape Architecture, University of
Maryland, College Park, MD 20742

ABSTRACT Potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is a well-known pest of alfalfa in eastern North America. Its unique injury is associated with changes in host plant physiology, yet research to date has focused on the whole plant response. We conducted studies to determine the feeding site preference of nymphs and adults on alfalfa plants, and to measure the impact of leaf and stem feeding on subsequent photosynthesis and stomatal conductance of leaves. Our observations of potato leafhopper nymphs in the field, and of nymphs and adults in the greenhouse, demonstrated contrasting (but variable) plant part preference for settling by the different leafhopper life stages: nymphs prefer leaves while adults prefer stems. In addition, CO₂ gas exchange measurements demonstrated that the location of leafhopper feeding on the plant surface affects the specific pattern of injury expressed in the physiology of alfalfa: leaf-feeding impacts photosynthesis rate and stomatal conductance of the injured leaf alone whereas stem-feeding impacts photosynthesis rate and stomatal conductance for leaves apical of the injured internode. Our results suggest that nymphs and adults likely cause different physiological effects on alfalfa, in spite of their equivalency in pest management recommendations. Furthermore, the relationship between potato leafhopper injury, photosynthesis, and hopperburn remain unclear, and understanding the mechanisms would aid in predicting the level of damage associated with injury.

KEY WORDS Homoptera, Cicadellidae, *Empoasca fabae*, potato leafhopper, alfalfa, *Medicago sativa*, feeding behavior, photosynthesis, stomatal conductance, plant-insect interactions

Although sap-feeding insects impact normal acquisition of resources by their host plants, relatively few species disrupt plant physiological functions (Raven 1983). Certain species of sap-feeders that do disrupt host plant physiology include those species associated with burning symptoms on leaves, such as brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), *Empoasca kraemeri* Ross & Moore (Homoptera: Cicadellidae), and potato leafhopper, *Empoasca fabae* (Harris) (Serrano & Backus 1998, Ecale Zhao & Backus 1999, Watanabe & Kitagawa 2000). Their feeding disrupts host physiology as a consequence of a cascade of morphological and physiological reactions in response to feeding injury (Welter 1989).

Potato leafhopper is a well-known pest of many eastern North American crops, including alfalfa, *Medicago sativa* L. (Lamp et al. 1994). Hopperburn of alfalfa is the result of injury induced by potato leafhopper feeding (Granovsky 1928). The leafhopper feeds on alfalfa by repeated injection of its stylets into the vascular tissue through which plant material is ingested (Backus & Hunter 1989, Kabrick & Backus 1990). Through a combination of mechanical and salivary stimuli, potato leafhopper feeding enhances a wound response in alfalfa that results in changes in the vascular tissue around the feeding site (Ecale & Backus 1995a, 1995b). When this occurs, photoassimilates transported through the phloem build up around the injured site (Johnson 1934, Hibbs et al. 1964, Nielsen et al. 1990, 1999), and rates of photosynthesis are reduced (Womack 1984, Flinn et al. 1990). Thus, leafhopper feeding initiates a cascade of changes in alfalfa, and ultimately, the injury is expressed as hopperburn, a characteristic yellowing of leaves

(Granovsky 1928) as well as delayed plant maturity, reduced nutritive components, stunted growth, and reduced yields (Kindler et al. 1973, Hower 1989, Hutchins & Pedigo 1989).

Currently, the cascade of physiological changes subsequent to vascular injury by potato leafhopper that leads to hopperburn is unclear. Research to date on the physiological response of plants to potato leafhopper injury has focused on whole plant responses (Womack 1984, Flinn et al. 1990). Yet, feeding site preference of adults depends on the host plant (Backus et al. 1990), and the pattern of distribution of individuals may be related to the pattern of physiological injury within a host plant. Also, the impact of feeding injury on CO₂ gas exchange rates may be localized around the feeding site, or may be generalized over the whole plant. Thus, the pattern of photosynthesis among leaves of an injured plant may also provide clues to the cause of hopperburn. Furthermore, research on the relationship between pest-induced stress and the physiological response of crops is critical in the development of pest management programs (Peterson & Higley 2001).

Because research to date has focused on the whole-plant response to leafhopper injury, and on adult behavior on individual alfalfa plants, we conducted studies to: 1) determine the feeding site preference of nymphs and adults on alfalfa plants, and 2) measure the impact of leaf and stem feeding on subsequent CO₂ gas exchange rates (photosynthesis and stomatal conductance) of leaves.

Materials and Methods

Feeding site preference - field observations. To determine the location of nymphs on alfalfa stems under field conditions, we observed nymphs on three separate dates (24-July-1996, 15-Aug-1996, 19-Aug-1996) at the Central Maryland Research and Education Center, Clarksville, Maryland. Fields were selected on the basis of alfalfa development (early bud) and leafhopper infestation (nymph counts in sweep samples exceeded 3 per sweep). Individual stems were carefully clipped at the base, and quickly scanned for the locations of nymphs. The stems were compared to a wire flag, marked every 10 cm, so that location was recorded as between 0-10 cm, 10-20 cm, etc. from the base of the stem. The location was further recorded as on one of the following plant parts: stem, petiole, adaxial (upper) leaf surface, or abaxial (lower) leaf surface. In some cases, the nymphs were actively moving on the stem; this observation was also recorded. Finally, the length of the stem was recorded.

Feeding site preference - greenhouse observations. To further elucidate the feeding locations of nymphs and adults we conducted a greenhouse experiment using a completely randomized design of all possible combinations of two alfalfa development stages and five leafhopper life stages. Leafhoppers of various life stages were reared on susceptible 'Ranger' alfalfa plants in the greenhouse. A series of cages was prepared by caging ovipositing females on plants for two d each week for 5 weeks and allowing eggs and nymphs to develop. Leafhopper life stage treatments were young nymphs (second or third instars), old nymphs (fourth or fifth instars), young female adults (pre-reproductive, less than 7 d after adult emergence), old female adults (reproductive, 7-14 d after adult

emergence), and male adults. Plant development was manipulated by cutting 20 plants each week during a cycle of five weeks.

For this experiment, 60 'Ranger' alfalfa plants in 15 cm diameter plastic pots were clipped to one stem per pot. Half of the pots contained vegetative stems, with 7-14 d of regrowth and no visible flower buds. The other half contained reproductive stems, with 21-28 d of regrowth and 2-3 visible buds. Each stem was caged using a 7.6 cm diameter cellulose acetate cylinder, provided with organically covered windows for ventilation. Adults were aspirated from the culture, gender determined with a microscope, and transferred to cages. Nymphs were transferred using a camel hair brush after categorization on the basis of size and wingpad development. Each cage received three leafhoppers of a given stage. After 24 h, we recorded the location of insects. Leaves were then removed, labeled, and leaf area determined using a Videomex digital analysis system (Columbus Instruments, Columbus, Ohio). Stem and petiole lengths and diameters were determined using a caliper. These measurements were used to calculate the relative leaf, stem, and petiole surface area available to leafhoppers.

Leaf injury experiment. We examined the impact of leaf feeding by potato leafhopper on photosynthesis rates of injured and other leaves on the alfalfa plant. Two leaf treatments, injured or healthy, were randomly assigned to separate 'Ranger' alfalfa plants, grown from individual cuttings in 15 cm plastic pots under greenhouse conditions, and cut back 14 d prior to the experiment. Each treatment had five replications in a blocked design. At the time of the start of the experiment, each plant had at least three stems in a vegetative stage, 15-25 cm in height. One stem was selected to have a leaf

cage and for CO₂ gas exchange measurements, while an adjacent stem was selected for CO₂ gas exchange measurements only. The leaf cage consisted of two 10 ml disposable plastic beakers with foam rings attached to the rims. The two beakers were placed together over a leaf, and held in place with tape and attached to a wire stake. The cage was mounted on the third (from the top) fully-expanded leaf on the stem. The experiment was conducted in a MB-60 plant growth chamber (Percival Scientific Inc., Boone, Iowa) at 14:10 (L:D) h, with light intensity at plant height of 120 micromoles/m²/sec during the day.

The experimental leafhoppers were reared on alfalfa as described above. Nymphs were allowed to grow to 4th or 5th instar, and two such nymphs were aspirated into cages of plants of the “injured” treatment. Cages on the “healthy” treatment remained without leafhoppers during the experiment and served as controls. After 24 h, all cages and all leafhoppers were removed.

CO₂ gas exchange was measured with a LI-6200 portable photosynthesis measurement system (Li-Cor Inc., Lincoln, Nebraska) using a 0.25 L chamber. Measurements were taken on the same day after removal of the cages (d 0), and on d 1, 2, 3, and 7. On each day, measurements were taken on leaf 1 (the uppermost fully-expanded leaf), 2 (the second leaf from the top), and 3 (the third leaf from the top) of the “treatment” stem, and on leaf 1 and 2 of the “adjacent” stem of each plant. In the LI-6200 system, rates of net photosynthesis are measured by calculating CO₂ exchanged per unit leaf area per second and expressed as $\mu\text{mol m}^{-2} \text{s}^{-1}$. Transpiration rate is measured from the change in humidity with time, and stomatal conductance is measured from the

transpiration rate, leaf temperature, and air temperature, and is expressed as cm s^{-1} . At the end of the experiment, leaves were removed, labeled, and leaf area determined using a Videomex digital analysis system (Columbus Instruments, Columbus, Ohio). CO_2 gas exchange measurements were then calculated on the basis of actual leaf areas.

Stem injury experiment. We examined the effect of stem feeding injury in a companion experiment simultaneous with the leaf injury experiment. Using identical plants and leafhoppers, two stem injury treatments, injured or healthy, were assigned to plants, with five replications per treatment. A cage, formed from two 10 ml disposable beakers as above, was mounted over the stem internode between the second and third fully-expanded leaves from the top of the stem. Two nymphs were placed in cages for the injured treatment, whereas cages for the healthy treatment remained without leafhoppers. All other conditions and measurements were identical to the leaf experiment.

Statistical analysis. Field observations of nymphal feeding sites were statistically analyzed using chi-square for a random distribution among height categories and among plant part categories. Each category of plant parts is not equally represented by area, and we did not record specific areas associated with each category. In contrast, the laboratory observations of feeding sites were analyzed using chi-square test of the hypothesis that leafhoppers were located on stems, leaves, and petioles in proportion to their surface area on the plants.

Data from each of the leaf and stem injury experiments were analyzed using analysis of variance of mixed models for randomized complete block with repeated

measures (Proc Mixed, SAS 1997). Photosynthesis rate and stomatal conductance for each leaf position and on “treated” and “adjacent” stems were compared across leafhopper treatments (injured and healthy) and d subsequent to injury (0, 1, 2, 3, and 7). Both leafhopper treatments and d subsequent to injury were considered fixed effects, while blocks were random. Because preliminary analysis indicated that the responses during the first three d subsequent to injury were similar within a treatment (nonsignificant treatment by d interaction), yet the responses on d 7 differed from d 0-3, a separate analysis was performed for d 0-3 and d 7. Using Akaike’s Information Criteria, un(1) variance structure was selected for the analysis.

Results

Feeding site preference - field observations. Within alfalfa fields, nymphs were observed (n = 308) most commonly on the lower surface of leaves (50.8%), but also on the upper surface of leaves (27.1%) and on stems (9.5%). In 12.6% of the observations, the nymphs were moving and their feeding site could not be determined. Instars varied little from the overall pattern, however few (4%) of early instar (I+II) nymphs while a larger portion (15%) of late instar (IV+V) nymphs were observed on stems (Fig. 1). Not weighting for surface area of plant parts, nymphs occurred significantly more frequently on leaves than stems (χ^2 for instar I+II = 63.5, $P < 0.001$; χ^2 for instar III = 45.8, $P < 0.001$; χ^2 for instar IV = 25.8, $P < 0.001$; χ^2 for instar V = 36.5, $P < 0.001$). When comparing the occurrence on upper leaf versus lower leaf surface of leaves, nymphs generally occurred more frequently on the lower surface (χ^2 for instar I+II = 14.2, $P <$

0.001; χ^2 for instar III = 4.0, $P < 0.05$; χ^2 for instar IV = 2.7, $P < 0.10$; χ^2 for instar V = 4.6, $P < 0.05$).

The majority of nymphs were observed between 10-20 cm (45.1%) or 20-30 cm (41.9%) (Fig. 2). On individual stems, nearly half (46.4%) of the nymphs occurred in the top 10 cm section, whereas only a few (2.9%) occurred in the bottom 10 cm section of stems.

Feeding site preference - greenhouse observations. In cages over greenhouse-grown alfalfa, numbers of adults were similar on stems and leaves (40.7% versus 38.9%, respectively), whereas numbers of nymphs were greater on leaves than on stems (94.4% versus 1.4%, respectively) (Fig. 3). A relatively small number of adults and nymphs were observed on petioles (6.7%). No nymphs, but 25% of the males and 11.1% of the females were observed off plants. Using plant surface area as a basis to measure preference, the settling preference depended on alfalfa development. Whereas females showed no preference on vegetative alfalfa (for young females, $\chi^2 = 0.3$, $P > 0.10$; for old females, $\chi^2 = 1.0$, $P > 0.10$), females preferred stems over leaves on reproductive alfalfa (for young females, $\chi^2 = 42.9$, $P < 0.001$; for old females, $\chi^2 = 12.9$, $P < 0.001$). In contrast, whereas nymphs preferred leaves over stems on vegetative alfalfa (for young nymphs, $\chi^2 = 5.0$, $P = 0.025$; for old nymphs, $\chi^2 = 3.9$, $P < 0.05$), nymphs showed no preference on reproductive alfalfa (for young nymphs, $\chi^2 = 3.6$, $P > 0.05$; for old nymphs, $\chi^2 = 1.2$, $P > 0.10$). For both plant stages, males preferred stems over leaves (for vegetative alfalfa, $\chi^2 = 11.1$, $P < 0.001$; for reproductive alfalfa, $\chi^2 = 9.1$, $P < 0.001$).

Leaf injury experiment. The pattern of CO₂ gas exchange responses of photosynthesis rate and stomatal conductance were consistent from the leaf experiment for 0-3 d post-injury: only the leaf that was caged with leafhoppers had a significant change, whereas the two leaves above the caged leaf and the two leaves of the adjacent stem were not affected (Table 1). Although photosynthesis rates varied significantly by d subsequent to injury, the interaction term between d and injury was not significant. Thus, no significant recovery was observed for the 3 d after leafhopper removal. The terms for d and d*injury were not significant for stomatal conductance.

After one d of exposure to leafhoppers, the photosynthesis rate of injured leaves was reduced by 54.6%, 49.9%, 52.8%, and 55.7% in comparison to the healthy control leaves on 0, 1, 2, and 3 d after leafhopper removal, respectively (averaged in Fig. 4). Similarly, stomatal conductance of injured leaves was reduced by 41.4%, 55.3%, 42.6%, and 33.8% in comparison to the healthy control leaves on 0, 1, 2, and 3 d after removal, respectively.

On d 7 post-injury of the leaf experiment, differences were not significant for either the photosynthesis rate (range of F values from 0.23 to 5.66 and P values from 0.08 to 0.64) or stomatal conductance (range of F values from 0.01 to 0.72 and P values from 0.33 to 0.92) between any of the corresponding leaves of plants caged with leafhoppers and caged without leafhoppers.

Stem injury experiment. The pattern of CO₂ gas exchange responses of photosynthesis rate and stomatal conductance were also consistent from the stem experiment: only the leaves above the internode that was caged with leafhoppers had a

significant change, whereas the leaf below the caged internode and the two leaves of the adjacent stem were not affected (Table 2). For both the photosynthesis and stomatal conductance models for leaf 1 and leaf 2, only injury was a significant term, while d or the interaction term were not. Similar to the leaf experiment, no significant recovery was observed for the 3 d after leafhopper removal.

After one d of exposure to leafhoppers, the photosynthesis rate of leaf 1 above the injured internode declined by 56.0%, 54.4%, 50.5%, and 39.9% in comparison to the healthy control leaves on 0, 1, 2, and 3 d after leafhopper removal, respectively (averaged in Fig. 4). For leaf 2, just above the injured internode, the photosynthesis rate declined 69.5%, 39.8%, 56.0%, and 43.0% in comparison to the healthy control leaves on 0, 1, 2, and 3 d after leafhopper removal, respectively. Similarly, stomatal conductance of leaf 1 declined by 47.6%, 68.6%, 51.1%, and 23.3%, and of leaf 2 declined by 53.3%, 51.2%, 58.4%, and 24.3%, in comparison to the healthy control leaves on 0, 1, 2, and 3 d after removal, respectively.

On d 7 post-injury of the stem experiment, differences were not significant for either the photosynthesis rate (range of F values from 0.33 to 1.82 and P values from 0.21 to 0.60) or stomatal conductance (range of F values from 0.01 to 2.51 and P values from 0.19 to 0.93) between any of the corresponding leaves of plants caged with leafhoppers and caged without leafhoppers.

Discussion

Our observations of potato leafhopper nymphs in the field, and of nymphs and adults in the greenhouse, demonstrated contrasting (but variable) plant part preference for

settling by the different leafhopper life stages: nymphs generally prefer leaves while adults generally prefer stems. In addition, our CO₂ gas exchange measurements demonstrated that the location of leafhopper feeding on the plant surface affects the specific pattern of injury expressed in the physiology of alfalfa: leaf-feeding impacts photosynthesis and stomatal conductance of the injured leaf alone whereas stem-feeding impacts photosynthesis and stomatal conductance for leaves apical of the injured internode. Thus, the variability of feeding behaviors as described by Backus & Hunter (1989) for potato leafhopper is exacerbated by the variability of plant part selection by leafhopper stages and the subsequent effect on host plant physiology.

When a leafhopper encounters a host plant, it proceeds through a sequence of stereotypical behaviors, including plant surface exploration, stylet probing, plant fluid ingestion, and probe termination (Backus 1985). Since plant fluid ingestion requires the most time, the observed locations of leafhoppers on the plant surface likely reflect the sites of feeding. However, the precise actions of stylet penetration are only discerned by methods such as electronic monitoring, thus the location of injured tissue may not reflect the observations of individuals on plants (Backus et al. 1990). For example, although Backus et al. (1990) demonstrated that potato leafhopper adults prefer stems of alfalfa plants, the severity of injury symptoms was not related to the time spent on alfalfa stems or leaves. Nevertheless, our observations of settling behavior that nymphs generally prefer leaves and adults prefer stems suggests that the different life stages injure different parts of the alfalfa plant. However, we did find evidence that stage of the alfalfa plant may modify preference (Fig. 3).

Injury by potato leafhopper over one day, confined to either a leaf or a stem internode, resulted in approximately 50% reduction in the photosynthesis rate of alfalfa. Similarly, Flinn et al. (1990) measured a 60% and 80% reduction in photosynthesis on whole plants caged with 4 or 8 potato leafhoppers. We further observed no recovery of photosynthesis for up to 3 d subsequent to the injury. On d 7 post-injury, no significant difference in photosynthesis of injured and healthy plants was present, suggesting that the plant repaired the injury within the week. Through a time course study, Ecale & Backus (1995a) demonstrated that alfalfa was capable of repairing vascular connections by 6 d subsequent to potato leafhopper injury.

Because other studies focused on the impact of potato leafhopper on photosynthesis of injured alfalfa using whole plants (Womack 1984, Flinn et al. 1990), this is the first report of the pattern of the physiological response of portions of the plant subsequent to localized injury. When one leaf was injured by potato leafhopper, the photosynthesis and stomatal conductance of only that leaf was affected. Leaves on the same stem and leaves of an adjacent stem on the same plant were not affected. In contrast, when a stem internode was injured, photosynthesis and stomatal conductance of all leaves apical to the internode was affected. The leaf below the internode and the leaves on an adjacent stem of the same plant were not affected. Thus, we found no evidence of compensation by other leaves on the plant for the loss of photosynthetic capacity of injured leaves or of leaves affected by stem-feeding.

The mechanism to explain how potato leafhopper injury is associated with reduced photosynthesis rate and stomatal conductance remains unclear, as is the

relationship between these physiological responses and the occurrence of hopperburn symptoms on leaves. Previous research has clearly shown that potato leafhopper injury to stem tissue causes reduced phloem translocation of photoassimilates (Nielsen et al. 1990, 1999), and that carbohydrate levels increase on injured leaves (Johnson 1938, Hibbs et al. 1964). One hypothesis for the reduction of photosynthesis is feedback inhibition (Hibbs et al. 1964). Basically, feedback inhibition (also called end product inhibition) results from the accumulation of starch subsequent to injury, caused by the restriction of sugar transport through the phloem and by the inhibition of the transport of sugars from the leaf. Other mechanisms may also be responsible, including those that operate at the molecular level (Pirone, et al. in press). Indeed, recent research of aphid injury suggests that vascular feeding may significantly affect either stomatal response or carboxylation reactions, and not light-harvesting reactions of photosynthesis (Macedo et al. 2003a, 2003b).

Our results have several implications for pest management, yet also highlight the need for additional research. First, the injury caused by stem-feeding was more significant to the whole plant physiology than leaf-feeding. Since adults, more so than nymphs, feed on stems, they are likely to cause greater damage than nymphs. This is especially significant because IPM programs use nymph and adult counts as equivalent in determining the threshold for pesticide applications (e.g., Townsend 2002), yet our study suggests the pattern of injury on an alfalfa plant differs between adults and nymphs. Second, we found that feeding by potato leafhopper reduces photosynthesis rate within 24 hrs, preceding the development of hopperburn symptoms by 6-8 d (Granovsky 1928),

and suggesting that suppression of feeding must be timely to prevent injury. Most importantly, we found that the dynamic behavior of individuals on where they feed on plants, combined with contrasting effects of the location of feeding on plant physiology, suggests that the need for suppression by pesticides is very difficult to assess. Indeed, and finally, the mechanistic relationship between injury, photosynthesis, and hopperburn remains unclear, and thus requires further study.

Acknowledgments

We thank David Liewehr for his statistical advice. Support for this research came in part from USDA-CSREES Project No. MD-H-176 (NC-193), USDA NE Regional IPM Project No. 93-34103-8418, and the Maryland Agricultural Experiment Station Grants Program.

References Cited

- Backus, E. A. 1985.** Anatomical and sensory mechanisms of leafhopper and planthopper feeding behavior, pp. 163-194. In L. R. Nault and J. G. Rodriguez (Eds.), *The Leafhoppers and Planthoppers*. John Wiley, New York, New York, 500 pp.
- Backus, E. A., N. M. Gruenhagen & S. A. Becker. 1990.** The potato leafhopper (Homoptera: Cicadellidae) exhibits different settling distributions on alfalfa and broad bean. *J. Econ. Entomol.* 83: 814-818.
- Backus, E. A. & W. B. Hunter. 1989.** Comparison of feeding behavior of the potato leafhopper *Empoasca fabae* (Homoptera: Cicadellidae) on alfalfa and broad bean leaves. *Environ. Entomol.* 18: 473-480.

- Ecale, C. L. & E. A. Backus. 1995a.** Time course of anatomical changes to stem vascular tissue of alfalfa, *Medicago sativa*, from probing injury by the potato leafhopper, *Empoasca fabae*. *Can. J. Bot.* 73: 288-298.
- Ecale, C. L. & E. A. Backus. 1995b.** Mechanical and salivary aspects of potato leafhopper probing in alfalfa stems. *Entomol. exp. appl.* 77: 121-132.
- Ecale Zhou, C. L. & E. A. Backus. 1999.** Phloem injury and repair following potato leafhopper feeding on alfalfa stems. *Can. J. Bot.* 77: 537-547.
- Flinn, P. W., A. A. Hower & D. P. Knievel. 1990.** Physiological response of alfalfa to injury by *Empoasca fabae* (Homoptera:Cicadellidae). *Environ. Entomol.* 19: 176-181.
- Granovsky, A. A. 1928.** Alfalfa “yellow top” and leafhoppers. *J. Econ. Entomol.* 21: 261-267.
- Hibbs, E. T., D. L. Dahlman & R. L. Rice. 1964.** Potato foliage sugar concentration in relation to infestation by the potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae). *Ann. Entomol. Soc. Amer.* 57: 517-521.
- Hower, A. A. 1989.** Potato leafhopper as a plant stress factor on alfalfa, pp. 35-39. In E. J. Armbrust and W. O. Lamp (Eds.), *History and Perspectives of Potato Leafhopper (Homoptera: Cicadellidae) Research*. Misc. Publ. Entomol. Soc. Am. 72, Entomological Society of America, Lanham, Maryland, 69 pp.
- Hutchins, S. H. & L. P. Pedigo. 1989.** Potato leafhopper-induced injury on growth and development of alfalfa. *Crop Sci.* 29: 1005-1011.

- Johnson, H. W. 1934.** Nature of injury to forage legumes by the potato leaf hopper. J. Agric. Res. 49: 379-406.
- Johnson, H. W. 1938.** Further determinations of the carbohydrate-nitrogen relationship and carotene in leafhopper yellowed and green alfalfa. Phytopathol. 28: 273-277.
- Kabrick, L. R. & E. A. Backus. 1990.** Salivary deposits and plant damage associated with specific probing behaviors of the potato leafhopper, *Empoasca fabae*, on alfalfa stems. Entomol. exp. appl. 56: 287-304.
- Kindler, S. D., W. R. Kehr, R. L. Ogden & J. M. Shalk. 1973.** Effect of potato leafhopper injury on yield and quality of resistant and susceptible alfalfa clones. J. Econ. Entomol. 66: 1298-1302.
- Lamp, W. O., G. R. Nielsen & S. D. Danielson. 1994.** Patterns among host plants of potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae). J. Kans. Entomol. Soc. 67: 354-368.
- Macedo, T. B., L. G. Higley, X. Ni & S. Quisenberry. 2003a.** Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. J. Econ. Entomol. 96: 188-193.
- Macedo, T. B., L. G. Higley, X. Ni & S. Quisenberry. 2003b.** Light activation of Russian wheat aphid-elicited physiological responses in susceptible wheat. J. Econ. Entomol. 96: 194-201.
- Nielsen, G. R., W. O. Lamp & G. W. Stutte. 1990.** Potato leafhopper (Homoptera: Cicadellidae) feeding disruption of phloem translocation in alfalfa. J. Econ. Entomol. 83: 807-813.

- Nielsen, G. R., C. Fuentes, B. Quebedeux, Z. Wang & W. O. Lamp. 1999.** Alfalfa physiological response to potato leafhopper injury depends on leafhopper and alfalfa developmental stage. *Entomol. exp. appl.* 90: 247-255.
- Peterson, R. K. D. & L. G. Higley. 2001.** Illuminating the black box: the relationship between injury and yield, pp. 1-12. R. K. D. Peterson and L. G. Higley (Eds.), *Biotic Stress and Yield Loss*. CRC Press, Boca Raton, Florida, 261 pp.
- Pirone, C. L., L. C. Alexander & W. O. Lamp.** Patterns of starch accumulation in alfalfa subsequent to potato leafhopper (Homoptera: Cicadellidae) injury. *Environ. Entomol.* (in press).
- Raven, J. A. 1983.** Phytophages of xylem and phloem: a comparison of animal and plant feeders. *Adv. Ecol. Res.* 13: 135-234.
- SAS Institute, Inc. 1997.** SAS/STAT Software: Changes and Enhancements through Release 6.12. Cary, North Carolina: SAS Institute. 1167 pp.
- Serrano, M. S. & E. A. Backus. 1998.** Differences in cellular abnormalities induced by the probing behaviors of *Empoasca kraemeri* (Homoptera: Cicadellidae) on tolerant and susceptible common beans. *J. Econ. Entomol.* 91: 1481-1491.
- Townsend, L. 2002.** Potato leafhoppers.
<http://www.uky.edu/Agriculture/Entomology/entfacts/fldcrops/efl15.htm>.
- Watanabe, T. & H. Kitagawa. 2000.** Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). *J. Econ. Entomol.* 93: 1192-1198.

Welter, S. C. 1989. Arthropod impact on plant gas exchange, pp. 135-150. In E. A. Bernays (Ed.), *Insect-Plant Interactions*, Vol. 1. CRC Press, Boca Raton, Florida, 164 pp.

Womack, C. L. 1984. Reduction in photosynthetic and transpiration rates of alfalfa caused by the potato leafhopper (Homoptera: Cicadellidae) infestations. *J. Econ. Entomol.* 77: 508-513.

Table 1. F values and statistical significance of CO₂ gas exchange responses of alfalfa subsequent to leafhopper injury to leaf 3 of the treated stem.

Stem ^a	Leaf ^b	Factor ^c	F value and significance ^d	
			Photosynthesis	Stomatal conductance
Trt	1	Inj	0.20	0.82
		Day	8.48**	0.45
		Inj*Day	0.55	1.18
	2	Inj	0.11	0.34
		Day	7.57**	1.55
		Inj*Day	0.25	0.80
	3	Inj	9.47**	16.87***
		Day	2.08	1.09
		Inj*Day	0.09	0.71
Adj	1	Inj	0.01	0.00
		Day	4.02*	2.18
		Inj*Day	0.21	0.12
	3	Inj	0.03	0.18
		Day	1.15	1.21
		Inj*Day	0.23	0.68

^a Measurements taken from leaves on the treated (Trt) stems or the adjacent (Adj)

untreated stems.

^b Leaf number is counted with the uppermost, fully-developed leaf labeled as 1.

^c Inj, presence or absence of leafhopper injury; Day, d subsequent to injury.

^d *, P<0.05; **, P<0.01, ***, P<0.001.

Table 2. F values and statistical significance of CO₂ gas exchange responses of alfalfa subsequent to leafhopper injury to the stem internode between leaf 2 and 3 of the treated stem.

Stem ^a	Leaf ^b	Factor ^c	F value and significance ^d	
			Photosynthesis	Stomatal conductance
Trt	1	Inj	10.57**	11.67**
		Day	0.73	1.25
		Inj*Day	0.14	0.94
	2	Inj	17.54***	21.02***
		Day	1.77	3.13
		Inj*Day	0.35	2.51
	3	Inj	0.32	3.63
		Day	0.70	0.58
		Inj*Day	2.05	0.98
Adj	1	Inj	0.39	1.29
		Day	0.89	0.85
		Inj*Day	1.33	0.72
	3	Inj	1.42	0.70
		Day	0.88	1.85
		Inj*Day	0.83	1.39

^a Measurements taken from leaves on the treated (Trt) stems or the adjacent (Adj), untreated stems.

^b Leaf number is counted with the uppermost, fully-developed leaf labeled as 1.

^c Inj, presence or absence of leafhopper injury; Day, d subsequent to injury.

^d *, P<0.05; **, P<0.01, ***, P<0.001.

Figure Captions

Fig. 1. Observed distributions of nymphal instars by plant surface type in alfalfa fields.

Fig. 2. Top, observed distributions of nymphal instars by height within the canopy; bottom, distributions of alfalfa stems by height.

Fig. 3. Settling distribution of adult and nymphal potato leafhoppers on vegetative and reproductive alfalfa after 24 hrs in the greenhouse. Old fem, females 7-14 d after adult emergence; yng fem, females less than 7 d after adult emergence; mal, males; old nym, fourth or fifth instar nymphs; yng nym, second or third instar nymphs.

Fig. 4. Mean \pm standard error gas exchange rates, averaged over d 0-3 subsequent to injury, for individual leaves of the treated stem for the leaf injury experiment. Units of net photosynthesis are $\mu\text{mol m}^{-2} \text{s}^{-1}$, and units of stomatal conductance are cm s^{-1} .

Fig. 5. Mean \pm standard error gas exchange rates, averaged over d 0-3 subsequent to injury, for individual leaves of the treated stem for the stem injury experiment. Units of net photosynthesis are $\mu\text{mol m}^{-2} \text{s}^{-1}$, and units of stomatal conductance are cm s^{-1} .

Running Head

Lamp et al.: Leafhopper Feeding Site Preference and Alfalfa Photosynthesis

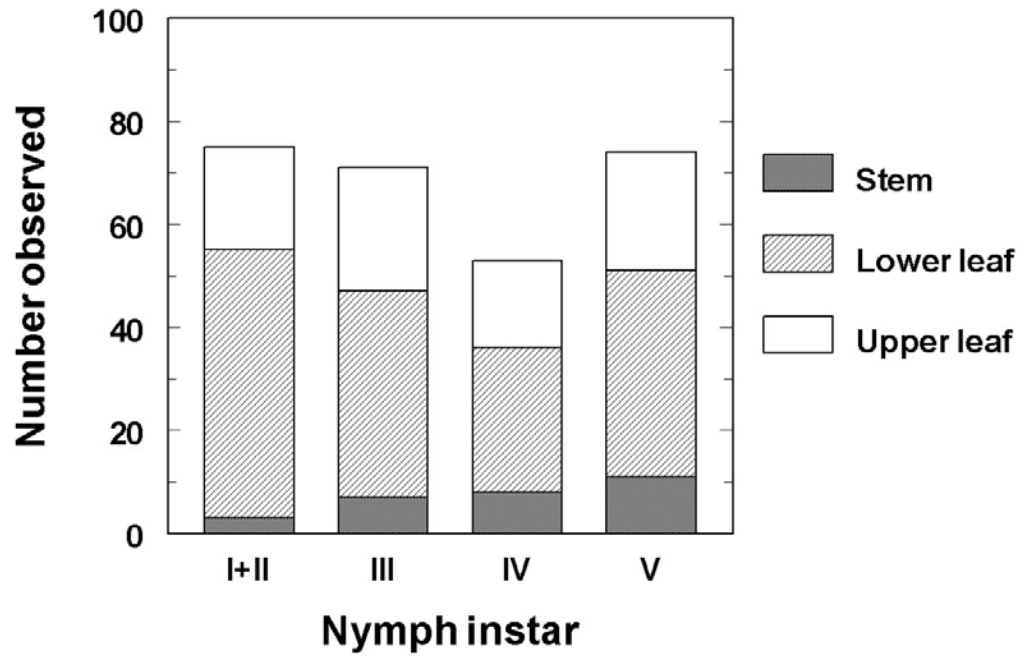


Fig. 1. Observed distributions of nymphal instars by plant surface type in alfalfa fields.

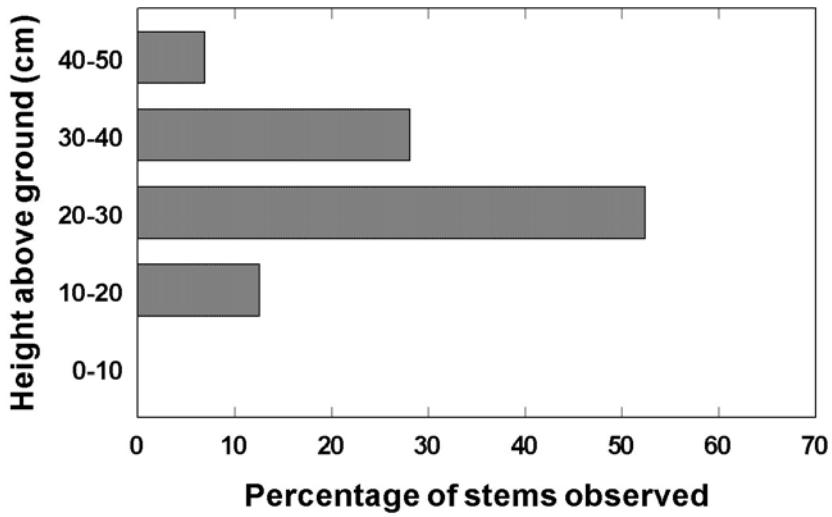
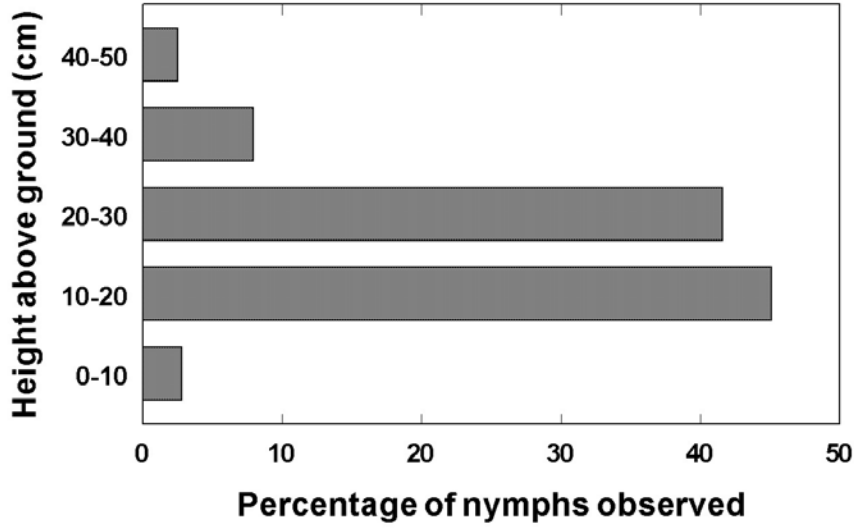


Fig. 2. Top, observed distributions of nymphal instars by height within the canopy; bottom, distributions of alfalfa stems by height.

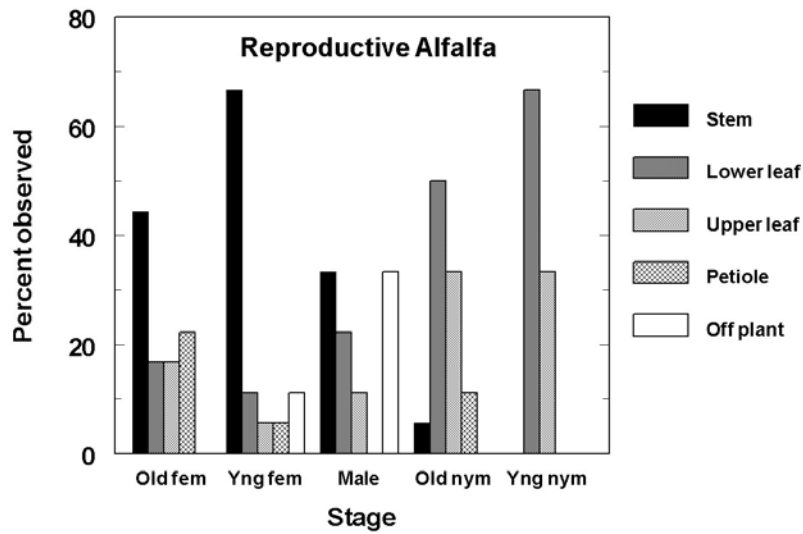
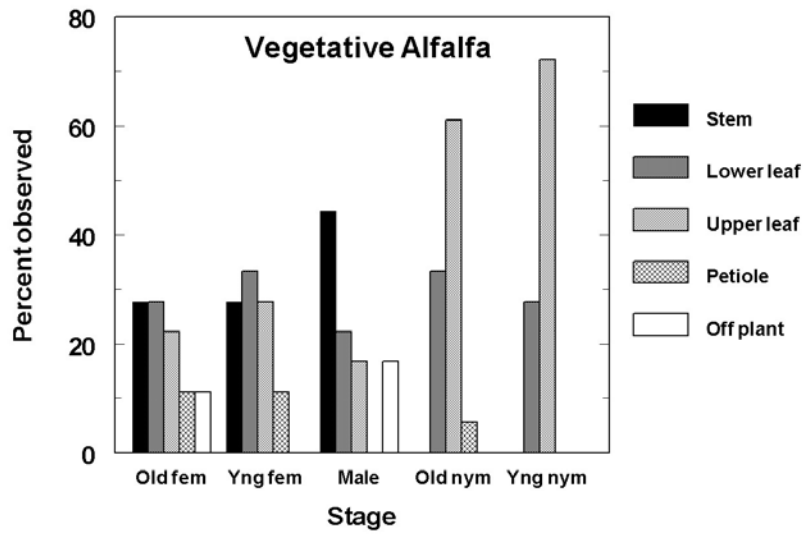


Fig. 3. Settling distribution of adult and nymphal potato leafhoppers on vegetative and reproductive alfalfa after 24 hrs in the greenhouse. Old fem, females 7-14 d after adult emergence; yng fem, females less than 7 d after adult emergence; mal, males; old nym, fourth or fifth instar nymphs; yng nym, second or third instar nymphs.

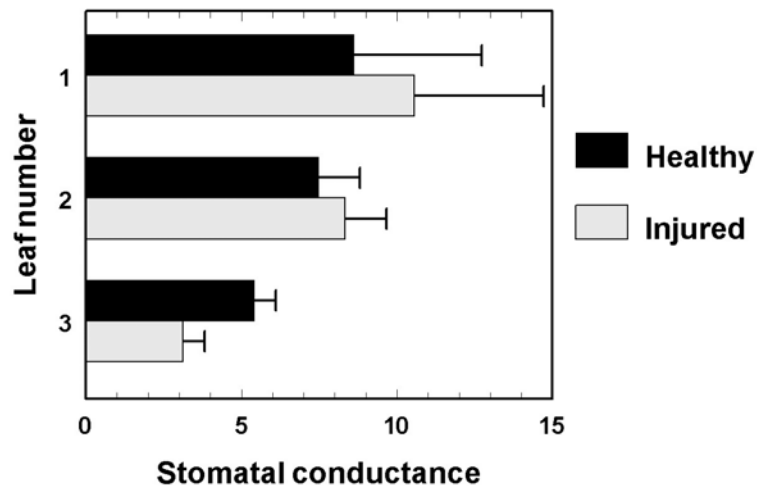
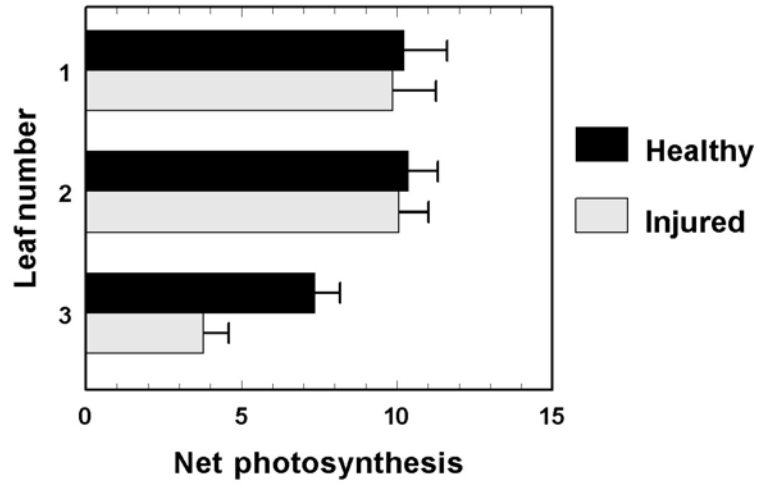


Fig. 4. Mean \pm standard error gas exchange rates, averaged over d 0-3 subsequent to injury, for individual leaves of the treated stem for the leaf injury experiment. Units of net photosynthesis are $\mu\text{mol m}^{-2} \text{s}^{-1}$, and units of stomatal conductance are cm s^{-1} .

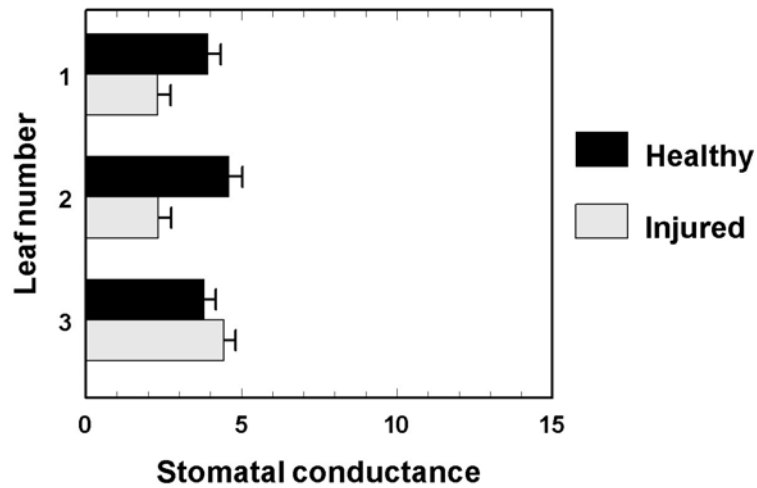
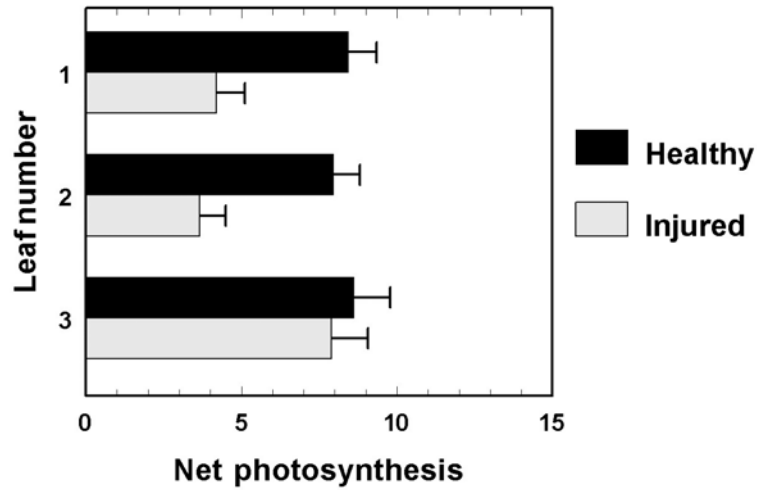


Fig. 5. Mean \pm standard error gas exchange rates, averaged over d 0-3 subsequent to injury, for individual leaves of the treated stem for the stem injury experiment. Units of net photosynthesis are $\mu\text{mol m}^{-2} \text{s}^{-1}$, and units of stomatal conductance are cm s^{-1} .