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Researchers at the University of Nebraska have been investigating the role of peroxidases in the defense response of resistant and susceptible warm-season turfgrasses to chinch bug feeding. Results of these studies suggest that the up-regulation of peroxidases in resistant buffalograsses is a direct response to chinch bug feeding and that peroxidases have the potential to be used as markers for selecting chinch bug-resistant turfgrasses.

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The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of \$30 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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# The Role of Peroxidases in the Defense Response of Warm-season Turfgrasses to Chinch Bugs

Tiffany Heng-Moss, Osman Gulsen, Thomas Eichkoff, Robert Shearman, and Frederick Baxendale

## SUMMARY

Researchers at the University of Nebraska have been investigating the role of peroxidases in the defense response of resistant and susceptible warm-season turfgrasses to chinch bug feeding. Their progress includes:

- Correlation analyses of 28 buffalograss genotypes with varying levels of chinch bug resistance and ploidy levels indicated that buffalograss total protein content was correlated to chinch bug injury, while basal peroxidase levels was not, suggesting that the up-regulation of peroxidases in resistant buffalograsses is a direct response to chinch bug feeding.

- Research also characterized peroxidase changes in resistant and susceptible buffalograsses and zoysiagrasses challenged by chinch bugs. These studies documented an increase in peroxidase activity in the resistant buffalograsses in response to insect feeding. These findings suggest that an initial plant defense response to chinch bug feeding may be to elevate levels of specific oxidative enzymes, such as peroxidase, to help detoxify peroxides that accumulate in response to plant stress.

- Native gel electrophoresis analysis identified differences in the isozyme profiles of infested and control buffalograsses 'Prestige' and 196, and the zoysiagrass 'Zorro'. These results suggest that peroxidases have the potential to be used as markers for selecting chinch bug resistant turfgrasses, and may help explain how plants defend themselves against biotic stresses, such as chinch bugs.

- Knowledge gained from this research will benefit golf course superintendents, sod producers, and other turfgrass managers by furnishing turfgrasses with improved resistance to chinch bugs.

Chinch bugs are pests of several cool- and warm-season turfgrasses. Recently, the western chinch bug (*Blissus occiduus* Barber) has been

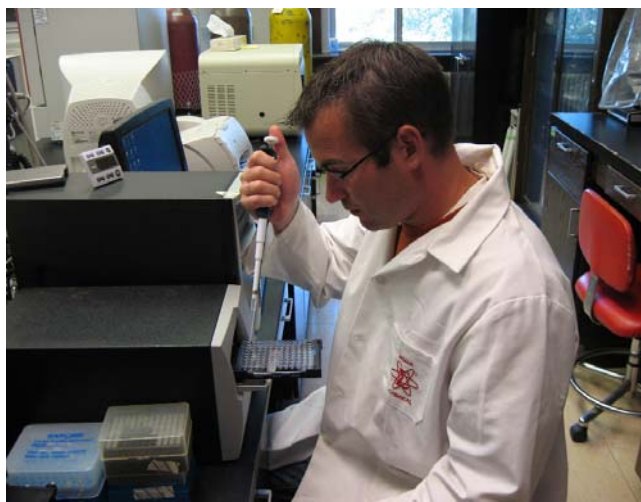
TIFFANY HENG-MOSS, Ph.D., Associate Professor, Department of Entomology, University of Nebraska, Lincoln, NE; OSMAN GULSEN, Ph.D., Department of Horticulture, Erciyes University, Kayseri, Turkey; THOMAS EICHKOFF, Ph.D., Monsanto Company, Monmouth, IL; ROBERT SHEARMAN, Ph.D., Professor, Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE; and FREDERICK BAXENDALE, Ph.D., Professor, Department of Entomology, University of Nebraska, Lincoln, NE.

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identified as an important insect pest of buffalograss and zoysiagrass (3, 5). Management strategies for the western chinch bug include the use of cultural practices to reduce thatch, proper fertilization, irrigation, use of resistant turfgrasses, and insecticides (2). Heng-Moss et al. (10), Gulsen et al. (6), and Eichkoff et al. (5) identified several buffalograsses and zoysiagrasses resistant to the western chinch bug including the buffalograsses 'Prestige' and 'Cody', and the zoysiagrasses 'Emerald' and 'Zorro'.

Additional research by Heng-Moss et al. (9) and Eichkoff et al. (4) investigated the categories (antibiosis, antixenosis, and tolerance) of buffalograss and zoysiagrass resistance to the western chinch bug and identified the buffalograsses 'Prestige' and 'Cody', and zoysiagrasses 'Emerald' and 'Zorro' as tolerant. Heng-Moss et al. (11) and Gulsen et al. (6) provided some insights into the role of peroxidase in the tolerance response of the resistant buffalograss, 'Prestige', to chinch bug feeding; however, the extent of this response needs to be examined in other resistant turfgrasses. The identification of mechanisms



Dr. Thomas Eichkoff, now with Monsanto, uses a commercially available protein assay kit to determine protein content of chinch bug-resistant and -susceptible buffalograss genotypes.





Dr. Osman Gulsen, now with Erciyes University, Kayseri, Turkey, selects diploid, triploid, tetraploid, pentaploid, and hexaploid buffalograss plants to determine total protein content and peroxidase activity of these buffalograss genotypes in the absence of chinch bug feeding.

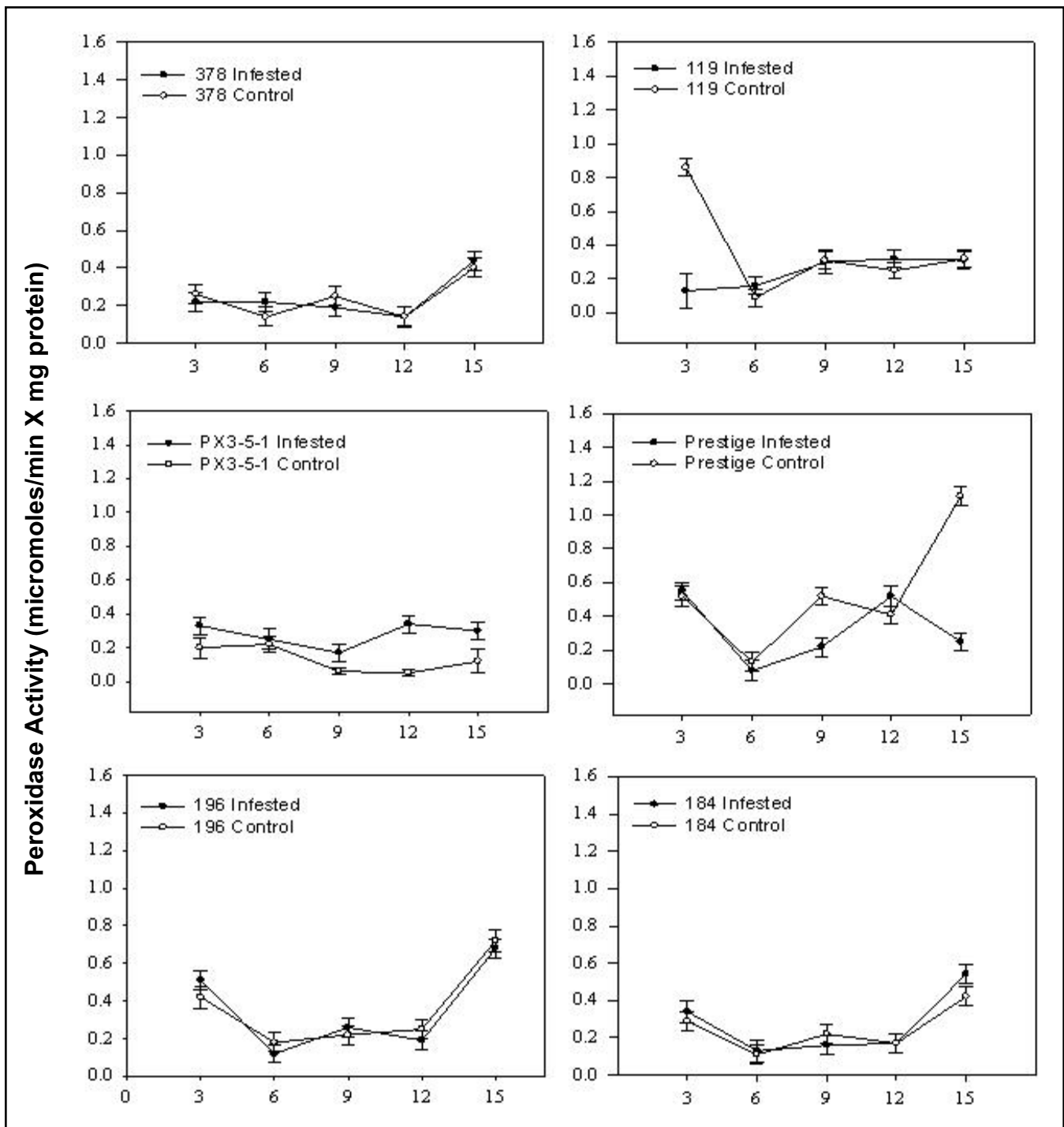
responsible for the observed tolerant response will aid in our understanding of plant-insect interactions, and in the defense responses of plants to biotic stresses.

The USGA is currently funding a project focusing on deciphering the role of peroxidases in the defense response of buffalograss and zoysiagrass to the western chinch bug. Specific objectives of this research include: 1) assessing the relationships among protein content, basal peroxidase levels, chinch bug injury, and ploidy levels of chinch bug-resistant and -susceptible buffalograsses; 2) comparing peroxidase activity levels of resistant and susceptible buffalograsses and zoysiagrasses in response to chinch bug feeding; and 3) analyzing extracted proteins from chinch bug-resistant and -susceptible buffalograsses and

zoysiagrasses by native gel electrophoresis to obtain information on the peroxidase profiles.

### **Peroxidases**

Peroxidases play an important role in plant stress-related interactions. The proposed functions of peroxidases in plants include lignification, suberization, somatic embryogenesis, auxin metabolism, wound healing, as well as defense against pathogens and other biotic and abiotic factors (1, 13, 15). Enzymes such as peroxidase reduce reactive oxygen species (ROS) accumulation and detoxify oxidation products when plants have been challenged with various stressors (16). It has been speculated that resistant genotypes may have the ability to increase peroxidase level

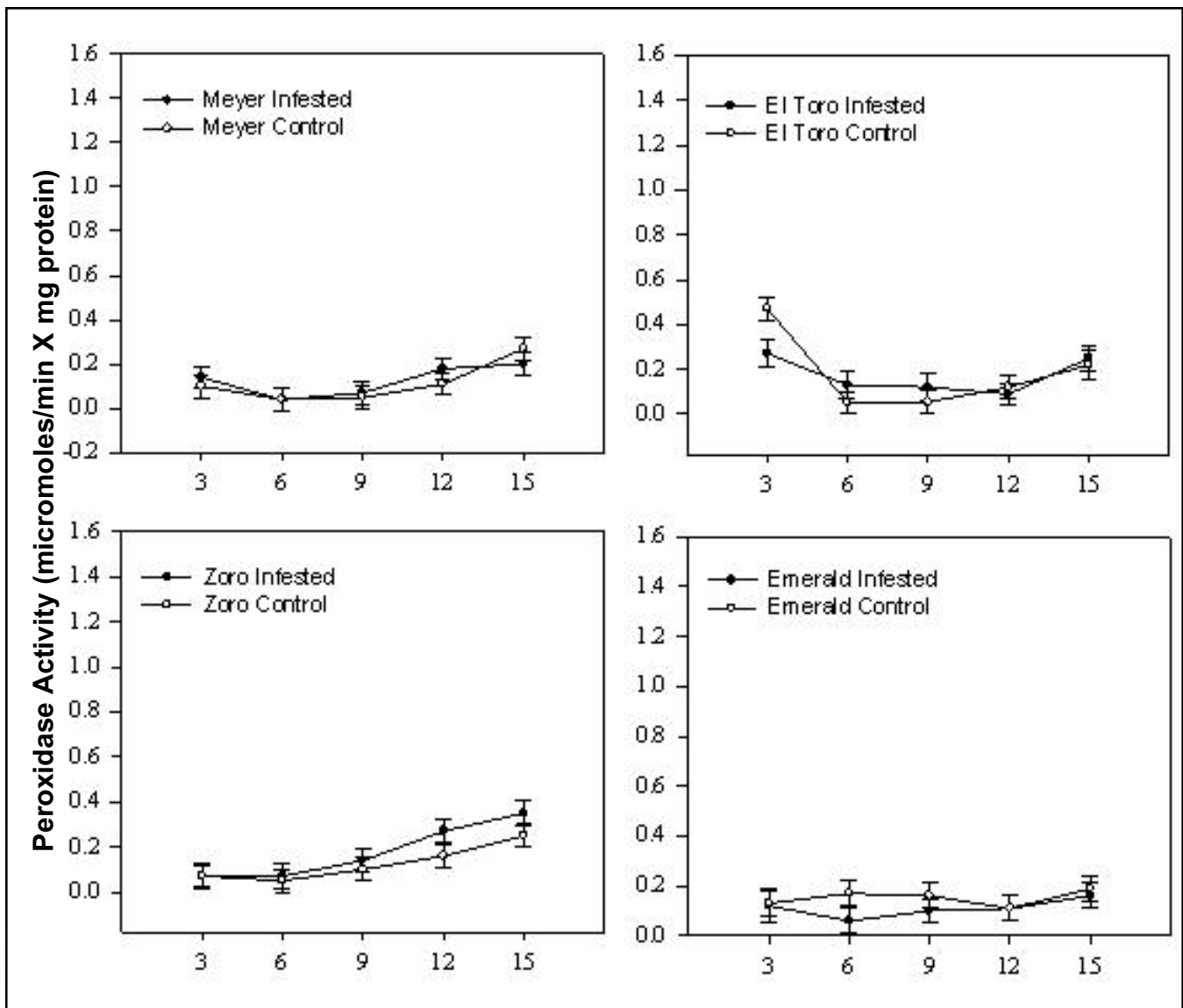


**Figure 1.** Peroxidase activity ( $\mu\text{mol}/\text{min} \times \text{mg protein}$ ) of resistant and susceptible buffalograsses at 3, 6, 9, 12, and 15 days after chinch bug introduction. Published in the *Journal of Arthropod-Plant Interactions* 4:45-55.

activity, and thereby detoxify the radicals and peroxides, whereas, susceptible plants are unable to detoxify those compounds (8, 11, 12). This suggests that genotypes with a higher level of resistance would have a higher up-regulation capacity for peroxidase, have a more sensitive up-regulation response, or both.

### Total Protein Content and Basal Peroxidase Levels Among Buffalograsses

As a first step toward understanding the role of peroxidase in the defense response of resistant turfgrasses to chinch bugs, total protein content and peroxidase activity of 28 buffalograss



**Figure 2.** Peroxidase activity ( $\mu\text{mol}/\text{min} \times \text{mg protein}$ ) of resistant and susceptible zoysiagrasses at 3, 6, 9, 12, and 15 days after chinch bug introduction. Published in the *Journal of Arthropod-Plant Interactions* 4:45-55.

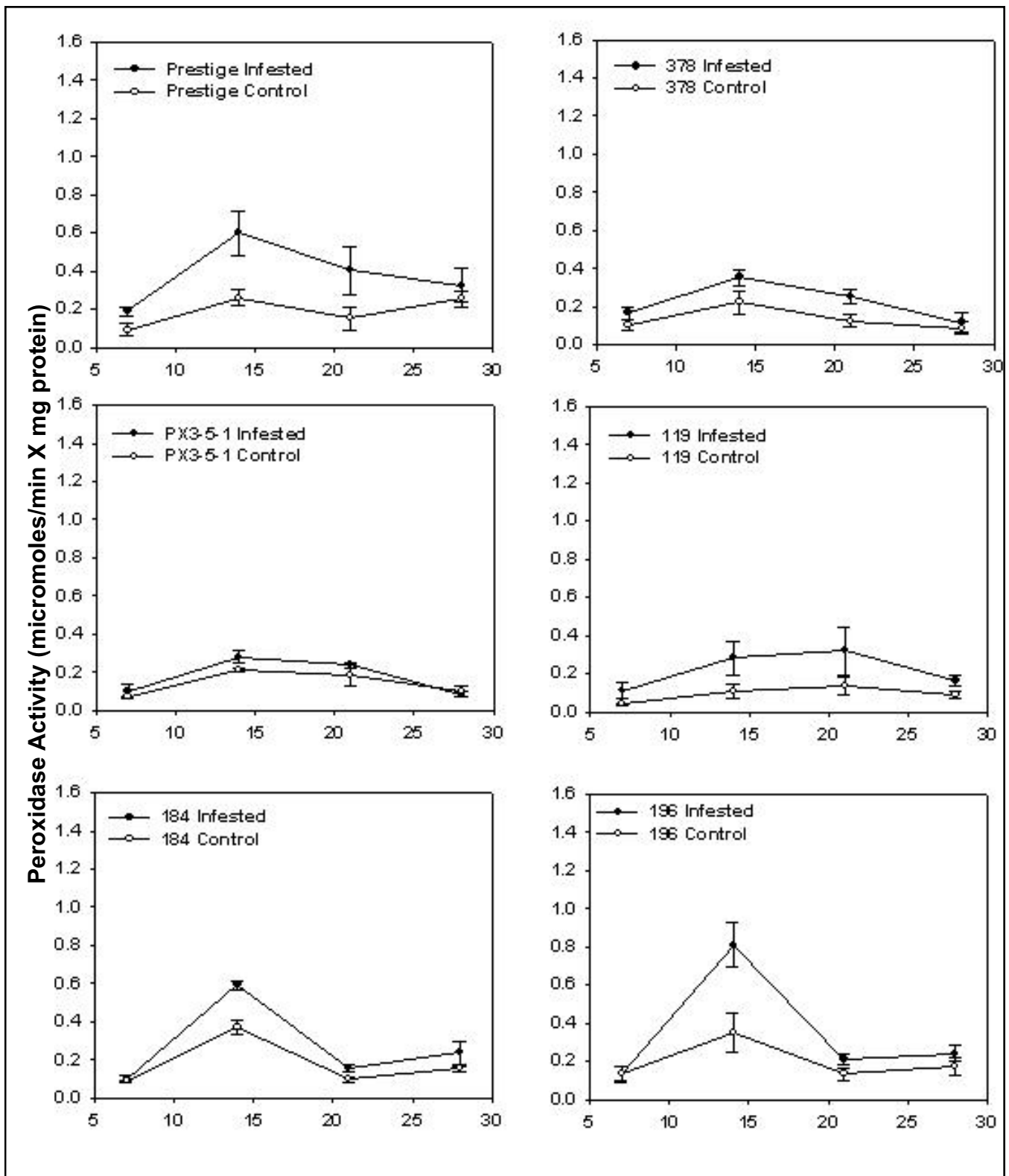
genotypes with varying levels of resistance to the western chinch bug were evaluated to document the relationship among total plant protein content, basal peroxidase levels, chinch bug injury, and ploidy level.

Of the 28 buffalograsses evaluated, four were highly resistant, 13 were moderately resistant, eight were moderately susceptible, and three were highly susceptible. This germplasm represented diploid, triploid, tetraploid, pentaploid, and hexaploid plants. Plants were grown in the absence of chinch bugs for this study.

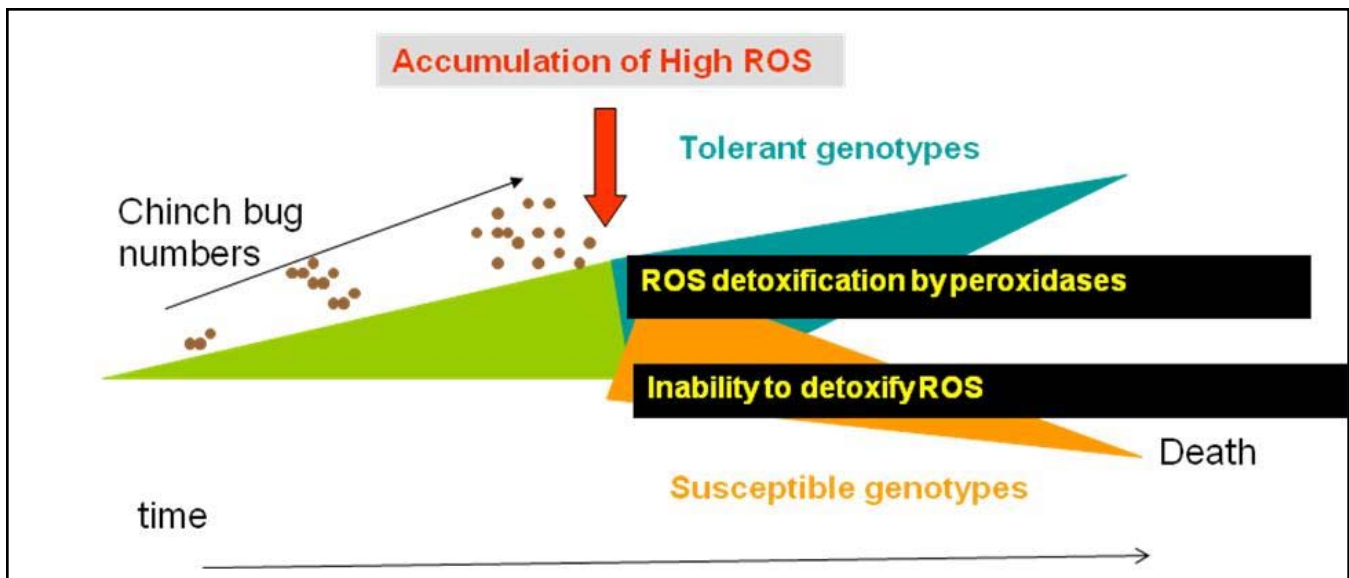
Soluble proteins were extracted from 20 mg of plant tissue using a standard sap extraction

method (modified from Heng-Moss et al., 11). Total protein content was determined using a commercially available (BCA) protein assay kit (Pierce, Rockford, IL) using bovine serum albumin as a standard. Triplicate aliquots of each sample were measured using a semi-automated microplate reader, PowerWave (BIO-TEK Instruments, Inc., Winooski, VT).

A significant correlation was detected between chinch bug injury and total protein content. The susceptible genotypes had higher protein content than the resistant genotypes. Although a significant correlation was detected, this value was low, which suggests that total pro-



**Figure 3.** Peroxidase activity ( $\mu\text{mol}/\text{min} \times \text{mg protein}$ ) of resistant and susceptible buffalograsses at 7, 14, 21, and 28 days after chinch bug introduction. Published in the *Journal of Arthropod-Plant Interactions* 4:45-55.



**Figure 4.** Proposed role of peroxidases in the defense response of resistant turfgrasses to chinch bugs. Resistant genotypes have the ability to increase peroxidase, and thereby detoxify the radicals and peroxides, whereas, susceptible plants, are unable to detoxify those compounds. Published in the *Journal of Arthropod-Plant Interactions* 4:45-55.

tein content is not an effective indicator of chinch bug resistance. This supports our working hypothesis that the up-regulation of specific proteins are linked to the resistance rather than up-regulation of the overall protein level. There was no significant correlation between total protein content and ploidy level.

Peroxidase activity was also assessed for the 28 buffalograsses. Peroxidase activity was measured by determining the increase in absorbance at 470 nm for 2 min using a protocol modified from Hildebrand et al. (12) and Heng-Moss et al. (11). The enzymatic reaction was initiated by adding 2  $\mu\text{L}$  of 30% hydrogen peroxide to wells of a 96-well microplate containing 60  $\mu\text{L}$  of 18 mM guaiacol, 20  $\mu\text{L}$  of 200 mM HEPES (pH 7.0), 117  $\mu\text{L}$  of distilled water, and 1  $\mu\text{L}$  of buffalograss extract. The specific activity of peroxidase was determined using the molar absorptivity of guaiacol at 470 nm ( $26.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ ). For each sample, peroxidase activity was measured four times using the microplate reader described above.

No significant correlations were found between basal peroxidase levels and chinch bug injury, or between basal peroxidase levels and ploidy level. This suggests that basal peroxidase

levels in buffalograss genotypes are not a useful indicator of chinch bug resistance and that the up-regulation of peroxidase in resistant buffalograsses is a direct response to chinch bug feeding.

#### **Changes in Protein Content and Peroxidase Activity in Response to Chinch Bug Feeding**

A second component of this research was to investigate changes in total protein content and peroxidase activity levels in selected resistant and susceptible buffalograsses and zoysiagrasses in response to chinch bug feeding. Three separate studies were conducted. Study 1 included four resistant ('Prestige', 196, PX3-5-1, and 184) and two susceptible (119 and 378) buffalograsses. Study 2 consisted of four zoysiagrasses, 'Meyer' (highly-moderately susceptible), 'El Toro' (moderately resistant to moderately susceptible), 'Emerald' (moderately resistant), and 'Zorro' (moderately resistant). Study 3 included the same buffalograsses used in study 1, but was conducted over a 28-day time frame to identify long-term changes in protein content and peroxidase activity. Studies 1 and 2 were conducted over a shorter 15-day period.





**Figure 5.** Native gel stained for peroxidase activity in control and infested Prestige buffalograss at 15 days after chinch bug introduction. I = infested, C = control. Arrows indicate notable differences in levels of peroxidase activity. Published in the *Journal of Arthropod-Plant Interactions* 4:45-55.

### Total Protein

For all three studies, protein changes in response to chinch bug feeding provided few consistent trends among resistant and susceptible genotypes in either buffalograss or zoysiagrass. This observation strengthens the hypothesis that total protein changes over time are not a viable indicator of chinch bug resistance.

### Peroxidase Activity

Study 1. The chinch bug-resistant buffalograss PX3-5-1 had higher levels of peroxidase

activity (Figure 1) in infested plants than in control plants at all time intervals. Peroxidase activity was significantly higher at day 12. Infested ‘Prestige’ plants had similar or higher levels of peroxidase activity on all days when compared to control plants, and were significantly higher on days 9 and 15. Peroxidase activity was similar between infested and control plants for the resistant buffalograsses, 184 and 196, and the highly susceptible buffalograsses, 378 and 119, at all harvest dates.

Study 2. Similar results were observed in zoysiagrass’ response to chinch bug feeding. ‘El Toro’ control plants had significantly higher levels of peroxidase activity (Figure 2) on day 3, but significantly lower activity levels on day 6. ‘Meyer’ (chinch bug-susceptible zoysiagrass) infested plants had significantly higher levels of peroxidase activity on day 12, while on day 15, infested plants had significantly lower peroxidase activity levels than the control plants. Control plants of the resistant zoysiagrass ‘Emerald’ had significantly higher levels of activity than the infested plants on day 6.

In general, the zoysiagrasses ‘Emerald’ and ‘Meyer’ had differing levels of peroxidase activity (Figure 2) between infested and control plants at all harvest dates. No consistent trends were evident among these grasses suggesting that resistant plants are not increasing peroxidase activity in response to chinch bug feeding. In the resistant zoysiagrass ‘Zorro’, however, after day 3 there were consistently higher levels of peroxidase activity in infested plants compared to control plants. These differences were significant on days 12 and 15. This observation indicates that ‘Zorro’ is able to elevate peroxidase levels in response to chinch bug feeding, which suggests a possible peroxidase role in the defense response mechanism for this grass.

Study 3. Overall, all infested buffalograss genotypes showed higher peroxidase activity levels when compared to control plants (Figure 3). Of the four chinch bug resistant genotypes, infested ‘Prestige’ plants had significantly higher levels of peroxidase activity than control plants on days 14 and 21. The resistant buffalograss 196, showed

significantly higher levels of peroxidase activity in infested plants on day 14. The highly susceptible genotypes, 378 and 119, had significantly higher levels of peroxidase activity on days 14 and 21.

The results of these experiments are consistent with our working hypothesis that peroxidases play a role in the response of resistant turfgrasses to chinch bugs (Figure 4). Similar trends in peroxidase activity were observed in both short-term and long-term studies in the buffalo-grasses 119, 378, and 'Prestige'. In all studies, peroxidase activity levels were elevated in Prestige, which supports the finding of Heng-Moss et al. (11) that peroxidases may be contributing to the chinch bug resistance observed in this genotype. It is important to note that peroxidase activity in the chinch bug-resistant buffalo-grasses 184 and 196 dramatically increased in both studies at day 14 and 15, respectively.

In Study 1, peroxidase activity also increased in these grasses; whereas, in Study 3 the elevated activity was not observed. The genotype PX3-5-1 had similar levels of peroxidase activity in the infested plants from days 6 to 15 in studies 1 and 3. However, these responses were not seen in the control plants (similar to genotypes 184 and 196). These differing responses may be the result of one or more of the numerous functions that have been associated with peroxidases. While peroxidases may be playing a role in the resistance of these genotypes to chinch bugs, they may also indicate that peroxidases play a more general role in the defense response of these grasses.

### **Peroxidase Profiles**

Isozyme profiles for peroxidase activity were visualized using histochemical methods. All native gels were evaluated for the presence or absence of bands and for band intensity. Gels were photographed after incubation and staining. The incubation and staining procedures were modified from Heng-Moss et al. (11).

Analysis of native gels stained for peroxidase activity displayed visual differences in the peroxidase expression levels among the six buf-



Similar trends in peroxidase activity were observed in both short-term and long-term studies in the buffalo-grasses 119, 378, and 'Prestige'. In all studies, peroxidase activity levels were elevated in 'Prestige' suggesting that peroxidases may be contributing to the chinch bug resistance observed in this genotype.

falograsses. Differences were also observed among the control plants for the buffalograsses reflecting genetic variability among genotypes. Native gels displayed visual differences in peroxidase expression levels between infested and control plants in 378, 119, 184, 196, and PX3-5-1. As expected, differences in the peroxidase profiles of infested and control ‘Prestige’ were identified (Figure 5).

These results support the findings of Heng-Moss et al. (11) who detected elevated levels of peroxidase activity in ‘Prestige’ in response to chinch bug feeding, while similar elevations were not observed in 378. Again, no general trends were observed among the remaining buffalograsses except for ‘Prestige’ at 15 days after chinch bug introduction. Future studies should focus on these differentially-expressed peroxidases and their role in the buffalograss defense systems.

Analysis of native gels stained for peroxidase activity displayed few visual differences in the expression levels of peroxidase among the four zoysiagrasses. Similar to the buffalograsses, differences were observed among control plants reflecting natural genetic variability among the zoysiagrasses. Native gels confirmed the trends for similar peroxidase expression levels between infested and control ‘Meyer’, ‘El Toro’, ‘Emerald’, and ‘Zorro’ plants.

### **Role of Peroxidases in the Defense Response of Turfgrasses to Chinch Bug Feeding**

Peroxidases serve an important role in the defense response of many plants to biotic and abiotic stresses (14, 15). Our data suggests that peroxidases could be playing multiple roles in a tolerant plant’s defense response to insect feeding, including the downstream signaling of plant defense reactions to chinch bug injury, efficient removal of reactive oxygen species, or both (8, 15). Most likely, certain peroxidases are responsible for chinch bug resistance, so future studies should focus on these specific peroxidases and their regulatory elements in relation to chinch bug resistance.

We also found that several resistant buffalograsses (184, 196, and PX3-5-1) and the zoysiagrass ‘Emerald’ do not consistently show an increase in peroxidase activity in response to chinch bug feeding despite being categorized as tolerant (4). These results suggest alternate mechanisms of resistance may be present in these grasses.

This research provides essential information for the development of chinch bug-resistant buffalograsses and zoysiagrasses for use on golf courses and other turfgrass areas, and for the implementation of chinch bug management decisions. Commercial production of warm-season turfgrasses with resistance to chinch bugs will offer turfgrass professionals and homeowners with a high quality turfgrass with enhanced resistance to chinch bugs.

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