2015

Transcriptional Profiling of Resistant and Susceptible Buffalograsses in Response to *Blissus occiduus* (Hemiptera: Blissidae) Feeding

Crystal M Ramm
University of Nebraska-Lincoln

Michael Wachholtz
University of Nebraska at Omaha

Keenan Amunsen
University of Nebraska-Lincoln

Teresa J. Donze
University of Nebraska-Lincoln, teresad11@hotmail.com

Tiffany Heng-Moss
University of Nebraska-Lincoln, thengmoss2@unl.edu

See next page for additional authors

Authors
Crystal M Ramm, Michael Wachholtz, Keenan Amunsen, Teresa J. Donze, Tiffany Heng-Moss, Paul Twigg, Nathan A. Palmer, Gautam Sarath, and Frederick P. Baxendale

Transcriptional Profiling of Resistant and Susceptible Buffalograsses in Response to *Blissus occiduus* (Hemiptera: Blissidae) Feeding

CRYSTAL RAMM,[1] MICHAEL WACHHOLTZ,[2] KEENAN AMUNDSEN,[3] TERESA DONZE,[1] TIFFANY HENG-MOSS,[1,4] PAUL TWIGG,[5] NATHAN A. PALMER,[6] GAUTAM SARATH,[6] AND FRED BAXENDALE[1]

J. Econ. Entomol. 108(3): 1354–1362 (2015); DOI: 10.1093/jee/tov067

ABSTRACT Understanding plant resistance mechanisms at a molecular level would provide valuable insights into the biological pathways impacted by insect feeding, and help explain specific plant tolerance mechanisms. As a first step in this process, we conducted next-generation sequencing using RNA extracted from chinch bug-tolerant and -susceptible buffalograss genotypes at 7 and 14 d after chinch bug feeding. Sequence descriptions and gene ontology terms were assigned to 1,701 differentially expressed genes. Defense-related transcripts were differentially expressed within the chinch bug-tolerant buffalograss, Prestige, and susceptible buffalograss, 378. Interestingly, four peroxidase transcripts had higher basal expression in tolerant control plants compared with susceptible control plants. Defense-related transcripts, including two peroxidase genes, two catalase genes, several cytochrome P450 transcripts, a glutathione s-transferase, and a WRKY gene were upregulated within the Prestige transcriptome in response to chinch bug feeding. The majority of observed transcripts with oxidoreductase activity, including nine peroxidase genes and a catalase gene, were downregulated in 378 in response to initial chinch bug feeding. The observed difference in transcript expression between these two buffalograss genotypes provides insight into the mechanism(s) of resistance, specifically buffalograss tolerance to chinch bug feeding.

KEY WORDS buffalograss, chinch bug, transcriptome, peroxidase, catalase

Buffalograss, *Buchloë dactyloides* (Nuttall) Engelmann, is a warm-season grass native to the North American Great Plains (Wenger 1943, Pozarnsky 1983). Buffalograss has gained popularity as an alternative turfgrass species for golf courses, home lawns, and public establishments because of its low maintenance requirements and relative freedom from diseases and arthropod pests (Pozarnsky 1983, Wu and Harivandi 1989, Riordan et al. 1996). Although relatively disease- and pest-free, the western chinch bug, *Blissus occiduus* (Barber) is an important insect pest of buffalograss (Baxendale et al. 1999). The continued use of this warm-season turfgrass is dependent on the development and implementation of effective management strategies for chinch bugs and other buffalograss-infesting arthropods.

Buffalograss resistance to chinch bugs, when employed in an integrated pest management program, has the potential to effectively and economically reduce chinch bug infestations, while minimizing pesticide inputs, costs, and maintenance effort. Previous research has identified buffalograsses with resistance to the western chinch bug (Heng-Moss et al. 2002, Gulsen et al. 2004, Serba et al. 2011). Of the identified resistant buffalograss cultivars, 'Prestige' exhibits the highest level of resistance even though it often became heavily infested with chinch bugs. Several buffalograsses, including the cultivar '378', have been identified as highly susceptible to the western chinch bug. Subsequent choice and no-choice studies characterized Prestige as tolerant to the western chinch bug (Heng-Moss et al. 2003).

Although resistant buffalograsses have been identified, limited information is available on the resistance (tolerance) mechanisms and the genes associated with plant tolerance to chinch bugs. Plant defense to phloem-feeding insects typically involves hundreds of genes responding not only to cellular disruption by the insect's piercing–sucking mouthparts but also to salivary toxins released during feeding (Miles 1999, Smith et al. 2007). The intricate relationship that phloem-feeding

[1] Department of Entomology, University of Nebraska, Lincoln, NE 68583.
[2] Department of Biology, University of Nebraska, Omaha, NE 68182.
[3] Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583.
[4] Corresponding author, e-mail: thengmoss2@unl.edu.
[5] Department of Biology, University of Nebraska, Kearney, NE 68449.
[6] Grain, Forage and Bioenergy Research Unit, USDA-ARS & Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68583.

insects share with their host plant may elicit multiple signaling pathways leading to general and specific defense-related responses (Thompson and Goggin 2006, Smith et al. 2007). These plant-signaling pathways are most commonly mediated by a number of different compounds including jasmonic acid, salicylic acid, and ethylene (Walling 2000, Thompson and Goggin 2006). Prior research has documented differential expression of transcripts between resistant and susceptible plants involved in oxidative burst, plant defense and signaling, photosynthesis, and cell maintenance in response to phloem-feeding insects (Zhang et al. 2004, Park et al. 2005, Botha et al. 2006, Boyko et al. 2006, Gutsche et al. 2009a). Understanding tolerance mechanisms at a molecular level will provide insights into the biological pathways impacted by chinch bug feeding and help illuminate plant tolerance mechanisms.

The transcriptome of two buffalograss cultivars, Prestige and 378, were recently sequenced using both Illumina and 454 sequencing platforms. These sequences revealed 325 differentially expressed (DE) genes between the two genotypes under basal conditions (not challenged with chinch bugs; Wachholtz et al. 2013). Investigating the differential gene expression between these two buffalograss genotypes in response to chinch bug feeding may provide insights into the mechanism(s) of tolerance. Accordingly, the objectives of this study were to examine the transcriptional profiles of Prestige and 378 in response to chinch bug feeding and identify specific DE transcripts between tolerant (Prestige) and susceptible (378) cultivars of buffalograss in response to chinch bug feeding.

Materials and Methods

Buffalograss sod plugs (10.6 cm diameter by 8 cm deep) of Prestige and 378 were collected from the John Seaton Anderson Turfgrass and Ornamental Research Facility (JSA Research Facility), University of Nebraska Agricultural Research and Development Center, near Mead, NE. Individual stolons of each genotype were planted in "SC-10 Super Cell" single-cell 3.8 cm diameter by 21 cm deep cone-tainers (Stuewe & Sons, Inc., Corvallis, OR) in a soil mixture with a ratio of 2:1:3:3 sand, soil, peat, and perlite. Plants were watered and fertilized (20N-10P-20K soluble) as needed and maintained at a temperature of 24 ± 3°C and a photoperiod of 16:8 (L:D) h under 400-watt high-intensity discharge lamps.

Chinch bugs were collected from buffalograss research plots at the JSA Research Facility by vacuuming the soil surface with a modified ECHO Shred N' Vac (Model 2400, ECHO Incorporated, Lake Zurich, IL). Chinch bugs were sifted through a 2-mm mesh screen, and fifth instars were collected with an aspirator. Chinch bugs were held in the laboratory for 24 h, and injured or dead insects were discarded prior to initiating the experiment.

The treatment design was arranged as a $2 \times 2 \times 2$ factorial with 2 buffalograss genotypes (Prestige and 378), 2 chinch bug infestation levels (control and infested), and 2 sampling dates (7 and 14 d after chinch bug introduction). Ten fifth-instar chinch bugs were placed on buffalograss plants randomly assigned to the chinch bug-infested treatment. Plants were covered with tubular Plexiglas cages (4 cm diameter by 30 cm height) to confine chinch bugs to the plants. Organdy fabric was secured to the tops of cages using rubber bands. Control plants were caged in the same manner. The experimental plants were arranged in a randomized complete block design with six replications. Chinch bug presence and associated plant damage ratings (1–5 scale, where 1 = 10% or less of the leaf area damaged) were recorded at each evaluation date following the methods described by Heng-Moss et al. (2002). Damage rating and chinch bug numbers were analyzed using a mixed model (PROC MIXED, SAS Institute 2008) to detect differences between the two buffalograsses. Means were separated using Fisher least significant difference (LSD) procedure when appropriate ($P < 0.05$). Chinch bug-infested and control plant tissue (leaf blades) was harvested and flash frozen in liquid nitrogen for RNA extraction.

Total RNA was extracted from 100 mg of plant leaf tissue from three biological replicates from each genotype and treatment. RNA was extracted using the FastTrackMAG maxi kit (Invitrogen #K158002), and cDNA was created using the QuantiTect Whole Transcriptome kit (Qiagen #207043). The cDNA was purified using the QiAamp DNA Blood mini kit (Qiagen #51104). Samples were submitted for sequencing on the Illumina GA sequencing platform and the 454 Titanium FLX sequencer.

Separate transcriptomes were assembled for each genotype using Trinity software (Grabherr et al. 2011). Sequencing reads for each individual sample were mapped to the Prestige-assembled transcriptome with Bowtie2 (Langmead and Salzberg 2012). Comparisons between control plants of the two genotypes and control and infested samples for each genotype at 7 and 14 d post infestation were made with the edgeR Bioconductor package (Robinson et al. 2010) to identify DE transcripts (Table 1). Differentially expressed genes determined by their false discovery rate (FDR < 0.05) were annotated with Blast2GO (Conesa et al. 2005). Buffalograss transcripts of interest were selected from the list of DE transcripts at each time point and for each genotype by filtering the Blast2GO "seq descriptions" and "GO IDs" using the following search terms: oxidase, oxide, catalase, GRAS, WRKY, kinase, cytochrome, defense, and stress.

Results

Damage Ratings. No visible differences ($P > 0.05$) in plant damage were observed between chinch bug-infested 378 and Prestige plants at 7 d after chinch bug introduction. By 14 d, 378 had an average damage rating (±SE) of 2.1 ± 0.3, while the average damage rating for Prestige plants was 1.0 ± 0 ($F = 4.7$; df = 1, 20; $P < 0.001$). No significant differences ($P > 0.05$) were detected between the number of chinch bugs on the two infested buffalograsses at 7 and 14 d after chinch bug introduction.

Table 1. Summary of sequencing and read mapping to the assembled Prestige transcriptome

		Avg total reads	Avg no. of reads mapped to the Prestige transcriptome	Avg total alignment (%)	Avg unaligned reads	Unaligned (%)
Day 7	Prestige Control	29039190.33	23788288	81.92	5250902.333	18.08
	Prestige Infested	27713341	21327254	76.965	6386087	23.04
	378 Control	28557695	24275724.67	85.015	4281970.333	14.99
	378 Infested	27410279.67	23405269.67	85.39	4005010	14.61
Day 14	Prestige Control	24504643.33	20326400	82.95	4178243.333	17.05
	Prestige Infested	26407683	21966082.67	83.18	4441600.333	16.82
	378 Control	29062279.33	24160179.67	83.13	4902099.667	16.87
	378 Infested	20998904	15865918	75.56	5132986	24.44

Levels of Transcript Expression: Control Versus Infested. At 7 d, Prestige showed 362 upregulated transcripts and 1,186 downregulated transcripts in response to chinch bug feeding. By 14 d, chinch bug-infested Prestige plants exhibited a reduction in the overall number of DE transcripts, with 129 upregulated and 515 downregulated transcripts. The susceptible genotype 378 had 104 upregulated transcripts and 776 downregulated transcripts in response to chinch bug feeding at 7 d. By 14 d, 378 exhibited 108 upregulated transcripts and 29 downregulated transcripts in response to chinch bug feeding (Table 2). Of the 2,192 DE Prestige transcripts, 310 were present at both 7 d and 14 d post infestation. For 378, 11 DE transcripts of the 1,017 were expressed at both time points.

Gene Ontology. Sequence descriptions and gene ontology terms were assigned to 1,032 of the 1,882 unique DE Prestige transcripts and to 669 of the 1,006 DE transcripts from 378. After filtering for defense-related terms of interest (oxidase, oxide, catalase, GRAS, WRKY, kinase, cytochrome, defense, and stress), 112 DE transcripts from 378 and 166 DE transcripts from Prestige were selected for further analysis in BLAST2GO. The distribution of transcripts in each GO term did not change significantly between genotypes. However, the analysis of the select set of stress response search terms revealed more DE transcripts that were downregulated than upregulated at 7 and 14 d in both cultivars, with Prestige having more downregulated transcripts than 378 (Fig. 1).

Genes of Interest.

Control Versus Control (378 vs. Prestige). At 7d, there were 7,665 DE transcripts between 378 and Prestige control plants. Of those 7,665 transcripts, 6,566 transcripts had higher read counts in Prestige. Among these 7-d DE transcripts, four peroxidase transcripts had higher expression levels in Prestige control plants when compared with 378 control plants (Table 3). At 14 d, 9,753 transcripts were DE between 378 and Prestige control plants. Of those 9,753 transcripts, 8,495 transcripts had higher read counts in Prestige. Among these 14-d DE transcripts, three peroxidase transcripts had higher expression levels in Prestige control plants when compared with 378 control plants (Table 3). It is interesting to note the difference in glutathione peroxidase expression between Prestige and 378. Of the four DE glutathione peroxidases, Prestige had a minimum Log2 fold expression change of 7.9 when compared with 378.

Table 2. Log2 fold expression change number of sequences (control vs. chinch bug-infested at FDR < 0.05)

Log2 fold expression change	Day 7 Prestige	Day 7 378	Day 14 Prestige	Day 14 378
1–2	6	1	2	1
2–3	5	3	6	4
3–4	9	1	4	5
4–5	13	1	3	13
5–6	8	6	8	14
6–7	13	1	5	9
<7	308	91	101	62
(−1)–(−2)[a]	5	2	2	0
(−2)–(−3)	9	43	2	0
(−3)–(−4)	10	66	7	0
(−4)–(−5)	28	89	10	0
(−5)–(−6)	102	87	20	0
(−6)–(−7)	131	51	30	0
>(−7)	901	438	444	29

[a]The negative Log2 fold expression change indicates downregulation.

Prestige: Control Versus Infested. There were 1,548 DE transcripts between control and infested Prestige plants at 7 d and 644 DE transcripts at 14 d. Among these DE transcripts, 20 defense-related transcripts were identified (Table 4). Of these select transcripts, three peroxidase and two catalase transcripts were upregulated in response to chinch bug feeding (Table 4). Several other defense-related genes were upregulated in chinch bug-infested plants, including five cytochrome P450 genes, a glutathione s-transferase and a WRKY gene (Table 4).

378: Control Versus Infested. There were 880 and 137 DE transcripts between control and infested 378 plants at 7 d and 14 d, respectively. In this chinch bug-susceptible buffalograss cultivar, nine peroxidase genes were downregulated at 7 d in response to chinch bug feeding (Table 5). In addition to these peroxidases, a catalase transcript and a scarecrow-like protein transcript were downregulated in 378 (Table 5). None of these transcripts were DE between control and infested plants at 14 d.

Discussion

Several defense-related transcripts were DE within the Prestige and 378 buffalograss transcriptomes. Comparison of data from control and chinch bug-infested plants, and subsequent comparison of tolerant and susceptible genotypes, allowed for the identification of

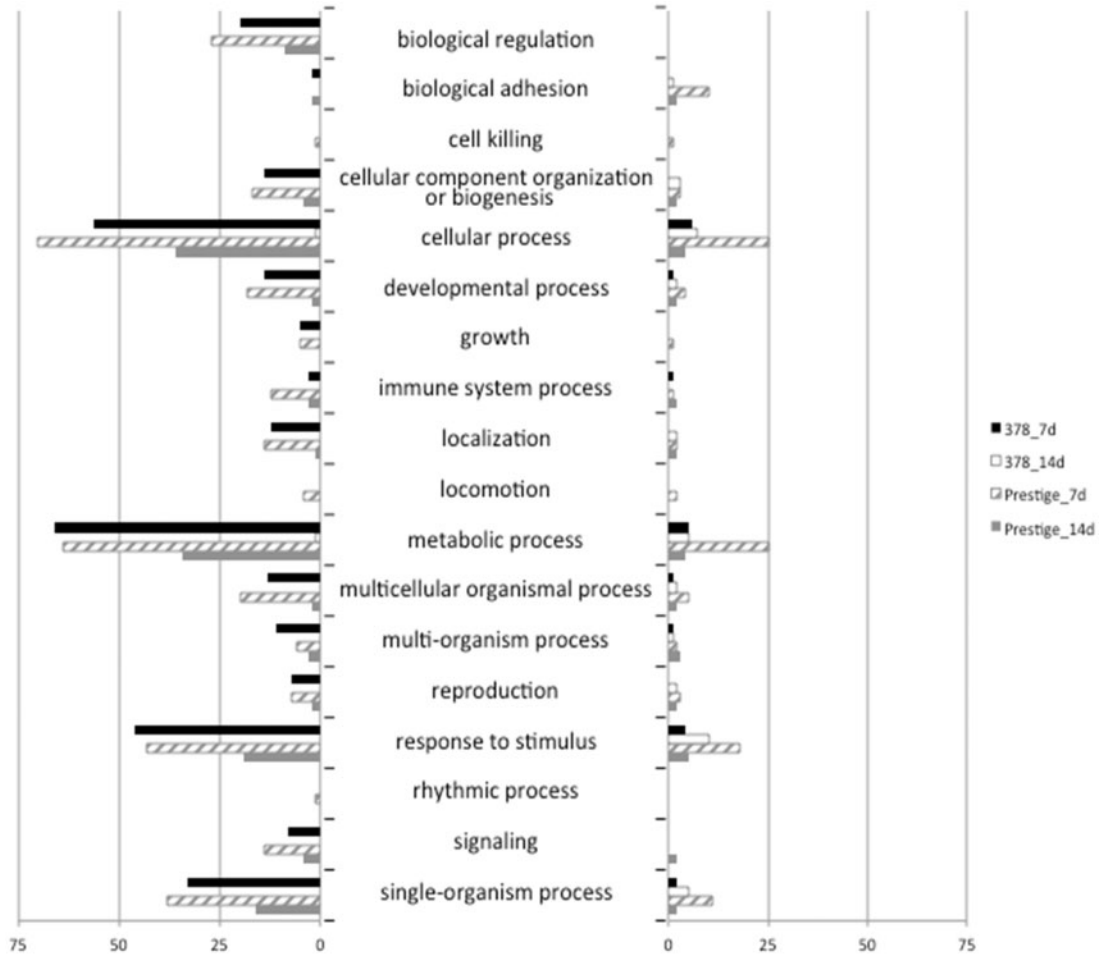

Fig. 1. Summary of level 2 biological process GO terms. The left panel shows the number of GO terms for transcripts that are downregulated in response to chinch bug feeding, while the right panel shows the number of GO terms for upregulated transcripts.

Table 3. Significantly expressed defense-related sequences (Prestige control vs. 378 control)

	Transcript	Best-hit description	7 d Log2 fold change	FDR	14 d Log2 fold change	FDR
1	pre_comp846520_c0_seq1	Glutathione peroxidase	9.072528149	0.007142497	–	–
2	pre_comp972845_c0_seq1	Glutathione peroxidase	8.340054052	0.043060119	–	–
3	pre_comp767937_c0_seq1	Glutathione peroxidase	8.227115658	0.047955888	–	–
4	pre_comp612889_c0_seq1	Phospholipid hydroperoxide glutathione peroxidase	7.911875325	0.015833603	–	–
5	pre_comp42361_c0_seq1	Glutathione peroxidase			11.2825293	0.004507019
6	pre_comp25238_c0_seq1	Peroxidase 27			8.325406364	0.019913684
7	pre_comp36874_c0_seq2	Peroxidase 72-like			4.356180153	0.017905058
8	pre_comp38446_c0_seq3	Peroxidase 12-like			−1.734206586	0.018268232
9	pre_comp37649_c0_seq2	Peroxidase 1 precursor	−1.879710127	0.032908626	−2.180875856	0.00860138
10	pre_comp35227_c0_seq1	Class iii peroxidase			−2.85412216	0.045179059

FDR < 0.05. Positive Log2 fold change values demonstrate higher transcript expression in Prestige (tolerant) control plants while negative Log2 fold change values demonstrate higher transcript expression in 378 (susceptible) control plants.

DE genes between control plants of the two genotypes and expression level changes in both the tolerant and susceptible buffalograsses in response to chinch bug feeding. Many of the changes documented in this study support those found in other studies investigating tolerant Prestige and susceptible 378 buffalograsses (Heng-Moss et al. 2004, Gulsen 2010, Ramm et al. 2013).

In total, seven peroxidases had higher expression in Prestige control plants than 378 control plants

Table 4. Buffalograss Prestige: significantly expressed defense-related sequences (control vs. chinch bug-infested)

	Transcript	Best-hit description	7 d Log2 fold change	FDR	14 d Log2 fold change	FDR
1	pre_comp6712_c0_seq1	Cytochrome p450-like tbp	11.17735797	0.010792971	–	–
2	pre_comp447775_c0_seq1	Glucose dehydrogenase	11.12909036	0.038277163	–	–
3	pre_comp220676_c0_seq1	Glutathione s-transferase	10.80935801	0.022094481	–	–
4	pre_comp28074_c0_seq1	Wrky74—superfamily of tfs having wrky and zinc finger domains	10.29827632	0.047770669	–	–
5	pre_comp630507_c0_seq1	Catalase a	10.04711188	0.02609458	–	–
6	pre_comp161847_c0_seq1	Glutathione s-transferase	−5.786195637	0.036669151	–	–
7	pre_comp324268_c0_seq1	Superoxide dismutase	−6.633253064	0.008654515	–	–
8	pre_comp34325_c0_seq1	Leucoanthocyanidin reductase	–	–	11.65849247	0.019631654
9	pre_comp27996_c0_seq1	Peroxidase 72	–	–	5.384801462	0.036419416
10	pre_comp2732_c0_seq1	Cytochrome p450	–	–	−3.533954564	0.022785525
11	pre_comp38948_c0_seq4	Cytochrome p450	–	–	−9.233671551	0.012899805
12	pre_comp605149_c0_seq1	Catalase 1	9.666579007	0.046915628	−4.191584652	NS[a]
13	pre_comp36191_c0_seq1	Peroxidase 12-like	0.88976928	NS	2.413824189	0.018033191
14	pre_comp198050_c0_seq1	Flavonoid o-methyltransferase	−4.953366099	0.047359912	−2.816575243	NS
15	pre_comp423119_c0_seq1	Thioredoxin reductase cytoplasmic	−5.895832012	0.045272034	4.980654631	NS
16	pre_comp38978_c0_seq1	Cytochrome p450 86b1-like	−6.015223692	0.008348797	−5.733293046	0.018033191
17	pre_comp201629_c0_seq1	Copper amine oxidase	−6.171738964	0.046738807	−9.883918184	0.029182496
18	pre_comp846520_c0_seq1	Glutathione peroxidase	−9.072528149	0.030600933	−4.095466948	NS
19	pre_comp522317_c0_seq1	Protein kinase xa21	−10.12597447	0.012675129	−9.503703993	0.033681278
20	pre_comp38948_c0_seq3	Cytochrome p450	−10.86334261	0.013844127	−11.73283888	0.010495158

FDR < 0.05. Positive Log2 fold change values demonstrate higher transcript expression in infested plants while negative Log2 fold change values demonstrate higher transcript expression in control plants.

[a]NS, not significantly different.

(Table 3). In comparison, only three peroxidases had higher expression in 378 control plants as compared with Prestige control plants between 7 and 14 d (Table 3). It is noteworthy to mention the large difference in expression of specific peroxidases that have higher expression in Prestige control plants as compared with 378 control plants. When comparing the Log2 fold change in expression between the two genotypes, the glutathione peroxidases (GPXs) have a minimum 7.9 Log2 fold change, with higher expression in Prestige (Table 3). Two of the seven peroxidases with higher expression in Prestige control plants were class III peroxidases while five of the seven were glutathione peroxidases. Class III plant peroxidases have been shown to play a role in wound healing, auxin catabolism, cell wall-building processes, oxidation of toxic reductants, the breakdown and removal of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), and defense against pathogen and insect attack (Hiraga et al. 2001, Ni et al. 2001, Kawano 2003, Heng-Moss et al. 2004, Saathoff et al. 2013). Glutathione peroxidases are antioxidants that catalyze the reduction of ROS such as H_2O_2 using glutathione and other reducing agents (Ursini et al. 1995). The main functions of plant GPXs are to protect cells from damage by peroxides and to play a role in signal transduction pathways under stress conditions (Suzuki et al. 2012). Specific GPX expression and activity increased in response to pathogen infection in barley (Navrot et al. 2006). High levels of specific GPX transcripts in Prestige control plants may be part of the cellular mechanisms affording better resistance to chinch bugs and potentially other biotic stressors.

In response to chinch bug feeding, nine peroxidases were downregulated in 378 (Table 5) and two peroxidases were upregulated in Prestige (Table 4). The peroxidase transcript, pre_comp36874_c0_seq1, downregulated in 378 in response to chinch bug feeding has sequence identity (identity: 72%, E value: 4e-148) with a DE peroxidase gene in *Ipomoea batatas* L. in response to pathogenic bacteria (Kim et al. 2008). One of the upregulated peroxidases encoded by transcript pre_comp36191_c0_seq1 has sequence identity (identity: 50%, E value: 8e-72) with a peroxidase gene that showed increased expression in response to pathogen attack in a resistant *Triticum monococcum* L. genotype (Liu et al. 2005). In addition, two catalases were upregulated in Prestige (Table 4) and one downregulated in 378 (Table 5) at 7 d after chinch bug introduction. The upregulated catalase transcript, pre_comp605149_c0_seq1, has sequence identity to catalase genes identified in *Cucurbita maxima* Duchesne (identity: 45%, E value: 1e-10) in response to heat stress (Ara et al. 2013) and *Solanum tuberosum* L. (identity: 47%, E value: 8e-11) (Niebel et al. 1995) that is induced in response to nematodes and bacterial infection.

Based on these findings, we propose that tolerant plants have the ability to elevate their level of ROS-scavenging enzymes, such as peroxidases and catalases, which may enable them to efficiently remove ROS that accumulate in response to insect feeding (Bi and Felton 1995, Leitner et al. 2005, Wu and Baldwin 2010). Furthermore, the upregulation and downregulation patterns of these peroxidases suggest that ROS accumulation and detoxification occurs simultaneously in response to chinch bug feeding (Park et al. 2005, Gutsche et al. 2009b). ROS may play multiple roles in the tolerant response of Prestige to chinch bug feeding (Ramm et al. 2013). Increased levels of ROS may act as a signaling molecule for increased activation of ROS-dependent genes, defense-related transcripts, and ROS scavengers such as peroxidases and catalases as

Table 5. Buffalograss 378: significantly expressed defense-related sequences (control vs. chinch bug-infested)

	Transcript	Best-hit description	7 d Log2 fold change	FDR	14 d Log2 fold change	FDR
1	pre_comp254128_c0_seq1	Low quality protein: serine threonine protein kinase	11.33520278	0.047658983	–	–
2	pre_comp36874_c0_seq1	Peroxidase 72-like	−2.836094486	0.037735077	–	–
3	pre_comp37276_c0_seq1	Peroxidase	−5.003885913	0.027453938	–	–
4	pre_comp22072_c0_seq1	Peroxidase 16-like	−5.436291344	0.046782767	–	
5	pre_comp106282_c0_seq1	Peroxidase	−7.220683326	0.003339836	–	–
6	pre_comp4620_c0_seq1	Peroxidase 64-like	−9.653028419	0.014817325	–	–
7	pre_comp447775_c0_seq1	Glucose dehydrogenase	−11.7308214	0.046714592	–	–
8	pre_comp43810_c0_seq5	g-Type lectin s-receptor-like serine threonine-protein kinase rks1-like	–	–	11.08440249	0.014964005
9	pre_comp34325_c0_seq1	Leucoanthocyanidin reductase	9.983390402	0.045840694	−1.602433114	NS[a]
10	pre_comp24663_c0_seq1	Xylanase inhibitor protein 1-like	0.975555575	NS	4.601106204	0.020539486
11	pre_comp38686_c0_seq1	Peroxidase 15-like	−3.384701624	0.020906307	−0.890296707	NS
12	pre_comp42361_c0_seq2	Glutathione peroxidase	−4.399110155	0.048203818	1.804807941	NS
13	pre_comp513475_c0_seq1	Stress response protein	−8.668964055	0.041492533	3.119625273	NS
14	pre_comp316044_c0_seq1	Peroxidase 27 precursor	−8.908964643	0.043315795	−4.308066724	NS
15	pre_comp44167_c0_seq3	Scarecrow-like protein 14-like	−9.975675179	0.042374184	0.697445036	NS
16	pre_comp520116_c0_seq1	Catalase a	−10.10204695	0.049061926	3.011132514	NS
17	pre_comp23155_c0_seq1	Peroxidase 16-like	−10.16859702	0.01753303	1.009572348	NS

FDR < 0.05. Positive Log2 fold change values demonstrate higher transcript expression in infested plants while negative Log2 fold change values demonstrate higher transcript expression in control plants.

[a]NS, not significantly different.

has been reported in other studies (Passardi et al. 2005, Miller et al. 2009, Torres 2010).

We found that the chinch bug-tolerant buffalograss has a greater number of peroxidase transcripts with higher basal expression levels when compared with the susceptible buffalograss 378 (Table 3). High basal read counts of select peroxidase transcripts in the Prestige transcriptome demonstrate that these two buffalograsses are physiologically very different with respect to the types and relative levels of oxidative enzymes. These data support the idea that Prestige is better prepared to deal with oxidative stresses associated with insect herbivory, and are consistent with previous research documenting significantly higher basal expression levels of specific defense-related transcripts such as peroxidase in Prestige control plants compared with 378 control plants (Ramm et al. 2013). Overall, these results indicate that elevated basal expression levels of peroxidases could be one facet of the mechanisms underlying tolerance to chinch bug feeding in Prestige as compared with the susceptible 378 genotype.

It is important to note that the majority of observed transcripts with oxidoreductase activity were downregulated in 378 in response to chinch bug feeding at 7 d (Table 5). Multiple oxidative enzymes, such as peroxidases and catalases, were downregulated within the 378 buffalograss transcriptome at 7 d after chinch bug feeding, but then upregulated at a later time (Table 5). These results support previous findings that 378 is not as well equipped with basal levels of oxidative enzymes. In addition, 378 is not able to upregulate production of these oxidative enzymes to deal with increasing oxidative stress resulting from chinch bug feeding (Ramm et al. 2013). As compared with tolerant Prestige (Table 4), the large number of downregulated transcripts coding for enzymes involved in redox metabolism in 378 (Table 5) suggests this genotype does not have the ability to readily detoxify increasing ROS at the onset of insect feeding, and could be a contributing factor in plant susceptibility. At 14 d, there was increased expression of specific peroxidases and catalases in 378 in response to chinch bug feeding (Table 5); however, the level of expression was insufficient in preventing accumulation of ROS and as a result, these plants experienced visible plant damage.

In addition to DE peroxidases and catalases, other defense-related transcripts displayed differential expression. Of particular interest was a scarecrow-like protein transcript that was downregulated in 378 in response to chinch bug feeding. Scarecrow proteins are part of the GRAS family of plant proteins. The absence of specific GRAS genes has been documented as playing a key role in the susceptibility of tomato to *Pseudomonas syringae* infection (Mayrose et al. 2006). This finding supports an earlier study by Ramm et al. (2013) who reported higher basal level expression of a specific buffalograss GRAS transcript in Prestige when compared with 378 and upregulation of the same GRAS transcript in the tolerant buffalograss in response to chinch bug feeding. Additional research needs to be conducted to determine if GRAS expression is directly linked to the tolerance response in Prestige.

Several cytochrome P450 transcripts were also DE in response to chinch bug feeding. Cytochrome P450s (cP450) are multifunctional enzymes involved in the synthesis of secondary metabolites, and have the ability to catalyze many different reactions and produce different products (Hamberger and Bak 2013, Neilson et al. 2013). These enzymes also play a role in the synthesis of signaling molecules such as jasmonic acid, a hormone that plays a critical role in the plant defense response (Werck-Reichhart and Feyereisen 2000, Mizutani and Ohta 2010, Heitz et al. 2012, Hamberger and Bak 2013). The upregulation of a cP450 gene in

resistant Prestige may play a role in the plant's ability to tolerate chinch bug feeding by downstream signaling for the production of defense-related transcripts.

In Prestige, two glutathione s-transferase (GST) transcripts were differentially expressed between control and infested plants. One GST transcript was downregulated and one was upregulated in response to chinch bug feeding. GST enzymes are best known for their role in detoxification, but they also have multiple functions in plants, including primary and secondary metabolism (Dixon et al. 2002). GSTs also play an important role in cellular signaling and stress tolerance (Dixon et al. 2002, Laborde 2010). GST production has been documented in response to both abiotic and biotic stresses (Dixon et al. 2002). Expression of specific GST transcripts has been documented in resistant barley as well as resistant sorghum in response to aphid feeding, further implicating the potential role of GST in the plant defense response to insect pressure (Park et al. 2005, Gutsche et al. 2009a).

WRKY transcription factors are a family of proteins that regulate various plant processes, but are most notably involved in managing a diverse array of biotic and abiotic stressors by way of downstream transcriptional reprogramming and modulation (Pandey and Somssich 2009). The transcript pre_comp28074_c0_seq1, showed sequence identity (identity: 68%, E value: 2e-14) to a WRKY in *Triticum aestivum* whose overexpression improves stress tolerance in transgenic *Arabidopsis* plants through regulation of downstream genes (Niu et al. 2012) as well as sequence identity (identity: 86%, E value: 2e-11) with WRKY genes in *Brassica napus* responding to fungal pathogens (Yang et al. 2009).

It is interesting to note that for 378 there is a noticeable decrease in the number of downregulated transcripts in each category of biological processes from 7 d to 14 d in response to chinch bug feeding (Fig. 1). Overall, the number of downregulated transcripts is closest to zero in 378 at 14 d in response to chinch bug feeding, whereas Prestige (although showing a decrease in the number of downregulated transcripts from 7 to 14 d) consistently shows an overall greater number of downregulated transcripts at 14 d. Although a fewer number of transcripts are upregulated in response to insect feeding for both genotypes at each time point, for the categories of biological processes, cellular processes, biological regulation, and response to stimulus, at 7 d there is a noticeable increase in the number of upregulated transcripts in Prestige. The noticeable difference in the number of downregulated transcripts in infested Prestige versus 378 supports the idea that Prestige is better equipped for chinch bug infestation at the basal level, and therefore, a large number of transcripts are highly expressed in control plants (downregulated in response to chinch bugs).

In conclusion, results from this study support our working hypothesis that differential expression of oxidative enzymes such as peroxidases and catalases is a key factor in buffalograss tolerance or susceptibility to chinch bug feeding. Our results provide the first analysis using next-generation sequencing to investigate chinch bug interactions with tolerant and susceptible buffalograsses. Our data also provide insight into the possible pathways recruited throughout the continuum of the tolerance response. Here, we have identified many genes that could be used as molecular markers in the identification of other tolerant buffalograsses. Ultimately, this research will facilitate development of improved buffalograsses with resistance to chinch bugs and shorten the timeframe needed to identify and improve buffalograsses with superior chinch bug resistance.

Acknowledgments

This research was supported in part by the Nebraska Research Initiative Grant and the United States Golf Association.

References Cited

Ara, N., K. Nakkanong, W. Lv, J. Yang, Z. Hu, and M. Zhang. 2013. Antioxidant enzymatic activities and gene expression associated with heat tolerance in the stems and roots of two cucurbit species ("*Cucurbita maxima*" and "*Cucurbita moschata*") and their interspecific inbred line "*Maxchata*". Int. J. Mol. Sci. 14: 24008–24028.

Baxendale, F. P., T. Heng-Moss, and T. Riordan. 1999. *Blissus occiduus* Barber (Hemiptera: Lygaeidae): A new chinch bug pest of buffalograss turf. J. Econ. Entomol. 92: 1172–1176.

Bi, J. L., and G. W. Felton. 1995. Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. J. Chem. Ecol. 21: 1511–1530.

Botha, A. M., L. Lacock, C. Van Niekerk, M. Matsioloko, F. du Preez, S. Loots, E. Venter, K. Kunert, and C. Cullis. 2006. Is photosynthetic transcriptional regulation in *Triticum aestivum* L. cv. 'TugelaDN' a contributing factor for tolerance to Diuraphis noxia (Homoptera: Aphididae)? Plant Cell Rep. 25: 41–54.

Boyko, E. V., C. Smith, B. Thara, J. Bruno, Y. Deng, S. Starkey, and D. Klaahsen. 2006. The molecular basis of plant gene expression during aphid invasion: What Pto-and Pti-like sequences are involved in interactions between wheat and Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 99: 1430–1445.

Conesa, A., S. Götz, J. Garcia-Gomez, J. Terol, M. Talon, and M. Robles. 2005. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21: 3674–3676.

Grabherr, M. G., B. Haas, M. Yassour, J. Levin, D. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29: 644–652.

Dixon, D. P., A. Lapthorn, and R. Edwards. 2002. Plant glutathione transferases. Genome Biol. 3: 3004.1–10.

Gulsen, O., T. M. Heng-Moss, R. Shearman, P. S. Baenziger, D. Lee, and F. P. Baxendale. 2004. Buffalograss germplasm resistance to *Blissus occiduus* (Hemiptera: Lygaeidae). J. Econ. Entomol. 96: 2101–2105.

Gulsen, O., T. Eickhoff, T. M. Heng-Moss, R. Shearman, F. P. Baxendale, G. Sarath, and D. Lee. 2010. Characterization of peroxidase changes in resistant and susceptible warm-season turfgrasses challenged by *Blissus occiduus.* Arthropod-Plant Interact. 4: 45–55.

Gutsche, A. R., T. M. Heng-Moss, L. Higley, G. Sarath, and D. Mornhinweg. 2009a. Physiological responses of resistant and susceptible barley, *Hordeum vulgare* to the Russian

wheat aphid, *Diurpahis noxia* (Mordvilko). Arthropod-Plant Interact. 3: 233–240.

Gutsche, A. R., T. Heng-Moss, G. Sarath, P. Twigg, Y. Xia, G. Lu, and D. Mornhinweg. 2009b. Gene expression profiling of tolerant barley in response to *Diuraphis noxia* (Hemiptera: Aphididae) feeding. Bull. Entomol. Res. 99: 163–173.

Hamberger, B., and S. Bak. 2013. Plant P450s as versatile drivers for evolution of species-specific chemical diversity. Philos. Trans. R Soc. Lond. B Biol. Sci. 368: 1612.

Heitz, T., E. Widemann, R. Lugan, L. Miesch, P. Ullmann, L. Désaubry, E. Holder, B. Grausem, S. Kandel, M. Miesch, et al. 2012. Cytochromes P450 CYP94C1 and CYP94B3 catalyze two successive oxidation steps of plant hormone jasmonoyl-isoleucine for catabolic turnover. J. Biol. Chem. 287: 6296–6306.

Heng-Moss, T. M., F. P. Baxendale, T. Riordan, and J. Foster. 2002. Evaluation of buffalograss germplasm for resistance to *Blissus occiduus* (Hemiptera, Lygaeidae). J. Econ. Entomol. 95: 1054–1058.

Heng-Moss, T. M., F. P. Baxendale, T. Riordan, L. Young, and K. Lee. 2003. Chinch bug-resistant buffalograss: an investigation of tolerance, antixenosis and antibiosis. J. Econ. Entomol. 96: 1942–1951.

Heng-Moss, T. M., G. Sarath, F. Baxendale, D. Novak, S. Bose, X. Ni, S. Quisenberry. 2004. Characterization of oxidative enzyme changes in buffalograsses challenged by *Blissus occiduus.* J. Econ. Entomol. 97: 1086–1095.

Hiraga, S., K. Sasaki, H. Ito, Y. Ohashi, and H. Matsui. 2001. A large family of class III peroxidases. Plant Cell Physiol. 42: 462–468.

Kawano, T. 2003. Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction. Plant Cell Rep. 21: 829–837.

Kim, Y. H., K. S. Yang, C. Y. Kim, S. H. Ryu, W. K. Song, S. Y. Kwon, H. S. Lee, J. W. Bang, and S. S. Kwak. 2008. Molecular cloning of peroxidase cDNAs from dehydration-treated fibrous roots of sweetpotato and their differential expression in response to stress. Biochem. Mol. Biol. Rep. 41: 259–265.

Laborde, E. 2010. Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death. Cell Death Differ. 17: 1373–1380.

Langmead, B., and S. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357–359.

Leitner, M., W. Boland, and A. Mithöfer. 2005. Direct and indirect defences induced by piercing–sucking and chewing herbivores in *Medicago truncatula*. New Phytol. 167: 597–606.

Liu, G., X. Sheng, D. L. Greenshields, A. Ogieglo, S. Kaminskyj, G. Selvaraj, and Y. Wei. 2005. Profiling of wheat class III peroxidase genes derived from powdery mildew-attacked epidermis reveals distinct sequence-associated expression patterns. Mol. Plant-Microbe Interact. 18: 730–741.

Mayrose, M., S. Ekengren, S. Melech-Bonfil, G. Martin, and G. Sessa. 2006. A novel link between tomato GRAS genes, plant disease resistance and mechanical stress response. Mol. Plant Pathol. 7: 593–604.

Miles, P. W. 1999. Aphid saliva. Biol. Rev. Camb. Philos. Soc. 74: 41–85.

Miller, G., K. Schlauch, R. Tam, D. Cortes, M. A. Torres, and V. Shulaev. 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Sci. Signal. 2: ra45.

Mizutani, M., and D. Ohta. 2010. Diversification of P450 genes during land plant evolution. Annu. Rev. Plant Biol. 61: 291–315.

Navrot, N., V. Collin, J. Gualberto, E. Gelhaye, M. Hirasawa, P. Rey, D. Knaff, E. Issakidis, J. P. Jacquot, and N. Rouhier. 2006. Plant glutathione peroxidases are functional peroxiredoxins distributed in several subcellular compartments and regulated during biotic and abiotic stresses. Plant Phys. 142: 1364–1379.

Ni, X., S. Quisenberry, T. M. Heng-Moss, J. Markwell, G. Sarath, R. Klucas, and F. P. Baxendale. 2001. Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. J. Econ. Entomol. 94: 743–751.

Neilson, E. H., J. Q. Goodger, I. E. Woodrow, and B. L. Møller. 2013. Plant chemical defense: at what cost? Trends Plant Sci. 18: 250–258.

Niebel, A., K. Heungens, N. Barthels, D. Inzé, M. V. Montagu, and G. Gheysen. 1995. Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. MPMI-Mol. Plant Microbe Interact. 8: 371–378.

Niu, C. F., W. Wei, Q. Y. Zhou, A. G. Tian, Y. J. Hao, W. K. Zhang, B. Ma, Q. Lin, Z. B. Zhang, J. S. Zhang, et al. 2012. Wheat WRKY genes TaWRKY2 and TaWRKY19 regulate abiotic stress tolerance in transgenic Arabidopsis plants. Plant Cell Environ. 35: 1156–1170.

Pandey, S. P., and I. E. Somssich. 2009. The role of WRKY transcription factors in plant immunity. Plant Physiol. 150: 1648–1655.

Park, S. J., Y. Huang, and P. Ayoubi. 2005. Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. Planta 223: 932–947.

Passardi, F., C. Cosio, C. Penel, and C. Dunand. 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep. 24: 255–265.

Pozarnsky, T. 1983. Buffalograss: home on the range, but also a turf grass. Rangelands Arch. 5: 214–216.

Ramm, C., A. Saathoff, T. Donze, T. Heng-Moss, F. Baxendale, P. Twigg, L. Baird, and K. Amundsen. 2013. Expression profiling of four defense-related buffalograss transcripts in response to chinch bug (Hemiptera: Blissidae) feeding. J. Econ. Entomol. 106: 2568–2576.

Riordan, T. P., F. Baxendale, R. Gaussion, and J. Watkins. 1996. Buffalograss: an alternative native grass for turf. Coop Ext, University of Nebraska-Lincoln, NE. G96-1297-A.

Robinson, M. D., D. McCarthy, and G. Smyth. 2010. EdgeR: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140.

Saathoff, A. J., T. Donze, N. A. Palmer, J. Bradshaw, T. Heng-Moss, P. Twigg, C. M. Tobias M. Lagrimini and G. Sarath. 2013. Towards uncovering the roles of switchgrass peroxidases in plant processes. Front. Plant Sci. 4: 202.

SAS Institute. 2008. PROC User's Guide. SAS Institute Inc, Cary, NC.

Serba, D., T. Heng-Moss, R. Shearman, B. Abeyo, S. Baenziger, and D. Lee. 2011. Evaluation of buffalograss genotypes and full-sibs for chinch bug resistance. J. Econ. Entomol. 104: 2073–2077.

Smith, C. M., and E. Boyko. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomol. Exp. Appl. 122: 1–16.

Suzuki, N., S. Koussevitzky, R. Mittler, and G. Miller. 2012. ROS and redox signaling in the response of plants to abiotic stress. Plant Cell Environ. 35: 259–270.

Thompson, G. A., and F. Goggin. 2006. Transcriptomics and functional genomics of plant defense induction by phloem-feeding insects. J. Exp. Botany 57: 755–766.

Torres, M. A. 2010. ROS in biotic interactions. Physiol. Plant. 138: 414–429.

Ursini, F., M. Maiorino, R. Brigelius-Flohe, K. D. Aumann. 1995. Diversity of glutathione peroxidase. Methods Enzymol. 252.

Wachholtz, M., T. Heng-Moss, P. Twigg, G. Lu, and K. Amundsen. 2013. Transcriptome analysis of two buffalograss cultivars. BMC Genomics 14: 613.

Walling, L. L. 2000. The myriad plant responses to herbivores. J. Plant Growth Regul. 19: 195–216.

Wenger, L. E. 1943. Buffalograss. Kansas Agric. Exp. Sta. Bull. 321.

Werck-Reichhart, D., and R. Feyereisen. 2000. Cytochromes P450: a success story. Genome Biol. 25: 128–145.

Wu, J., and I. T. Baldwin. 2010. New insights into plant responses to the attack from insect herbivores. Annu. Rev. Genet. 44: 1–24.

Wu, L., and A. Harivandi. 1989. Buffalograss: promising, drought-resistant-and here now. Golf Course Manage. 57: 42–54.

Yang, B., Y. Jiang, M. Rahman, and M. Deyholos. 2009. Identification and expression analysis of WRKY transcription factor genes in canola (*Brassica napus* L.) in response to fungal pathogens and hormone treatments. BMC Plant Biol. 9: 68.

Zhang, F., L. Zhu, and G. He. 2004. Differential gene expression in response to brown planthopper feeding in rice. J. Plant Physiol. 161: 53–62.

Received 24 October 2014; accepted 26 February 2015.