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Source: Florida Entomologist, 96(2) : 458-462

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0210

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DAMAGE BY *NEOCHETINA* WEEVILS (COLEOPTERA: CURCULIONIDAE) INDUCES RESISTANCE IN *EICHHORNIA CRASSIPES* (COMMELINALES: PONTEDERIACEAE)

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Abstract

Induced resistance in invasive plants might influence herbivore performance or preference, and hamper herbivore-based biological control management. Specialist weevils *Neochetina eichhorniae* Hustache (Coleoptera: Curculionidae) and *N. bruchi* Warner (Coleoptera: Curculionidae) are two herbivores commonly used to control *Eichhornia crassipes* (Mart.) Solms (Commelinales: Pontederiaceae), a destructive and widespread invasive plant. However, we do not know whether *Neochetina* weevils induce resistance in *E. crassipes*. I report results from excised-leaf bioassay and whole-plant choice experiments testing adult weevil preference and performance in response to previous damage. I found that adult weevil damage induced resistance in *E. crassipes*, decreasing weevil performance, but simulated damage did not influence weevil performance in a comparable way. Simulated damage did, however, influence weevil preference. This study is the first test for induced resistance to *Neochetina* weevil herbivory in *Eichhornia crassipes*.

Key Words: *Neochetina eichhorniae, Neochetina bruchi*, water hyacinth, herbivory, biological control

Resumen

La resistencia inducida en plantas invasoras puede influir en el desempeño de los herbívoros o preferencia el desempeño o la preferencia de los herbívoros y ser un obstaculo para el control biológico basado en los herbívoros . Los gorgojos especializados, *Neochetina eichhorniae* Hustache (Coleoptera: Curculionidae) y *N. bruchi* Warner (Coleoptera: Curculionidae), son dos herbívoros comúnmente utilizados para controlar la *Eichhornia crassipes* (Mart.) Solms (Commelinales: Pontederiaceae), una planta invasora y destructiva de amplia distribución. Sin embargo, no sabemos si los gorgojos *Neochetina* inducen resistencia en *E. crassipes*. Se informan los resultados de un bioensayo de hojas extirpadas y experimentos de opciónes con plantas enteras que probaron la preferencia y el desempeño de adultos de gorgojos en respuesta al daño hecho anteriormente. Se encontró que el daño del adulto del gorgojo indujo resistencia en *E. crassipes*, disminuyendo el desempeño del gorgojo, pero el daño simulado no influyó en el desempeño del gorgojo de una manera comparable. El daño simulado, sin embargo, influyó en la preferencia del gorgojo. Este estudio es la primera prueba de la resistencia inducida por el gorgojo *Neochetina* en *Eichhornia crassipes*.

Palabras Clave: *Neochetina eichhorniae*, *Neochetina bruchi*, el jacinto de agua, herbivoría, biocontrol biológico

Water hyacinth (*Eichhornia crassipes*, Pontederiaceae) is an important invasive species in North America and many regions of the world (Penfound & Earle 1948), and much effort has been devoted to *E. crassipes* management. Water hyacinth weevils (*Neochetina eichhorniae* and *N. bruchi*, Coleoptera: Curculionidae), hereafter *Neochetina*, are specialist herbivores that feed on *E. crassipes* as larvae and as adults (Stark & Goyer 1983; Center et al. 2005). The weevils are frequently used as biocontrol agents, and have been found to at least partly control *E. crassipes* populations (Center et al. 1999; Center et al. 2005).

Understanding the basic biology of the interaction between *Neochetina* and *E. crassipes* might improve our ability to make good decisions for management of *E. crassipes* populations. *Neochetina* weevil damage can alter *E. crassipes* traits, increasing phosphorus, potassium and nitrogen levels (Center & Van 1989), and increasing levels of peroxidase, an anti-pathogen defense (Moran 2005). One important aspect of the interaction between herbivores and their host plants is induced resistance to herbivory (e.g., Bernays & Chapman 1994; Tiffin 2000; Kessler & Baldwin 2002), which can produce negative feedbacks to insect popula-

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tion growth (e.g., Underwood 1999; Underwood & Rausher 2002). However, no study has yet quantified how *Neochetina* damage affects subsequent *Neochetina* feeding on *E. crassipes*, which would indicate induced resistance to herbivory.

In three greenhouse experiments, I examined *E. crassipes* response to adult *Neochetina* damage and to simulated *Neochetina* adult and larval damage. I tested the effect of real and simulated damage on subsequent weevil performance (adult feeding damage in no-choice bioassays) and weevil preference (weevil number or damage in whole-plant choice tests).

Materials and Methods

I conducted these experiments in the spring and summer of 2010 and 2011 in tanks at the Florida State University Mission Road Research Facility in Tallahassee, Florida. Adult *Neochetina eichhorniae* and *N. bruchi* have similar life histories (Deloach & Cordo 1976; Stark & Goyer 1983). Adults of both species leave characteristic feeding scars on the leaf blade surface and lay eggs in leaf petioles, where larvae burrow through leaf tissue, feeding on and damaging both apical and axillary (lateral) meristems (Stark & Goyer 1983; Center et al. 2005). I used both species interchangeably in these experiments. I collected the weevils used to impose insect damage and for bioassays from plants on the St. Marks River (N 30.225978° W 84.156243°) and Weems Pond, Tallahassee (N 30.455525° W 84.222849°). Natural populations of *Neochetina* generally have a 1:1 sex ratio (Deloach & Cordo 1976; Stark & Goyer 1983), and while I did not control for sex ratio in damage treatments or bioassays, because damage amount inflicted by individual male and female *Neochetina* differs only slightly (Stark & Goyer 1983) I do not believe the sex of weevils used in my experiments substantially influenced experimental results. I collected *E. crassipes* from the Crooked River (N 29.92872° W 84.62568°), Lake Munson (N 30.37000° W 84.31465°), and the Wacissa River (N 30.34384° W 83.99923°).

Experiment 1: Effects of Simulated and Real Adult Damage on Weevil Performance and Preference (2010)

I maintained 30 plants from the Crooked River population in ten cages in a greenhouse. Each cage contained three plants, and each plant per cage received either simulated weevil damage, actual weevil damage, or no damage. To simulate adult weevil damage, I used a razor blade to remove approximately 25-30% of the plant's leaf surface tissue in small patches evenly distributed across expanded leaves, leaving the underlying fibrous tissue. Damage was approximately 2.5-3 times greater than the maximum observed damage in local populations (unpublished data). To impose actual weevil damage I enclosed entire plants in mesh bags and allowed 10-15 adult weevils per plant to feed for 4 d. Final damage levels were approximately 10% damage per leaf, close to average damage levels in local populations (unpublished data). While female weevils may have oviposited during the damage period, eggs would not have had time to hatch and feed before the bioassay (Deloach & Cordo 1976; Stark & Goyer 1983). I physically handed control plants but did not damage them.

I measured systemic induced resistance after damage with weevil performance in no-choice bioassays. I excised the youngest expanded leaf of each plant and placed the leaf in a 5.5 fluid ounce (163 mL) plastic cup, filled with water and a paper towel to elevate the leaf blade above the water; each cup was enclosed in a mesh bag. I introduced four weevils onto each leaf to feed overnight for 14 h. I measured induced resistance as the difference in scar number before and after the bioassay. I measured adult whole-plant preference by introducing 10-15 adult weevils into each of the three-plant cages after damage and bioassay leaf excision. I allowed the weevils to forage for 24 h then counted the weevil number per plant.

Experiment 2: Effects of Simulated Larval and Adult Damage on Weevil Preference (2010)

I maintained 120 plants from the Crooked River (n = 38), Wacissa River (n = 74) and Lake Munson (n = 8) in outdoor tanks. I imposed simulated adult weevil damage as described in Experiment 1, and I added two larval damage treatments and a no-damage control treatment (n = 30 per damage treatment). *Neochetina* larvae feed inside leaf tissue and can damage internal plant structures, including apical and axillary (lateral) meristems (Stark & Goyer 1983; Center et al. 2005). To simulate larval damage I used forceps to destroy either the single apical meristem or all visible axillary meristems. Sixty to 72 h after all damage treatments were completed, I caged the plants approximately evenly across damage treatment and source population into 12 groups of 10. Half the cages received twenty *Neochetina* weevils which were allowed to forage for 4 days. I introduced no weevils into the remaining cages. After 4 days, I removed the weevils with a visual search and counted leaf scars. I quantified herbivore preference as weevils per plant and scar number per plant. I repeated these procedures again 12 days after initial damage treatments, reversing weevil and no-weevil treatments so that the plants that were previously unexposed received weevils.

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Experiment 3: Effects of Simulated Larval and Adult Damage on Weevil Performance (2011)

I maintained 189 plants from the Crooked River (n = 108) and Wacissa River (n = 81) in indoor aquaria. I simulated adult and larval weevil damage as in Experiment 2, but in this experiment I imposed axillary damage over 10 days, rather than a single day. I measured induced resistance with bioassays as described in Experiment 1, conducted 2 days after damage completion (n = 109) and 10 days after damage completion (n = 80) on 2 different subsets of plants. I measured bioassay damage in this experiment by photographing the excised leaf before and after the bioassay and quantifying damaged leaf area using ImageJ software (Rasband 2012).

Statistical Analyses

For each experiment, I used Type III SS analysis of variance (ANOVA) or covariance (ANCOVA). In Experiment 1, I analyzed effects of damage treatment on (1) the square-root transformed difference in scar number including cage as a fixed block effect, and (2) the proportion of weevils found on each plant including cage as a fixed block effect and plant wet biomass as a covariate. In Experiment 2, I analyzed the effect of damage treatment on (1) the log transformed change in scar number including cage as a fixed block effect, and the number of weevils recovered from (2) the early-exposure group (log transformed) and (3) the late-exposure group (square-root transformed). In Experiment 3, I analyzed the effect of damage treatment on scar area per bioassay weevil including date of bioassay, source population, and aquaria as fixed effects covariates. Where ANOVA or ANCOVA results indicated significant damage effects, I used Tukey's HSD to determine differences in treatment groups.

In each case, cage or aquaria was a fixed effect because I was not concerned with interpreting effects of block identity. Population was fixed because multiple populations were sampled only to increase genetic diversity of the experimental plants, not to investigate population differences. Variables were transformed as necessary to meet assumptions of the analysis as necessary. All analyses were done in R 2.13.0 (R Development Core Team 2011) with the package "car" (Fox & Weisberg 2011).

Results and Discussion

Adult weevil damage induced resistance in *Eichhornia crassipes* as measured by weevil performance, but simulated adult damage did not (Experiment 1). Damage treatment affected subsequent damage ($F_{2,18}$ = 8.37, P < 0.01), with insect damage decreasing subsequent damage relative to control plants by 57%, with no effect of simulated damage on subsequent bioassay damage (Fig. 1). This is to my knowledge the first test for induced resistance to *Neochetina* weevils in *E. crassipes*. It is worth noting that the experimental plants were collected from the wild and likely experienced damage by *Neochetina* and other herbivores before being used in these experiments. This means that the plants might still have had some level of induced resistance before damage treatments were imposed, but since previous induced resistance was likely generally equal for all damage treatments, treatment effects will still show whether damage type further induces resistance differentially. *Neochetina* weevils have been shown to alter plant nutritional quality after 12 weeks of infestation (Center & Van 1989), but in that case damage increased nutrient content. While my study cannot determine the mechanism responsible for the induced resistance, it is possible that shorter-term nutritional changes decrease weevil feeding. Regardless of mechanism, because induced resistance can produce negative feedbacks to insect population growth (e.g., Underwood 1999; Underwood & Rausher 2002), my results could indicate that larger or repeated inputs of biocontrol herbivores are required to maintain damage levels sufficient to influence plant population growth.

Neither insect nor manual leaf damage influenced whole-plant herbivore preference as measured by number of weevils per plant in Experiment 1 ($F_{2,25}$ = 0.9149, P = 0.41). This is in contrast to findings that *Neochetina* weevils are more attracted to damaged plants (Perkins et al., 1976) due to the release of volatiles from damaged tissue (Del Fosse & Perkins 1977). It is possible that damaged plants in my study did not attract more

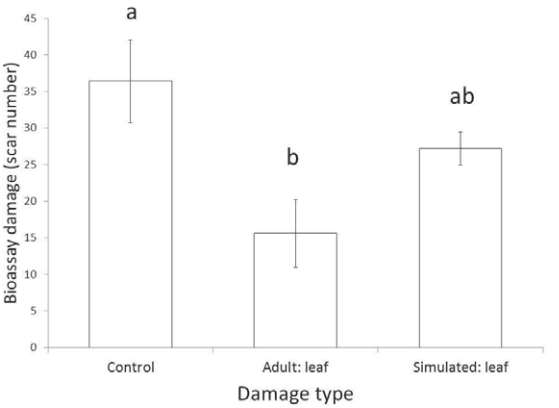


Fig. 1. Weevil performance in no-choice bioassays (measured by scar number) indicates that plants induce resistance to insect leaf damage but not to manual leaf damage. Error bars show ± 1 SE; letters indicate significant Tukey's HSD values.

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weevils than undamaged plants due to the close proximity of plants from all damage treatments, which may have hindered the ability of weevils to track airborne volatiles to damaged plants. The increased attraction of *Neochetina* to *E. crassipes* demonstrated by Perkins et al. (1976) does not necessarily imply that induced resistance in response to damage is not present. Perkins et al. (1976) and Del Fosse & Perkins (1977) measured weevil abundance on plants, not weevil damage, and so those studies cannot rule out that damage reduces subsequent damage per herbivore. In my study, the bioassay presented herbivores with undamaged tissue from damaged plants, and so isolated responses to systemic induced resistance response from attraction to volatiles.

Simulated damage treatment did influence whole-plant preference as measured by scar number in Experiment 2 ($F_{3, 114} = 4.34, P < 0.01$). Forty-five percent fewer feeding scars were present on plants with simulated adult (leaf) damage (Fig. 2). This result suggests that herbivores were less likely to feed on a leaf-damaged plant due to the presence of the damaged tissue itself rather than any systemic induced resistance, which was not found in response to simulated leaf damage (Experiment 1). There are several potential mechanisms for this result that are beyond the scope of the current study. First, insects may be deterred by changes to the leaf surface, like the chemical composition of leaf surface wax (e.g., Bernays & Chapman 1994), even if the plant does not induce systemic resistance. Second, *E. crassipes* leaves produce phenolic compounds that are resistant to fungal pathogens (Martyn & Cody 1983) and may be repellant to insect herbivores. Third, the insects might simply find less leaf material to eat on a previously damaged plant and choose a less damaged plant.

Simulated damage type did influence weevil performance (Experiment 3, $F_{3, 183} = 3.61$, $P = 0.02$), however no treatment group differed from the control group (Fig. 3). Plants with axillary damage received significantly less damage than plants with leaf damage, and plants with leaf damage generally received more damage than plants in other treatment groups overall, supporting the conclusion that the preference results from Experiment 2 were due to the presence of scarred tissue itself rather than systemic induced resistance.

Although *Neochetina* adults can cause substantial tissue damage, and can facilitate pathogen spread among plants (Maron 2005), larvae are likely more detrimental to *E. crassipes* population growth than adult weevils because larvae tunnel through plant tissues (Cilliers 1991) and can damage meristems (Stark & Goyer 1983; Center et al. 2005). Because mechanical damage like that used to simulate *Neochetina* larval damage in my experiments sometimes does not elicit the same plant responses as real damage (Walling 2000; Massey et al. 2007), my experiments cannot rule out induction in response to real larval damage. Developing methods to manipulate larval damage would be necessary to determine whether *Neochetina* larvae might influence *E. crassipes* induced resistance and potentially biocontrol efforts.

Overall, the results of my study indicate that adult *Neochetina* damage does influence subsequent adult damage. While the amount of herbi-

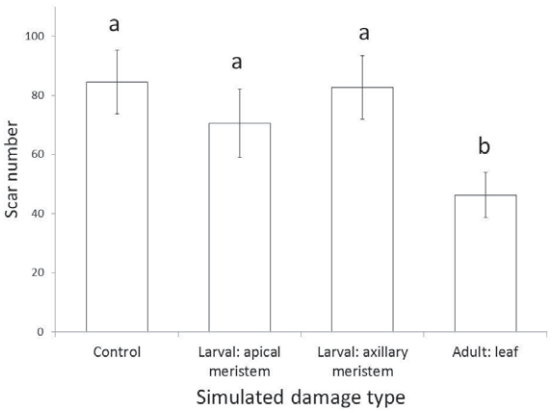


Fig. 2. Weevil preference for whole plants (measured by scar number) indicates that weevils feed less on plants with simulated adult (leaf) damage than on plants with simulated larval (axillary or axial) damage or undamaged plants. Error bars show ± 1 SE; letters indicate significant Tukey's HSD values.

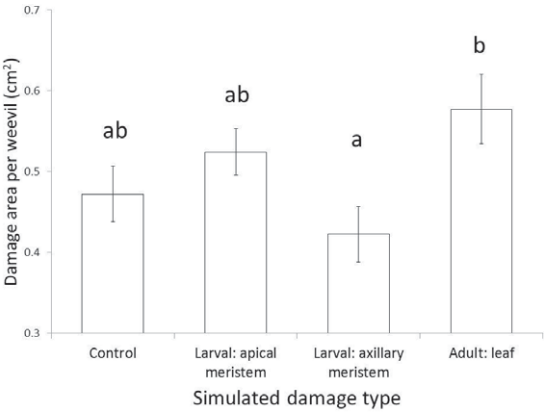


Fig. 3. Weevil performance in no-choice bioassays (measured by damaged leaf area per weevil) indicates that weevils perform better on plants with simulated adult (leaf) damage than on plants with simulated larval (axillary) damage, but weevils performed equally well on all treatment groups relative to undamaged plants. Error bars show ± 1 SE; letters indicate significant Tukey's HSD values.

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vore damage plants receive and how damage affects plant reproduction are influenced by many factors, such as plant age (Boege & Marquis 2005) and resources (Gutbrodt et al. 2012), induced resistance could contribute to reduced weevil biocontrol efficacy over time (e.g., Center et al. 1999).

Acknowledgments

Thanks to N. Underwood, B. Inouye, A. Winn, and J. Grinath for comments, D. McNutt for field assistance, and J. Travis for cattle tanks. Portions of this project were funded by the Florida Exotic Pest Plant Council Julia Morton Invasive Plant Research Grant.

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