Potato Leafhopper (Homoptera: Cicadellidae) Injury Disrupts Basal Transport of ^{14}C-Labelled Photoassimilates in Alfalfa

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ABSTRACT The potato leafhopper, *Empoasca fabae* (Harris), is a key pest of alfalfa, *Medicago sativa* L., in part because of the leafhopper's ability to disrupt upward translocation within phloem tissues. To determine if leafhopper injury also disrupts basal translocation necessary for regrowth and perenniality of alfalfa, we used radiolabeled $^{14}CO_2$ to measure the basal transport of photoassimilates in injured and healthy plants. In one experiment, less ^{14}C was transported to lower stem tissue of leafhopper-injured plants in comparison to the same tissue of healthy plants in early vegetative and early reproductive stages of alfalfa development. In a second experiment, less ^{14}C was transported to lower stem, crown, and root tissues of injured plants in comparison to the same tissues of healthy, early reproductive plants. The disruption of basal transport caused by potato leafhopper may impact carbon storage and mobilization subsequent to defoliation, winter survival, and nitrogen fixation.

KEY WORDS *Empoasca fabae*, phloem translocation, alfalfa

TRANSLOCATION IS THE process by which plants distribute carbon from the site of fixation to sites of assimilate storage and use. Piercing-sucking herbivores exploit this sugar and nutrient distribution process by ingesting from phloem tissues (Raven 1983). However, in the process, some of these herbivores may alter the rate of translocation within vascular tissues. The disruption of apical transport of photoassimilates by piercing-sucking herbivores has been studied especially because of the negative impact on crop growth (Cagampang et al. 1974, Nielsen et al. 1990). Yet, perennial crops like alfalfa, *Medicago sativa* L., undergo cyclic changes in the direction and magnitude of assimilate transport (Pearce et al. 1969, Ueno and Smith 1970). During the 10 d following defoliation (i.e., harvest), root starch declines rapidly as carbon is transported apically to regrowing shoots (Smith 1962). After approximately 21 d following defoliation, carbon is transported basally to root and crown tissues. This basal transport of carbohydrates impacts subsequent regrowth rates, and is implicated in the long-term survival of alfalfa plants (Smith 1964; but see also Volenec et al. 1996). Yet, no studies have shown a direct influence of piercing-sucking insects on the disruption of basal transport of photoassimilates in alfalfa.

The potato leafhopper, *Empoasca fabae* (Harris), is a key pest of alfalfa in the eastern half of the United States (Lamp et al. 1991). Through an unusual feeding strategy known as lacerate-and-flush (Kabrick and Backus 1990), potato leafhoppers rapidly puncture multiple phloem cells and remove cell contents, thereby promoting cell collapse (Ecale and Backus 1995a, 1995b, Ecale Zhou and Backus 1999) and disrupting translocation (Nielsen et al. 1990). Subsequent growth of neighboring cells in a saliva-enhanced wound response (Ecale and Backus 1995b) further disrupts translocation.

By labeling photoassimilates with radioactive CO_2, we have demonstrated that potato leafhopper injury to alfalfa disrupts transport of photoassimilates from leaves near the middle of the plant to the apical growing tips, especially on early vegetative plants, seven to 10 d after defoliation (Nielsen et al. 1990, 1999). Other evidence suggests that basal transport of photoassimilates from leaf tissue to root and crown tissues may also be affected by leafhopper injury. For example, Shaw and Wilson (1986) found reduced carbohydrate levels in alfalfa root tissue subsequent to leafhopper injury in comparison to insecticide-treated alfalfa. Also, studies have shown a carryover effect of leafhopper injury on alfalfa yield from one summer to the first harvest during the subsequent spring, using both field cages (Poos and Johnson 1936) and insecticides (Vough et al. 1992) to manipulate leafhopper densities. Yet, no studies have directly shown that potato leafhopper injury disrupts basal translocation. Thus, to determine if the leafhopper disruption of alfalfa causes reduced basal translocation, we used $^{14}CO_2$ to document the transport of photoassimilates in injured and healthy alfalfa plants. Ultimately, this information will permit the design of more realistic, biologically based, economic injury levels that incorporate the long-term impact of leafhopper injury.

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Materials and Methods

Two types of experiments were conducted in midsummer. In the first, radiolabeled $^{14}CO_2$ was provided to a single leaf distal to leafhopper-injured stem tissue on stems at three developmental stages. In the second, $^{14}CO_2$ was provided to all leaf and stem tissues distal to the injured tissue on early reproductive plants only. Also, ^{14}C scintillation counts were made on root and crown tissues during the second experiment, but not during the first experiment. For clarity and ease of presentation we will refer to leafhopper-exposed stems as "injured" and leafhopper-free stems as "healthy."

Experiment 1. To test the effect of leafhopper injury on basal phloem translocation as influenced by the developmental stage of alfalfa, we conducted a factorial combination of three stages of alfalfa development and two levels of leafhopper injury. Individual plants, 'WL 320', were grown from seed in 10-cm ceramic pots with 300S Pro-Gro (E.C. Geiger, Harleysville, PA) potting mix under greenhouse conditions for ≈9 mo before the experiment. The greenhouse was operated with natural light, with temperatures ranging from 15 to 28°C. Plants were watered once or twice daily, and applications of 20-20-20 (N–P–K) full strength liquid fertilizer were made every other week. Plants were defoliated approximately every 5 wk on a staggered basis. Plants that had grown for 7, 14, and 24 d after defoliation represented early vegetative, late vegetative, and early reproductive stages, respectively. The two levels of injury were with and without leafhopper feeding. One stem was selected from each pot for the injury treatment. Stems within each development treatment were selected for uniformity. Height and developmental stage (scale of Kalu and Fick 1981) were recorded, and a cage was placed around the stem just below the third or fourth fully developed leaf from the top of the stem as the internode length would permit. The cage consisted of two 10-ml plastic beaker cups with a foam ring between them, held together with tape. The cup cage had a diameter of 27 mm, and was placed between two petioles to confine the leafhoppers to stem tissue. Two leafhopper nymphs, third or fourth instars, were aspirated into half of the cages for each developmental stage. Nymphs were used to standardize the degree of leafhopper injury. Leafhoppers were reared on alfalfa in the greenhouse from adults collected earlier from a local alfalfa field (Clarksville, MD). Each developmental stage and leafhopper injury treatment was replicated five times.

After 22 h, cages were removed from all stems. Previous studies had demonstrated that this length of feeding exposure was sufficient to disrupt normal physiological function of the stem (Nielsen et al. 1990, 1999; W.O.L., unpublished data). The leaf above each cage was used as the source leaf for $^{14}CO_2$ labeling. The petioles of source leaves were covered with two strips of cellophane tape to stabilize them and protect them from injury in handling. Radiolabeling the translocation stream with ^{14}C took place outside the greenhouse in available natural light and CO_2 concentrations. Plants were placed horizontally and each designated source leaf sealed in a seven by 10 cm plastic bag. Two milliliters of air containing $^{14}CO_2$ (50 μCi) was injected into each bag and the injection hole sealed. The source leaf was exposed to $^{14}CO_2$ for 15 min. After the fixation period, the bags were removed and the plants were returned to the greenhouse for an additional 24 h to permit translocation of the radiolabeled photoassimilates.

After translocation, shoots were cut into four parts. The parts consisted of the shoot tips (all tissues above the first fully expanded leaf), the upper stem (stem tissue below the tip and above the source leaf; leaf tissue was discarded), the injured region (the internode that had been caged), and the lower stem (stem tissue below the injured region and above the crown; leaf tissue was discarded). Each part was weighed, cut into 2- to 3-mm pieces, and placed immediately in 1 ml of tissue solubilizer (Scintigest, Fisher, Pittsburgh, PA, USA) in scintillation vials and incubated for four to 5 d at room temperature. After the tissues had dissolved, aqueous scintillation cocktail (Sigma-Fluor, S4273, Sigma, St. Louis, MO) was added to each vial and the ^{14}C radioactivity of the samples was measured in a liquid scintillation counter (Pharmacia LKB, Rockville, MD).

Experiment 2. To test the effect of leafhopper injury on basal phloem translocation, we confined leafhoppers to alfalfa stems, labeled the translocation stream from apical leaves with ^{14}C, and measured the subsequent ^{14}C concentration in five parts of leafhopper-infested and leafhopper-free stems.

Thirty long plastic cone pots (4 cm diameter by 21 cm) were used, each containing one plant of the leafhopper susceptible alfalfa cultivar 'Ranger'. Plants had been defoliated ≈3 wk earlier and were selected so that stems were in an early bud stage (Stage three on scale of Kalu and Fick 1981). A disposable beaker cup cage was placed on the internode just below the fourth fully expanded leaf below the stem apex. Tape attached each cage to a supporting stake.

Two potato leafhopper nymphs, third or fourth instars, obtained as before, were introduced into half of the cages. The other caged stems were the uninjured controls. Cages and leafhoppers remained on plants overnight (23 h). Leafhopper treatments were replicated 14 times.

Radiolabeling the translocation stream with ^{14}C took place outside the greenhouse as described above, except that a larger plastic bag (13 by 22 cm) was used to cover all of the leaf and stem tissue above the caged internode. Also, the concentration of ^{14}C was increased to 67 μCi per plant. Preliminary experiments demonstrated that the small amount and variance of radiolabeled photoassimilate recovered in the underground portions of alfalfa plants required a larger leaf area for assimilation and greater experimental replication to demonstrate differences.

After the fixation period of 15 min, the bags were removed and returned to the greenhouse for an additional 24 h of translocation. After translocation,

Table 1. ANOVA for experiment 1, showing the effect of alfalfa stage of development and leafhopper injury on the translocation of ^{14}C photoassimilates

Source	Degrees of freedom (NDF, DDF)	*F* value	$P > F$
Alfalfa stage (STG)	2, 26.1	12.50	0.0002
Leafhopper injury (INJ)	1, 25.1	1.17	0.2893
Plant part (PART)	3, 43.1	28.37	0.0001
STG*INJ	2, 26.1	0.55	0.5849
STG*PART	6, 33.6	1.65	0.1646
INJ*PART	3, 43.1	2.77	0.0532
STG*INJ*PART	6, 33.6	1.72	0.1461

NDF, numerator degrees of freedom; DDF, denominator degrees of freedom from PROC Mixed, SAS (SAS Institute 1997).

above- and below-ground portions of plants were cut into five parts and each part weighed. All leaf tissue was discarded. The parts consisted of the stem tips (all tissues above the caged area), the injured region (internode covered by the cage), the lower stem (stem tissue below the source leaf and above the crown), the crown, and the root. Each part was processed as described above for experiment 1.

Statistical Analysis. Analysis of variance (ANOVA) was performed on the data from both experiments with the MIXED procedure (SAS Institute 1997). For experiment 1, we tested for the fixed effects of alfalfa stage (early vegetative, late vegetative, and early reproductive), leafhopper injury (no leafhopper injury and leafhopper injury), and stem part (shoot tip, upper stem, injured region, and lower stem) on the concentration of the labeled assimilate (expressed as log dpm per mg fresh weight). For experiment 2, we tested for the fixed effects of injury (no leafhopper injury and leafhopper injury), and plant part (stem tip, injured region, lower stem, crown, and root) on the concentration of the labeled assimilate (expressed as log dpm per mg fresh weight). For both experiments, replication served as a random effect, and stem part was nested within alfalfa stage and injury treatment for experiment 1, and plant part was nested within injury treatment for experiment 2. To account for correlation among the residuals, various covariance structures were modeled and AIC was used to ascertain which of the covariance structures fit best (Littell et al. 1996). We compared the amount of labeled assimilate in the plant part of injured plants to the same part of healthy plants and made pairwise comparisons for significant differences ($\alpha = 0.05$) using Fisher least significant difference (LSD) test. Only meaningful comparisons were made (i.e., $^{14}CO_2$ concentration within healthy plant part versus the concentration in the matching injured plant part).

Results

Experiment 1. The total ^{14}C assimilated per plant did not differ among plant stage and injury treatments (ANOVA, $F = 1.1$; df = 5, 29; $P = 0.39$). However, concentration of ^{14}C photoassimilates varied significantly with both alfalfa stage of development and plant part (Table 1). Mean and standard error concentrations across plant stages and plant parts were 3.62 ± 0.15, 3.70 ± 0.08, and 2.35 ± 0.23 log dpm/mg for early vegetative, late vegetative, and early reproductive plants, respectively, and 3.49 ± 0.23, 3.61 ± 0.18, 3.61 ± 0.15, and 2.18 ± 0.21 log dpm/mg for shoot tip, upper stem, injured region, and lower stem parts, respectively. Although the main effect of leafhopper injury did not significantly affect ^{14}C concentration, the injury × part interaction indicates that leafhopper injury did significantly affect the distribution of ^{14}C concentrations across plant parts ($P = 0.0532$, Table 1).

Table 2. Mean comparisons for experiment 1, showing the effect of alfalfa stage of development and leafhopper injury on the translocation of ^{14}C photoassimilates

Developmental stage of alfalfa	Shoot part	Mean ± SEM of recovered ^{14}C photoassimilate (log DPM per fresh mg)		LSD test ($P > t$)
		Healthy	Injured	
Early vegetative	Shoot tip	3.48 ± 0.39	4.28 ± 0.12	0.0540
	Upper stem	3.92 ± 0.18	4.29 ± 0.14	0.2227
	Injured region	3.99 ± 0.10	4.10 ± 0.14	0.7113
	Lower stem	3.10 ± 0.06	1.83 ± 0.61	0.0001
Late vegetative	Shoot tip	3.80 ± 0.23	4.01 ± 0.19	0.4788
	Upper stem	3.95 ± 0.16	3.97 ± 0.09	0.9483
	Injured region	4.02 ± 0.07	3.82 ± 0.13	0.5065
	Lower stem	3.13 ± 0.12	2.90 ± 0.17	0.4418
Early	Shoot tip	2.88 ± 0.72	2.50 ± 0.98	0.6816
	Upper stem	2.99 ± 0.56	2.56 ± 0.67	0.6425
	Injured region	3.08 ± 0.50	2.67 ± 0.55	0.6688
	Lower stem	1.58 ± 0.33	0.52 ± 0.27	0.0457

Specific comparisons of concentrations in injured and healthy plants demonstrated that injury significantly reduced photoassimilate transport to lower stem tissue of early vegetative and early reproductive plants, but not of late vegetative plants (Table 2). In addition, significantly greater ^{14}C was found in the shoot tip of early vegetative plants of injured stems in comparison to the tip of healthy stems ($P < 0.05$, Table 2).

Experiment 2. As in the first experiment, the total ^{14}C assimilated per plant did not differ among injury treatments (*t*-test, $t = 1.0$, df = 14, $P = 0.33$). For the concentration of ^{14}C photoassimilates, main effects (leafhopper injury and plant part) and their interaction were highly significant ($P < 0.0001$, Table 3), in part because of the increased replication and the increase in photosynthetic tissue exposed to $^{14}CO_2$ compared with experiment 1. Mean concentrations were

Table 3. ANOVA for experiment 2: effect of leafhopper injury on the basal translocation of ^{14}C photoassimilates from the apical tip of alfalfa to above- and belowground portions of early reproductive stage alfalfa

Source	Degrees of freedom (NDF, DDF)	*F* value	$P > F$
Leafhopper injury (INJ)	1, 36	441.7	0.0001
Plant part (PART)	4, 81.4	386.5	0.0001
INJ*PART	4, 81.4	53.3	0.0001

Table 4. Mean comparisons for experiment 2: effect of leafhopper injury on the translocation of photoassimilate within above- and belowground portions of early reproductive stage alfalfa

Stem section	Mean and SEM of recovered ^{14}C photoassimilate (log DPM/mg)		LSD test ($P > t$)
	Healthy	Injured	
Stem tip	4.74 ± 0.07	4.76 ± 0.05	0.9051
Injured region	3.97 ± 0.07	3.54 ± 0.19	0.0107
Lower stem	3.49 ± 0.15	0.80 ± 0.08	0.0001
Crown	2.39 ± 0.21	−0.06 ± 0.07	0.0001
Root	1.93 ± 0.22	−0.19 ± 0.05	0.0001

3.30 and 1.77 log dpm/mg for healthy and injured plants, respectively, and 4.75, 3.76, 2.15, 1.17, and 0.87 log dpm/mg for stem tip, injured region, lower stem, crown, and root parts, respectively. Although the specific comparison of ^{14}C concentration demonstrated no difference in stem tips of healthy versus injured plants, all other sampled tissues of injured plants had significantly lower ^{14}C concentrations compared with the same tissues of healthy plants (Table 4). The concentrations in basal plant tissues were especially affected, with real (not logarithmic) concentrations in lower stem, crown, and root tissues reduced by a factor >100 as a result of leafhopper injury.

Discussion

Our results demonstrated that potato leafhopper injury to stem tissue reduced the basal translocation of photoassimilates in alfalfa. In the first experiment, less ^{14}C derived from photosynthesis was transported to lower stem tissue of injured plants compared with healthy plants in the early vegetative and early reproductive stages of development. Levels of ^{14}C in root and crown tissues were not measured in experiment 1. In the second experiment, less ^{14}C was transported to lower stem, crown, and root tissues of injured plants compared with healthy plants in the early reproductive stage of development. Although reduced photosynthesis rates on injured plants may contribute to these differences, ^{14}C assimilation per plant did not differ among treatments in either experiment, and concentrations did not differ significantly on tissues near the apex of injured and healthy plants of experiment 2. The interference of basal transport of carbon by leafhopper injury may especially impact three critical physiological functions of alfalfa: carbon storage and mobilization subsequent to defoliation; winter survival; and nitrogen fixation.

The role of nonstructural carbohydrates in regrowth and persistence has been studied extensively. In a classic study, Graber et al. (1927) reported that nonstructural carbohydrate concentrations in alfalfa roots declined in spring as plants resumed growth and also after defoliation. The concept of reserve carbohydrates, more than any other single concept, has driven alfalfa management decisions. Briefly, concentrations of sugars, and especially starch (collectively referred to as total nonstructural carbohydrates, or TNC) decline rapidly in alfalfa roots during the 10 d immediately after defoliation as new shoots begin development (Smith 1962). A portion of the carbon made available by degradation of root starch is used for shoot growth, while part is used to maintain roots and crowns (Pearce et al. 1969; Smith and Silva 1969; Rapoport and Travis 1984). The TNC concentrations remain low between days 10 and 20 postdefoliation, after which root carbohydrates reaccumulate as photoassimilate production exceeds that required for shoot growth. Plants that do not reaccumulate root TNC due to pest-induced stress may have reduced growth rates following defoliation, or worse, fail to survive. However, recent evidence suggests that proteins, not carbohydrates, are especially important for legume survival and regrowth subsequent to defoliation (Volenec et al. 1996). The impact of potato leafhopper injury on nitrogen (N) transport and root N accumulation has not been investigated, and long-term studies are currently underway to address these issues.

Rates of nitrogen fixation also depend on the stage of alfalfa development and the transport of photoassimilates. Just after defoliation, nitrogen fixation falls 78–88% and slowly recovers in relation to the supply of photoassimilates transported to root tissue (Vance et al. 1988). Although root-feeding herbivores are known to impact nitrogen fixation (Hower and Leath 1989), foliar-feeders that affect photosynthetic rates and photoassimilate transport are predicted to also impact nitrogen fixation. Researchers have documented that leaf defoliation by chewing herbivores reduces nitrogen fixation of legumes (e.g., soybean looper, *Pseudoplusia includens* (Walker), on soybean, *Glycine max* (L.), Wier and Boethel 1996); however, no documentation of the impact of piercing-sucking herbivores on nitrogen fixation has been published.

In our experiments, we confined leafhopper injury to one internode of stem tissue. In the field, leafhoppers are free to choose among other tissues (e.g., leaves and petioles) and among internodes (e.g., near the apex of the stem instead of the center). Adult leafhoppers prefer to settle on stem tissue (Backus et al. 1990); however, leafhopper nymphs are more often found on leaf tissue (W.O.L., unpublished data). Thus, injury to plants in the field is likely to be much less than the levels we observed, and field studies are needed to support our laboratory data.

Nevertheless, our results suggest a mechanism (i.e., disruption of basal transport) for severe potato leafhopper injury to reduce the rate of carbohydrate loading of crown and root tissues. Based on our knowledge of alfalfa physiology, the reduction of carbohydrate loading of crown and root tissues causes a reduction in the rate of nitrogen fixation late in the growth cycle, the rate of regrowth after defoliation, and the ability of the plant to survive defoliation and winter. None of these effects are reflected in current integrated pest management recommendations, which focus on the impact of the leafhopper on the current crop growth (e.g., Cuperus et al. 1983), and not on the carry-over effects on subsequent crop growth (e.g., Vough et al. 1992), nor on the persistence of the life of alfalfa stands

(Beuselinck et al. 1994). Thus, verification of these physiological responses of alfalfa should lead to improved economic thresholds and more realistic estimates of pest-induced losses that incorporate the long-term effects of potato leafhopper injury.

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