12-2006

Microarray Analysis of Late-season Velvetleaf (Abutilon theophrasti) Effect on Corn

David P. Horvath
USDA, Agricultural Research Service, david.horvath@ars.usda.gov

Robert Gulden
University of Guelph

Sharon A. Clay
South Dakota State University, sharon.clay@sdstate.edu

Weed Science, 54:983–994. 2006

Microarray analysis of late-season velvetleaf (*Abutilon theophrasti*) effect on corn

David P. Horvath
Corresponding author. Bioscience Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Fargo, ND 58105-5674; horvathd@fargo.ars.usda.gov

Robert Gulden
Department of Plant, Agriculture, University of Guelph, Guelph, ON N1G 2W1, Canada

Sharon A. Clay
Department of Plant Science, South Dakota State University, Brookings, SD 57007

Microarray analysis was used to identify changes in gene expression in corn leaves collected from plants at the V11–14 growth stage that resulted from competition with velvetleaf. The plants were grown in field plots under adequate N (addition of 220 kg N ha^{-1}) and irrigation to minimize N and water stress. Consequently, only differences resulting from competition for micronutrients, light, and perhaps allelopathic stress were anticipated. Genes involved in carbon and nitrogen utilization, photosynthesis, growth and development, oxidative stress, signal transduction, responses to auxin and ethylene, and zinc transport were repressed in corn growing in competition with velvetleaf. Very few genes were induced because of competition with velvetleaf, and those that were provided little indication of the physiological response of corn. No differences were observed in genes responsive to water stress or sequestering/transporting micronutrients other than zinc, indicating that these stresses were not a major component of velvetleaf competition with corn at the developmental stage tested.

Nomenclature: Velvetleaf, *Abutilon theophrasti* L. ABUTH; corn, *Zea mays* L.

Key words: Weed competition, microarray, genomics.

Weeds substantially reduce crop yield for a number of reasons. Yield loss caused by weed interference can be the result of reduced availability to the crop of one or more resources (such as light, water, and nutrients) necessary for optimum production. The reduced availability is assumed to be the result of preemptive use of the resource by the weed (Kropff and van Laar 1993; Zimdahl 2004), and this competition has been shown to be overcome by the addition of water or fertilizer (Blackshaw et al. 2002; Tollenaar et al. 1994). In addition, weeds can negatively affect crop yield through the production of allelopathic chemicals that can interfere with specific physiological processes of the crop (Weston and Duke 2003). Finally, there is substantial evidence that crop growth can be negatively altered by developmental signals induced by shade avoidance physiology when crops are grown in close proximity to weeds (Rajcan et al. 2004). Evidence also suggests that weeds only affect crop growth and yield during early stages of growth and that late-emerging weeds have little or no effect on growth or yield (Clay et al. 2005b; Cousens 1985; Cousens et al. 1987). However, because of the lack of tools to observe the global physiological status of crops grown in the presence of weeds, all previous studies have resorted to observing specific physiological responses or the use of crop growth and yield as gross indicators of the effect of weeds under varying field conditions. The availability of high-density microarrays for many crop species has opened the opportunity to determine expression levels of thousands of well-characterized genes simultaneously. The function of those genes that have different expression levels in the presence or absence of weeds can provide both expected and unexpected insight into many of the physiological processes affected by weed competition. Such information should prove useful to both modelers and breeders by highlighting various responses and genes that play a role in crop–weed interactions.

Velvetleaf is an introduced dicotyledenous weed that has large leaves, can grow up to 2.4 m tall, and is thought to release allelopathic chemicals (Colton and Einhellig 1980). Velvetleaf competition has been shown to decrease corn grain yield from 0 to 80% depending on field conditions and weed density (Lindquist et al. 1998; Scholes et al. 1995; Werner et al. 2004). The interaction between velvetleaf and corn has served as a model for crop–weed competition in numerous studies (Lindquist 2001; McDonald et al. 2004; Sattin et al. 1992; Teasdale 1995). A critical period of weed control has been observed in many crops–weed interactions, including corn and velvetleaf (Bryson 1990; Hall et al. 1992; Norsworthy and Oliviera 2004; Van Acker et al. 1993). In corn, the critical period of weed control typically ranges from the three- to eight-leaf stage (V3–V8; Hall et al. 1992). The bulk of the negative effects of competition and interference on corn yield occurs during this period. Weeds that emerge before and after this period, have a marginal effect on yield in comparison because weeds are either not competing with the crop (before) or weeds have emerged too late to affect yield of the over-towering crop significantly (after). The critical period and weed competition is not a static phenomenon and is influenced by many factors, such as nutrient status (Evans et al. 2003), management factors (Norsworthy and Oliviera 2004), and time of emergence (Bosnic and Swanton 1997). Many of the factors that strongly influence the competitive outcome between crops and weeds (e.g., time of emergence and weed density) are well documented and have specific empirical functions associated with them (e.g., weed density effects on yield loss [Cousens 1985], relative time of weed emergence on yield loss [Cousens et al. 1987]).

Rajcan et al. (2004) have suggested a role for phytochrome in the process through which weeds can alter crop developmental patterns during the critical period of crop–weed interaction. Reflected light from weeds alters the ratio of red to far red light (R/FR), inducing signaling processes

mediated by phytochrome (Ballaré and Casal 2000; Ballaré et al. 1987, 1990). The differences in R/FR induce morphological changes in crops associated with weed infestations because of shade avoidance responses, including alterations in leaf area index and shoot/root ratios (Samarakoon et al. 1990; Wong and Wilson 1980). Shade avoidance responses affect numerous hormone signaling pathways that are similar to developmental changes observed in crop plants growing in the presence of weeds (Devlin et al. 2003; Pierik et al. 2004; Steindler et al. 1999; Tian and Reed 2001; Vandenbussche et al. 2003). Some of these responses include alteration of photosynthetic activity and differences in auxin and ethylene signaling. With the use of macroarray analysis (similar to microarray analysis but with the use of larger quantities of target DNAs spotted onto membrane filters rather than glass slides), Fey et al. (2005) showed that gene expression in mouse-ear cress [*Arabidopsis thaliana* (L.) Heynh. ARBTH] changed almost immediately after exposure to a lower R/FR ratio. Phenotypic consequences of these changes were typically not immediately apparent. Although the phenotypic response of crop plants to weed interference and to variation in resource supply and R/FR has been well studied, how these factors actually affect plant metabolic and signaling pathways and bring about reduced crop yield or altered growth is poorly understood.

It is evident also that weeds can affect crop growth and yield later in the growing season. Subcanopy weeds can have an effect on crops during development. Studies by Clay et al. (2005a, 2006) and Bonifas and Lindquist (2006) indicated that detectable differences in nitrogen and potassium levels in mature leaves of late-season corn plants grown in the presence of weeds are unlikely to be the result solely of early influences of the weeds on crop growth and development.

Differences in the physiological state of the crops grown with or without weeds can be assessed by microarray analysis. Microarray analysis simultaneously assesses differences in expression of thousands of previously characterized genes after any given treatment. By examining the function of genes with expression patterns that are affected by a treatment, it is possible to identify the physiological processes or signaling pathways that are altered. If genes involved in a particular biochemical pathway are altered, then likely the treatment results in specific changes in that biochemical process. However, no previous papers have reported the use of microarrays to directly study crop–weed interactions. The objective of this study was to use microarray technology and nitrogen and carbon accumulation to determine whether physiological differences could be detected between corn grown under weed-free conditions or in competition with velvetleaf at a late vegetative stage of growth (when detectable differences were expected to be the most subtle). It is expected that information gained from these observations will test the hypothesis that velvetleaf has a continuous effect on corn growth and development past the critical stage and will be used to develop testable hypotheses concerning alterations of specific physiological functions or gene expression affected by weed competition.

Materials and Methods

Plant Material

Corn (DKC46-22) was grown in field plots at Aurora, SD, on a Brandt silty clay loam soil (fine-silty, mixed superactive, frigid Calcic Hapludoll). The sand, silt, and clay contents were 390, 383, and 226 g kg^{-1}, respectively, with pH 6.0 and an organic matter content of 35 g kg^{-1}. Corn was planted May 4, 2005, at a population of 10 plants m^{-2}, and treatments were assigned in a randomized complete block design to plots of 18 m^2. Velvetleaf was sown on the same day as corn in interrow areas about 20 cm from the crop row with a seed drill. Granular urea was broadcast at 224 kg N ha^{-1} after planting. Velvetleaf was hand-thinned to 8 plants m^{-2} after germination (first thinning occurred ~ 15 d after planting) and maintained at this density for the duration of the season. Plots were 6 m long by four rows wide with a row spacing of 76 cm and four replicates per treatment. About 214 mm of natural rainfall was received between planting and sampling with an additional 38 mm of irrigation water applied on July 1. Exposure from the time of planting to sampling totaled 472 growing degree days (base 10 C), which was 12% greater than the 30-yr average.

Corn leaves were harvested on July 11 and 12, 2005. Corn plants in the velvetleaf-free treatment (control) were generally larger and developed more leaves than plants competing with velvetleaf. At this point, the corn plants had overtopped the velvetleaf (Figure 1). The top fully expanded leaf was collected from four randomly selected plants from each plot and pooled. The resulting material was immediately frozen in liquid nitrogen and stored at −80 C until RNA extraction. Samples were obtained from two replicates of velvetleaf-infested (W1 and W2) and two control (C1 and C2) plots between 2:00 and 3:00 P.M. on July 11, 2005, and a second set of replicates (W3, W4, C3, and C4) was collected the following morning between 9:00 and 10:00 A.M. It rained off and on the afternoon and night with a total rainfall accumulation of 8.4 mm between sampling times.

Corn ears along two 3-m-long sections of the center two rows of each plot were hand-harvested after physiological maturity (black layer) in October. After drying and shelling the ears, grain yield was estimated after correction to 15.5% water content.

Nitrogen Analysis

A sample of the same leaf tissue collected in July and used for microarray analysis was taken from each treatment, dried at 65 C, and ground to a fine flour texture in a cyclone-type mill. A subsample of the leaf tissue (~ 2.5 mg) was analyzed for total N and $\delta^{15}N$ with an isotope ratio mass spectrometer[1] (Clay et al. 2005a). In addition, soil samples, taken at planting, and urea fertilizer were characterized for $\delta^{15}N$. Samples were run in duplicate with at least 30% standards for calibration purposes.

The $\delta^{15}N$ values (‰) were calculated with the equation

$$\delta^{15}N = [(^{15}N/^{14}N_{sample} - {}^{15}N/^{14}N_{standard}) \div (^{15}N/^{14}N_{standard})] \times 1{,}000$$

where $^{15}N/^{14}N_{sample}$ is the isotopic ratio of nitrogen in a sample and $^{15}N/^{14}N_{standard}$ is the isotopic ratio of the standard (air, 0.0036765). Positive $\delta^{15}N$ values indicate that sources are enriched in ^{15}N, and negative values indicate a depletion of ^{15}N relative to air.

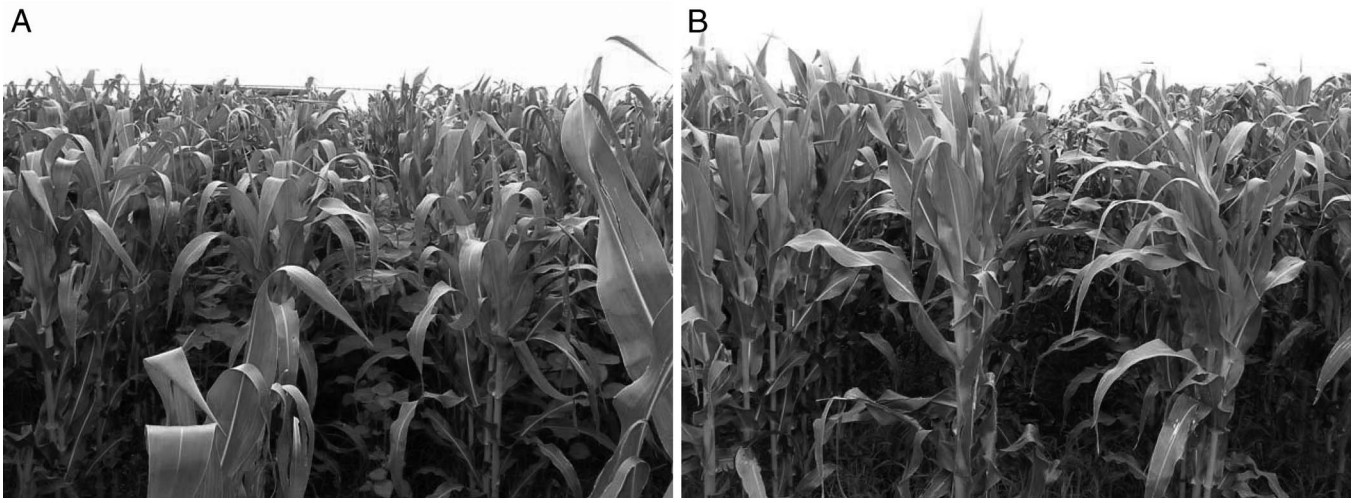

FIGURE 1. Photo of representative plots of corn with velvetleaf (A) and corn alone (B). The photo was taken just before leaf material was collected. The grass visible in the velvetleaf-free picture is from edge contamination and does not extend more than a few rows into the plot.

RNA Extraction and Microarray Analysis

Frozen plant material was ground to a fine powder in liquid nitrogen with a mortar and pestle. RNA was extracted from the resulting powder by the pine tree extraction method (Chang et al. 1993). Labeled cDNA was prepared from 30 μg of total RNA with the use of the Alexa Fluor cDNA labeling kit[2] according to manufacture's protocols. Labeled cDNA was hybridized to the 19,200-element SAM1.1 microarray chip for corn developed and distributed by Iowa State University[3] according to the manufacture's protocols. A partial rolling circle dye swap hybridization scheme (Churchill 2002) was used to compare gene expression between velvetleaf-free and velvetleaf-infested samples. Ten different two-dye hybridizations were performed that compared velvetleaf-infested vs. velvetleaf-free treatments. Five hybridizations directly compared samples collected on different days, and five compared samples collected on the same day. Chips were hybridized and washed following manufacture's protocols. Hybridization intensities (on the basis of florescence) for each probe (spotted clone) were visualized and quantified with an Affy 428 scanner[4] and Jaguar software.[4]

Statistical Analysis of Microarray Data

As per standard methods of analyzing microarray results, the M value (measure of differential gene expression) and A value (measure of how strongly a given gene is expressed) were determined for each probe. The M value is calculated as the $\log_2$ of the hybridization intensity ratios of velvetleaf-infested over velvetleaf-free. The A value is calculated as the $\log_2$ of the square root of the product of the hybridization intensity values from each probe. The hybridization intensity is determined by the level of fluorescence resulting from the excitation of the labeled cDNA that "sticks" to each DNA-containing probe. The resulting MA plots (M value by A value for each probe) were Loess-normalized by the GeneMath XT1 program[5] to produce a normalized M value for each gene. This procedure is done to remove differences that might be caused by differential labeling of the sample RNA as opposed to differential expression of individual genes. Because low-intensity probes are often difficult to interpret and are a major source of meaningless variation, expression ratios from these probes are customarily deleted from the data sets before analysis. Thus, probes with A values of less than one standard deviation over the mean for non–DNA-containing controls, as well as all probes that did not contain corn DNA, were deleted. Because 10 replicate hybridizations were done for each probe, if a given probe was deleted in three or more replicates, then the probe was considered unreliable and all data from that probe was deleted from further statistical analysis. The normalized ratios of hybridization intensities for all of the remaining probes were analyzed for statistical significance by the SAM 1.22 software[6] set for one class with 100 iterations to assign a Q value as an estimate of the false discovery rate for each gene.

To verify the results from the SAM analysis, an ANOVA approach was employed for each probe used in the SAM analysis only to determine a statistical probability associated with differential gene expressing in the control vs. weedy treatments. For each individual probe on the array, the $\log_2$ fluorescence values that had been normalized for dye (i.e., the fluorescence of the control and the weedy treatments for each probe on each of the 10 arrays once the variation associated with the reporter dyes had been removed) were tested for normality, and a separate one-way ANOVA with two treatments (weedy $n = 10$, control $n = 10$) was conducted for each probe with the MIXED procedure of SAS (Littell et al. 1996). In this analysis, treatment was considered a fixed effect, whereas variation associated with replicates of the array was removed by declaring array a random effect (Wolfinger et al. 2001). The probability (P value) of each of these individual F tests was reported and regressed against the Q value provided by the SAM analysis to compare the two approaches. In addition, the Bonferroni and the less conservative Bonferroni–Holm and Sidak–Holm step-down methods were used for familywise error correction of P values obtained from the Mixed Model ANOVA.

Results and Discussion

Effect of Velvetleaf on Growth

At the time of sampling, corn had overtopped the velvetleaf in the velvetleaf treatment (Figure 1). Corn plants

TABLE 1. Nitrogen and ^{15}N concentrations in the uppermost corn leaf on July 11, 2005, and corn grain yield at harvest (October 2005) grown with or without velvetleaf at Aurora, SD.

Treatment	Leaf tissue values, July 11		Grain yield
	N %	$\delta^{15}N$ ‰	kg ha^{-1}
Velvetleaf-free	3.84	−0.60	16,800
Velvetleaf present	3.56	0.25	12,300
P value	0.04	0.07	0.001

grown in the presence of velvetleaf were about 1.8 m tall and, on average, were at the V11 stage of growth. Corn plants grown in the velvetleaf-free treatment were more developed than plants grown with velvetleaf, as evidenced by plants that were both taller (about 2 m) and had more leaves (V14 stage of growth). Visual evaluation of plants in both treatments indicated that tassels had developed within the apical leaf sheath.

Soil samples taken before fertilization were enriched in ^{15}N and averaged 3.5‰, whereas urea was a depleted ^{15}N source with a measured $\delta^{15}N$ value of −1.45‰ (data not shown). The total nitrogen concentration of the top leaf of velvetleaf-free corn was greater than that of corn competing with velvetleaf (Table 1). The $\delta^{15}N$ values of the sampled leaf tissue indicated that corn in both treatments took up a mixture of fertilizer- and soil-derived N, but the mixture differed ($P = 0.07$) by treatment. The negative $\delta^{15}N$ value of corn tissue from the velvetleaf-free treatment indicated that the main source of N was urea fertilizer. Corn competing with velvetleaf was more reliant, but not totally reliant, on soil-mineralized N, as indicated by a more positive $\delta^{15}N$.

Grain yield was 27% lower in corn growing with velvetleaf compared with velvetleaf-free corn (Table 1). The 3.4% yield loss per plant in this study is somewhat less than that observed in earlier corn–velvetleaf competition studies in South Dakota (Scholes et al. 1995), in which reported incremental yield loss per velvetleaf plant was about 4.4%. These results indicate that in this single-year treatment, velvetleaf competition reduced corn growth rate, decreased total N and influenced the source of N in sampled leaf, and ultimately, decreased grain yield. However, the results provide no information on how velvetleaf affected corn at the molecular level.

Microarray Analyses of Gene Expression in Leaves of Velvetleaf-Infested and Velvetleaf-Free Corn

Microarray analysis is also referred to as "global gene expression" or "transcriptome" analysis. It is important to note that any given gene might or might not be considered differentially expressed depending on the samples being compared and the arbitrary cutoff of the statistical significance used. However, often multiple probes representing the same gene, groups of differentially expressed genes involved in particular physiological functions, or genes that are known to be regulated by particular signaling processes (sometimes referred to as functional analysis) are identified as differentially expressed among several independently replicated comparisons. Also, the probability of multiple probes representing the same gene falling into the statistically significant category by chance alone can be exceptionally small. When these functional clusters are observed, a picture of the physiological differences resulting from a particular treatment can be visualized that generally transcends minor environmental or statistical differences.

In these experiments, microarray analysis provided the relative expression levels of 19,200 probes (sometimes referred to as elements) on the corn microarrays. Of the 19,200 probes, ~ 14,000 were considered informative and contained a single PCR product with interpretable sequence data. All other probes were blank or contained otherwise undesirable PCR products (weak or multiple bands). The informative DNA-containing probes represented ~ 5,700 genes with BlastX hits to previously characterized genes (some of which were spotted more than once). The comparison of leaf material sampled from four replicated field plots of corn grown in the presence or absence of velvetleaf identified only 253 probes that were consistently differentially hybridizing (Q value [false discovery rate] of < 15%) and expressed at levels that provided confidence in the analysis (A values consistently > 1 SD more than background; Table 2). These probes represented about 188 different genes (or about 3% of the total number of informative probes) on the basis of the function derived from the top BlastX hit.

Combining the two different statistical approaches for determining significant differential gene expression (i.e., the permutation-based SAM analysis and the Mixed Models ANOVA) between the treatments averts some of the limitations inherent in either method and reduces the likelihood of false discovery of differentially expressed genes. Correct identification of differentially expressed genes is likely greater if they possess low Q values in combination with low *t* test probabilities compared with genes with low values for only one of these two statistical methods (Table 2). The Pearson correlation coefficient between SAM-derived Q values and ANOVA-derived P values was 0.901 (data not shown) and indicated a high degree of similarity in the identification of significantly different expressed genes between these methods. Therefore, we base the discussion on differential gene expression identified with the use of SAM. This combination of statistical approaches negates the need for familywise error correction of P values because identification of significant genes was based primarily on SAM. It is important to note that familywise error correction of P values rendered all gene expression differences not significant. Therefore, with the use of P values alone for gene identification, a cutoff of $P \leq 0.001$ should be used (= 17.8 false positive identifications by chance on average).

Of the 253 differentially hybridizing probes identified by the SAM method, 240 were down-regulated and 13 were up-regulated in corn growing in the presence of velvetleaf. Although the range in fold differences (absolute ratios in gene expression between treatments) was subtle, only about −1.7 to +1.4 (Table 2 shows $\log_2$ of the fold changes), the small differences could be reflective of minimal changes in gene expression in all of the leaf cells or greater differences within a subtype of the tissues sampled (i.e., vascular tissues, etc.). It is also possible that the subtle nature of the differences is the result of residual effects of competition early in development that are known to occur. This study represents data from a single time point chosen to make differences particularly challenging to identify. Indeed, the relatively mi-

nor differences in the magnitude of gene expression differences is not surprising because morphological differences were not detectable between the leaf tissues from the two samples, and the leaves did not experience any obvious competition for light. Additional studies are clearly needed to determine whether more dramatic differences can be observed at earlier time points because it is also possible that the subtle nature of the differences is the result of residual effects of competition early in development that are known to occur. However, detectable differences in nitrogen levels are noteworthy in the leaf material from weedy and weed-free corn (Table 1). Also, differences in groups of genes involved in carbon utilization, growth, nitrogen utilization, oxidative stress, protein catabolism and synthesis, signal transduction, and cell transport and communication are consistent. These observations are consistent with the hypothesis that weeds can affect multiple physiological processes well after the critical period of weed control.

Velvetleaf Competition Inhibited Genes Involved in Carbon Utilization and Photosynthesis

Assuming similar harvest index among treatments, total biomass at physiological maturity was about 26% greater in weed-free corn. Therefore, CO_2 assimilation was reduced by weed competition during the growing season. Microarray analysis indicated that genes involved in photosynthetic processes were clearly reduced in leaves collected in the late vegetative stage of corn. This is somewhat surprising because the corn had substantially overtopped the velvetleaf, and the sampled leaf material collected was from the top of the plant. Because no shading was taking place, and with previous studies indicating permanent effects of weeds on crops early in the growing season, it is tempting to speculate that velvetleaf competition early in development induced a permanent inhibition of photosynthesis. If this was the case, one possible and exciting possibility is that the shade avoidance response altered the chromatin structure in such as way as to alter the expression of these photosynthetic genes long after the weed was overtopped by the crop. Chromatin alterations (sometimes referred to as chromatin remodeling) involve the modification of histones that bind to the DNA and can stably alter expression of specific genes through numerous iterations of cell division (Reyes et al. 2002). However, it is also possible that velvetleaf was simply responsible for the reduction of N (Table 1) or some other nutrient needed for normal photosynthetic capacity. Additional studies are needed to determine the mechanisms by which weeds inhibit the expression of, or permanently alter, photosynthetic genes.

Velvetleaf Competition Reduced Expression of Growth-Related Genes

The relatively lower level of expression in various histones and other genes involved in cell growth and division in corn competing with velvetleaf suggests that cell cycle activity was negatively influenced by competition. The relatively taller size of the weed-free corn is consistent with reduced cell division. The likely reduced rate of cell division in corn competing with velvetleaf suggests that competition inhibited leaf growth at the late developmental stage tested. However, no earlier time points were tested in these experiments. The velvetleaf did not overtop the corn at any time because they were planted on the same date. Thus, it could be that the reduced negative effect of velvetleaf competition on growth was similar at both early and late time points.

Very few genes involved in cell division were observed to be differentially expressed in mouse-ear cress growing under low R/FR conditions at either early or late time points (Devlin et al. 2003). Thus, it seems likely that the reduced cell division was due to some effect of velvetleaf on the corn other than shade avoidance. It is possible that the effect of velvetleaf on corn growth might be the result of allelopathic effects or deprivation of specific nutrients. However, it is also possible that the long-term inhibition of photosynthesis could be negatively affecting cell growth (and subsequent division).

Velvetleaf Competition Repressed Genes Responsive to Oxidative Stress

The repression of several genes known to be responsive to oxidative stress suggests that weed competition actually lowered the level of oxidative stress in corn. Oxidative stress is implicated in many physiological processes, including disease resistance, abiotic stress, and programmed cell death (Mittler 2002). This result was surprising. One possible explanation is that the reduction in photosynthetic capacity results in fewer oxidative radicals. It is also possible that the velvetleaf actively repressed oxidative responses systemically in the crop. Evidence indicates that velvetleaf is allelopathic to other plants (Colton and Einhellig 1980; Sterling et al. 1987). Negatively regulating the ability of the crop to respond to oxidative stresses would provide velvetleaf with a significant growth advantage over a crop. Again, further studies are needed to determine whether corn was simply experiencing less oxidative stress when in competition with velvetleaf or whether it had a reduced ability to respond to oxidative stress.

Velvetleaf Competition Repressed Genes Involved in Protein Synthesis and Metabolism

A number of the genes down-regulated in corn competing with velvetleaf were ubiquitin related or known chaperonins such as HSP70. These genes are known to be involved in protein degradation/stabilization processes. There was also reduced expression in numerous genes encoding proteins involved in protein synthesis, such as the translation elongation factors and ribosomal proteins. In addition, nitrogen utilization genes such as asparagine synthetase and nitrate reductase were similarly down-regulated. Why these genes would be down-regulated in corn grown in the presence of velvetleaf is not known. However, nitrogen content in corn grown in competition with velvetleaf was clearly reduced. It might be possible that mechanisms were in place to reduce protein turnover and synthesis when the crop is in a competitive environment.

Velvetleaf Competition Affected Genes Involved in Specific Signal Transduction Processes

Several auxin-regulated genes were also down-regulated by the presence of velvetleaf. Additionally, ubiquitin/ribosomal protein S27a was also down-regulated by velvetleaf compe-

TABLE 2. Differentially expressed genes regulated by corn–velvetleaf interactions. The columns show the probe identification number for the array, the gene accession number of the expressed sequence tag for the spotted cDNA, the average normalized ratio of expression levels from 10 hybridizations, the relative probability that the gene is differentially expressed on the basis of *t* tests, the probability that the gene is a false positive, the putative function on the basis of the BlastX hit containing descriptive information, and the gene ontology designation. Gene functions marked with an asterisk (*) represent those cDNAs for which sequence data was too short or otherwise not noted in the GEO platform and thus had to be matched to contigs present in the TIGR maize database before BlastX analysis. The line segregates those genes that were preferentially expressed in the control plants (top) and those preferentially expressed in the plants grown in the presence of velvetleaf (bottom).

GEO gene ID no.	Accession no.	Average M value	*t* test P value	Q value	Function[a]	Gene ontology
9049	BQ620881	−0.26	0.0019	9.23	ADP-ribosylation factor	Carbon utilization
17405	AI665577	−0.43	0.0272	14.10	Argininosuccinate lyase	Carbon utilization
12341	BG842749	−0.31	0.0015	6.31	Ascorbate peroxidase	Carbon utilization
4344	BM340172	−0.20	0.0019	10.43	ATP synthase F1, gamma subunit	Carbon utilization
4728	BM078717	−0.29	0.0063	10.43	Carbohydrate binding/transferase	Carbon utilization
1148	BM348874	−0.44	0.0167	10.43	Chlorophyll *a/b* binding protein 1*	Carbon utilization
12348	BG842102	−0.41	0.0156	10.43	Ferredoxin-NADP (H) oxidoreductase*	Carbon utilization
11553	BM349987	−0.40	0.0088	9.23	Fructose-bisphosphate aldolase class-I	Carbon utilization
4476	CB381162	−0.59	0.0297	10.43	Glyceraldehyde 3-phosphate dehydrogenase	Carbon utilization
4365	BM080287	−0.47	0.0199	9.23	Glyceraldehyde-3-phosphate dehydrogenase*	Carbon utilization
2743	BM266895	−0.38	0.0035	7.98	Light-induced protein	Carbon utilization
2560	CB886218	−0.33	0.0027	7.46	Malate dehydrogenase	Carbon utilization
6148	CD568964	−0.20	0.0068	14.45	NADH dehydrogenase	Carbon utilization
3123	BM350316	−0.29	0.0052	9.23	NADH dehydrogenase (ubiquinone)	Carbon utilization
4053	CB351512	−0.54	0.0359	14.10	NADH dehydrogenase subunit 3	Carbon utilization
6174	CD058800	−0.48	0.0299	12.52	NADP-dependent malate dehydrogenase*	Carbon utilization
6768	BG840456	−0.60	0.0093	7.46	NADP-dependent malic enzyme	Carbon utilization
762	BM382616	−0.63	0.0205	9.23	Phosphoenolpyruvate carboxykinase*	Carbon utilization
5768	CD484712	−0.60	0.0004	0.00	Phosphoenolpyruvate carboxylase	Carbon utilization
7279	CB604487	−0.49	0.0109	7.98	Phosphoenolpyruvate carboxylase	Carbon utilization
400	BM347611	−0.41	0.0088	7.46	Phosphoenolpyruvate carboxylase	Carbon utilization
12795	AI855092	−0.41	0.0043	7.46	Phosphoenolpyruvate carboxylase	Carbon utilization
3266	CB605122	−0.30	0.0073	10.43	Phosphoglycerate dehydrogenase	Carbon utilization
8576	CD484764	−0.42	0.0176	10.43	Phosphoglycerate kinase	Carbon utilization
4100	CB411211	−0.30	0.0076	10.43	Phosphoribosylaminoimidazole carboxylase	Carbon utilization
6311	AW054121	−0.57	0.0072	6.31	Photosystem I J-protein	Carbon utilization
5146	BM267937	−0.40	0.0149	10.43	Photosystem I reaction center subunit II	Carbon utilization
3964	BM381413	−0.43	0.0178	10.43	Photosystem I reaction centre subunit *n*	Carbon utilization
4724	BG841274	−0.69	0.0099	6.31	Photosystem II type II chlorosphyll *a/b* binding	Carbon utilization
16448	AW352495	−0.50	0.024	10.43	Ribulose 1,5-bisphosphate carboxylase/oxygenase	Carbon utilization
6533	CB886070	−0.26	0.003	10.43	Serine carboxypeptidase II	Carbon utilization
2742	BM268439	−0.47	0.0091	7.46	Sugar starvation–induced protein	Carbon utilization
394	AI734499	−0.30	0.0182	14.45	Triose phosphate/phosphate translocator	Carbon utilization
4775	BM073494	−0.35	0.0013	6.31	Undecaprenyl pyrophosphate synthase*	Carbon utilization
9977	BM072913	−0.30	0.0084	10.43	DNA-3-methyladenine glycosidase I–like*	Growth
13056	AI714655	−0.37	0.022	10.43	MA3 domain–containing protein, topoisomerase	Growth
5803	BQ172663	−0.56	0.0104	7.46	Actin	Growth
8519	CB816029	−0.33	0.0206	14.45	Actin	Growth
4799	BM074264	−0.55	0.0176	9.23	Actin*	Growth
3998	BM078973	−0.39	0.0233	10.43	Actin*	Growth
367	BM080755	−0.43	0.0124	9.23	Alpha tubulin*	Growth
4056	CB331722	−0.37	0.0195	14.10	Alpha2-tubulin	Growth
3176	BM079223	−0.24	0.0074	12.52	Early nodulin 75–like protein*	Growth
6004	CB280772	−0.35	0.0071	9.23	Histone H2A	Growth
6194	CD527785	−0.31	0.0051	9.23	Histone H2A	Growth
8937	CB886104	−0.35	0.0071	9.23	Histone H2B	Growth
9285	CB816764	−0.26	0.0047	10.43	Histone H2B	Growth
7730	CB885407	−0.25	0.0033	10.43	Histone H2B	Growth
7684	CB816284	−0.45	0.0079	7.46	Histone H2B	Growth
7635	CB329465	−0.30	0.0063	10.43	Histone H2B	Growth
4531	CB885837	−0.37	0.0003	0.00	Histone H2B.4*	Growth
9293	CB816741	−0.28	0.0075	11.25	Histone H3	Growth
8835	CB329635	−0.21	0.0036	10.43	Histone H3	Growth
4403	CB240209	−0.50	0.0045	6.31	Histone H3.2	Growth
967	CD573156	−0.29	0.0177	14.45	Histone H4	Growth

TABLE 2. Continued

GEO gene ID no.	Accession no.	Average M value	*t* test P value	Q value	Function[a]	Gene ontology
3126	BM335824	−0.26	0.0032	9.23	Histone H4	Growth
4009	CA998687	−0.25	0.0116	14.10	Histone H4	Growth
3952	BM340564	−0.31	0.0038	9.23	Microtubial binding protein	Growth
3027	BQ401175	−0.28	0.0093	12.52	Myosin VII ZMM3*	Growth
6134	CB885567	−0.28	0.0109	14.10	Nucleosome/chromatin assembly factor group D	Growth
5376	CD058792	−0.25	0.0039	11.19	Nucleosome/chromatin assembly factor group D	Growth
2554	CD568644	−0.31	0.0123	12.52	Nucleosome/chromatin assembly factor group D	Growth
3287	CB617255	−0.43	0.0128	9.23	Pectin methylesterase	Growth
4678	CD651304	−0.35	0.0088	10.43	Translationally controlled tumor protein-like	Growth
1739	CB885730	−0.35	0.0134	10.43	Wiskott–Aldrich syndrome homolog protein*	Growth
6908	CB833484	−0.28	0.0205	12.52	Asparagine synthetase	Nitrogen utilization
1177	BM072930	−0.40	0.0116	9.23	Glutamate binding	Nitrogen utilization
1194	AF153448	−0.61	0.0352	10.43	Nitrate reductase	Nitrogen utilization
7689	CB617316	−0.19	0.0004	10.43	Nitrilase-associated protein	Nitrogen utilization
6324	CB351321	−0.39	0.011	10.43	Molybdopterin synthase large subunit	Nitrogen utilization
352	BM337403	−0.77	0.0374	10.43	Aluminum-induced protein	Nitrogen utilization
8981	CD651060	−0.40	0.0066	7.46	Phosphate translocator	Nitrogen utilization
6026	CB334432	−0.31	0.0165	10.43	Zinc binding protein	Nitrogen utilization
5568	AI586580	−0.49	0.0022	6.31	Zinc transporter	Nitrogen utilization
11968	AI586580	−0.45	0.0131	10.43	Zinc transporter	Nitrogen utilization
3171	BG840334	−0.39	0.0031	6.31	Ascorbate peroxidase	Oxidative stress
3179	BM074336	−0.25	0.0081	14.45	Catalytic/lactoylglutiathione lyase	Oxidative stress
6753	BM336474	−0.28	0.0141	14.45	Copper chaperone	Oxidative stress
7151	BM340758	−0.33	0.0042	9.23	Cu/Zn-superoxide dismutase copper chaperone	Oxidative stress
13516	AI948344	−0.32	0.0065	12.52	Cytochrome *b*5*	Oxidative stress
1975	BM073983	−0.29	0.0011	7.46	Cytochrome *c* oxidase subunit 6b-1*	Oxidative stress
9577	BM079052	−0.20	0.0005	7.98	Cytochrome *c* oxidase subunit VIa precursor	Oxidative stress
12942	AI715084	−0.34	0.0022	7.46	Cytochrome P450*	Oxidative stress
2367	AI586609	−0.27	0.0148	14.10	Cytochrome P450*	Oxidative stress
12834	AW288857	−0.58	0.0092	7.46	Ferredoxin	Oxidative stress
4426	CB334246	−0.44	0.0048	7.46	Glutaredoxin protein*	Oxidative stress
13337	AI714396	−0.36	0.0101	10.43	Heme oxygenase 2	Oxidative stress
11264	CB604596	−0.25	0.008	14.10	Mn-superoxide dismutase	Oxidative stress
2800	AI64924	−0.31	0.0007	6.31	Peroxidase TPA: class III	Oxidative stress
8000	BM073887	−0.34	0.0046	7.46	Peroxiredoxin Q	Oxidative stress
1526	BM078890	−0.39	0.0147	10.43	Phytoene desaturase*	Oxidative stress
5720	CB815651	−0.60	0.03	10.43	Violaxanthin de-epoxidase–related-like*	Oxidative stress
5357	CD568756	−0.46	0.035	14.45	DnaJ-related protein ZMDJ1	Protein catabolism
13746	AI715063	−0.55	0.0033	6.31	HSP 70	Protein catabolism
4205	BQ293373	−0.45	0.0182	10.43	HSP 70	Protein catabolism
4164	CD527170	−0.45	0.0272	14.10	HSP 70	Protein catabolism
4575	CD485168	−0.44	0.0172	10.43	HSP 70	Protein catabolism
8475	CB604177	−0.27	0.0061	11.25	HSP 70	Protein catabolism
6636	BQ172675	−0.55	0.0033	6.31	HSP 70*	Protein catabolism
3127	BG841073	−0.61	0.0362	11.19	HSP 82	Protein catabolism
5817	CD651664	−0.51	0.0136	9.23	HSP 82	Protein catabolism
955	CD001643	−0.32	0.0017	7.46	HSP 82	Protein catabolism
10448	CB381314	−0.34	0.0183	14.10	HSP 90-2	Protein catabolism
7342	CD058812	−0.54	0.0368	14.10	Leucyl/phenylalanyl–tRNA protein transferase*	Protein catabolism
3066	AW000454	−0.51	0.0201	10.43	Ribosomal protein L37a*	Protein catabolism
2779	BM073850	−0.26	0.0197	14.10	Ribosomal protein S0-B 40S*	Protein catabolism
5612	CA829909	−0.79	0.0086	6.31	Ubiquitin	Protein catabolism
191	CD573341	−0.63	0.0128	7.46	Ubiquitin	Protein catabolism
10086	CB605169	−0.51	0.0278	11.25	Ubiquitin	Protein catabolism
9327	CD568790	−0.39	0.0132	10.43	Ubiquitin	Protein catabolism
3226	CB329758	−0.30	0.0117	12.52	Ubiquitin 2	Protein catabolism
4163	CD527419	−0.59	0.0061	6.31	Ubiquitin 6	Protein catabolism
6494	CB617104	−0.34	0.0103	10.43	Ubiquitin 6	Protein catabolism
8146	CD001503	−0.25	0.0032	10.43	Ubiquitin 6	Protein catabolism
10931	CB885948	−0.55	0.0096	7.46	Ubiquitin 6*	Protein catabolism
5929	BM334196	−0.25	0.0137	14.45	Chloroplast 50S ribosomal protein L31	Protein synthesis
10929	CB885957	−0.33	0.0181	14.45	Elongation factor 1 alpha	Protein synthesis
2976	CD058872	−0.33	0.0028	6.31	Ribosomal protein 40S	Protein synthesis

Table 2. Continued

GEO gene ID no.	Accession no.	Average M value	*t* test P value	Q value	Function[a]	Gene ontology
4467	CB604886	−0.46	0.0085	7.46	Ribosomal protein L19	Protein synthesis
6115	B815941	−0.39	0.0105	9.23	Ribosomal protein L22 60S	Protein synthesis
7966	BM380712	−0.33	0.0147	9.23	Ribosomal protein L30 60S	Protein synthesis
1078	CD662115	−0.33	0.0089	10.43	Ribosomal protein L37a	Protein synthesis
3995	AI692072	−0.31	0.0076	10.43	Ribosomal protein S10	Protein synthesis
9297	CB616808	−0.28	0.0128	14.45	Ribosomal protein S15 40S	Protein synthesis
4139	CB885795	−0.21	0.0007	10.43	Ribosomal protein S19	Protein synthesis
4500	CB411212	−0.36	0.009	9.23	Ribosomal protein S2 40S	Protein synthesis
5522	BM350491	−0.29	0.0032	10.43	Ribosomal protein S5	Protein synthesis
9333	CB886116	−0.28	0.0047	10.43	Ribosomal protein S4	Protein synthesis
5771	CD527443	−0.25	0.0159	14.45	Translation initiaton factor 5A	Protein synthesis
2054	CB331357	−0.39	0.0012	6.31	Ankyrin protein	Signal transduction
7237	CB380560	−0.26	0.0081	10.43	Calmodulin*	Signal transduction
10894	CB605525	−0.30	0.0056	10.43	Cellular retinaldehyde–binding protein	Signal transduction
3160	BM347410	−0.40	0.0023	6.31	Cysteine-rich protein 2 binding protein*	Signal transduction
3533	BM080170	−0.39	0.0097	9.23	GF14-d protein	Signal transduction
6122	CB886313	−0.29	0.0018	7.46	GTP-binding ATARFC1	Signal transduction
4776	BM073471	−0.53	0.0025	0.00	GTP-binding protein typA	Signal transduction
779	BM073285	−0.35	0.0128	10.43	Guanylate cyclase	Signal transduction
17339	AI691787	−0.31	0.0062	14.10	Mitogen-activated protein kinase kinase 2*	Signal transduction
3298	CB411213	−0.35	0.0127	11.19	MYB family transcription factor*	Signal transduction
4408	CB239909	−0.29	0.0122	14.10	Pre-mRNA splicing factor*	Signal transduction
6052	CB351717	−0.28	0.005	10.43	Protein kinase	Signal transduction
6002	CB280782	−0.27	0.0013	7.46	Ras-GTPase activating protein SH3	Signal transduction
7664	CB605047	−0.27	0.0083	14.10	RNA methyltransferase*	Signal transduction
4658	BQ295746	−0.33	0.0103	10.43	RNA recognition motif	Signal transduction
15714	BE056987	−0.26	0.0155	14.45	RNA recognition motif*	Signal transduction
17715	BE056184	−0.25	0.0038	10.43	RNase L inibitor–like protein*	Signal transduction
1130	BM080478	−0.33	0.0045	10.43	S2P metalloprotease*	Signal transduction
6036	CB334257	−0.29	0.0044	10.43	Signal recognition particle receptor	Signal transduction
7541	BM340524	−0.36	0.003	7.98	TPR repeat:response regulator receiver*	Signal transduction
10532	CB885463	−0.26	0.0147	11.19	Ubiquitin/ribosomal protein CEP52	Signal transduction
6077	CB604306	−0.37	0.0077	9.23	Ubiquitin-conjugating enzyme	Signal transduction
4427	CB329392	−0.41	0.0045	7.46	Ubiquitin-conjugating enzyme	Signal transduction
6558	CB886348	−0.40	0.0269	14.45	Ubiquitin-conjugating enzyme	Signal transduction
6187	CD661947	−0.39	0.0036	7.46	Ubiquitin-conjugating enzyme	Signal transduction
8909	CB885271	−0.37	0.0245	14.45	Ubiquitin-conjugating enzyme	Signal transduction
6903	CB885254	−0.31	0.0128	12.52	Ubiquitin-conjugating enzyme OsUBC5a	Signal transduction
5629	CB329440	−0.44	0.0162	10.43	Ubiquitin-conjugating enzyme*	Signal transduction
1234	CB380710	−0.22	0.0077	14.10	Aux/IAA protein*	Signal transduction—auxin
1551	BM340861	−0.47	0.0008	0.00	Auxin-regulated protein	Signal transduction—auxin
7121	BM337420	−0.38	0.02	10.43	Auxin-regulated protein	Signal transduction—auxin
10634	BQ294261	−0.31	0.0012	6.31	Auxin-regulated protein–like*	Signal transduction—auxin
3141	BM072774	−0.27	0.0056	12.52	Xaa-Pro aminopeptidase 2 (auxin transport)*	Signal transduction—auxin
13689	AW455611	−0.46	0.0232	12.52	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
10939	CB886146	−0.44	0.0048	7.46	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
4648	CD662157	0.42	0.0066	7.46	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
9751	CD058650	−0.40	0.0094	9.23	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
9018	CD61506	−0.39	0.0024	6.31	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
3111	AW120026	−0.37	0.0061	7.46	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
7612	CA829996	−0.37	0.006	9.23	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
11254	CB334680	−0.34	0.001	6.31	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
7380	CD059053	−0.50	0.0029	6.31	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
7632	CB331830	−0.40	0.0159	10.43	Ubiquitin/ribosomal protein S27a	Signal transduction—auxin/sugar
3270	CB381177	−0.48	0.0113	9.23	Ubiquitin/ribosomal protein S27a*	Signal transduction—auxin/sugar
11614	CA829559	−0.34	0.0178	14.10	Ubiquitin/ribosomal protein S27a*	Signal transduction—auxin/sugar
11601	CB351414	−0.32	0.0062	9.23	Ubiquitin/ribosomal protein S27a*	Signal transduction—auxin/sugar
8878	CB381594	−0.27	0.0052	10.43	Ubiquitin/ribosomal protein S27a*	Signal transduction—auxin/sugar
5207	CB251906	−0.33	0.0183	14.10	*Erwinia*-induced protein 2*	Signal transduction—defense
7943	BG842691	−0.33	0.0111	11.19	Hydroxyproline-rich glycoprotein*	Signal transduction—defense
4448	CB351691	−0.37	0.011	10.43	Pectate lyase*	Signal transduction—defense
10335	BM080845	−0.41	0.0198	10.43	Pto kinase interactor 1–like	Signal transduction—defense
7600	BM340161	−0.41	0.0363	14.10	Floral organ regulator 1	Signal transduction—development

Table 2. Continued

GEO gene ID no.	Accession no.	Average M value	*t* test P value	Q value	Function[a]	Gene ontology
4524	CB886251	−0.34	0.022	14.45	Barren stalk 1	Signal transduction—development
2760	BG841647	−0.30	0.0229	14.10	Female sterile homeotic-related protein Frg-1*	Signal transduction—development
4435	CB329328	−0.36	0.0121	10.43	KNOX class homeodomain protein	Signal transduction—development
7011	BQ293485	−0.38	0.0011	6.31	Muscleblind-like 1 isoform b	Signal transduction—development
2793	AY313903	−0.31	0.0075	9.23	Yabby9 protein	Signal transduction—development
3135	BG840533	−0.40	0.0038	6.31	ASR2	Signal transduction—ethylene
8412	CA829370	−0.40	0.0371	14.45	EREBP-4–like protein	Signal transduction—ethylene
1596	AI691697	−0.30	0.0141	14.45	Ethylene-intensive 2	Signal transduction—ethylene
6175	CD585982	−0.39	0.0033	7.46	Ethylene-responsive transcriptional coactivator	Signal transduction—ethylene
766	BM379481	−0.51	0.0008	0.00	GDA2 Protein*	Signal transduction—ethylene
10577	CD484365	−0.29	0.0014	7.46	*S*-adenosylmethionine synthetase	Signal transduction—ethylene
7683	CB815961	−0.27	0.0085	12.52	DRE binding factor 2*	Signal transduction—stress
5158	BM339638	−0.26	0.0082	14.10	bHLH transcription factor PTF1	Signal transduction
4334	BM080214	−0.31	0.0132	10.43	DNA binding protein	Signal transduction
3174	BM079397	−0.27	0.006	9.23	Histone deacetylase complex subunit SAP18	Signal transduction
6038	CB280839	−0.38	0.0241	14.10	Methyl-binding domain protein	Signal transduction
2082	CB815511	−0.29	0.0162	11.19	Methyl-binding domain protein MDB106	Signal transduction
3627	CB329389	−0.34	0.0112	10.43	Nuclear movement protein–like	Signal transduction
4774	BM073558	−0.31	0.0017	6.31	Nucleic acid binding	Signal transduction
11668	CB604795	−0.37	0.0157	11.19	WRKY transcription factor 79	Signal transduction
5928	BM334349	−0.34	0.0257	12.52	Zinc finger protein–like	Signal transduction
379	BM072807	−0.29	0.0028	10.43	Zinc finger transcription factor ZFP30	Signal transduction
4731	BG840283	−0.26	0.0018	7.46	ABC transporter*	Transport/cell communication
8907	CB833905	−0.25	0.0019	9.23	Anchored to membrane*	Transport/cell communication
15211	AW621102	−0.33	0.0176	14.45	Apical junction molecule protein1*	Transport/cell communication
13920	AI948168	−0.43	0.0195	14.10	Bet1/Sft1-related SNARE*	Transport/cell communication
6223	BQ401137	−0.65	0.0279	10.43	Beta-1 3-glucanase–like*	Transport/cell communication
4605	BQ293597	−0.38	0.0138	10.43	Beta-1 3-glucanase*	Transport/cell communication
901	CB833833	−0.38	0.0063	7.98	Beta-1 3-glucanase*	Transport/cell communication
4587	CD662054	−0.32	0.004	9.23	Beta-1 3-glucanase*	Transport/cell communication
6190	CD527814	−0.30	0.0028	9.23	Integral membrane protein–like	Transport/cell communication
5473	AW000452	−0.27	0.022	14.45	Phospholemman chloride channel*	Transport/cell communication
5135	BM333785	−0.48	0.0305	14.10	Plasma membrane integral protein ZmPIP1-4	Transport/cell communication
8078	CB411061	−0.41	0.027	14.10	Plasma membrane MIP protein	Transport/cell communication
6136	CB885560	−0.24	0.0032	10.43	Epa4p*	Transport?
2012	CA829309	−0.30	0.0123	14.10	Epa5p*	Transport?
7825	BQ401315	−0.53	0.0208	9.23	Unknown function	Unknown
12889	AW455638	−0.49	0.0105	7.46	Unknown function	Unknown
3194	DQ017583	−0.47	0.0021	6.31	Unknown function	Unknown
9330	CB885440	−0.42	0.0259	14.10	Unknown function	Unknown
1153	BM336765	−0.39	0.0003	0.00	Unknown function	Unknown
4351	BM074715	−0.37	0.0194	10.43	Unknown function	Unknown
1578	BM073403	−0.36	0.0189	14.10	Unknown function	Unknown
1572	BM073604	−0.36	0.0132	10.43	Unknown function	Unknown
6130	CB885590	−0.35	0.02	14.10	Unknown function	Unknown
6374	BM079429	−0.35	0.0059	9.23	Unknown function	Unknown
3938	AW120367	−0.32	0.0015	6.31	Unknown function	Unknown
1629	CB331594	−0.31	0.0194	14.45	Unknown function	Unknown
5492	AW000149	−0.27	0.0168	14.45	Unknown function	Unknown
4359	BM332887	−0.26	0.0005	6.31	Unknown function	Unknown
3166	AI622802	−0.24	0.0175	14.45	Unknown function	Unknown
12373	BM074113	−0.39	0.0011	6.31	AprA*	
2763	AW331264	−0.38	0.0196	11.25	Esterase/lipase/thioesterase–like protein*	
5160	BM339253	−0.29	0.0211	14.10	FKBP-type peptidyl-prolyl *cis-trans* isomerase 3	
7737	CB886063	−0.33	0.0062	9.23	Gag-pol polyprotein*	
18065	BE012190	−0.27	0.007	12.52	Methyltransferase*	
18878	AW438275	−0.35	0.0243	14.45	r40cl protein*	
7696	CB617103	−0.59	0.0112	7.46	r40c2 protein	
6092	CB605136	−0.18	0.0005	12.52	Vgr-related protein*	
19167	AW062030	0.44	0.0002	0.00	Cinnamyl-CoA reductase*	Defense
17901	AI600652	0.43	0.0006	7.94	Wiskott–Aldrich syndrome protein interacting*	Growth
17899	AI861189	0.41	0.0005	0.00	HVA22 family*	Membrane transport
17969	AW066145	0.48	0.0029	10.43	Mitochondrial ribosomal protein L5*	Protein synthesis
18744	AI657257	0.47	0.0031	11.25	RNase L inhibitor protein–related*	Signal transduction

Table 2. Continued

GEO gene ID no.	Accession no.	Average M value	*t* test P value	Q value	Function[a]	Gene ontology
19170	AW244929	0.48	0.0008	0.00	Trithorax group portein osa*	Signal transduction
17958	AI657220	0.52	0.0005	0.00	Zein-alpha precursor*	Storage protein
11504	AW331807	0.37	0.0005	7.94	Conserved hypothetical membrane protein*	Transport
18354	AI649963	0.42	0.0008	10.43	Unknown function	Unknown
17979	AW066383	0.47	0.0025	10.43	Unknown function	Unknown
18774	AW066036	0.47	0.0008	0.00	Unknown function	Unknown
16292	AI948113	0.59	0.0059	11.25	Unknown function	Unknown
17968	AW060065	0.81	0.0006	0.00	Unknown function	Unknown

[a] Abbreviations: ABC, ATP-binding cassette; ADP, adenosine 5′-diphosphate; ATP, adenosine triphosphate; bHLH, basic helix–loop–helix; DRE, Drought Responsive Element; EREBP, ethylene-responsive element binding protein; GTP, guanosine-5′-triphosphate; NAD, nicotinamide adenine dinucleotide; NADH, reduced NAD; NADP, NAD phosphate; NADPH, reduced NADP; TPA, tissue plasminogen activator; TPR, tetratrico peptide repeat.

tition. Analysis of expression data in mouse-ear cress from genes similar to the differentially expressed ubiquitin/ribosomal protein S27a suggests that this gene is regulated by auxin and possibly cytokinin (data not shown). Evidence also suggests that ubiquitin/ribosomal protein S27a might be regulated by various treatments that specifically affect cell division (Bassani et al. 2004). Genes involved in ethylene signaling/responses were also down-regulated by velvetleaf competition (Table 2). Previous findings indicated that both auxin and ethylene signaling responses are altered by shade avoidance responses (Devlin et al. 2003; Pierik et al. 2004; Vandenbussche et al. 2003). Because leaves sampled in this study were above the velvetleaf canopy, either the observed differences were maintained after the crop had escaped the shading effect of the weed or shading of the lower portions of the plant is capable of inducing systemic responses. On the basis of his observations, C. J. Swanton (personal communication) hypothesizes that many detrimental effects of weeds induced early in crop–weed interactions are permanent, even if the weeds subsequently are removed. Indeed, some observations suggest that weeds cause an irreversible reduction in yield early in development (Zimdahl 2004).

Gene Expression Induced by Velvetleaf Competition

It is significant that many fewer genes were induced by the presence of velvetleaf in the topmost leaf than those that were repressed. This suggests a very limited direct response of corn to deal with competition from velvetleaf. On the basis of recommendations of the producers of the corn microarrays (Iowa State University), the genes up-regulated by velvetleaf competition had no known functions on the basis of their sequence analysis. However, when the publicly available sequence data from the cDNAs that were spotted were used to identify longer expressed sequence tags from other databases, several genes were identified. Cinnamoyl–coenzyme A reductase is known to play a role in lignification and cell defense (Lacombe et al. 1997). Additionally, the induction of mitochondrial ribosomal protein L5a might imply a possible alteration in posttranscriptional regulation of some ribosomal proteins, indicating a potential mechanism by which velvetleaf competition could alter gene expression in corn that might not be detectable at the transcriptional level.

A gene encoding a protein that interacts with the Wiskott–Aldrich syndrome protein was up-regulated. It is particularly interesting because a putative homologue of the Wiskott–Aldrich syndrome protein was down-regulated. Both of these genes are involved in growth and cytoskeletal reactions in animal systems (Symons et al. 1996). This suggests that the Wiskott–Aldrich syndrome signaling system might be conserved in corn and that it is altered in velvetleaf competition responses. Given the function of these genes in regulation of growth in animals and the observations of growth perturbation in corn competing with velvetleaf, it is possible that these genes play a role in corn growth as well. Unfortunately, too few genes are up-regulated to see any clear pattern of physiological processes being activated by velvetleaf competition. More information is needed on the function of the genes that are up-regulated by velvetleaf competition, and additional up-regulated genes are needed to develop a clearer picture of the direct effect of velvetleaf competition. Hopefully, future studies will provide a better characterization of the function of the other genes induced by velvetleaf competition.

Conclusions and Future Perspectives

These data indicate that changes in gene expression were observable in corn during late-season velvetleaf competition. These differences were observed in about 3% of the genes with BlastX hits despite that sampled leaves were not under direct competition for light, and corn was well past the critical period for weed effects and plants were sampled at different times and field locations. Significant clusters of genes involved in specific physiological processes were identified. Genes involved in carbon and nitrogen utilization and photosynthesis, cell growth and development, signal transduction, and oxidative stress were shown to be preferentially repressed in corn plants grown in competition with velvetleaf. Surprisingly, with the exception of a few genes involved in zinc transport that showed repression by velvetleaf, relatively few genes involved in micronutrient sequestration or accumulation were induced by velvetleaf competition. These data suggest that velvetleaf might not compete strongly with corn for micronutrients or phosphorous. However, ample evidence suggests that velvetleaf, even at this late developmental stage can negatively effect N levels and metabolic activity in the crop. However, additional experiments in which velvetleaf removal occurs before or just after the critical weed-free period will be required to determine when

and whether these changes are caused by direct competition of the crop with velvetleaf or whether they are the result of chromatin remodeling or initial damage caused by exposure to weed competition.

The availability of microarrays for corn and other crops should open up the possibility to answer many of the fundamental questions concerning crop–weed interactions that have been intractable until now. For example, such data can be used for hypothesis generation and to design more specific experiments to study the physiological mechanisms of crop–weed interactions at the molecular level. Differential gene expression patterns could also be useful in discerning the relative importance of multiple resources for which crops compete. Such experiments should provide much-needed information to improve the competitive ability of crop genotypes. In addition, this information is needed to develop robust models of crop–weed interactions to better understand and predict the consequences of weed competition and interference. Such models will be invaluable tools for delineating critical weed-free periods, enhancing timing of weed control measures, and reducing interference of weeds in the crop by modifying management practices.

Sources of Materials

[1] Europa 20-20 ratio mass spectrometer, SerCon, Wistaston Road, Crewe, Cheshire CW2 7RP, U.K.

[2] Alexa Fluor cDNA labeling kit (A32755), Invitrogen Life Technologies Inc., 1600 Faraday Avenue, Carlsbad, CA 92008.

[3] SAM1.1 19,200-element corn chips (SAM1.1) Center for Plant Genomics, Iowa State University, Ames, IA 50011.

[4] Affy428 scanner and Jaguar software, Affymetrix Inc., 3380 Central Expressway, Santa Clara, CA 95051.

[5] GeneMath XT 1.5, Applied Maths Inc., 512 East 11th Street, Suite 207, Austin, TX 78701.

[6] SAM 1.22 (http://www-stat.stanford.edu/~tibs/SAM/index.html) Stanford University Labs, Stanford, CA 94305.

Acknowledgments

We thank Jon Kleinjan for his technical assistance. This research was partially funded by South Dakota Agricultural Experiment Station, South Dakota Corn Council, and the South Dakota State University Griffith Faculty Research Award.

Literature Cited

Ballaré, C. L. and J. J. Casal. 2000. Light signals perceived by crop and weed plants. Field Crops Res. 67:149–160.

Ballaré, C. L., R. A. Sánchez, A. L. Scopel, J. J. Casal, and C. M. Ghersa. 1987. Early detection of neighbor plants by phytochrome perception of spectral changes in reflected sunlight. Plant Cell Environ. 10:551–557.

Ballaré, C. L., A. L. Scopel, and R. A. Sánchez. 1990. Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. Science 247:329–332.

Bassani, M., P. M. Neumann, and S. Gepstein. 2004. Differential expression profiles of growth-related genes in the elongation zone of corn primary roots. Plant Mol. Biol. 56:367–380.

Blackshaw, R. E., G. Semach, and H. H. Janzen. 2002. Fertilizer application method affects nitrogen uptake in weeds and wheat. Weed Sci. 50:634–641.

Bonifas, K. D. and J. L. Lindquist. 2006. Predicting biomass partitioning to root versus shoot in corn and velvetleaf (*Abutilon theophrasti*). Weed Sci. 54:133–137.

Bosnic, C. A. and C. J. Swanton. 1997. Influence of barnyardgrass (*Echinochloa crus-galli*) time of emergence and density on corn (*Zea mays*). Weed Sci. 45:276–282.

Bryson, C. T. 1990. Interference and critical time of removal of hemp sesbania (*Sesbania exaltata*) in cotton (*Gossypium hirsutum*). Weed Technol. 4:833–837.

Chang, S., J. Puryear, and J. Cairney. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep. 11:113–116.

Churchill, G. A. 2002. Fundamentals of experimental design for cDNA microarrays. Nat. Genet. 32(Suppl.):490–495.

Clay, D. E., S. A. Clay, D. L. Lyons, and J. M. Blumenthal. 2005a. ^{13}C discrimination in corn grain can be used to separate and quantify yield losses due to water and nitrogen stresses. Weed Sci. 53:23–29.

Clay, S. A., K. R. Banken, F. Forcella, M. M. Ellsbury, D. E. Clay, and A. E. Olness. 2006. Influence of yellow foxtail on corn growth and yield. Comm. Soil Sci. Plant Anal. 37:1421–1435.

Clay, S. A., J. Kleinjan, D. E. Clay, F. Forcella, and W. Batchelor. 2005b. Growth and fecundity of several weed species in corn and soybean. Agron. J. 97:294–302.

Colton, C. E. and F. A. Einhellig. 1980. Allelopathic mechanisms of velvetleaf (*Abutilon theophrasti* Medic., Malvaceae) on soybean. Am. J. Bot. 67:1407–1413.

Cousens, R., P. Brain, J. T. O'Donovan, and P. A. O'Sullivan. 1987. The use of biologically realistic equations to describe the effects of weed density and relative time of emergence on crop yield. Weed Sci. 35: 720–725.

Cousens, R. D. 1985. A simple model relating yield loss to weed density. Ann. Appl. Biol. 107:239–252.

Devlin, P. F., M. J. Yanovsky, and S. A. Kay. 2003. A genomic analysis of the shade avoidance response in *Arabidopsis*. Plant Physiol. 133:1617–1629.

Evans, S. P., S. Z. Knezevic, J. T. Lindquist, and C. A. Shapiro. 2003. Influence of nitrogen and duration of weed interference on corn growth and development. Weed Sci. 51:546–556.

Fey, V., R. Wagner, K. Braütigam, M. Wirtz, R. Hell, A. Dietzmann, D. Leister, R. Oelmüller, and T. Pfannschmidt. 2005. Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*. J. Biol. Chem. 280:5318–5328.

Hall, M. R., C. J. Swanton, and G. W. Anderson. 1992. The critical period of weed control in grain corn (*Zea mays*). Weed Sci. 40:441–447.

Kropff, M. J. and H. H. van Laar. 1993. Modelling Crop-Weed Interactions. Wallingford, Oxon, Great Britain: CAB International. 274 p.

Lacombe, E., S. W. Van Hawkins, J. Doorsselaere, J. Piquemal, D. Goffner, O. Poeydomenge, A. M. Boudet, J. Grima-Pettenati. 1997. Cinnamoyl-CoA reductase, the first committed enzyme of the lignin branch biosynthetic pathway: cloning, expression and phylogenetic relationships. Plant J. 11:429–441.

Lindquist, J. L. 2001. Performance of INTERCOM for predicting corn–velvetleaf interference across north-central United States. Weed Sci. 49:195–202.

Lindquist, J. L., D. A. Mortensen, and B. E. Johnson. 1998. Mechanisms of corn tolerance and velvetleaf suppressive ability. Agron. J. 90:787–792.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS systems for mixed models. Cary, NC: SAS Institute Inc. 633 p.

McDonald, A. J., S. J. Riha, and C. L. Mohlerl. 2004. Mining the record: historical evidence for climatic influences on maize–*Abutilon theophrasti* competition. Weed Res. 44:439–445.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405–410.

Norsworthy, J. K. and M. J. Oliveira. 2004. Comparison of the critical period for weed control in wide- and narrow-row corn. Weed Sci. 52: 802–807.

Pierik, R., M.L.C. Cuppens, L.A.C.J. Voesenek, and E.J.W. Visser. 2004. Interactions between ethylene and gibberellins in phytochrome-mediated shade avoidance responses in tobacco. Plant Physiol. 136:2928–2936.

Rajcan, I., K. J. Chandler, and C. J. Swanton. 2004. Red–far-red ratio of reflected light: a hypothesis of why early-season weed control is important in corn. Weed Sci. 52:774–778.

Reyes, J. C., L. Hennig, and W. Gruissem. 2002. Chromatin-remodeling and memory factors. New regulators of plant development. Plant Physiol. 130:1090–1101.

Samarakoon, S. P., J. R. Wilson, and H. M. Shelton. 1990. Growth, morphology and nutritive quality of shaded *Stenotaphrum secondatum*, *Axonopus compressus* and *Pennisetum clandestinum*. J. Agric. Sci. 114:161–169.

Sattin, M., G. Zanin, and A. Berti. 1992. Case study for weed competition/

population ecology: velvetleaf (*Abutilon theophrasti*) in corn (*Zea mays*). Weed Technol. 6:213–219.
Scholes, C., S. A. Clay, and K. Brix-Davis. 1995. Velvetleaf (*Abutilon theophrasti*) effect on corn (*Zea mays*) growth and yield in South Dakota. Weed Technol. 9:665–668.
Steindler, C., A. Matteucci, G. Sessa, T. Weimar, M. Ohgishi, T. Aoyama, G. Morelli, and I. Ruberti. 1999. Shade avoidance responses are mediated by the ATHB-2 HD-Zip protein, a negative regulator of gene expression. Development 126:4235–4245.
Sterling, T. M., R. L. Houtz, and A. R. Putnam. 1987. Phytotoxic exudates from velvetleaf (*Abutilon theophrasti*) glandular trichomes. Am. J. Bot. 74:543–550.
Symons, M., J. M. Derry, B. Karlak, S. Jiang, V. Lemahieu, F. McCormick, U. Francke, and A. Abo. 1996. Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. Cell 8:723–734.
Teasdale, J. R. 1995. Influence of narrow row/high population corn (*Zea mays*) on weed control and light transmittance. Weed Technol. 9:113–118.
Tian, Q. and J. W. Reed. 2001. Molecular links between light and auxin signaling pathways. J. Plant Growth Regul. 20:274–280.
Tollenaar, M., S. P. Nissanka, A. Aguilera, S. F. Weise, and C. J. Swanton. 1994. Effects of weed interference and soil nitrogen on four maize hybrids. Agron. J. 62:15–17.
Van Acker, R. C., C. J. Swanton, and S. F. Weise. 1993. The critical period of weed control in soybean [*Glycine max* (L.) Merr.]. Weed Sci. 41: 194–200.
Vandenbussche, F., W. H. Vriezen, J. Smalle, L.J.J. Laarhoven, F.J.M. Harren, and D. Van Der Straeten. 2003. Ethylene and auxin control the *Arabidopsis* response to decreased light intensity. Plant Physiol. 133: 517–527.
Werner, E. L., W. S. Curran, J. K. Harper, G. W. Roth, and D. P. Knievel. 2004. Velvetleaf (*Abutilon theophrasti*) interference and seed production in corn silage and grain. Weed Technol. 18:779–783.
Weston, L. A. and S. O. Duke. 2003. Weed and crop allelopathy. Crit. Rev. Plant Sci. 22:367–389.
Wolfinger, R. D., G. Gibson, E. D. Wolfinger, L. Bennett, H. Hamadeh, P. Bushel, C. Afshari, and R. S. Pauls. 2001. Assessing gene significance form cDNA microarray expression data via mixed models. J. Comp. Biol. 8:625–637.
Wong, C. C. and J. R. Wilson. 1980. Effects of shading on the growth and nitrogen content of green panic and siratro in pure and mixed swards defoliated at two frequencies. Aust. J. Agric. Res. 31:269–285.
Zimdahl, R. L. 2004. Weed–Crop Competition. A Review. 2nd Ed. Ames, IA: Blackwell Publishing. 220 p.

Received June 2, 2006, and approved July 26, 2006.