Plant-mediated interactions between Neochetina spp. weevils and the fungal pathogen Cercospora piaropi on Eichhornia crassipes (water hyacinth)*

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Summary

Insect biological control agents of weeds may aid infection by plant pathogens by generating wounds or by vectoring. Pathogen infection may lead to plant biochemical changes that alter host suitability for insects. In Eichhornia crassipes (water hyacinth), Neochetina bruchi and N. eichhorniae adult weevils feed mostly on immature leaves, while symptoms of infection by Cercospora piaropi, a fungal pathogen, occur mostly on old leaves. This study examined associations between weevil scarring and fungal spotting (necrosis) on leaves, and determined if necrosis is associated with levels of biochemical factors that may influence weevil feeding. Scarring and necrosis scores were positively correlated across four field sites sampled at four times. At individual sampling times, two of the four sites tended to have higher scarring and necrosis scores, but scores were not correlated. Total available carbohydrate, potassium and phenolic contents did not vary across sites in the same manner as did necrosis scores. Peroxidase activities and potassium levels in furled leaves were positively correlated to necrosis scores in oldest non-senescent leaves. Phenolic content in late-season samples was also correlated to necrosis. Prior C. piaropi symptom production on old leaves of cultivated plants did not influence weevil feeding on young leaves. Leaf scarring and necrosis were related, but fungal infection did not alter the feeding of E. crassipes weevils by changing plant biochemical components.

Keywords: defence, induction, insect–pathogen synergism, nutrition, plant stress.

Introduction

Insects and plant pathogens have been employed as biocontrol agents against the same weed species. Examples include Carduus spp. (musk thistle) (Charudattan 2001) and Chondrilla juncea (skeletonweed) (Julien & Griffiths 1998). Many more weeds have been targeted with pathogens alone (Charudattan 2001). Little is known about the integrative effects of biocontrol by combinations of insects and pathogens (Zidack 1999; Caesar 2000). Many pathogens gain entry into plants via wounds made by insect feeding. Such ‘direct’ interactions (Hatcher 1995) could generate additive or synergistic biocontrol effects (Caesar 2000). Biological control by insects could be influenced by plant biochemical responses to pathogen infection (Zidack 1999). Fungal infection increases peroxidase activity and phenolic defences (Hammerschmidt & Kuć 1995) and alters the protein and carbohydrate nutritional composition of tissues in ways known to influence insect feeding and survival (Hatcher 1995).

Biological control of Eichhornia crassipes (Mart) (water hyacinth) in the south-eastern United States has involved, among other agents, two introduced weevil species, Neochetina eichhorniae (Warner) and Neochetina bruchi (Hustache), and a native fungal pathogen, Cercospora piaropi Tharp. Adult weevil feeding on mostly furled and young unfurled leaves generates scarring damage, and fungal infection accelerates leaf senescence, leading to the

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development of necrotic lesions on mostly old leaves. Scarring and necrosis vary among field sites and environments (Freeman et al. 1981; Center et al. 1999). The objectives of this study were to determine if scarring and necrosis were associated in young and old leaves of field plants, and if necrosis influenced plant biochemical factors or altered weevil feeding.

Materials and methods

Field sampling

Four field sites in the Lower Rio Grande Valley of Texas were sampled four times: June–July (summer) 2001, November (fall) 2001, April (spring) 2002, and July (summer) 2002. Site ‘Lake Canal’ (LC) was a continuously flowing canal connecting two sides of a reservoir. Site ‘Rio Grande’ (RG) was located directly on the Rio Grande, at the mouth of a pumping station inlet. Site ‘Inlet’ (IN) was a closed, disused inlet, adjacent but not connected to the Rio Grande. Site ‘Resaca Canal’ (RC) was an irrigation canal adjacent to series of small reservoirs. Plastic pipe squares (0.5 × 0.5 m) were thrown into mats of plants to define sampling units (4–5 units per site per sampling time). The two youngest and two oldest unfurled leaves were collected from five plants in each unit. The percentage of the adaxial leaf laminar surface coverage with necrotic spots, indicative of C. piaropi infection, was visually estimated on the second-oldest unfurled leaf. All colorimetric analyses were performed on a Spectronic Genesys-2 spectrophotometer.

Biochemical analyses

The furled leaf and the youngest unfurled leaf were collected from three plants per unit and were pooled and frozen at –80°C. All colorimetric analyses were performed on a Spectronic Genesys-2 spectrophotometer. Total soluble protein content and peroxidase activity were examined as in Showler & Moran (2003) using Bradford reagent (Sigma) at 595 nm for protein and guaiacol reagent at 470 nm for peroxidase. Total available carbohydrate (TAC) content was determined in 30 mg lyophilised samples as in Center & Van (1989) using anthrone reagent and glucose standard at 625 nm. To determine potassium content, 0.15 g lyophilized samples were ashed at 600°C for 6 hours, dissolved in 0.02N HCl and filtered through Whatman #4 paper. Samples were read with a Jenway PFP 7 flame photometer using potassium chloride solutions as standards. Water-soluble phenolic content was assayed using procedures modified from Center & Van (1989). Lyophilized samples (50 mg) were extracted once with 5 mL diethyl ether and three times with 5 mL 80% methanol: 1% HCl (99:1). Pooled methanolic supernatants were extracted with 6 mL hexane and concentrated under reduced pressure at 35°C. Phenolics were quantified with the Folin-Ciocalteu reagent (Sigma-Aldrich) at 760 nm. Chlorogenic acid, a common phenolic in E. crassipes leaves (Martyn & Cody 1983; Center & Wright 1991), was used as a standard.

Inoculations and bioassays in greenhouse

Eichhornia crassipes plants were grown in 1000 L outdoor tanks filled with irrigation water containing 5–7 ppm nitrogen (not augmented), 5 ppm phosphorus (P₂O₅) and 1 ppm iron (chelated ferric form). Six five- to eight-leaf plants were placed into 20 L tanks containing water from the 1000 L tanks. Cercospora piaropi was cultured on potato dextrose agar. Conidia and mycelial fragments from two-week-old cultures were scraped off and suspended in water (1 × 10⁶ fragments per mL) containing 0.1% Tween-20. The youngest unfurled leaves on E. crassipes plants in the 20 L tanks were gently abraded with sandpaper and inoculum applied with a pump aerosol sprayer. Mock-inoculated leaves received water containing 0.1% Tween 20. After two weeks of symptom development, tanks were caged with fine mesh, and 75 field-collected N. bruchi and N. eichhorniae beetles (species ratio approximately 2:1) were added to each cage. After one week, feeding scars on both surfaces of the laminae of inoculated leaves (four–five positions down from the shoot apex) and the two youngest unfurled leaves were counted. Leaf area was determined with a Li-Cor LI-3100 leaf area meter. Feeding was expressed as scars per cm² area.

Statistics

Poisson regression in PROC GENMOD (SAS Institute 1999) was used to determine the effects of field site and time on scarring and necrosis scores across all sampling times, and the effect of site on scores within each time. Pearson Chi-square adjustments to standard errors were used to correct for overdispersion when needed (Allison 2001). Pairwise contrasts tested for differences among sites, with P for significance adjusted to 0.0083 based on six independent comparisons. TAC and potassium content variation across sites and over time were examined with repeated measures analysis using PROC MIXED. Akaike’s finite sample Information Criterion (AICC) (SAS Institute 1999) was minimized by specifying unstructured covariance with banding. Within sampling times, two- and one-factor analyses of variance in PROC GLM evaluated leaf age and site effects, and feeding in the greenhouse bioassay. Spearman rank correlations examined associations between scarring and necrosis scores and biochemical factors.
Results

Scarring and necrosis scores

Across all sampling times, summed scarring scores for youngest and second-youngest unfurled leaf laminae were strongly correlated ($r = 0.68$, $n = 64$, $P < 0.001$), as were summed necrosis scores on the second-oldest and oldest leaves ($r = 0.68$, $n = 64$, $P < 0.001$). Across all sampling times, scarring scores were positively, significantly correlated to necrosis scores, (e.g. scarring on the second-youngest leaf to necrosis on the second-oldest leaf, $r = 0.40$, $n = 64$, $P = 0.001$; to oldest leaf necrosis, $r = 0.52$, $n = 64$, $P < 0.001$). Scarring and necrosis scores were higher (1.4-fold and 1.8-fold, respectively) on plants from sites IN and RC than from sites LC and RG, although these two pairs of sites differed significantly only for necrosis on the oldest leaf (Fig. 1A). Scarring scores on second-youngest unfurled leaves varied across sites ($\chi^2 = 18.0$, df = 3, $P < 0.001$) and sampling times ($\chi^2 = 28.9$, df = 3, $P < 0.001$), as did necrosis scores on oldest leaves (site effect, $\chi^2 = 48.3$, df = 3, $P < 0.001$, time effect, $\chi^2 = 114.6$, df = 3, $P < 0.001$). However, site-to-site variation in scarring on second-youngest leaves did not usually occur at individual sampling times (Fig. 1B). Necrosis scores varied significantly by site at all four sampling times ($\chi^2 \geq 8.8$, df = 3, $P < 0.05$), but were never higher at both sites IN and RC than at sites LC and RG (Fig. 1C). The significant time effects and site-by-time interactions (necrosis only) ($\chi^2 \geq 55.4$, df = 9, $P < 0.001$) reflected increases in damage and necrosis between the summer and fall 2001 sampling times, and decreases by spring 2002 (Fig. 1B, 1C). Scarring and necrosis scores were not correlated at individual time points ($P > 0.05$).

Biochemical measures and necrosis

Total available carbohydrate content did not vary by site in furled leaves ($P > 0.05$) (data not shown). In youngest unfurled leaves, TAC content varied across sites ($F = 11.4$, df = 3, 16, $P < 0.001$) and sampling times ($F = 34.1$, df = 1, 40, $P < 0.001$) with linear ($F = 3.6$, df = 3, 40, $P = 0.02$) and quadratic ($F = 53.7$, df = 1, 40, $P < 0.001$) site-by-time interactions. However, patterns of variation among sites in TAC content were not consistent with the trend of higher scarring and necrosis at sites IN and RC (Fig. 2A). Potassium content varied by site in both furled leaves ($F = 7.3$, df = 3, 15, $P = 0.003$) and youngest unfurled leaves ($F = 5.6$, df = 3, 16, $P = 0.008$) but did not vary according to sampling time ($P > 0.05$). In youngest unfurled leaves, potassium never varied across sites in a manner consistent with trends in scarring or necrosis scores (Fig. 2B).

Figure 1. Leaf scarring and necrosis scores summed within samples of *Eichhornia crassipes* plants. A. Scarring scores on the youngest (Young-1) and second-youngest (Young-2) unfurled leaves and necrosis scores on the second-oldest (Old-2) and oldest (Old-1) leaves, averaged across all sampling times. B. Scarring scores on second-youngest unfurled leaves. C. Necrosis scores on oldest leaves. Values represent mean ± SE; $n = 4–5$ samples per site per time. Bars within leaf ages (A) or sampling times (B, C) with different letters are significantly different ($P < 0.05$). NS = no significant differences among means.
Phenolic content was examined in samples collected in fall 2001, the time at which necrosis scores were highest (Fig. 1C). Phenolic content varied significantly between sites LC and IN in youngest unfurled leaves ($F = 6.4$, df = 7, 24, $P < 0.001$; site effect, $F = 7.6$, $P = 0.001$) (Fig. 3). Phenolics were higher in furled leaves than in youngest unfurled leaves ($F = 22.0$, $P < 0.001$) in contrast to TAC and potassium contents, which did not consistently vary between leaf ages. Soluble protein, TAC and potassium contents, and soluble peroxidase activity were positively correlated between furled and youngest unfurled leaf ages ($r > 0.31$, $n = 55–64$, $P < 0.05$), as were phenolic contents in Fall 2001 samples ($r = 0.56$, $n = 16$, $P = 0.02$). Soluble peroxidase activity ($r = 0.35$, $n = 60$, $P = 0.005$) (Fig. 4A) and potassium content ($r = 0.44$, $n = 55$, $P = 0.009$) (Fig. 4B) in furled leaves were correlated to necrosis scores in oldest non-senescent leaves. Correlations between necrosis and soluble protein and TAC contents were not significant. In fall 2001 samples, necrosis scores in second-oldest leaves were positively correlated to phenolics in youngest unfurled leaves ($r = 0.56$, $n = 16$, $P = 0.02$).

Bioassays with Neochetina spp. weevils

Inoculated leaf laminae had light symptom coverage ($\leq 15\%$) 2 weeks after inoculation. Other leaves on inoculated plants were free of symptoms. Leaf scarring by Neochetina spp. weevils was not significantly different between infected and mock-inoculated plants on either inoculated leaves or on the two youngest unfurled leaves (data not shown).

Discussion

Leaf scarring on young E. crassipes leaves and necrosis on old leaves showed a positive association when examined across four field sites and four sampling times, even though scarring and necrosis were spatially separated. Most laminar scarring by adult Neochetina spp. weevils occurs on furled and newly unfurled, young leaves (Center & Wright 1991). Old leaves receive little new adult weevil damage (Center 1985) but show necrotic spotting resulting from earlier C. piaropi infection more commonly than do young leaves (Conway 1976). The bioassay and biochemical results suggest that C. piaropi symptom production did not alter weevil feeding, even though necrosis was associated with increased potassium and phenolics, potential determinants of feeding (Center & Van 1989). The field results may thus reflect chronic weevil scarring and the direct associations between scarring and infection observed previously (Charudattan 1986).

Variation in damage by Neochetina spp. and in stress related to pathogen infection or abiotic conditions are common among E. crassipes populations (Freeman et al. 1981; Center et al. 1999), including those in the Lower Rio Grande Valley of Texas (Moran unpublished data). Elevated feeding on furled and young unfurled leaves by Neochetina spp. at sites IN...
and RC may have increased fungal infection, leading to greater necrosis on these leaves when they were older (Charudattan 1986). A similar damage–pathogen association occurred between E. crassipes mites and the fungus Acremonium zonatum (Charudattan et al. 1978). Abiotic site characteristics common to sites IN and RC could have decreased plant growth and increased biological control (Charudattan 1986; Center et al. 1999). However, the two sites differed in disturbance related to water flow and mechanical control, which were present at site RC but absent at site IN. The scarring-necrosis correlation involving all time points was likely a function of variable weevil and fungal activity in individual sampling units, rather than site characteristics. The buildup of necrosis at most sites late in the field season in 2001 and the subsequent decline are consistent with previous studies of C. piaropi (Conway 1976; Cofrancesco et al. 1985).

Total available carbohydrate and potassium content in furled and young leaves, and water-soluble phenolic content in all 2001 samples were not related on a site-by-site basis with necrosis scores. However, peroxidase activities and potassium content in furled leaves were positively correlated with necrosis in oldest leaves across all sites and times, and late-season phenolic content showed an association in one of four possible young-old leaf combinations. Infection by a foliar necrosis-inducing fungal or bacterial pathogen often leads to increases in plant proteins and sugars (Hatcher 1995), phenolics (Nicholson & Hammerschmidt 1992) and peroxidase activities (Hammerschmidt & Kuć 1995). These responses are dynamic over time. The timing of infection and symptom production relative to field sampling of E. crassipes is unknown. Peroxidases may increase the toxicity of phenolics via oxidation (Nicholson & Hammerschmidt 1992). Polyphenol-oxidases also contribute to oxidation in E. crassipes (Martyn & Cody 1983). The higher phenolic content in furled than in unfurled leaves agrees with past results (Center & Wright 1991). Nitrogen and potassium content in healthy plants is also highest in the furled leaves preferred by adult weevils (Center & Wright 1991).

Although necrosis may have increased potassium, peroxidase and phenolics in furled leaves, the bioassay results suggest that these effects did not lead to indirect, plant-mediated influences of prior infection on weevil feeding. Additive or synergistic biocontrol impacts of Neochetina spp. weevils and C. piaropi on E. crassipes (Charudattan 1986) can occur in the absence of fungus-induced changes in host plant suitability. Other biotic or abiotic sources of plant stress could influence the wounding-related weevil-fungus interaction if they alter host suitability.

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**References**


![Figure 4](https://example.com/fig4.png)

**Figure 4.** Correlations between necrosis scores in oldest leaves of water hyacinth and biochemical factors in furled leaves. A. Peroxidase activity, n = 60. B. Potassium content, n = 56. Each point represents one sample of leaves collected at any of four sampling times. FW = fresh weight; DW = dry weight.


