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ORIGINAL PAPER

Characterization of peroxidase changes in resistant and susceptible warm-season turfgrasses challenged by *Blissus occiduus*

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Abstract Peroxidases play an important role in plant stress related interactions. This research assessed the role of peroxidases in the defense response of resistant and susceptible buffalograsses [*Buchloe dactyloides* (Nutt.) Engelm] and zoysiagrasses (*Zoysia japonica* Steudel) to the western chinch bug, *Blissus occiduus* Barber. The objectives were: (1) to assess the relationships among protein content, basal peroxidase levels, chinch bug injury, and ploidy levels of chinch bug-resistant and -susceptible buffalograsses; (2) to compare peroxidase activity levels of resistant and susceptible buffalograsses and zoysiagrasses in response to chinch bug feeding; (3) and to analyze extracted proteins from chinch bug-resistant and -susceptible buffalograsses and zoysiagrasses by native gel electrophoresis to obtain information on the peroxidase profiles. Correlation analyses of 28 buffalograss genotypes with varying levels of chinch bug resistance and ploidy levels indicated that buffalograss total protein content was correlated ($r = 0.47$, $P = 0.01$) to chinch bug injury, while basal peroxidase levels was not ($r = 0.19$, $P = 0.29$), suggesting that the up-regulation of peroxidases in resistant buffalograsses is a direct response to chinch bug feeding. Three of the four chinch bug-resistant buffalograss genotypes evaluated had higher peroxidase activity in the infested plants compared to control plants. Peroxidase activity levels were similar between infested and control plants of the two highly susceptible buffalograss genotypes. Zoysiagrasses had lower peroxidase activity in general when compared to buffalograss control plants, and only 'Zorro' consistently showed higher peroxidase activity in the infested plants. Native gel electrophoresis analysis identified differences in the isozyme profiles of infested and control buffalograsses 'Prestige' and 196, and the zoysiagrass 'Zorro'. Results from this study 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.

Keywords Buffalograss · Chinch bug · Zoysiagrass · Oxidative enzymes · Plant resistance

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Introduction

Chinch bugs are serious pests of many cool- and warm-season turfgrasses. Recently, the chinch bug, *B. occiduus* Barber was identified as an important insect pest of buffalograss and zoysiagrass (Baxendale et al. 1999; Eickhoff et al. 2006). The reported host range of *B. occiduus* includes barley (*Hordeum* spp.), corn (*Zea mays* L.), oats (*Avena sativa* L.), sorghum [*Sorghum bicolor* (L.) Moench], wheat (*Triticum aestivum* L.), bromegrass (*Bromus* spp.), several

cool- and warm-season turfgrasses, and various native grasses (Ferris 1920; Parker 1920; Farstad and Staff 1951; Eickhoff et al. 2004). Currently, *B. occiduus* has been reported in Arizona, California, Colorado, Kansas, Montana, Nebraska, New Mexico, and Oklahoma in the United States, and in Alberta, British Columbia, Manitoba, and Saskatchewan Canada (Bird and Mitchner 1950; Slater 1964; Baxendale et al. 1999).

Management strategies for *B. occiduus* include the use of cultural practices to reduce thatch, proper fertilization, irrigation, use of resistant turfgrasses, and insecticides (Baxendale et al. 1999). Heng-Moss et al. (2003), Gulsen et al. (2004), and Eickhoff et al. (2006) 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. (2003) and Eickhoff et al. (2008) investigated the categories of buffalograss and zoysiagrass resistance to western chinch bug and identified the buffalograsses Prestige and Cody, and zoysiagrasses Emerald and Zorro as tolerant. Tolerance responses to insect feeding may include morphological traits, such as meristem sequestration and reactivation, or photoassimilate storage by the plant (Kessler and Baldwin 2002), as well as physiological or biochemical responses such as increased photosynthetic capacity, nutrient uptake, or oxidative enzyme activity. The identification of mechanisms 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.

Several researchers (Van Loon 1976; Castillo et al. 1984; Hildebrand et al. 1986; Zhang and Kirkham 1994; Felton et al. 1994a, b; Ni et al. 2001; Allison and Schultz 2004; Dowd et al. 2006; Heng-Moss et al. 2006) have suggested that peroxidases play an important role in a plant's response to abiotic and biotic stresses. These researchers suggest that increased peroxidase levels in specific plant compartments may enhance the plant's ability to tolerate insect feeding, and/or play a critical role in the plant's defense system.

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 (Hiraga et al. 2001; Allison and Schultz 2004; Passardi et al. 2005). Enzymes such as peroxidase reduce reactive oxygen species (ROS) accumulation and detoxify oxidation products when plants have been challenged with various stressors (Willekens et al. 1994; Schenk et al. 2000). It has been speculated that resistant genotypes may have the ability to increase peroxidase level activity, and thereby detoxify the radicals and peroxides, whereas, susceptible plants, are unable to detoxify those compounds (Hildebrand et al. 1986; Heng-Moss et al. 2004; Gutsche et al. 2009). This suggests that genotypes with a higher level of resistance would either have a higher up-regulation capacity for peroxidase or have a more sensitive up-regulation response or both.

Although prior research (Heng-Moss et al. 2004; Gulsen et al. 2007) provided some insights into the potential defensive role of peroxidases in Prestige buffalograss in response to chinch bug feeding, the extent of this response needs to be examined in other resistant turfgrasses. Therefore, the objectives of this study were: (1) to assess the relationships among protein content, basal peroxidase levels, chinch bug injury, and ploidy levels of chinch bug-resistant and -susceptible buffalograsses; (2) to compare peroxidase activity levels of resistant and susceptible buffalograsses and zoysiagrasses in response to chinch bug feeding; (3) and to analyze extracted proteins from chinch bug-resistant and -susceptible buffalograsses and zoysiagrasses by native gel electrophoresis to obtain information on the peroxidase profiles.

Materials and methods

Experiment #1: total protein content and basal peroxidase levels of 28 buffalograss genotypes

This study was conducted to document the relationship among total plant protein content, basal peroxidase levels, chinch bug injury, and ploidy level. Peroxidase levels of 28 buffalograss genotypes with varying levels of resistance to *B. occiduus* were evaluated in this study. Damage ratings and ploidy levels for these buffalograsses were evaluated (Table 1) by Gulsen et al. (2005).

Plant materials

Genotypes selected for this study represented both germplasm diversity in buffalograss and diversity in chinch bug feeding response. 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. The 28 genotypes included three commercial cultivars and 25 selections representing natural buffalograss populations collected from the North American Great Plains. Single plants cloned from each of the 28 genotypes were planted in 15 cm diameter by 15 cm deep pots with a soil mixture of 35% peat, 32% vermiculite, 9% soil, and 24% sand, and were maintained at 25 ± 1°C with metal halide supplemental lightning on a 16:8 h (L:D) photoperiod. Soil was saturated bi-weekly with a soluble fertilizer (21N–3.5P–

Table 1 Twenty-eight buffalograss genotypes with varying levels of chinch bug resistance and ploidy levels

Genotypes	Ploidy level[c]	Chinch bug injury[e]	Relative level of resistance[f]
184[a,b]	Hexaploid	1.5	HR
'Prestige'[a,b]	Tetraploid	1.6	HR
196[a]	Hexaploid	1.6	HR
PX3-5-1[a,b]	Triploid[d]	1.7	HR
240[b]	Hexaploid	2.2	MR
III-4-9[b]	Diploid	2.2	MR
193[b]	Hexaploid	2.3	MR
209[b]	Hexaploid	2.4	MR
170[b]	Hexaploid	2.5	MR
83[b]	Hexaploid	2.6	MR
203[b]	Hexaploid	2.6	MR
47[b]	Hexaploid	2.7	MR
III-6-6[b]	Diploid	2.7	MR
DP-47G[b]	Diploid	2.7	MR
II-6-6[b]	Diploid	3.0	MR
'Density'[b]	Diploid	3.0	MR
174[b]	Tetraploid	3.0	MR
45B[b]	Tetraploid	3.3	MS
132[b]	Tetraploid	3.4	MS
97[b]	Hexaploid	3.8	MS
95-55[b]	Hexaploid	3.8	MS
87A[b]	Pentaploid	3.8	MS
28[b]	Hexaploid	3.8	MS
'378'[a,b]	Pentaploid	3.9	MS
223A[b]	Hexaploid	3.9	MS
4A[b]	Hexaploid	4.2	HS
188[b]	Hexaploid	4.4	HS
119[ab]	Tetraploid	4.5	HS

[a] The genotypes assessed for alterations in total protein content, peroxidase kinetics, and native gel analyses in response to chinch bug infestation

[b] The genotypes used in correlation analysis among total protein content, ploidy level, basal peroxidase activity, chinch bug injury

[c] Ploidy levels were previously determined by Johnson et al. (2001)

[d] Ploidy level determined by Gulsen et al., unpublished

[e] Chinch bug resistance of 28 buffalograsses was evaluated by Gulsen et al. (2004) as follows: 1 = 10% or less damage, 2 = 11–30% damage, 3 = 31–50% damage, 51–70% leaf damage

[f] Relative level of resistance of the 28 genotypes was grouped as follows: highly resistance (HR) = 2 or less; moderately resistance (MR) = 2.1–3.0; moderately susceptible (MS) = 3.1–4.0; highly susceptible (HS) = 4.1–5.0

15K) solution at 200 mg l^{-1} nitrogen. Plants were grown in the absence of chinch bugs for this study. The experimental design was a completely randomized design with three replications.

Sample collection

The established buffalograsses were trimmed at 3 cm to ensure that plants were of a similar growth stage. The leaf blades, sheaths, and stems were collected 15 days after trimming for analysis of peroxidase activity. Harvested plant material was stored at −80°C until processing.

Preparation of samples

Soluble proteins were extracted from 20 mg of plant tissue, using a standard sap extraction method (modified from Heng-Moss et al. 2004). Plant tissues were placed between two rollers of a sap extraction apparatus (Ravenel Specialities Co., Seneca, SC). One and a half mL solution of 20 mM HEPES buffer at pH 7.2, containing a protease inhibitor cocktail [0.3 g/1 g of tissue of 4-(2-aminoethyl) benzenesulfonyl fluoride, bestatin, pepstatin A, E-64, leupeptin, 1,10-phenanthroline (Sigma, St. Louis, MO)] and 1% polyvinylpyrrolidone (PVP) was dropped on the top of the roller. The homogenate was collected from the bottom of the roller and centrifuged at 10,000×*g* for 10 min at 4°C. The supernatant was collected and placed at 4°C (<4 h) until analyzed.

Total protein measurement

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).

Peroxidase activity

Peroxidase activity was measured by determining the increase in absorbance at 470 nm for 2 min using a protocol modified from Hildebrand et al. (1986) and Heng-Moss et al. (2004). The enzymatic reaction was initiated by adding 2 µl of 30% hydrogen peroxide to wells of a 96-well microplate containing 60 µl of 18 mM guaiacol, 20 µl of 200 mM HEPES (pH 7.0), 117 µl of distilled water, and 1 µl of buffalograss extract. The specific activity of peroxidase was determined using the molar absorptivity of guaiacol at 470 nm (26.6×10^3 M^{-1} cm^{-1}). For each sample, peroxidase activity was measured four times using the microplate reader described above.

Statistical analysis

Correlations among total protein content, basal peroxidase activity levels, chinch bug injury, and ploidy levels were

analyzed using PROC CORR nested in SAS Version 8.0 (SAS Institute, Cary, NC).

Experiment #2: changes in protein content and peroxidase activity in response to chinch bug feeding

Three studies were conducted to investigate changes in total protein content and peroxidase activity levels in selected resistant and susceptible buffalograss and zoysiagrass germplasm to evaluate changes in response to chinch bug feeding. 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.

Plant materials

Studies 1 and 2 The buffalograss and zoysiagrass plants used were propagated using stolons/rhizomes and were grown in 'SC-10 Super Cell' single cell cone-tainers (3.8 cm diameter by 21 cm depth) (Stuewe & Sons, Inc. Corvallis, OR) containing a soil mixture of peat 35%: vermiculite 32%: soil 9%: sand 24%. Cone-tainers were placed in 7 by 14 cone-tainer trays (Stuewe & Sons, Inc. Corvallis, OR). Plants were irrigated as needed using water trays (35 cm by 50 cm) and fertilized every 2 weeks with a soluble fertilizer (21N–3.5P–15K) solution, containing 200 mg l^{-1} nitrogen. Plants were maintained in the greenhouse at 25 ± 1°C with metal halide supplemental lighting on a 16:8 h (L:D) photoperiod for 4 weeks before starting the study.

The previously described screening procedure developed by Heng-Moss et al. (2002) was used to evaluate plants. Grasses were trimmed to the soil surface 2 weeks prior to initiation of experiment to ensure a similar growth stage across each experiment.

Blissus occiduus were collected from buffalograss research plots at the University of Nebraska's John Seaton Anderson (JSA) Research Facility near Mead, Nebraska by vacuuming the soil surface with a modified ECHO Shred 'N Vac (Model #2400, ECHO Incorporated, Lake Zurich, IL). Chinch bugs were preconditioned by starving them under laboratory conditions (26 ± 3°C 16:8 photoperiod) for 24 h prior to initiation of the experiment.

The experimental design for each study was completely randomized with four replications. Treatments for study 1 were arranged in a 6 × 2 × 5 factorial (6 buffalograsses, infested and control plants, and 5 sampling dates). Treatments for study 2 were arranged in a 4 × 2 × 5 factorial (4 zoysiagrasses, infested and control plants, and 5 sampling dates). Plants were randomly designated to be a control plant or an infested plant. At the start of the experiment, 10 (fourth instar, fifth instar, or adult) chinch bugs were introduced onto the leaf blades of each designated infested plant. Chinch bugs were confined on the plants using tubular plexiglass cages (4 cm diameter by 30 cm height) with organdy fabric fastened by rubber bands at the top. Control plants were also caged for consistency. After chinch bug introduction, plants were maintained in the greenhouse until each respective sampling date.

Study 3 The buffalograss plants used in this study were propagated, maintained, and screened as previously described. All plants were trimmed to the soil surface prior to chinch bug introduction to ensure a similar growth stage of all plants.

The experimental design was completely randomized with three replications. Treatments were arranged in a 6 × 2 × 4 factorial (6 buffalograsses, infested and control plants, and four sampling dates). Plants were randomly designated a control plant or an infested plant. Chinch bugs for this study were collected with an aspirator from pots of buffalograss maintained in the greenhouse. At the start of the experiment, 10 (fourth instar, fifth instar, or adult) chinch bugs were introduced onto the leaf blades of each designated infested plant. Chinch bugs were confined on the plants as previously described.

Sample collection

Studies 1 and 2 Buffalograss and zoysiagrass samples consisting of leaf blades and lower leaf sheaths were collected for protein analyses. Tissues were collected at 3, 6, 9, 12, and 15 days after introduction (DAI) of chinch bugs. Before collecting the plant material for protein analyses, chinch bugs were removed and chinch bug damage ratings were performed, using a 1–5 visual scale with 1 = 0–10%, 2 = 11–30%, 3 = 31–50%, 4 = 51–70%; and 5 = >70% damage and plant close to death (Heng-Moss et al. 2002). Plant material was frozen in liquid nitrogen and stored at −80°C.

Study 3 Grass samples consisting of leaf blades and lower leaf sheaths were collected as previously described for protein analyses. Tissues were collected at 7, 14, 21, and 28 DAI.

Preparation of samples

Studies 1, 2, and 3 Plant extraction methods and total plant protein content and peroxidase activity measurements were

carried out as described for the 28 buffalograss protein profile study. Buffalograss and zoysiagrass plant proteins were also analyzed by native gel electrophoresis to obtain information on peroxidase isozyme patterns as described by Heng-Moss et al. (2004). The samples were analyzed for peroxidase profiles by native gel electrophoresis on a Bio-Rad Criterion gel apparatus (Bio-Rad, Richmond, CA) using precast 18-well 7.5% polyacrylamide gels (Bio-Rad, Richmond, CA) as described previously (Heng-Moss et al. 2004). Twenty-five micrograms of protein, as determined by the BCA protein assay (Pierce, Rockford, IL), were loaded in each lane. Samples were diluted 1:1 with a gel loading buffer consisting of 62.5 mM Tris–HCl (pH 6.8), 40% glycerol, and 0.01% bromophenol blue prior to loading. Gels were electrophoresed at 120 V for 1.5 h at 4°C.

Isozyme profiles for peroxidase activity were visualized using histochemical methods. All 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 Vallejos (1983). In our procedure, gels were soaked at room temperature for 10 min in 40 ml of 50 mM sodium acetate buffer (pH 5.0). After this initial incubation period, 10 mg of 4-chloronapthanol (dissolved in 0.5 ml of methanol) and 20 µl of 30% hydrogen peroxide were added to the buffer. Zones of peroxidase activity appeared as dark blue bands after approximately 10–15 min.

Statistical analysis

Total protein content and peroxidase activity values were analyzed using SAS Version 8.0 Mixed model analysis (SAS Institute 2002, Cary, NC) to detect differences in total protein content and peroxidase activity levels among treatments. Means were separated using Fisher's least significant difference.

Results and discussion

Experiment 1: total protein content and basal peroxidase levels of 28 buffalograss genotypes

A significant correlation was detected between chinch bug injury and total protein content ($r = 0.47$, $P < 0.01$) (data not shown). The susceptible genotypes had higher protein content than the resistant genotypes. Heng-Moss et al. (2004) found higher protein levels in the susceptible genotype 378 than the resistant genotype Prestige, which was consistent with our findings. Although a significant correlation ($r = 0.47$) was detected, this value was low, which suggests that total protein 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 ($r = 0.2$, $P = 0.3$) between total protein content and ploidy level (data not shown).

Peroxidase activity was also assessed for the 28 genotypes. No significant correlations ($r = 0.19$, $P = 0.29$) were found between basal peroxidase levels and chinch bug injury (data not shown), or between basal peroxidase levels and ploidy level ($r = -0.24$, $P = 0.22$) (data not shown). This suggests that basal peroxidase levels in buffalograss genotypes are not a useful indicator of chinch bug resistance.

Experiment 2: changes in protein content and peroxidase activity in response to chinch bug feeding

Chinch bug damage

Studies 1 and 2 No visible chinch bug damage was observed on any of the buffalograsses or zoysiagrasses at 3 or 6 DAI. All grasses showed varying degrees of chinch bug damage by 15 DAI. Mean damage ratings ± SE at 15 DAI were as follows: buffalograsses: Prestige = 1.2 ± 0.2, 378 = 2.4 ± 0.8, PX3-5-1 = 1.0 ± 0, 119 = 2.2 ± 0.9, 184 = 1.5 ± 0.3, and 196 = 1.4 ± 0.3; zoysiagrasses: Zorro = 1.3 ± 0.2, Emerald = 1.1 ± 0.1, El Toro = 2.0 ± 0, and Meyer 2.3 ± 0.2. The results for buffalograss were consistent with previous findings reported by Heng-Moss et al. (2002) and Gulsen et al. (2004).

Study 3 The six buffalograss genotypes infested with chinch bugs showed no visible chinch bug damage at 7 DAI. The two susceptible genotypes, 378 and 119, had damage ratings of 1.5 and 1.4, respectively, at 14 DAI. All genotypes showed varying degree of chinch bug damage at 28 DAI: Prestige = 1.3 ± 0.4, 378 = 2.0 ± 0.3, PX3-5-1 = 1.2 ± 0.7, 119 = 3.8 ± 0.6, 184 = 1.3 ± 0.4, 196 = 1.5 ± 0.2. These results compared favorably to earlier findings (Gulsen et al. 2007; Heng-Moss et al. 2002).

Total protein

Study 1 A genotype × treatment × sampling date interaction was detected for total protein content ($P = 0.0001$) among the buffalograsses evaluated (data not shown). The infested resistant buffalograsses PX3-5-1, 184, 196, and Prestige had higher protein contents than uninfested control plants except for PX3-5-1 at day 9 and 15, 184 at day 3, 12, and 15, 196 at day 3 and 12, and Prestige at day 3 and 9. The infested susceptible buffalograsses 378 and 119 had lower total protein contents than the control plants at all harvest dates except day 15 for '378' and day 3 for 119.

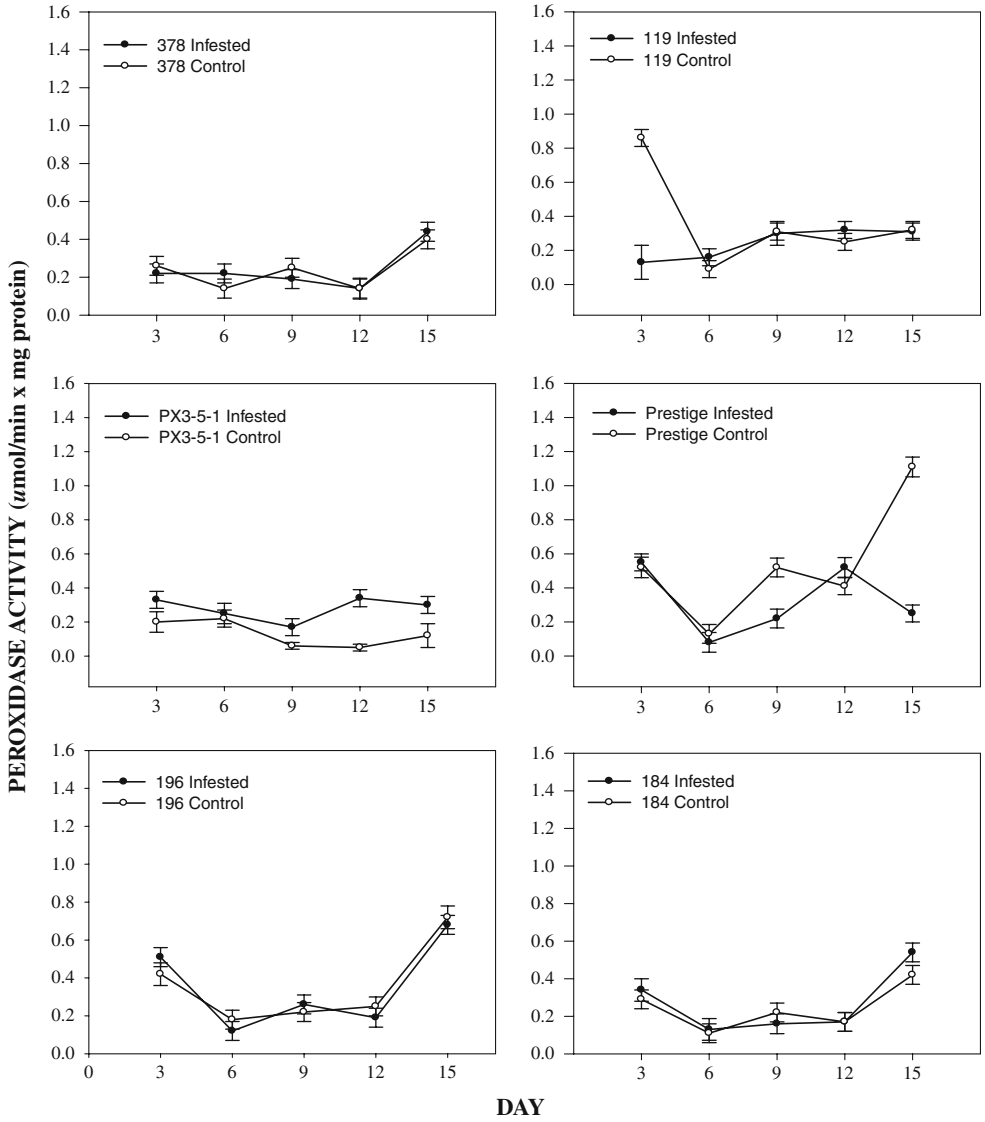

Fig. 1 Peroxidase (μmol/min × mg protein) of resistant and susceptible buffalograsses at 3, 6, 9, 12, and 15 days after chinch bug introduction

While significant differences were detected in total protein content among the buffalograsses, there were no consistent trends between infested and control plants. These results were also consistent with the findings of Heng-Moss et al. (2004) and Gulsen et al. (2007).

Study 2 A genotype × treatment × sampling date interaction was also detected for total protein content ($P = 0.0001$) among the zoysiagrasses evaluated (data not shown). The control plants of El Toro, Emerald, and Meyer had lower total protein contents than infested plants at all times except for El Toro on day 6, and Emerald on day 3. The infested Zorro plants had higher total protein contents at all times except for day 6. No consistent trends were evident in total protein content in the resistant and susceptible zoysiagrasses.

Study 3 A genotype × treatment interaction were detected for total protein content ($P = 0.02$) (data not shown). Infested Prestige plants had significantly higher total protein contents at day 7, 14, and 21, while control plants of PX3-5-1, 119, and 196 at day 7, and 184 at day 14 had significantly higher total protein contents than infested plants. Heng-Moss et al. (2004) found similar changes in total protein content in Prestige, which is consistent with the results reported here.

In summary, 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 A significant genotype × treatment × sampling date interaction was detected for peroxidase activity (df = 20, 542; $F = 3.97$; $P < 0.0001$) among the evaluated buffalograsses in response to *B. occiduus* feeding. The chinch bug-resistant buffalograss PX3-5-1 had higher levels of peroxidase activity (Fig. 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 A result similar to study 1 was observed in zoysiagrass' response to chinch bugs. A significant genotype × treatment × sampling date interaction was detected for peroxidase activity (df = 12, 354; $F = 4.6$; $P < 0.0001$) among the zoysiagrasses in response to *B. occiduus* feeding. El Toro control plants had significantly higher levels of peroxidase activity (Fig. 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 (Fig. 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 Significant genotype × treatment (df = 5, 94; $F = 4.15$; $P = < 0.0001$), sampling date × genotypes (df = 15, 94; $F = 11.63$; $P = < 0.0001$), and sampling

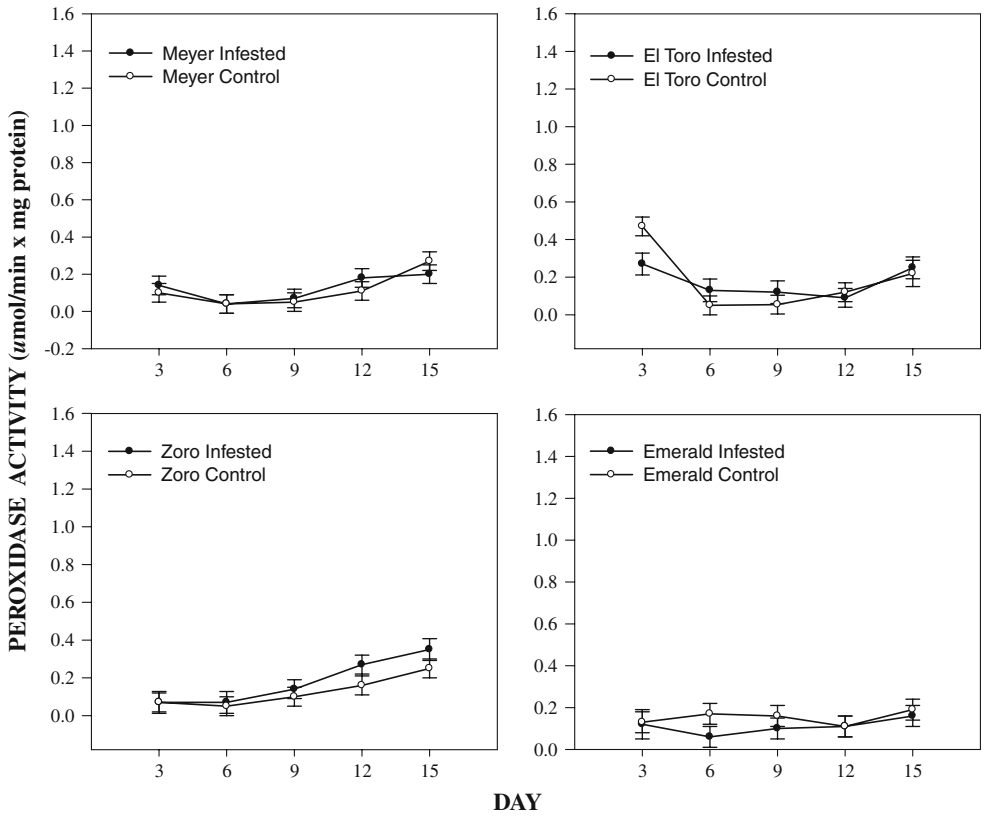

Fig. 2 Peroxidase activity (μmol/min × mg protein) of resistant and susceptible zoysiagrasses at 3, 6, 9, 12, and 15 days after chinch bug introduction

date × treatment (df = 3, 94; $F = 7.27$; $P = < 0.0001$) interactions were detected for peroxidase activity in response to chinch bug feeding. Overall, all infested buffalograss genotypes showed higher peroxidase activity levels when compared to control plants (Fig. 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 (Fig. 4).

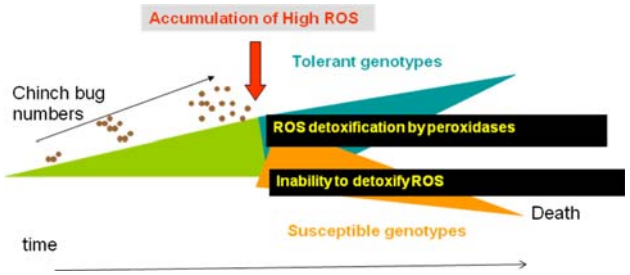

Fig. 4 Proposed role of peroxidases in the defense response of resistant buffalograsses to the western chinch bug. For both susceptible and resistant buffalograsses, chinch bug feeding results in increased levels of reactive oxygen species (ROS). Resistant buffalograsses have the ability to increase peroxidase, and thereby detoxify the radicals and peroxides, whereas, susceptible plants, are unable to detoxify these compounds

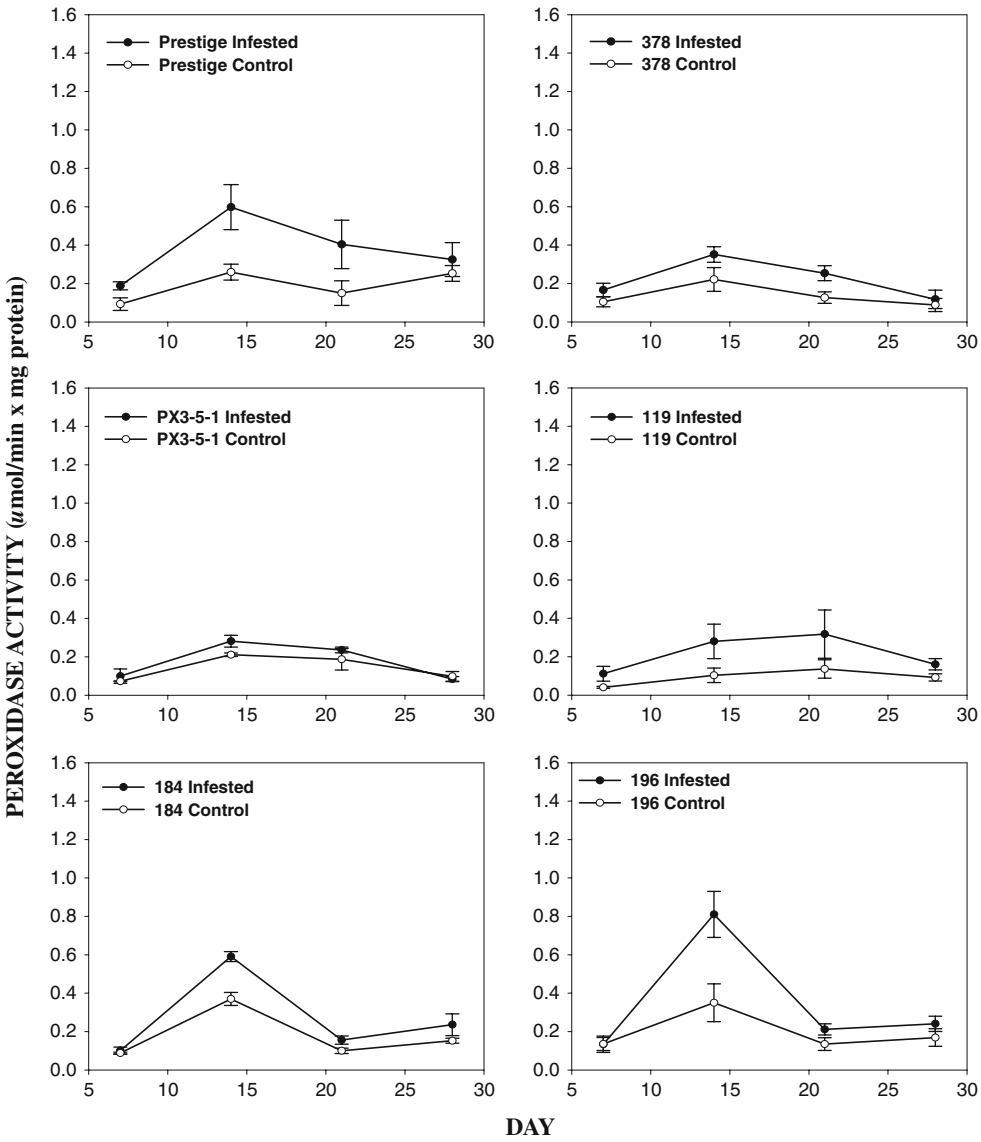

Fig. 3 Peroxidase activity (µmol/min × mg protein) of resistant and susceptible buffalograsses at 7, 14, 21, and 28 days after chinch bug introduction

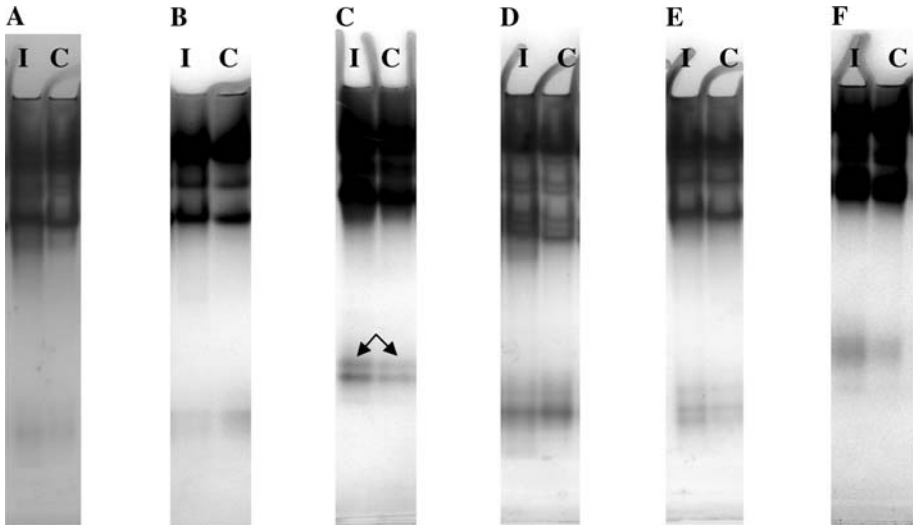

Fig. 5 Native gels stained for peroxidase activity among resistant and susceptible buffalograsses at 15 days after chinch bug introduction. *I* infested, *C* control. *A* '378', *B* 119, *C* 'Prestige', *D* 184, *E* 196, *F* PX3-5-1. *Arrows* indicate notable differences in levels of peroxidase activity

Similar trends in peroxidase activity were observed in both short-term and long-term studies in the buffalograsses 119, 378, and Prestige. In all studies, peroxidase activity levels were elevated in Prestige, which supports the finding of Heng-Moss et al. (2004) 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 buffalograsses 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.

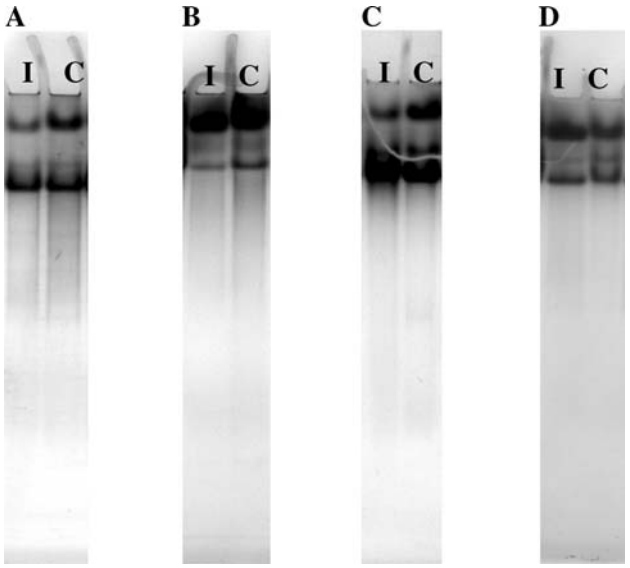

Fig. 6 Native gels stained for peroxidase activity among resistant and susceptible zoysiagrasses 15 days after chinch bug introduction. *I* infested, *C* control, *A* 'Meyer', *B* 'El Toro', *C* 'Emerald', *D* 'Zorro'. No notable differences in levels of peroxidase activity were detected

Native gel analyses

Study 1 Analysis of native gels stained for peroxidase activity displayed visual differences in the peroxidase expression levels among the six buffalograsses. Differences were also observed among the control plants for the buffalograsses reflecting genetic variability among genotypes. Native gels (Fig. 5) displayed visual differences in peroxidase expression levels between infested and control plants in 378, 119, 184, 196, and PX3-5-1. These reflect the activities measured in experiment 1 (Fig. 1). As expected, differences in the peroxidase profiles of infested and control Prestige were identified (indicated by arrows). These results support the findings of Heng-Moss et al. (2004) who detected elevated levels of peroxidase activity in Prestige in response to *B. occiduus* 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.

Study 2 Analysis of native gels stained for peroxidase activity displayed few visual differences in the expression levels of peroxidase among the four zoysiagrasses (Fig. 6). 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 (Fig. 5).

Study 3 Native gel analysis of peroxidases showed differences in the expression levels of peroxidases between infested and control plants among and within genotypes (Figs. 7, 8). Consistent with the peroxidase activity described above, expression levels on native gels were comparable between infested and control plants. As with the peroxidase activity levels (Fig. 3), differences in the band intensity on the native gel profiles were more notable at 14 DAI, especially in Prestige and 196 (Fig. 7). Differences in band intensities between infested and control plants decreased at 21 and 28 DAI (Fig. 8). Reduced peroxidase activity at 21 and 28 DAI was likely associated with increasing chinch bug feeding in infested plants of all six genotypes.

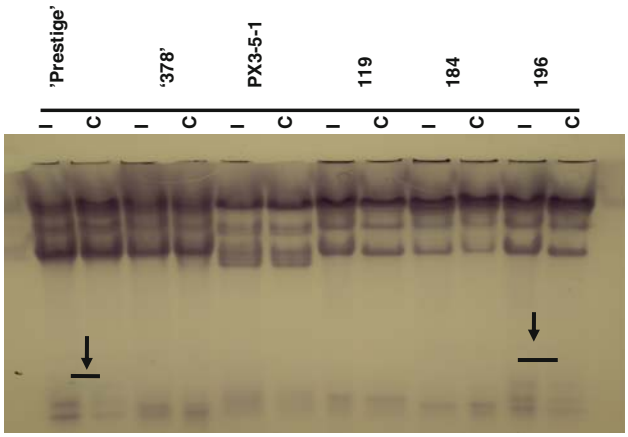

Fig. 7 Native gels stained for peroxidase activity among resistant and susceptible buffalograsses at 14 days after chinch bug introduction. *I* infested, *C* control. *Arrows* indicate notable differences in levels of peroxidase activity

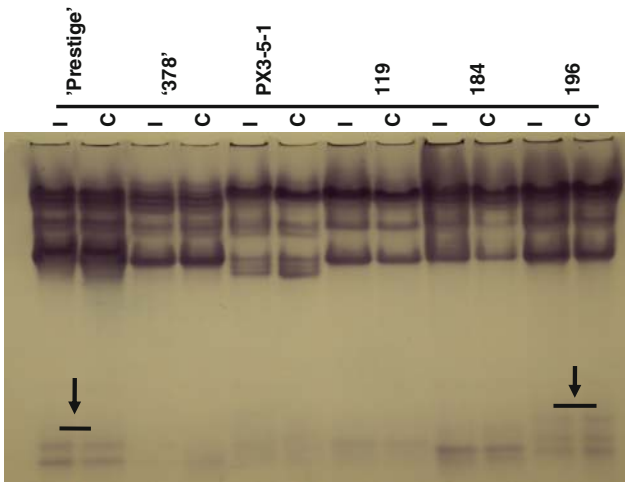

Fig. 8 Native gels stained for peroxidase activity among resistant and susceptible buffalograsses at 21 days after chinch bug introduction. *I* infested, *C* control

Conclusions

Peroxidases serve an important role in the defense response of many plants to biotic and abiotic stresses (Mavelli et al. 1982; Passardi et al. 2005; Welindere et al. 2002). 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 (Passardi et al. 2005; Gutsche et al. 2009). 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.

This research 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 *B. occiduus* feeding despite being categorized as tolerant (Eickhoff et al. 2008). These results suggest alternate mechanisms of resistance may be present in these grasses.

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