Interaction Between Flooding or Drought Stress and Potato Leafhopper Injury in Alfalfa

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Abstract
Potato leafhopper is a predominant biotic stress in alfalfa production, whereas drought and flooding are frequent abiotic stresses. Controlled environment studies were conducted to examine the interaction between flooding or drought stress and potato leafhopper (PLH, Empoasca fabae [Harris]) feeding damage on seedlings of two cultivars of alfalfa (Medicago sativa L): one susceptible to PLH, one resistant to PLH. Flooding treatments reduced shoot dry matter accumulation approximately 50% in the absence of PLH in both cultivars and significantly reduced root dry matter in one cultivar. Drought stress significantly reduced shoot dry matter and increased root dry matter. PLH feeding combined with either flooding or drought stress reduced root dry matter accumulation but not shoot dry matter. Flooding injury and PLH feeding injury showed an additive negative response for root dry matter accumulation. Flooded and non-stressed plants accumulated less root total non-structural carbohydrates (TNC) when subjected to PLH feeding. Responses of the two cultivars were similar across all biotic and abiotic treatments. The results clearly show that the most damaging physiological response of seedling alfalfa to PLH feeding is a sharp decline in root growth and TNC accumulation. This suggests that seedlings that have experienced even minor root waterlogging damage may be especially susceptible to damage by PLH feeding.

Introduction
Seedling establishment is a critical phase in alfalfa production. Young plants may be exposed to many stresses including shoot- and root-feeding insects, pathogens, weed competition, inadequate soil fertility and soil pH, and soil moisture extremes (i.e., drought or flooding). Seedlings can be injured by even a few days of soil waterlogging (16). Spring weather in the north central and northeastern USA often is characterized by periods of intense rain over several days that result in waterlogging or ponding in areas of poor soil drainage. Extended periods of moderate to severe drought also are common during summer growth, often coinciding with potato leafhopper (PLH) feeding.

Potato leafhopper is a serious insect pest of alfalfa in the north central and northeastern USA that affects both forage yield and quality. PLH feeding in alfalfa causes phloem damage and reduced assimilate transport to the shoot apex and to root and crown tissue (5,8,10). Seeding alfalfa seems to be more susceptible to PLH damage than established, older plants (4,6). Economic loss from PLH derives primarily from shoot stunting and reduced biomass accumulation, with yield reductions of 10 to 40% reported (3,9). Shoot regrowth rate and subsequent yield have been reduced following severe PLH damage (11, 14). Root growth also is affected by PLH feeding. An infestation of eight leafhoppers per plant over two growth cycles decreased root dry matter by 50% (2). However, when alfalfa growth was already stunted from inadequate potassium (K) fertility, growth reduction by PLH was less (4). Some studies have reported reduced root total non-structural carbohydrates (TNC) levels, an
important reserve energy source for shoot regrowth and overwintering, following severe PLH infestations (4,14); other studies have shown little or no effect of PLH injury on root TNC concentration (2,9). These variable responses of root TNC to PLH injury suggest that experimental protocol and the different environments plants were grown under may have had variable effects on root TNC accumulation.

Although the importance of understanding how the interaction between abiotic and biotic stress affects alfalfa growth and persistence has been noted (12), relatively few studies have addressed these interactions. Schroeder et al. (13) reported a possible interaction between drought stress and PLH injury and concluded that abiotic stress should be considered when determining economic thresholds for PLH control in alfalfa. No research has been reported on the interaction of flooding stress and PLH damage in alfalfa. The objective of this research was to examine the interaction between abiotic (flooding and drought) stress and biotic (PLH feeding) stress on alfalfa seedlings (seeded plants that had not gone through a cutting and regrowth cycle). Experiments were conducted in controlled environment chambers to reduce variations in light, temperature, and humidity. A secondary objective was to examine the stress response interactions of two cultivars that differ in PLH resistance/tolerance.

**Root Flooding and Water Deficit Effects on Alfalfa**

Two alfalfa cultivars were used in these studies: WL 323, a commercial PLH-susceptible cultivar, and 3S77, an experimental glandular-haired cultivar (supplied by M. McCaslin, Forage Genetics Inc., West Salem, WI) selected for high levels of PLH resistance. Seed was planted in pots containing a sterilized sand:soil (2:1, v:v) mixture. Seedlings were thinned to 10 per pot. Plants were watered as needed with nutrient solution. Plants were grown in environmental chambers at constant 23ºC, a 15-hr photoperiod, and 80% relative humidity.

The experimental design was a randomized complete block with a factorial combination of three watering treatments (control, waterlogged [i.e., flooded], and drought stress) and two PLH treatments (with and without PLH) in three replicates. The 3-day root waterlogging treatment was initiated by placing the pot in a larger container and adding water until the water level was approximately 1 cm above the media surface. Pots were drained at the end of the 3-day treatment. Drought stress was attained by reducing water supply to individual pots so that plants were at incipient wilting. This was accomplished by supplying small amounts of water, i.e., 20 to 50 ml, over short time intervals to replace water lost through transpiration. The control water treatment was accomplished by watering plants as needed to maintain vigorous plant growth. Flooding, drought, and non-water-stress control treatments (with and without PLH feeding) were started 21 days after planting (DAP) when 4 to 5 petioles were present. Plants were harvested 8 days later, 29 DAP, when plants were showing about 5% bloom. Shoots were collected and dry weights were determined. Roots were separated from the growth media using a stream of water. Root tissue was freeze-dried, weighed, and ground to pass a 30-mesh screen. Subsamples were extracted for total carbohydrate analysis as previously described (1).

Flooding and drought stress decreased shoot dry matter accumulation approximately 50% relative to the control plants in the absence of PLH (Table 1). Flooded plants were visibly shorter than non-stressed control plants and exhibited significant leaf yellowing (Fig. 1). Plants from drought treatments also were shorter in length with smaller leaves of deep green color. Root dry matter accumulation also was influenced by flooding or drought stress (Table 1).
Flooding caused a 33% decline in root dry matter accumulation for WL 323, but the 14% reduction in root dry matter for 3S77 was not significant. Drought stress increased root dry matter by approximately 50%. No obvious root rotting or decay was observed following the flooding treatment. Secondary effects of flooding, i.e., root pathogen damage, was not a factor because the rooting media had been sterilized before planting. An increase in root dry matter under drought stress is a common physiological response and functions to alleviate the stress by generating more roots to absorb available water. These experiments document that short periods of root waterlogging used in this study can significantly damage roots and result in reduced root dry matter accumulation. The 3-day flooding period used in these studies reflects a level of short-term stress that might be encountered in a field environment in spring or early summer.

Table 1. Net dry matter accumulation 8 days after being subjected to flooding or drought stress treatments at 21 days after planting in the absence of potato leafhopper. WL 323 and 3S77 are potato leafhopper susceptible and resistant cultivars, respectively.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Control</th>
<th>Flooded</th>
<th>Drought</th>
<th>LSD (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL 323</td>
<td>15.18</td>
<td>6.99 (46%)*</td>
<td>8.55 (56%)</td>
<td>1.82</td>
</tr>
<tr>
<td>3S77</td>
<td>12.54</td>
<td>5.48 (44%)</td>
<td>5.35 (43%)</td>
<td>1.96</td>
</tr>
<tr>
<td>Mean</td>
<td>13.86</td>
<td>6.24 (45%)</td>
<td>6.95 (50%)</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL 323</td>
<td>2.41</td>
<td>1.60 (66%)</td>
<td>3.86 (160%)</td>
<td>0.38</td>
</tr>
<tr>
<td>3S77</td>
<td>2.24</td>
<td>1.93 (86%)</td>
<td>3.10 (138%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean</td>
<td>2.33</td>
<td>1.77 (76%)</td>
<td>3.48 (149%)</td>
<td></td>
</tr>
</tbody>
</table>

* Data in parentheses represent treatment values as percentage of control. WL 323 is a non-PLH resistant cultivar; 3S77 is a glandular haired, PLH resistant cultivar.

**PLH Feeding Damage in Flood- or Drought-Stressed Alfalfa**

Alfalfa seedlings were exposed to PLH by caging each pot and introducing 20 unsexed adults at the same relative age per cage. The cages (Fig. 1) were constructed with fine mesh screen (to minimize heat build-up) on all sides except the front panel. A clear vinyl film on the front panel facilitated observation of the plants. Potato leafhoppers were collected from a greenhouse colony reared on lima bean plants. Treatments not exposed to PLH were enclosed in identical cages. PLH treatments were imposed concurrently with the water-stress treatments 21 days after planting. After PLH feeding for 8 days, all pots were harvested. Caged plants were maintained in the 23°C constant temperature growth chamber under high light intensity and 80% relative humidity.

An experiment consisted of water stress (flooding or drought stress) and PLH feeding treatments on one cultivar. Three replications were used in each experiment, with each pot of 10 plants constituting one experimental unit. The experiment was performed twice for each cultivar (two runs). Mixed model methodology, as implemented in SAS PROC MIXED (7), was used to analyze response data from these experiments. The analysis was conducted on data for each cultivar separately because cultivars were tested in separate experiments. Treatment effects were similar across the two experiments for each cultivar; therefore, the data were analyzed across experiments for each cultivar. Statistical significance was based on $P = 0.05$.

PLH feeding on WL 323 caused visible shoot stunting (Figs. 2, 3, and 4) and
leaf yellowing or bronzing (Fig. 5). It should be noted that even though the PLH treatments shown in Figs. 3 and 4 suggest differences in shoot dry matter accumulation, no significant difference due to PLH feeding was noted for the two water stress treatments (Fig. 6). We have observed that alfalfa shoots stressed by flooding, drought, or PLH often exhibit a higher percentage of shoot dry matter (data not shown) that would tend to equalize total shoot dry matter yield between stress and non-stress treatments.

Fig. 2. Well-watered control plants (WL 323) with and without potato leafhopper (PLH) feeding.

Fig. 3. Flood stressed plants (WL 323) with and without potato leafhopper (PLH) feeding (click image for larger view).

Fig. 4. Drought stressed plants (WL 323) with and without potato leafhopper (PLH) feeding.

Fig. 5. Potato leafhopper induced leaf yellowing and bronzing of alfalfa.
The degree of visible PLH feeding injury on 3S77 was not appreciably different from that observed on WL 323. There was a water stress x PLH treatment interaction for shoot dry matter of both cultivars. Shoot dry matter accumulation during the treatment period was affected by PLH only in non-water-stressed plants (Fig. 6; top). Plants stressed by drought or flooding showed no significant additional shoot damage, i.e. reduced dry matter accumulation, due to PLH feeding. Response patterns were similar in both cultivars. These results are consistent with the results of Kitchen et al. (4) on PLH damage in K-stressed alfalfa and suggest that PLH induced shoot damage, principally stunted growth, might not be as obvious if plant growth has already been retarded by flooding or drought stress.

Root dry matter accumulation was reduced in response to PLH feeding, regardless of cultivar or water-stress treatment (Fig. 6; middle). Non-water-stressed control plants displayed PLH-induced root dry matter reductions of 72% and 48% for WL 323 and 3S77, respectively. The water stress x PLH treatment interaction was significant for WL 323, but was not significant for 3S77. Flooding stress was particularly detrimental in combination with PLH stress; root dry matter accumulation declined 85% and 69% in WL 323 and 3S77, respectively, relative to the non-PLH feeding controls. The data suggest an additive response of PLH feeding and flooding injury contributing to a very low rate of root dry matter accumulation. Net accumulation of root dry matter for flooded 3S77 plants with PLH feeding was somewhat greater than WL 323; however, a statistical comparison between the cultivars is not reliable because the cultivars were not tested within the same experimental run. Although root dry matter accumulation in drought-stressed plants also was sharply depressed by PLH feeding (77% and 59%, respectively, for WL 323 and 3S77 relative to the non-PLH control), the net effect on root dry matter was much less than that for the flooding plus PLH treatment. Because drought stress tends to increase root dry matter and PLH decreases root dry matter (Table 1 and Fig. 6), the net effect with both stresses is root dry matter accumulation similar to the non-water-stress control with PLH. This suggests that alfalfa seedlings stressed by drought may be able to recover more readily from PLH damage than alfalfa seedlings stressed by flooding.
There was a water stress x PLH treatment interaction for root total non-structural carbohydrate content in both cultivars. Feeding by PLH resulted in a 40% reduction (mean of both cultivars) in carbohydrate accumulation in flooded roots relative to the non-PLH flooding treatment (Fig 6; bottom). In watered controls, the reductions were approximately 36% for both cultivars. PLH feeding did not have a significant effect on root TNC of drought-stressed plants, averaging only an 11% reduction over both cultivars. Because root TNC in Fig. 6 is expressed as percentage dry weight and root dry weight decreased in response to PLH feeding, total available root carbohydrates per plant were greatly reduced by PLH feeding. Calculations of the total TNC available per root at harvest indicate approximately 55 mg glucose equivalent for the control (i.e., no water stress, no PLH) roots and 12 mg glucose equivalent in flooded plants exposed to PLH. Because of the reported importance of root TNC in modulating PLH damage (9) and regrowth recovery (14), the additive negative effects of flooding and PLH feeding stress on TNC levels suggest a very severe stress on the seedling plant.

**Summary**

These results indicate that in seedling alfalfa, the major effect of PLH feeding on plant growth and development results from reduced assimilate transport to roots for growth and carbohydrate accumulation. Because PLH injury often is apparent as leaf yellowing or bronzing and reduced forage yield and quality, the more severe effects of PLH feeding on root growth and assimilate accumulation may be overlooked or underestimated. This is unfortunate because reduced root dry matter and TNC seem to be much more critical to the long-term health and persistence of the stand than PLH effects on reduced shoot dry matter accumulation (4).

When seedling alfalfa plants were exposed to root flooding or drought stress plus PLH feeding, shoot dry matter was affected only by abiotic stress, similar to the response noted in K-deficient alfalfa (4). This indicates PLH injury may be difficult to detect visually in seedling plants that have undergone growth reduction due to abiotic stress. In contrast to the effect on shoots, PLH feeding consistently reduced root growth in water-stressed and non-stressed plants. The reduction in root dry matter and TNC accumulation was especially acute in plants that had been flooded for three days, indicating that even minor flooding injury, when combined with PLH feeding, can have major negative effects on roots. Subsequent plant vigor and competitive response may be greatly diminished. Drought-stressed roots may be able to recover more readily from PLH feeding damage because root dry matter and TNC are maintained at higher levels than they are in flooded roots.

It has been reported that alfalfa cultivars with improved resistance to PLH damage may not fully express that resistance until plants have gone through several growth cycles (6). Sulc et al. (15) reported that yield loss due to PLH in resistant cultivars was less than half that observed in susceptible cultivars in seedling stands when PLH density was high. However, even the resistant cultivars were higher yielding in that study when treated with an insecticide. This study supports the observation that resistant cultivars in the seedling stage can suffer significant damage from PLH feeding, as demonstrated by the response of 3S77. Even short periods of PLH feeding in this study caused major loss of assimilates in roots, especially if the plants were exposed to flooding or drought stress. Therefore, we conclude that regular scouting of newly established stands for PLH build-up and timely application of appropriate control measures represent important components of successful stand establishment. The data in this study also suggest that close monitoring and control of PLH in PLH-resistant cultivars may be warranted during the seedling phase of establishment.

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Literature Cited