Varying Weed Densities Alter the Corn Transcriptome, Highlighting a Core Set of Weed-Induced Genes and Processes with Potential for Manipulating Weed Tolerance

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ABSTRACT The phenological responses of corn (Zea mays L.) to competition with increasing densities of winter canola (Brassica napus L.) as the weedy competitor were investigated. Changes in the corn transcriptome resulting from varying weed densities were used to identify genes and processes responsive to competition under controlled conditions where light, nutrients, and water were not limited. Increasing densities of weeds resulted in decreased corn growth and development and increased the number and expression intensity of competition-responsive genes. The physiological processes identified in corn that were consistently induced by competition with weeds included protein synthesis and various transport functions. Likewise, numerous genes involved in these processes, as well as several genes implicated in phytochrome signaling and defense responses, were noted as differentially expressed. The results obtained in this study, conducted under controlled (greenhouse) conditions, were compared with a previously published study where the response of corn to competition with other species was evaluated under field conditions. Approximately one-third of the genes were differentially expressed in response to competition under both field and controlled conditions. These competition-responsive genes represent a resource for investigating the signaling processes by which corn recognizes and responds to competition. These results also highlight specific physiological processes that might be targets for mitigating the response of crops to weeds or other competitive plants under field conditions.

Core Ideas

- Corn increases the number of differentially expressed genes and the intensity of differential gene expression in response to increasing weed density.
- Genes associated with kinase signaling and transport functions are upregulated by weeds.
- Genes associated with protein production are downregulated by weeds.
- A sugar transporter (PMT5) and NUCLEOREDOXIN 1 are upregulated by weeds under diverse conditions.

Cover crops are increasingly being used to reduce weeds and mitigate the loss of soil nutrients (Daryanto et al., 2018). However, cover crops, like weeds, can reduce yield in double- and relay-cropping systems if they are left on the field during the critical period for weed control. Yield loss can be compensated if the cover crop itself has economic value as a cash crop or provides value-added ecosystem services (Gesch et al., 2015). Because of their early maturation and over-wintering abilities, brassica oilseed crops, such as winter canola and...
winter camelina \(\text{Camelina sativa} \ (L.) \text{Crantz.}\), have been suggested as potential cover crops (Eberle et al., 2015). Although such cash cover crops can increase the growers’ profits, reducing yield losses in double- or relayed-crop systems because of competition is desirable and would enhance growers’ adoption of these critical practices for establishing sustainable agricultural intensification.

Understanding how weeds or cover crops interact with and reduce crop yield is important for developing methods and selective regimes for mitigating yield losses. Several studies have indicated the weeds can reduce crop yields through mechanisms other than direct competition for resources (Page et al., 2009; Afifi and Swanton, 2012). There is a long-observed phenomenon known as the critical period for weed control, which indicates weeds that have their greatest impact on crop yield early in the growing season. Competition for resources are low during this period, as nutrient and water levels in the soil are not limiting and weeds are generally of smaller stature than the crops and thus are not competing for light (Zimdahl, 1988). However, even if weeds are removed from the field after the critical period for weed control, crops are unable to recover and often show developmental delays compared with crops grown under weed-free conditions (Knezevic et al., 2002; Moriles, 2011; Page et al., 2012; Horvath et al., 2018). Additionally, it has often been observed that weeds can have significant impacts on growth and yield at low densities. However, crop responses to competition tend to reach a maximum and further increases in weed density beyond that point have minimal impact on crop growth and yield (Cousens, 1985). If weed-induced yield losses were a direct result of resource competition, yield loss would be more linearly associated with weed density. Therefore, weed-induced yield loss is hypothesized to occur as a result of crop developmental responses following perception of nearby weeds (Liu et al., 2009). Blocking the ability of crops to perceive nearby weeds could reduce yield losses. Gaining a better understanding of the nature of crop–weed interactions could provide targets for manipulating this process.

Far-red light is the best studied signal associated with plant–plant competition. Indeed, increased ratios of far-red light perceived by crops growing in the presence of weeds induce oxidative stress responses that damage the plant and inhibit photosynthetic processes (Ballaré and Pierik, 2017). Other studies have indicated that enrichment of far-red light from nearby weeds increases the concentrations of singlet oxygen (McKenzie-Gopsill et al., 2019). These responses occur even when there is no direct contact or shading between the crops and the weeds (Liu et al., 2009). However, there are other signals, including soil and volatile signals, that are likely to impact crop–weed competition (Ballaré and Pierik, 2017).

Transcriptomic studies provide an appropriate approach to investigating the physiological, developmental, and signaling processes associated with environmental stresses. Transcriptomic analyses of corn growing in the presence or absence of weeds under field conditions have implicated defense responses including salicylic acid (SA) signaling, phytochrome signaling, and nitrogen utilization and transport as processes that are altered by plant competition (Moriles et al., 2012; Horvath et al., 2018). However, the variation in global gene expression between years probably masks many significant transcriptome differences and thus very few genes, physiological processes, and signaling processes have been identified. Although these differences observed are highly robust, it is likely that many less robust weed-induced responses have been missed.

Previous field studies have indicated that increasing weed densities beyond a certain threshold level did not result in increased developmental responses (Cousens, 1985). However, it is still unclear if higher weed densities would cause more intense transcriptomic responses or alter additional physiological processes under controlled conditions. To avoid the issues regarding variability in field conditions and to further explore the processes that are responsive to weed densities, we performed transcriptomic analyses of corn growing under differing weed densities under controlled conditions.

**MATERIALS AND METHODS**

**Plant Material**

Corn and canola were planted simultaneously and grown in 4-L pots in potting soil (Sunshine Mix #1, Sun Gro Horticultural Distributions Inc., Bellevue, WA) in a greenhouse with supplemental lighting under a 14 hr light–10 hr dark photoperiod. Plants were fertilized weekly with half-strength Hoaglands solution (Hoagland and Arnon, 1950). For analysis of variation in response to weed or crop genotype, and for the statistical power analysis, two commercial corn lines (‘13D91’ and ‘16S92’) were used. Likewise, the competitor lines were one of two commercial varieties of winter canola (‘Lembkes’ and ‘Wichita’). For analysis of weed density responses, only corn line 13D91 and canola line Wichita were used, since there was no significant difference between lines or their interactions. The experimental design for the initial test for statistical power analysis and genotype interaction was a random complete block design with eight replicate blocks of three treatments (two different varieties of canola as the “weed” plus a no-weed control) and two crop genotypes for a total of six pots per block. For the weed density study, the experiment was a random complete design with six replicate blocks of four treatments (zero, two, four, or six weeds) for a total of four pots per block (see Supplemental Fig. S1 for a photograph of a representative block). All experiments were replicated twice. When plants were 8 wk old, the stem diameter (the widest dimension of the stem at the middle of the internode on the second internode above the cotyledon) was measured with calipers. Plant height was measured from the soil level to the tip of the youngest partially expanded leaf. Leaf number, not counting the cotyledon leaf, was counted if the leaf had a visible collar. Leaf area was measured with a LiCor 2000 instrument (Li-Cor, Lincoln,
NE) (all counted leaves were measured along with emerging leaves for corn from each individual plant). The fresh weight of aboveground plant parts was also measured.

RNA-seq Analysis
The distal 15 cm of the topmost fully expanded leaf of the 8-wk-old corn plants were harvested between 10:00 AM and 12:00 PM into liquid N₂. RNA was extracted from each plant via a modified pine tree RNA extraction protocol (Chang et al., 1993). Equal amounts of total RNA from two plants were pooled for each biological replicate. Three biological replicates for each treatment were collected. RNA from each biological replicate was used to create individually tagged RNAseq libraries using the New England Biolabs Next ultra-directional RNAseq library kit for Illumina (New England Biolabs, Ipswich, MA). These libraries were pair-ended sequenced with 100 bases per end with Illumina 2500 technology (Novogene Corporation Inc. Beijing, China). The raw data and metadata are available for download from the National Center for Biotechnology Information (accession # PRJNA542358). The resulting sequences were quality trimmed via the HTProcess trimming pipeline in the CyVerse discovery environment (Oliver et al. 2013) with parameters set to a minimum quality of 20 and a minimum length of 70 bases. The resulting reads were mapped to the reference corn genome by the RMTA_v1.6 program (Li and Dewey, 2011) (with the reference genome input being Zea.AGPv4. fasta and the reference annotations being Zea.AGPv4.gff3) in the CyVerse discovery environment. The resulting merged gtf file and individual bam files were used to run Cuffdiff-16-way-max-2.2.1 to identify significant pairwise expression differences (q-values) of all merged transcripts. Genes were considered differentially expressed if they were significant (q-values less than 0.05) and had a fragments per kilobase per million reads value of >2 for all biological replicates of at least one treatment in both repeats of the experiment in any given comparison to the control treatment (two weeds vs. control, four weed vs. control, and six weeds vs. control).

Gene Set and Subnetwork Enrichment Analysis
Fragments per kilobase per million reads values for the expressed transcripts (>2) from individual replicates for each treatment were fed into the Pathway Studio version 9.6 program (Elsevier, Amsterdam, The Netherlands) for analysis of significant over-representation of ontologies associated with various biochemical and signaling pathways and functions.

Statistical Analysis
Prospective power and sample size analysis was performed with an exemplary pilot dataset to optimize the resource usage and design of this study. We used the GLMPOWER procedure in SAS version 9.4 SAS/STAT 14.3 (SAS Institute Inc., Cary, NC) to perform prospective power and sample size analyses based on linear models, which also included post-hoc between-subject contrasts of the treatment effects of interest. The minimum power of the test was established at 0.8 but a range of power of 0.5 to 0.9 was also explored. For all other statistics, mean and SD for six replicates per measured parameter was determined using Microsoft excel.

RESULTS
Statistical Power Analysis and Differences between Crop and Weed Genotypes
The results indicated that six blocks provided sufficient statistical power to observe all treatment differences (Table 1 and Supplemental File SI). These initial analyses also indicated that any genotypic differences between the two crop varieties tested were insignificant. Likewise, responses to the different genotypes of canola were also usually insignificant (Supplemental File SI). Treatments generally resulted in insignificant differences when measured at 4 wk after planting; however, all attributes except leaf number had significant treatment effects at 8 wk. Based on these results, all further experiments were carried out with six replicates and a single corn and a single canola genotype and all subsequent plant attributes were measured at 8 wk after germination.

Increasing Weed Density Results in Greater Impacts on Corn Growth and Development
For plant height, leaf area, and fresh weight, all weed densities were significantly different from the control, with six competing plants having a greater impact than just two (Fig. 1A,D,E; Supplemental File S2). Stem diameter showed significant differences from the control in all treatments in Experiment 1 (Fig. 1C). However, in Experiment 2, the difference did not meet our significance criteria for the same comparison, although the trend was similar to that in Experiment 1. For leaf number, minimal differences were observed (Fig. 1B). These results indicate that increasing weed density incrementally reduces growth and development in corn.

Weed Presence Alters Transcriptome Responses in Corn
Approximately 22,000 transcripts mapped to the maize reference genome with an fragments per kilobase per
million reads value of >2 in all replicates of at least one treatment in both experimental repeats (Supplemental File S2). Of these, only 1045 were not mapped to previously characterized genes in the Maize version 4 assembly (Jiao et al., 2017). Among the 22,000 expressed transcripts, 875 were differentially expressed genes (q-value < 0.05) (Fig. 2), relative to the no-competition control in both experimental runs (Supplemental File S3). Of the 875 differentially expressed corn transcripts, 22, 111, and 360 were uniquely expressed at two, four, and six weeds per pot respectively. Twenty-one were differentially expressed at two and four weeds per pot and 34 were differentially expressed at two and six weeds per pot. One hundred and fifty-seven were differentially expressed at both four and six weeds per pot. Among the differentially expressed genes, 170 were common in any tested comparison to the control. Of the 875 differentially expressed genes, the majority (88–65% in Experiments 1 and 2 respectively) had the same trend in both experimental replications and had a higher magnitude of gene expression in response to increasing weed density. The absolute expression differences (treated vs. control) for all such genes had a general upwards change in expression intensity from two weeds to six weeds (Fig. 3).

**Gene Set Enrichment Analysis**

Gene set enrichment analysis identified 133 and 150 different ontologies that were significant on the basis of the differential expression levels of all identified and annotated genes in Experiments 1 and 2, respectively (Supplemental File S3). Of these, 56 were identified as significant in
both experiments, although 11 of these had differences in the direction of their mean expression pattern. When the expression was segregated into genes that were upregulated, 88 and 93 ontologies were significantly associated with upregulated genes in Experiments 1 and 2 respectively, and 81 and 205 ontologies were significantly associated with downregulated genes in Experiment 1 and 2 respectively. Of these, only 24 and 22 were associated in both experiments for up- and downregulated genes respectively (Table 2).

Weed-Induced Corn Responses under Field and Controlled Conditions Identifies a Core Set of Overlapping Genes and Processes

The transcriptome changes associated with weed presence under field conditions have been reported previously (Horvath et al., 2018). In the previous study, very few genes (six upregulated and 19 downregulated) were significantly differentially expressed in both 2007 and 2008. However, of these 25 differentially expressed genes, eight were also differentially expressed with the same trend in the present controlled studies (Table 3). Likewise, 11 and 9 ontologies were significantly associated with up- and downregulated genes, respectively, in both the field and controlled studies (Table 4). These genes and ontologies are thus considered to be robustly weed-responsive under many different environmental conditions and represent targets for investigating and manipulating the response of corn to weeds.

DISCUSSION

Increasing Weed Density Leads to Increased Changes in Gene Expression

Here, we examined the differential expression of genes that occurs when corn plants were grown in the same pots with varying numbers of competitors: winter canola in this case. It should be noted that in these greenhouse studies, differences in gene expression could be caused by any variation in the corn’s environment caused by the weeds. This may include direct and indirect responses to weed-produced signals or changes in the available soil volume. To identify the genes that are responsive specifically to weed-produced signals, it is important to identify the genes that were differentially regulated in both pot- and field-grown plants, as noted in our comparison studies. That said, in this study, over 800 genes were differentially expressed in response to weed competition under controlled conditions. Almost 250 differentially expressed genes were observed when just two weeds were grown with corn in the same pot. Further,
as the intensity of the weed pressure on corn increased, so did the number of genes and the magnitude of their differential expression. This data may indicate that gene expression is directly proportional to the intensity of the competition stress, implying that receptor mechanisms responsive to the weed signal(s) can detect the density of weeds present. Cousins (1985) reported that the growth inhibition response to weed pressure generally appears to have a peak threshold. Although differences in gene expression continued to increase in response to weed density, for most tested growth parameters, having six weeds per pot was not significantly different from four weeds per pot. Thus, further research is needed to determine if we reached the threshold noted by Cousins.

### Consistent Gene Ontologies are Associated with the Corn Response to Weeds

Under field conditions, corn’s responses to weeds implied the induction of defense responses, probably likely mediated by SA, phytochrome signaling, and downregulation of N utilization and growth (Horvath et al., 2018). Neither SA nor phytochrome signaling were strongly associated with responses to weeds under controlled conditions, according to this gene set enrichment analysis. Because the database used by the gene set enrichment program was modified since the field studies by Horvath et al. (2018) were done, the field data were rerun with the new database. The new analysis did not identify SA and phytochrome signaling as being associated with the responses of corn to competition under either field or controlled conditions. Despite this discrepancy, some ontologies were still significantly associated with weed presence under both field and controlled conditions. These included ontologies associated with transport functions and kinase activity among the upregulated genes and ontologies associated with protein translation among the downregulated genes (Table 4). Kinase activity is often involved in signaling processes in both plant and animal systems. However, there is little information available for discerning the specific signaling processes impacted by weed presence. Interestingly though, a fair number of lectin kinases were consistently upregulated in response to weeds under controlled conditions (Supplemental File S3). These genes are associated with defense responses that include responses to SA in Arabidopsis thaliana (L.) Heynh. according to information on The Arabidopsis Information Resource website (https://www.arabidopsis.org/, accessed 2 Oct. 2019). This observation is thus consistent with the previously observed association between SA and weed presence (Horvath et al., 2018; Rivas-San Vicente and Plasencia, 2011; de Wit et al., 2013) and perhaps adds some additional information regarding the mechanisms by which SA impacts physiological processes when weeds are present. Multiple ontologies associated with transporter functions were identified as significant under both field and controlled conditions. One noteworthy gene identified as being upregulated in both field and controlled conditions encodes polyol/monosaccharide transporter 5 (PMT5). There were at least two other sugar transporter genes that were differentially expressed under controlled conditions [one golgi nucleotide sugar transporter 3 (GONST3), and an apparent paralog of PMT5]. Furthermore, a number of amino acid and protein transporters were differentially expressed under our controlled conditions. This result is consistent with previous observations indicating that N accumulation is disrupted by weed presence under field conditions (Horvath et al., 2018). Finally, a fair number of metal transporters were also significantly induced in this study (Supplemental File S3). These metal transporters may implicate the potential impact of weed pressure on nutrient movement in corn, which could limit growth and development. As was the case with N, it seems unlikely

### Table 4. Over-represented ontologies among genes that were differentially expressed (q < 0.05) under both controlled and field conditions.

<table>
<thead>
<tr>
<th>Function</th>
<th>Expression trend†</th>
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<tbody>
<tr>
<td>Polyol/monosaccharide transporter 5</td>
<td>Up</td>
</tr>
<tr>
<td>DC1 domain-containing protein</td>
<td>Up</td>
</tr>
<tr>
<td>Ribosomal protein S21 family protein</td>
<td>Down</td>
</tr>
<tr>
<td>Tetrameric repeat-like superfamily protein</td>
<td>Down</td>
</tr>
<tr>
<td>Tetrameric repeat-like superfamily protein</td>
<td>Down</td>
</tr>
<tr>
<td>Chloroplast 3-amylose</td>
<td>Down</td>
</tr>
<tr>
<td>Regulator of chromosome condensation family protein</td>
<td>Down</td>
</tr>
<tr>
<td>Unknown</td>
<td>Down</td>
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<tr>
<td>Unknown</td>
<td>Down</td>
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† The expression trend is compared with the no-weed control.
that these nutrients were limiting, as plants were fertilized weekly throughout their growth.

Gene set enrichment analysis of the corn genes down-regulated by weed pressure under both field and controlled conditions identified several ontologies implicated in various processes involving protein production (Table 2, Table 4). Previous studies have noted considerable similarities in the transcriptomes of corn responding to low N and transcriptomes responding to weed pressure (Moriles et al., 2012). Given the role of N availability in protein production, these observations may be related to the amino acid and protein transport-associated ontologies noted above. However, only one putative protein production-associated gene (encoding a ribosomal protein S21 family protein, also annotated as GLUCOSE HYPERSENSITIVE 1) was significantly downregulated under both controlled and field conditions. Most the 28 differentially regulated ribosomal protein encoding genes were downregulated under controlled conditions (Supplemental File S3), suggesting a potential shift in conformation or availability of the translation machinery in response to weed pressure. This would also be consistent with the observation that growth is generally inhibited by weed presence, since protein production is required for growth. In most cases, this one included, the loss of N as a result of weed presence occurred even though N was supplemented and did not appear to be limiting (Bandeen and Buchholtz, 1967).

Phytochrome Signaling is Implicated in Corn’s Response to Weeds

Considerable work has focused on the phytochrome and red to far-red light signals in crop–weed competition studies (Page et al., 2009; Ballaré and Pierik, 2017). Indeed, under field conditions genes associated with phytochrome responses in corn were impacted in response to weeds (Horvath et al., 2018). There is considerable evidence to support the hypothesis that far-red-enriched light caused by the presence of nearby weeds results in enhanced oxidative stress through the generation of singlet oxygen in the chloroplasts (McKenzie-Gopsill et al., 2019). Previous studies in corn grown in the presence of weeds also supported this hypothesis, in that the genes involved in Photosystem I protection were consistently upregulated by weed presence (Horvath et al., 2018). Some oxidative stress related genes are differentially expressed under controlled conditions; however, they were present in both the up- and downregulated gene sets. In the current study, no obvious differences in red or far-red light quality was noted near the top of the plant where the leaf material was collected (data not shown), probably because there was no possibility of the top of the corn being shaded by the canola growing near its base. However, although gene set enrichment did not consistently implicate phytochrome responses, one gene (Zm00001D024783), encoding a phytochrome-interacting factor 3-like protein, was consistently upregulated under greenhouse conditions. A similar gene was implicated in weed responses in soybean [Glycine max (L.) Merr.] (Horvath et al., 2015).

Differences in the Expression of Specific Genes are Associated with Corn’s Response to Weeds

Most genes differentially regulated under both field and controlled conditions were downregulated in response to weeds. Among these genes are the regulator of chromosome condensation (RCCI) family genes (Table 3). In yeast (Saccharomyces cerevisiae), the encoded protein acts as a signal to detect unreplicated DNA and inhibits mitosis (Dasso, 1993). In A. thaliana, loss-of-function mutants have reduced cell cycle activity (Su et al., 2017). Thus down-regulation of RCCI would be consistent with the reduced growth observed when weeds were present. Since yield loss was probably not caused by reduced soil water, nutrients, or reduced light availability, the regulatory factors controlling the downregulated genes may provide targets for blocking the weed-induced growth-inhibiting signals.

Only two specific genes were consistently upregulated by weeds under both field and control conditions. One was annotated as a PMT5 and the other as a gene encoding a DC1 domain-containing protein (also annotated as NUCLEOREDOXIN 1) (Table 4). In A. thaliana, the homolog of PMT5 encodes a highly promiscuous sugar transporter capable of transporting a diverse range of linear and circular polysaccharides containing ribulose, myo-inositol, and monosaccharides (Klepek et al., 2005). Some of these could serve as signaling molecules. For example, myo-inositol is a well-known signaling compound in plants (Gillaspy, 2011). PMT5 is induced by cold, osmotic stress, and UV-B and in senescing leaves in A. thaliana. It is also induced by some biotic factors and silver nitrate and slightly upregulated by brassinolides in A. thaliana. It appears to be downregulated in A. thaliana by cytokinin. Intriguingly, the homolog of PMT5 has been observed to be coordinately regulated by the aquaporin-encoding gene NOD26-LIKE INTRINSIC PROTEIN 2:1 (Yue et al. 2012). Consistent with this earlier study, NOD26-LIKE INTRINSIC PROTEIN 2:1 was also significantly upregulated by weed presence in our study (Supplemental File S3). This observation indicates that some of these genes may be controlled by common regulatory factors.

In A. thaliana, the homolog of the NUCLEOREDOXIN 1 is known to play a role in redox homeostasis and oxidation-reduction process and is required for normal pollen tube growth. Unlike PMT5, NUCLEOREDOXIN is not regulated by abiotic stresses in A. thaliana but is induced by several biotic stresses, elicitors, and senescence. Interestingly, it is also induced by SA but is not strongly repressed by cytokinin as was observed for the homolog of PMT5. Thus although both of these genes are upregulated by weeds, they do not appear to be consistently controlled by all of the same physiological cues.

Neither PMT5 nor NUCLEOREDOXIN 1 are recognized regulatory proteins. However, their consistent induction in leaves under competition in various environments and in response to different weed species and densities indicates that these genes contain regulatory elements that are responsive to weed competition. Thus, once identified, the regulatory elements contained within
these genes should assist in understanding the signaling pathways by which weeds regulate gene expression in corn and provide potential targets for reducing the response of corn to weeds, thus increasing weed tolerance. These regulatory elements could also be used to reduce the response of corn genes to weeds, drive the production of genes to produce bioherbicides, or induce products or signals (e.g., florescence) that provide an early warning system for weed presence or impact.

CONCLUSION

Even when light, nutrients, and water are not limiting factors, weeds (in this case winter canola) still induced significant growth reduction in corn. Increasing weed density induced more intense changes in the corn transcriptome. Several of the physiological processes implicated include kinase signaling, transport, and protein production. A comparison of the transcriptome responses to weed pressures under controlled and field conditions identified a small set of genes with expression levels that are robustly regulated by competition. Such genes could serve as markers for competition and provide a system to identify both cis- and trans-signaling factors that are responsive to competition in corn. This information could be useful in developing weed tolerance and competition response monitoring. These observations provide insights into the mechanisms by which crop–weed interactions impact crop yield even when resources are not limiting. Modifying corn’s response to weeds could significantly enhance the potential for inter-cropping with other agronomically and ecologically valuable species such as winter brassica crops like canola and camelina.

Supplemental Information

Supplemental Fig S1. Photo of a block of corn plants growing with various numbers of weeds.

Supplemental File S1. Excel file showing statistics from the power analysis.

Supplemental File S2. Excel file showing the averages and SDs of phenological measurements from plants growing with various numbers of competitors.

Supplemental File S3. Excel file showing annotation, expression, and significance values for all transcripts identified in each library.

Supplemental File S4. Excel file showing complete gene set enrichment analyses. Separate pages show the results for each experimental run; the final page shows ontologies that overlapped in both experimental runs.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS

We thank Laura Kelly and Cheryl Huckle for their invaluable assistance in data collection and refining the experimental procedure.

REFERENCES


