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Title

Tecia solanivora infestation increases tuber starch accumulation inPastusa Suprema potatoes

Running Title

Potato responses to tuber moths

Authors

Pavan Kumar[1,4], Etzel Garrido[2,5], Kun Zhao[1], Yi Zheng[1], Saleh Alseekh[3], Erandi Vargas-Ortiz[1,6], Alisdair R. Fernie[3], Zhangjun Fei[1], Katja Poveda[2] and Georg Jander[1*]

[1]Boyce Thompson Institute for Plant Research, 533 Tower Road, Ithaca, NY 14853, USA

[2]Department of Entomology, Cornell University, Ithaca, NY 14853, USA

[3]Max Planck Institute of Molecular Plant Physiology, Wissenschaftspark Golm, Am Mühlenberg 1, 14476 Potsdam, Germany

[4]Present address: Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, USA

[5]Present address: Unidad de Microbiología Básica y Aplicada, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Av. de las Ciencias S/N, Juriquilla, Querétaro, México

[6]Present address: Department of Molecular Biology, Institute for Scientific and Technological Research of San Luis Potosí, San Luis Potosí, México

*Correspondence**:**Georg Jander (gj32@cornell.edu)

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Abstract

In response to infestation with larvae of the Guatemalan tuber moth (*Tecia solanivora*), some *Solanum tuberosum* (potato) varieties exhibit an overcompensation response, whereby the total dry mass of uninfested tubers is increased. Here, we describe early responses, within the first few days, of *T. solanivora* feeding, in the Colombian potato variety Pastusa Suprema. Non-targeted metabolite profiling showed significant secondary metabolism changes in *T. solanivora*-infested tubers, but not in uninfested systemic tubers. In contrast, changes in primary metabolism were greater in uninfested systemic tubers than in the infested tubers, with a notable 80% decline in systemic tuber sucrose levels within one day of *T. solanivora* infestation. This suggested either decreased sucrose transport from the leaves or increased sink strength, i.e. more rapid sucrose to starch conversion in the tubers. Increased, sucrose synthesis was indicated by higher rubicso activase and lower starch synthase gene expression in the leaves of infested plants. Elevated sink strength was demonstrated by 45% more total starch deposition in systemic tubers of *T. solanivora*-infested plants compared to uninfested control plants. Thus, rather than investing in increased defense of uninfested tubers, Pastusa Suprema promotes deposition of photoassimilates in the form of starch as a response to *T. solanivora* infestation.

Key Words

Tecia solanivora, *Solanum tuberosum*, Guatemalan tuber moth, potato, primary metabolism, overcompensation

INTRODUCTION

Insect feeding induces plant defense responses beyond those that result from mechanical damage (Howe and Jander 2008). In addition to direct physical and chemical defenses that deter herbivory, many plants exhibit tolerance, the ability to sustain significant herbivore damage without a yield loss (Agrawal 2000; Stowe et al. 2000; Tiffin 2000; Vargas-Ortiz et al. 2013). For instance, although *Empoasca fabae* (potato leafhoppers) generally reduce *Solanum tuberosum* (potato) yield, individual potato cultivars vary greatly in the amount of damage that they tolerate before there is a yield penalty (Kaplan et al. 2008). Similarly, potato cultivars vary in their tolerance of damage by *Tecia solanivora* (Guatemalan tuber moth) (Garrido et al. 2017), the most devastating potato pest in Central and South America (Salazar and Escalante 1984; Niño 2004). However, increased tolerance of *T. solanivora* damage may come at the cost of reduced yield in the absence of infestation (Garrido et al. 2017).

Perhaps more surprising than tolerance is the observation that, in some cases, tissue removal through herbivory can ultimately result in a higher biomass or seed production,compared to uninfested plants (Belsky 1986; Paige and Whitham 1987; Lennartsson et al. 1998; Strauss and Agrawal 1999), an effect that is commonly referred to as overcompensation.Some potato cultivars, notably Pastusa Suprema, a commercially grown Colombian potato variety, show significant increases in total tuber dry mass in response to infestation with *T. solanivora* larvae (Poveda et al. 2010; Poveda 2018). On ten different farms, plants that had 1 to 10% of their tubers damaged by *T. solanivora* larvae produced ~2.5-fold more marketable (undamaged) yield than potato plants that were completely undamaged. Even for plants with 11 to 20% of their tubers damaged, the total undamaged potato tuber yield increased nearly two-fold. Greenhouse experiments confirmed overexpression responses that were observed in the field (Poveda et al. 2012). Although increased yield in response to low-level *T. solanivora* infestation is consistently observed in Pastusa Suprema and certain other potato varieties, the initiation and molecular mechanisms of this tuber growth response have not been investigated.

Potato tuberization is a complex and incompletely understood process that involves reciprocal communication between potato foliage and the developing tubers. A phyB-dependent signal, possibly a phloem-mobile RNA originating from the shoot, promotes tuberization (Sarkar 2008). Plant hormones such as gibberellin, abscisic acid, strigolactones, and auxins have been shown to inhibit or promote dormancy release of axillary stolon bud growth, which is required for subsequent tuber formation, thereby affecting potato yield (Xu et al. 1998; Carrera et al. 2000; Roumeliotis et al. 2012). Connections between plant defense against herbivory and tuber growth are suggested by the observation that jasmonic acid, a defense signaling molecule, is metabolized to tuberonic acid, which has strong tuber-inducing activity (Koda 1997). Gene expression silencing experiments also show that lipoxygenase, a jasmonic acid

biosynthetic enzyme, influences potato tuber development (Kolomiets et al. 2001). Therefore, insect herbivory, which typically involves upregulation of jasmonic acid biosynthesis, could have indirect effects on tuber formation.

The transport of carbohydrates by the phloem to the tubers is a gradient-driven process whereby photoassimilates can be actively or passively loaded by transporters from source tissues and passively unloaded into sink tissues (Slewinski and Braun 2010). Sink strength, which is partially regulated by enzymes of sucrose metabolism (*e*.*g*. invertase and sucrose synthase), helps to drive this process (Roitsch 1999; Van Bel 2003). During initiation of tuberization, there is a metabolic switch in elongating stolons, whereby sucrose synthase increases and invertase activity decreases (Fernie and Willmitzer 2001). Overexpression of sucrose synthase can lead to an increased starch and total tuber yield in potato plants (Baroja-Fernandez et al. 2009). In another study, potato tuber starch yield was doubled by: (i) increasing carbohydrate export from the leaves through overexpression of pyrophosphatase or inhibition of ADP-glucose pyrophosphorylase, and (ii) increasing sink strength in the tubers by overexpressing plastidic glucose 6-phosphate/phosphate and adenylate translocators (Jonik et al. 2012).

Sink strength and photosynthesis are often tightly correlated, with tuber-derived signals influencing the photosynthetic rate. When potato plants initiate tuber formation, photosynthesis increases two- to threefold, whereas the removal of tubers reduces photosynthetic rates (Nosberger and Humphries 1965; Moorby 1968). In response to *T*. *solanivora* infestation of tubers, there is increased abundance of α-solanine, α-chaconine, and chlorogenic acid in the potato leaves (Kumar et al. 2016), an indication that not only signals for inducing sucrose transport, but also defense-related signals move from tubers to vegetative tissue.

Two proposed mechanisms that explain how plants increase yield,after herbivore damage, could apply in the case of potatoes infested with *T*. *solanivora*: (i) carbon reallocation from vegetative toreproductive tissue after herbivore damage (Hochwender et al. 2000; Stowe et al. 2000; Tiffin 2000; Schwachtje et al. 2006), or (ii) up-regulation of primary metabolism, *i*.*e*. photosynthetic ratein source tissue (Agrawal 2000; Tiffin 2000). Experiments with several Colombian potato varieties showed no shift from aboveground to belowground biomass in response to *T*. *solanivora* infestation (Garrido et al. 2017), suggesting that increased photosynthesis and subsequent increases in carbon transport as the more likely explanation of potato overcompensation. Endoreduplication, which has been associated with plant overcompensation responses (Scholes and Paige 2014), has been observed in potatoes (Uijtewaal 1987; Pijnacker et al. 1989), but has not been linked to the observed overcompensation response in this species.

To gain more insight into the molecular mechanisms of overcompensation in the Pastusa Suprema potato variety, we characterized local and systemic responses to *T*. *solanivora* infestation of a

single tuber. Whereas most prior research on plant overcompensation has been focused on the end result, *i.e*. increased reproductive fitness or yield, we testedthe hypothesis thatsystemic metabolic changes, leading to overcompensation and increased yield, occur within the first few days of tuber moth infestation. Our results show significant metabolic changes in uninfested tubers within one day after the initiation of *T*. *solanivora* feeding on a focal tuber, and increased starch deposition after eight days.

RESULTS

As illustrated in Figure 1, a single focal tuber of each Pastusa Suprema plant was infested with fifteen*T*. *solanivora* larvae. One, two, and eight days after the start of infestation, focal tubers, systemic tubers, leaves, and stems were harvested for metabolite analysis by high performance liquid chromatography - mass spectrometry(HPLC-MS) and gas chromatography – mass spectrometry (GC-MS), as well as gene expression analysis by Illumina sequencing (RNA-Seq) and quantitative reverse transcriptase-PCR (qRT-PCR).

We used an HPLC-MS metabolite profiling assay, which detects mostly non-polar secondary metabolites, to measure defensive responses that were induced by *T*. *solanivora* in potato tubers. Partial least squared discriminant analysis (PLS-DA) of the entire metabolomic data set, for each pairwise comparison,showed significant changes in the overall tuber metabolome in focal but not in systemic tubers (Figure 2A, Table S1). Also in focal, but not in systemic tubers, the defensive metabolites α-solanine, α-chaconine, and chlorogenic acid were significantly decreased one day after the initiation of *T*. *solanivora* feeding (Figure 2B, $P < 0.05$,two-way ANOVA with tuber treatment and metabolite as factors). This observation suggested suppression of plant defenses by the initiation of larval feeding. On days two and eight, there was no significant *T*. *solanivora*-induced change in the abundance of these defensive metabolites in either focal or systemic tubers.

We used targetedGC-MS analysis to measure the abundance of primary metabolites in leaves, stems, local tubers, and systemic tubers after *T*. *solanivora* infestation (Table S2). PLS-DA of all 63 detected metabolites, for each pairwise comparison (Figure 3), showed that systemic tubers have the greatest separation of the primary metabolite content between control and infested plants. Specific analysis of sucrose content, which would play a central role in potato overcompensation responses, showed an 80% decrease in systemic tubers, but not in focal tubers, leaves, or stems (Figure 4A-D).

Decreased tuber sucrose content could be the result of increased sink strength,*i.e*. conversion of phloem-transported sucrose into stored starch. Starch content was not increased significantly in focal tubers at any time point (Figure 5A). Although the average starch content, measured as release of glucose in an enzymatic assay, was about 30% higher in systemic tubers from infested plants, on day eight, this

effect was not significant (P= 0.07; Figure 5B). However, the total mass of systemic tubers from infested plants was greater than that of uninfested plants (P< 0.05; Figure 5C).Total starch deposition, per potato plant, calculated as tuber starch content × total systemic tuber mass, was 45% higher after eight days of*T*. *solanivora* feeding than in uninfested control plants (P< 0.01; Figure 5D).

RNA-Seqanalysis showed limited but significant gene expression changes in systemic tubers after one and two days of *T*. *solanivora* feeding. About 75% of thefiltered RNA-Seq reads could be aligned to thepublished potato genome (The Potato Genome Sequencing Consortium 2011; http://solanaceae.plantbiology.msu.edu/; Table S3),and 23,178 genes were expressed at >0.1 reads per kilobase exon model per million mapped reads (RPKM) in at least one sample. After one day of *T*. *solanivora* feeding, reads mapping to 126 of these genes were more abundantand 65were less abundant in systemic tubers (Table S4). Fewer changes were observed after two days, withexpression of threegenes increased andsevengenes decreased in systemic tubers of infested plants. Among the genes with increased expression levels in systemic tubers, several are likely to be defense-related (*e*.*g*. lipoxygenase and patatin).

Whereas the current potato genome is derived from a doubled haploid that was made from a diploid*Solanum tuberosum* group Phureja isolate (The Potato Genome Sequencing Consortium 2011), Pastusa Suprema is a heterozygous tetraploid. Therefore, we conducted a systematic search for genes that are expressed in Pastusa Suprema, but which are not present inthe annotated potato genome. A total of 9,652 assembled contigs had no significant homologyto annotated potato genes (Table S5). Sequence analysis of these contigs suggests that one third are likely to be non-coding due to frame shifts and/or stop codons. Most of the remaining contigs have significant homology to genes from other species, suggesting that they could be functional genes in Pastusa Suprema. Expression levels of 98 contigs were significantly changed after *T*. *solanivora* feeding, with 46 being up-regulated and 42 being down-regulated after one day (FC> 2 and adjust P-value < 0.05). As in the case of contigs with homology to the potato genome, these included likely defense-related genes, *e*.*g*. predicted lipoxygenases and protease inhibitors(Table S5).

To determine whether tuber gene expression changes could account for the observed sucrose decrease and starch increase in systemic tubers (Figures4 and 5), expression of genes involved in sucrose to starch conversion (Figure 6) was analyzed individually. This showed no significant changes in overall starch biosynthesis pathways in systemic tubers of infested plants (Table S6), suggesting that upregulation of sucrose-to-starch conversion was not occurring at the transcriptional level. The absence of significant expression changes in four genes encoding enzymes of the starch biosynthesis pathway in

systemic tubers, sucrose synthase, ADP-glucose pyrophosphorylase, starch synthase, and starch branching enzyme, was confirmed by qRT-PCR with independent biological samples (Figure 7A-D).

Gene expression analysis also was used to determine whether increased sucrose synthesis in leaves could contribute to elevated carbon flow to systemic tubers. Consistent with a hypothesis of increase sucrose production in the leaves of infested plants, gene expression analysis by qRT-PCR showed increased rubisco activase expression (Figure 8A) and decreased starch synthase expression (Figure 8B) in plants where one tuber was infested with *T*. *solanivora*. However, expression of two other starch biosynthesis genes was not significantly altered in the leaves of infested plants (Figure 8C,D).

DISCUSSION

Consistent with the documented overcompensation responses in Pastusa Suprema (Poveda et al. 2010; Poveda et al. 2012; Poveda 2018), we observed changes in both gene expression and primary metabolism in systemic tubers resulting from *T*. *solanivora* infestation. The 80% reduction in sucrose content in the systemic tubers, which occurred within one day of larval infestation (Figure 4B), could be due to either reduced transport of sucrose to these tubers or increased conversion of sucrose into starch. The following observations suggest that increased starch synthesis is the more likely scenario. (i) Increased transcription of rubisco activase (Figure 8A) and decreased transcription of starch synthase (Figure 8B) in leaves of infested plantsindicate that more sucrose is available for transport from source tissue.(ii) There is no decrease in the sucrose levels of focal tubers (Figure 5A), which would be expected if there were a general decrease in transport.(iii) After eight days of larval feeding, systemic tubers on *T*. *solanivora*-infested plants are larger than those on control plants (Figure 5C).(iv) Increased flux into starch is consistent with the increased total per-plant starch deposition (Figure 5D) that is observed in Pastusa Suprema after *T*. *solanivora* feeding.

Alterations in the sugar metabolism of systemic tubers do not seem to be the result of transcriptional changes in starch biosynthesis (Figure7, Table S6). Other studies have shown that starch synthesis during potato tuber growth can be regulated at the post-transcriptional level (Tiessen et al. 2002).Thus, we hypothesize that increased starch synthesis in systemic tubers of *T*. *solanivora*-infested plants is the result of post-transcriptional regulation.

Increased starch synthesis in systemic tubers is associated with altered, though not necessarily lower, plant defenses. The initial drop in tuber α-solanine, α-chaconine, and chlorogenic acid in the focal tubers of infested plants (Figure 2B) could represent either a suppression of plant defenses by *T*. *solanivora* or a reallocation of resources by the infested plants. This is a marked contrast to the significant increases in the abundance of these defensive metabolites in the leaves of *T*. *solanivora*-infested Pastusa

Suprema plants (Kumar et al. 2016), Although no significant changes in α-solanine, α-chaconine, and chlorogenic acid were observed in systemic tubers, (Figure 2C), defense-related gene expression in systemic tubers was induced after one day of *T*. *solanivora* feeding (Tables S4 and 5). Both effects are relatively short-term.Neither focal nor systemic tubers showed significant differences in defensive metabolite content after two and eight days of larval feeding. Only 279 out of 32,830 total detected transcripts were differentially expressed in systemic tubers after one day of *T*. *solanivora* feedingand, after two days of feeding, this had decreased to eleven differentially expressed genes (Tables S4 and 5). More robust defense induction in systemic tubers might not be an effective response because, as a specialist potato herbivore, *T*. *solanivora*is impervious to known potato chemical defenses (Poveda et al. 2012). It is perhaps notable that lipoxygenases are among the genes that are significantly overexpressed in the systemic tubers (Tables S4 and 5). As this enzymatic activity has been associated with potato tuber growth (Kolomiets et al. 2001), upregulation of lipoxygenase gene expression might contribute to the observed overcompensation response in Pastusa Suprema.

Together, our results show that tuber overcompensation responses in Pastusa Suprema begin rapidly after the onset of herbivory. Rather than increasing the accumulation of defensive metabolites in systemic tubers in response to *T*. *solanivora* feeding, these potato plants instead invest in tuber growth by up-regulating starch deposition. Within one day after the initiation of *T*. *solanivora* feeding, there is evidence of increased sucrose to starch conversion in systemic tubers. Potato photosynthetic rate is linked to the tuber sink strength (Nosberger and Humphries 1965; Moorby 1968). Thus, a likely scenario is that *T*. *solanivora* feeding increases sink strength and, thereby, carbon fixation in the infested plants. Further research to identify *T*. *solanivora*-derived signals that increase tuber sink strength has potential practical applications for increasing potato yield.

METHODS

Plant material and insect culture

*Solanum tuberosum*variety Pastusa Suprema (US Germplasm Resources Information Network accession number PI 661990), which was released by the Grupo de Investigación en Papa at the Universidad Nacional de Colombia in 2002, was used for all experiments. Plants were grown under a 12:12 h light:dark cycle at 25°C in a greenhouse at the Sarkaria Arthropod Research Laboratory (SARL) on the Cornell University campus (Ithaca, NY, USA). All plants were vegetatively propagated in Sunshine Soil Mix #1 (Sun Grow Horticulture, Bellevue, WA, USA) supplemented with 15 g of 10:10:10 N:P:K slow-release fertilizer (American Green, Pottsville, PA, USA). A *T*. *solanivora* colony (Poveda et al. 2012) was

maintained at SARL on Pastusa Suprema tubers in complete darkness at 25°C in an incubator (Percival Scientific, Perry, IA, USA).

T. solanivora infestation

Tubers of 90-day-old plants were exposed to *T. solanivora* larvae, as described previously(Poveda et al. 2012; Kumar et al. 2016; Figure 1). A single tuber from each plantwas moved to the soil surface without damaging the stolon or neighboring tubers. Fifteen newly hatched *T. solanivora*larvae were placed on the exposed tuber, referred to as the focal tuber, using a small paint brush. The undamaged tubers of these plants were referred to as systemic tubers. Control plants receive a similar treatment, but without the addition of *T. solanivora* larvae. Focal tubers, of control and *T. solanivora*-infested plants were protected from light by covering them with a piece of black cloth. After one, two, and eight days of infestation, leaf, stem, focal and systemic tuber samples from infested and control plants were collected in liquid nitrogen and stored at -80°Cpriorto metabolite and transcript analyses. Each individual potato plant was harvested at only one time point and was then discarded. Plantswhere*T. solanivora* larvae had moved from the focal tuber and had damaged systemic tubers also were discarded andnot used for further assays.

Analysis of primary and secondary metabolites

Primary metabolites were extracted and analyzed from leaf, stem, and tuber tissue samples, as reported previously (Lisec et al. 2006). Fifty mgsamplesof freshly frozen tissue weremixed with 730µL of 100% methanol containing 0.2mg/mLribitol (Sigma-Aldrich, St. Louis, MO, USA) as an internal standard. Samples were incubated in an incubator shaker at 70°C for 15 min and centrifuged at 13,000 × g for 10 min. The collected supernatant was mixed with 375µL of chloroform and 750µL of water by vortexing, followed bycentrifugation at 13,000 × g for 10 min. The polar aqueous phase was collected and derivatized as reported previously (Lisec et al. 2006). Gas chromatography - time of flight mass spectrometry (GC-TOF-MS) chromatograms were analyzed using TagFinder (Luedemann et al. 2008). The analytes were manually identified using the TargetFinder plug-in of the TagFinder software and compared with the reference library of the Golm Metabolome Database (GMD, http:// gmd.mpimp-golm.mpg.de/) Kopka et al. 2005; Schauer et al. 2005). The metabolite levels were calculated relative to the internal standard ribitol and normalized with the sample weight. The resulting data were analyzed using Metaboanalyst 3.0 (http://www.metaboanalyst.ca/faces/home.xhtml; Xia and Wishart 2016), with the following program parameters:upload data = peak intensity table, missing value estimation = skip, data filtering = mean intensity value, data transformation = logarithmic, and data scaling = auto scaling. Starch abundance in systemic tubers of*T. solanivora*-infested plants was measured usinga previously

reported colorimetricmethod (Vargas-Ortiz et al. 2013). Targeted (chlorogenic acid, α-solanine, and α-chaconine) and non-targeted secondary metabolite extraction and analyses by HPLC-MS of tuber samples were performed as reported previously Kumar et al. (2016).

RNA sequencing (RNA-Seq) and quantitative reverse transcriptase PCR (qRT-PCR)

Total RNA from systemic tubers of *T*. *solanivora*-infested and control plants was extracted using a previously described protocol (Kumar et al. 2007). To investigate the early transcriptomic response, systemic tubers after 1 and 2 days of *T*. *solanivora* infestation were harvested for RNA-Seq analysis. For each sample, two µg of purified total RNA was used for the preparation of strand-specific RNA-Seq libraries with 14 cycles of final amplification. Three biological replicates were prepared for each sample.The purified libraries were multiplexed and sequenced with 101 bp single-end read length in a single lane on an Illumina HiSeq2000 instrument (Illumina, San Diego, CA) at the Cornell University Biotech Facility.

The raw reads were processed by removing the adapters and low-quality bases using Trimmomatic (Bolger et al. 2014), and reads shorter than 40 bp after processing were discarded. The remaining high-quality reads were mapped to the SILVA ribosome RNA database (Quast et al. 2013a) using Bowtie (Langmead et al. 2009), allowing up to three mismatches. These unmapped reads were regarded as filtered for subsequent analyses. RNA-Seq data from two samples were excluded of further analysis due to a high portion of ribosomal RNA reads or a high frequency of reverse-strand sequences (Table S3)

The filtered reads were aligned to the potato reference genome (http://solanaceae.plantbiology.msu.edu; The Potato Genome Sequencing Consortium 2011) using HISAT (Kim et al. 2015). The number of reads mapped to each gene in each samplewas counted, and expression data were normalized as Reads Per Kilobase of transcript per Million mapped reads (RPKM). Raw count data were used as inputfor DESeq2 (Love et al. 2014) to identify differentially expressed genes. Genes with at least two-fold changes in expression level and adjusted p-values less than 0.05 were considered to be differentially expressed. RNA-Seq data were submitted to the National Center for Biotechnology Information (NCBI) Short Read Archive under accession number PRJNA431579.

The filtered reads were also *de novo* assembled into contigs using Trinity (Grabherr et al. 2011) with "min kmer cover" set to 5. The assembled contigs were compared to SILVA ribosome RNA database (Quast et al. 2013b) and GenBank Nucleotide (nt) database using BLAST+ with e-value cutoff of 1e-5(Camacho et al. 2009).Contigs having matches to ribosomal RNA sequences, or having hits from viruses, bacteria, or fungi rather than plant species, were considered to be contaminants and were

excluded. The redundant contigs were removed using CD-HIT with 95% sequence identify threshold(Fu et al. 2012). The filtered contigs were aligned to the potato genomes using GMAP (Wu and Watanabe 2005), with default parameters. Contigs that mapped to intergenic regions or which did not have significant homology to the potato genome were considered to be novel sequences. The functional annotations of these new contigs were predicted using automated assignment of human readable descriptions (AHRD: https://github.com/groupschoof/AHRD). The filtered reads were mapped to these contigs using BWA (Li and Durbin 2009). Statistical analysis to identify contigs that represent differentially expressed was performed as described above.

Transcript accumulation of targeted genes in leaf and tuber samples was measured by qRT-PCR. Total RNA from leaves and tubers was isolated using the SV total RNA isolation kit (Promega, Madison, WI, USA) and a protocol reported by Kumar et al.(2007). cDNA synthesis and transcript quantification were performed as reported previously (Kumar et al. 2016). Primers for gene amplification for qRT-PCR are listed in the Table S7.

Dataanalysis

Statistical comparisons, including t-tests, one-way ANOVA, and two-way ANOVA, were conducted using JMP software (SAS Institute, Miami, FL, USA). Targeted primary metabolite, non-targeted secondary metabolite data, and gene expression data were processed online using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca)(Xia and Wishart 2016). For PLS-DA, the following program parameters were used: upload data - peak intensity table; data filtering - mean intensity value; data transformation - log; data scaling - auto scaling; and P value threshold set to 0.05.

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AUTHOR CONTRIBUTIONS

P.K., E.G., S.A., K.Z., Y.Z., S.A., and E.V.O. conducted experiments and analyzed data. P.K., A.R.F., Z.F., K.P., and G.J. designed experiments and analyzed data. G.J. and P.K. wrote the manuscript.

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Figure Legends

Figure 1. Experimental setup for *Tecia solanivora* infestation of potatoes

A focal tuber was carefully brought to the surface, infested with 15 *T*. *solanivora* larvae, and covered with black cloth. Control tubers received the same treatment but no larvae. After one, two, and eight days, focal tubers, systemic tubers, leaves, and stems were harvested for metabolite and gene expression assays.

Figure 2. Changes in secondary metabolism in focal and systemic tubers

(**A**) Partial least squares discriminant analysis (PLS-DA) plots comparing the principal components of the of molecular ions identified by combined positive and negative ionization HPLC-MS in focal and systemic tubers of *T*. *solanivora*-infested (green) and control (red) plants. Each data point represents an independent plant sample, and the shaded areas represent 95% confidence regions. The observed variances are shown in parentheses. (**B**) Levels of α-solanine, α-chaconine, and chlorogenic acid in focal and systemic tubers of control and *T*. *solanivora*-infested plants. Asterisks indicate significant differences ($P < 0.05$) by two-tailed Student's *t*-test.

Figure 3. Analysis of primary metabolism after *T. solanivora* feeding

Partial least squares discriminant analysis (PLS-DA) plots comparing the principal components of primary metabolites measured by GC-MS. Control samples are in green and *T*. *solanivora*-infested samples are in red. Colored ovals indicate 95% confidence regions.

Figure 4. Sucrose content after *T. solanivora* infestation

Sucrose content was measure in (**A**) focal tubers, (**B**) systemic tubers, (**C**) leaves, and (**D**) stems. Mean ± S.Eof N = 5. Significant differences in the sucrose content of systemic tubers were assessed by two-way ANOVA, with day and treatment as factors.

Figure 5. Starch content after *T. solanivora* infestation

Starch content in (**A**) focal and (**B**) systemic tubers of control and *T*. *solanivora*-infested plants, as measured by release of glucose in enzymatic assays. Mean ±S.E of N = 6. (**C**) Total mass of systemic tubers. Mean ±S.E of N = 7. (**D**) Total starch accumulation in systemic tubers, calculated as starch concentration × the total mass of systemic tubers on each plant. Mean ±S.E of N = 7. P values were

calculated with two-tailed Student's *t*-tests, comparing control and infested samples at the same time point.

Figure 6. Enzymatic steps of starch synthesis from sucrose in potato tubers

Fru, fructose; UDPglc, UDP glucose; F6P, fructose-6-phosphate; G1P, glucose-1-phosphae; G6P, glucose-6-phosphate; ADPgluc, ADP glucose; 1, Sucrose synthase; 2, UDP-Glc pyrophosphorylase; 3, phosphoglucomutase; 4, fructokinase; 5, phosphoglucoisomerase; 6, phosphoglucomutase; 7, ADP-Glc pyrophosphorylase; 8, starch synthase, 9 starch branching enzyme.

Figure 7. Gene expression in systemic tubers, with and without *T. solanivora* infestation

Expression levels of (**A**) sucrose synthase, (**B**) ADP-glucose pyrophosphorylase, (**C**) starch synthase, and (**D**) starch branching enzyme were measured by qRT-PCR. Mean ±S.Eof N = 4-6. No significant differences, $P > 0.05$ by Student's *t*-tests comparing induced and uninduced samples at the same time point.

Figure 8. Leaf gene expression after *T. solanivora* infestation

Expression of (**A**) rubisco activase, (**B**) starch synthase, (**C**) ADP-glucose pyrophosphorylase, and (**D**) starch branching enzyme were measured by qRT-PCR in leaves, with and without *T. solanivora* infestation of one tuber. Mean ±S.E. of N = 4-6. Significant differences in gene expression over the course of the experiment were assessed by two-way ANOVA, with day and treatment as factors.

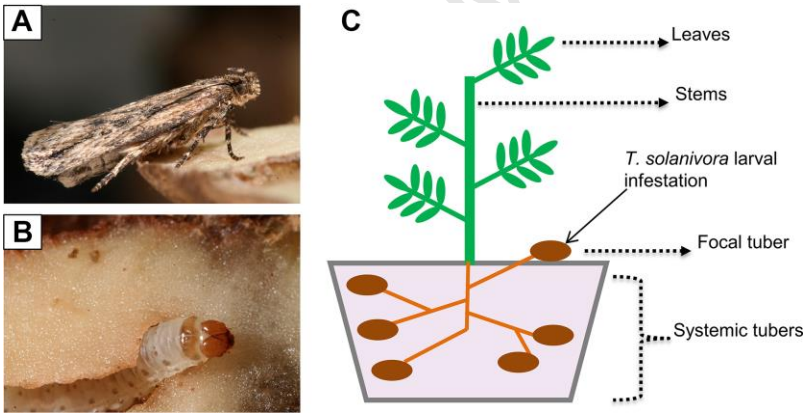
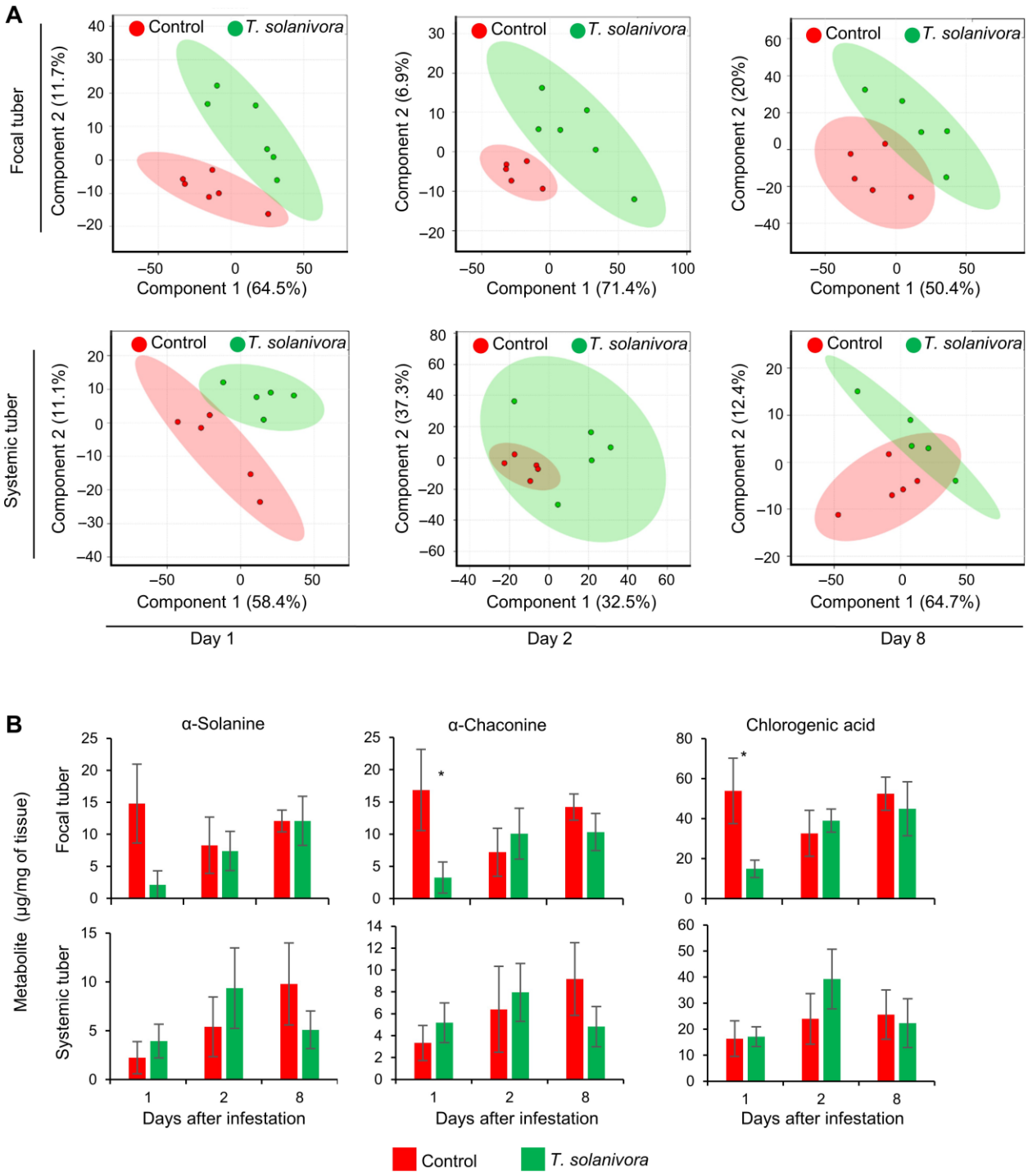


Figure 1



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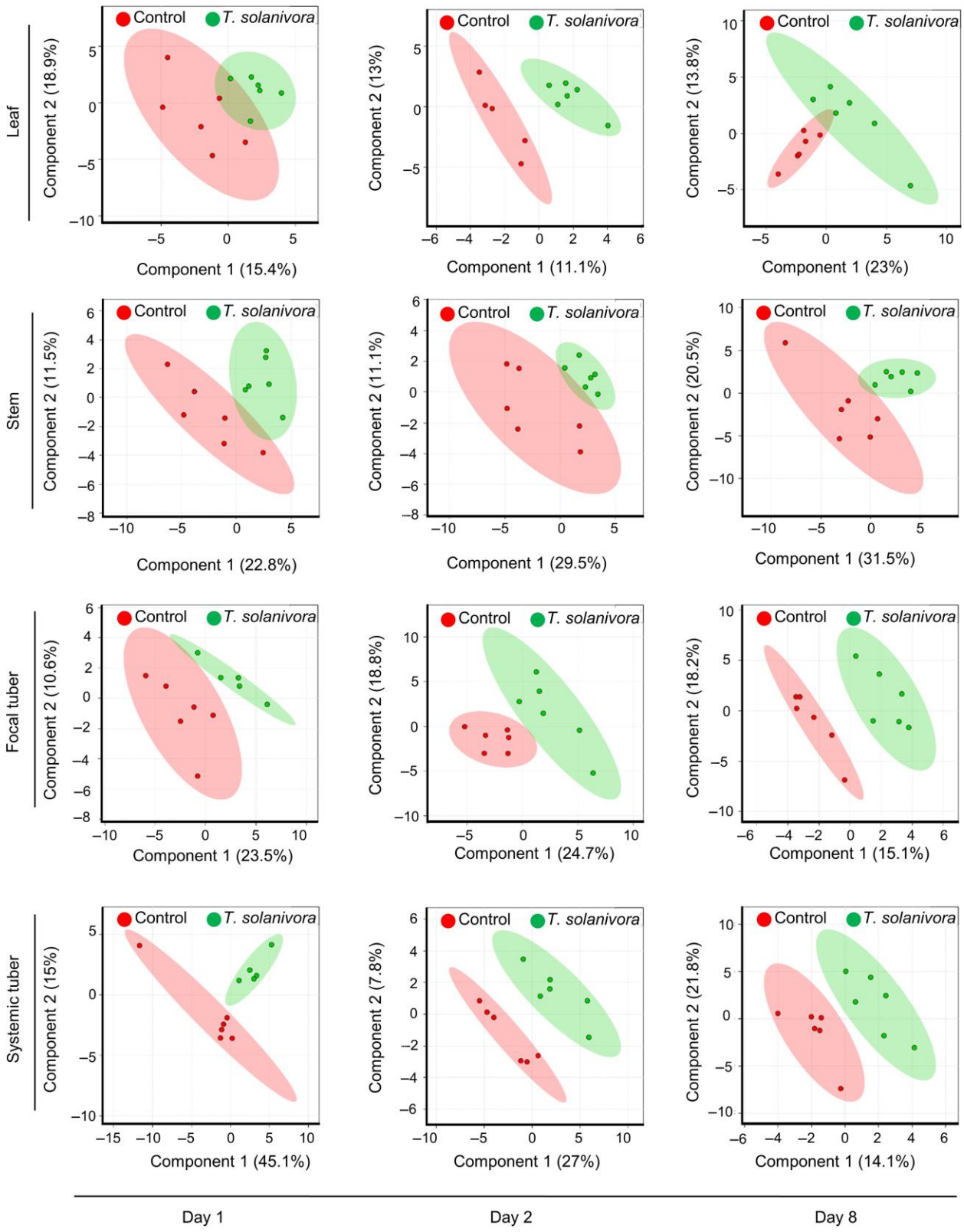

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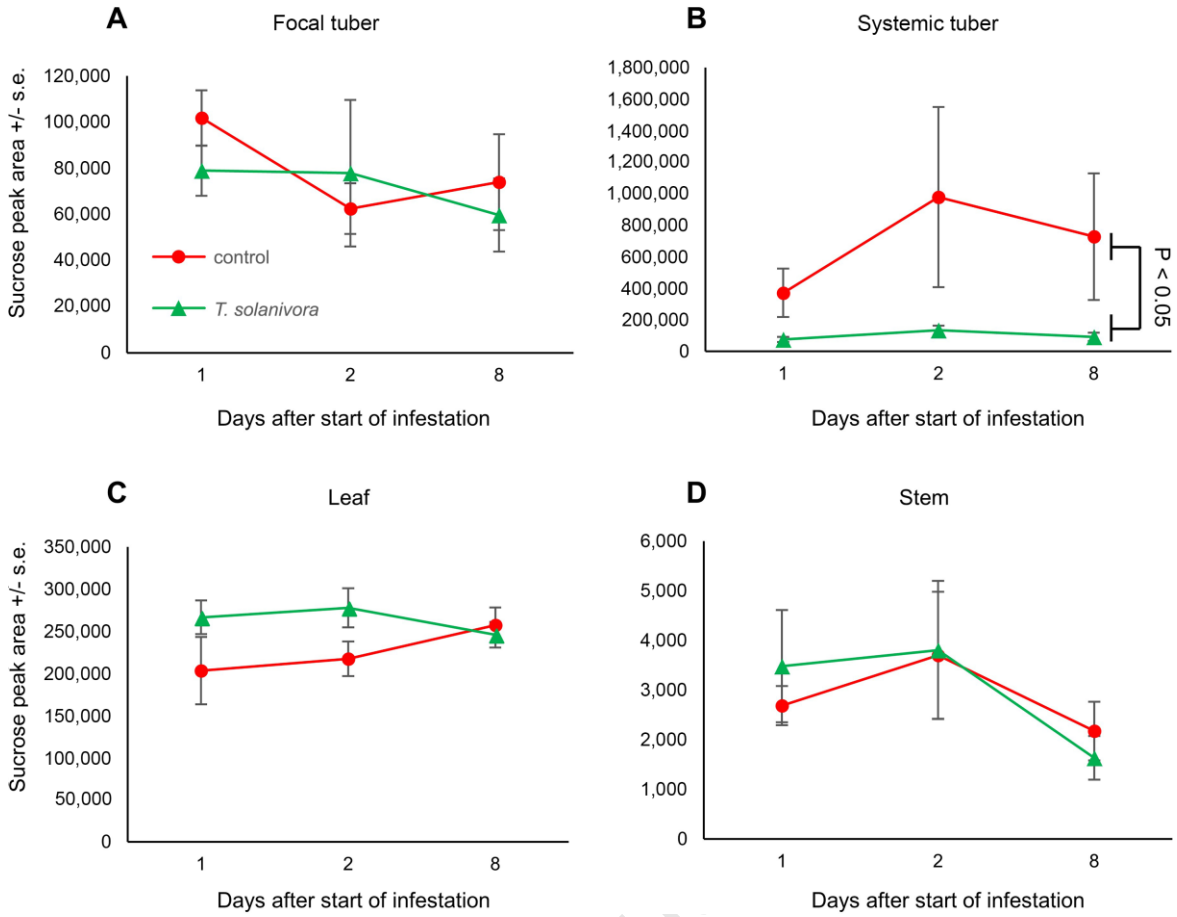

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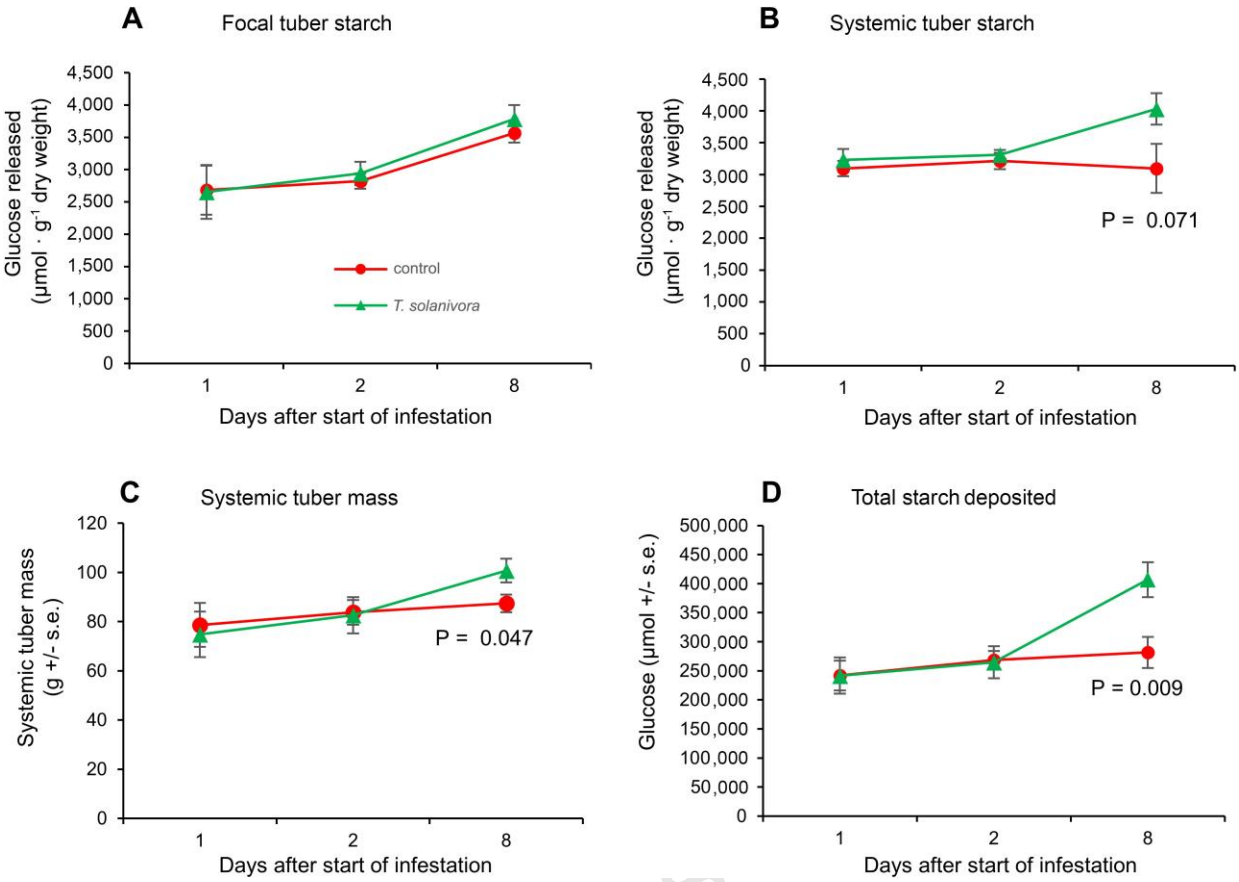

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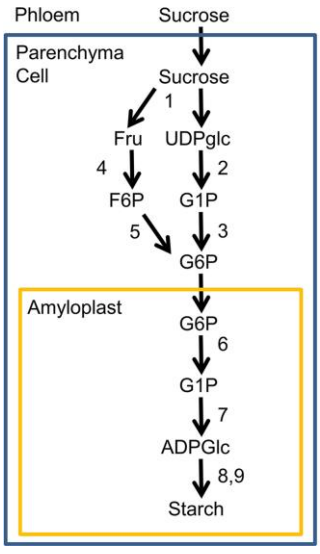

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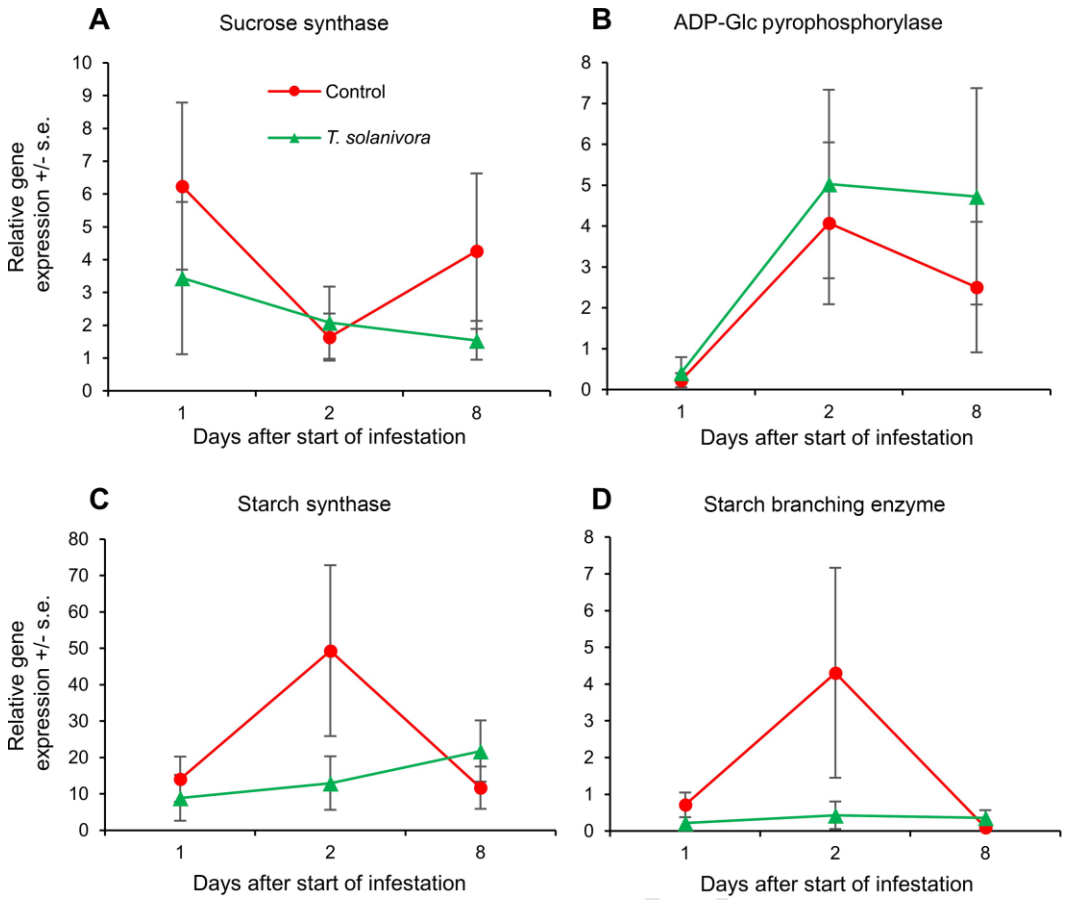

Figure 7

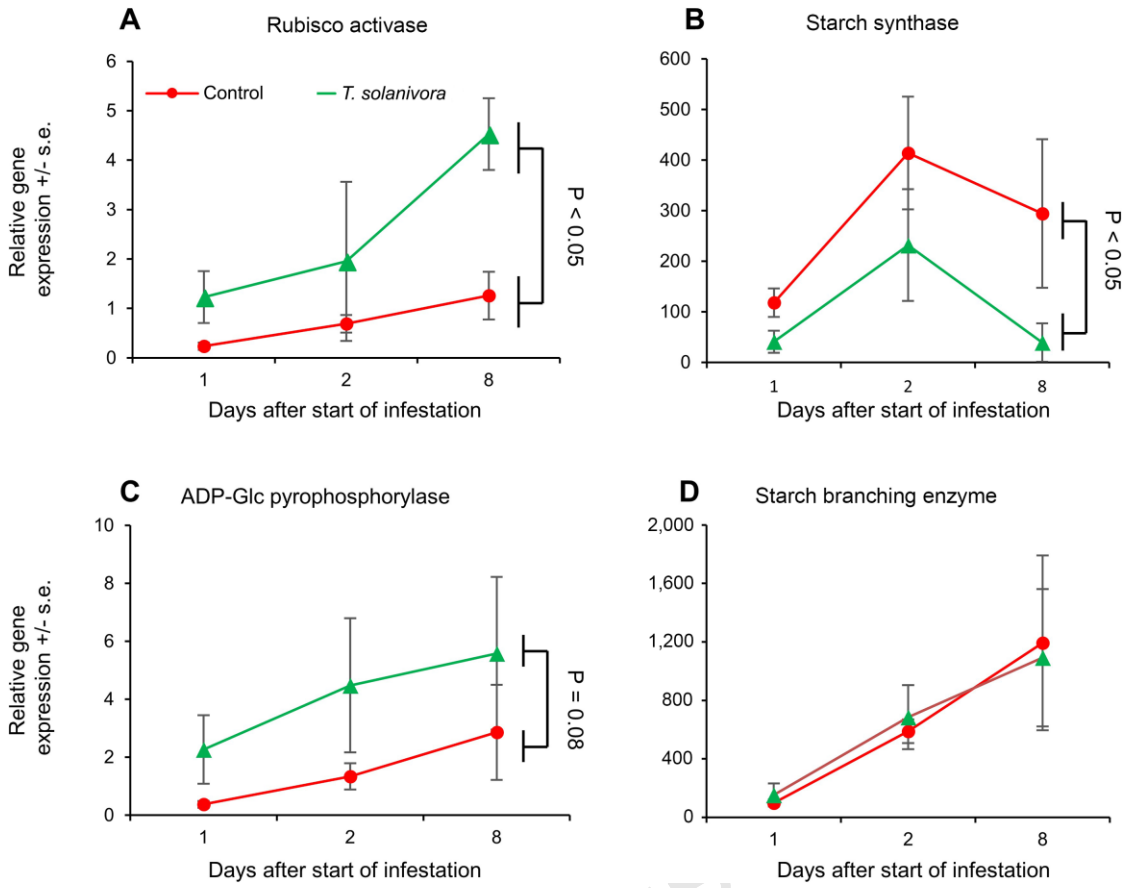

Figure 8