JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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# Differential Metabolic Responses Caused by the Most Important Insect Pest of Coffee Worldwide, the Coffee Berry Borer (*Hypothenemus hampei*)

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Cite This: *J. Agric. Food Chem.* 2020, 68, 2597−2605 Read Online

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**ABSTRACT:** The world's coffee supply is threatened by the coffee berry borer, *Hypothenemus hampei,* the most destructive pest affecting coffee production and quality. This study hypothesized that coffee berry borer infestation induces distinct metabolic responses in the green coffee seeds of *Coffea arabica* and *Coffea canephora* (robusta). A targeted metabolomics approach was conducted using liquid chromatography tandem mass spectrometry to quantify intracellular metabolites in infested and uninfested arabica and robusta green seeds. In parallel, the seed biomass content and composition were assessed for the same conditions. Coffee berry borer attack induced increases in the levels of chlorogenic acids in arabica seeds, whereas organic acids and sugar alcohols were more abundant in infested robusta seeds. Most importantly, a set of compounds was identified as biomarkers differentiating the metabolic response of these taxa to the coffee berry borer.

**KEYWORDS:** *arabica, la broca del cafe, green coffee seeds, metabolomics, plant−insect interaction, robusta*



## INTRODUCTION

The coffee berry borer (*Hypothenemus hampei*; Coleoptera: Curculionidae: Scolytinae) is the most destructive insect pest of coffee worldwide, infesting both *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Frohner (also known as robusta).[1] The insect, commonly known as "la broca del café" in Spanish, is endemic to Africa and has now been reported in most coffee-producing countries.[1] In 1998, over 715 000 ha were reported to be infested with the insect in Colombia,[2] and this figure had increased to over 800 000 ha by 2002.[3] Yearly losses caused by the coffee berry borer in Brazil have been estimated at $215−358 million (U.S. dollar)[4] and infestation levels in Jamaica have been reported at up to 85%.[5]

Infestation starts when adult female coffee berry borers enter coffee berries ca. 120−150 days after flowering, which corresponds to at least 20% dry weight in the berry.[6] Eggs are laid in galleries built throughout the seed,[7] followed by larval consumption and a subsequent reduction in yields and quality.[1] Despite the fact that the insect does not affect leaves, stems, nor branches, it damages green and mature berries.[8] Therefore, damage caused by the insect not only impairs coffee quality but also makes berries more vulnerable to other pests and diseases because of physical injury.[9]

Even though the insect has been studied for more than 100 years,[10,11] it continues to be very difficult to control because of its cryptic life cycle; that is, it spends most of its life inside the coffee berry, thus making traditional pest management strategies unreliable.[1] In addition, it is not uncommon for coffee to be grown in steep terrain, where access to water might be difficult. Consequently, the application of techniques that require spraying are a challenge not only because of the water constraint but also because a full 5-gal backpack sprayer weighs 41.7 lbs., making applications quite strenuous. Other pest management practices include the use of natural enemies such as parasitoids and fungal entomopathogens, but reliable management based on these techniques remains elusive.[1]

Changes in seed metabolism in arabica and robusta coffee as a result of feeding by the insect have not yet been elucidated. The objective of this work is to compare the metabolic profiles of coffee berry borer infested and uninfested seeds in arabica coffee from Colombia and in robusta coffee from Vietnam and India. Metabolomics is an approach that allows the detection of molecules at very low concentrations as it relies on state-of-the-art mass spectrometry techniques.[12] Although metabolomics has been widely applied to pharmaceutics and other areas of biomedical research, it is now becoming essential for an in-depth understanding of plant−insect interactions. For instance, signaling molecules related to plant defense against herbivory attack have been proposed, but their identity and biochemical synthesis have yet to be elucidated.[13,14] Nonetheless, metabolomics has shown that glucosinolates are produced by broccoli plants as a defense against Lepidoptera infestation[15] and that caffeoylquinic acids are associated with caterpillar herbivory in cabbage.[16] Although some studies have been performed on coffee varieties fingerprinting through metabolomics,[17,18] reports on the metabolome of coffee plants−insect interactions are still lacking.

In this study, we hypothesized that coffee berry borer attack alters seed metabolism. To test this hypothesis, we quantified biomass components such as protein, fatty acids, starch, and

Received: November 22, 2019
Revised: February 3, 2020
Accepted: February 10, 2020
Published: February 10, 2020





https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

cell walls in coffee berry borer infested and uninfested *C. arabica* and *C. canephora* seeds. Additionally, their metabolite profile was assessed by targeted metabolomics to determine the levels of amino acids, sugars and sugar alcohols, organic acids and phosphorylated compounds, phenolic compounds, flavonoids, and hormones. The importance of these results in terms of biochemical markers induced by the coffee berry borer are discussed.

## MATERIALS AND METHODS

**Seeds.** The coffee berry borer infests coffee seeds during development. The colonizing female bores an entrance hole into the coffee berries and oviposits in galleries built in the seeds.[7] As a result, it is possible to visually sort infested from uninfested seeds on the basis of the damage caused by the insect. Seeds were harvested in Colombia, India, and Vietnam, countries where the coffee berry borer is known to thrive. Arabica Colombia Supremo was purchased from Coffeemaria.com. Robusta from India and Vietnam were purchased from eBay vendors. Upon arrival, seeds were stored at room temperature since 2002 (India robusta), 2009 (Vietnam robusta), and 2013 (arabica Colombia Supremo). Before analysis, seeds were visually sorted as infested or uninfested. All seeds were processed for metabolomic analyses in 2017 and 2018. The number of green seeds and amount of material used is detailed below for each analysis.

**Fatty Acid and Protein Extraction and Quantification.** Fatty acids and proteins were sequentially extracted from 15 mg of dried green coffee seeds as described by Cocuron et al.[19] Three biological replicates were analyzed, each consisting of a pool of three pulverized seeds. Gas chromatography coupled to mass spectrometry (GC-MS) was used to determine fatty acid content and composition as in de Souza et al.[20] Protein content and quantification was performed according to Cocuron et al.[19]

**Carbohydrate Extraction and Quantification.** Soluble sugars, starch, hemicellulose, and crystalline cellulose were sequentially extracted from 10 mg of seed powder obtained from individually ground coffee seeds. Soluble sugars were extracted with 0.5 mL of 50% ethanol (v/v) after addition of 50 nmol of [U-$^{13}C_6$]-glucose as an internal standard and agitated at 30 Hz for 5 min using a mill grinding jar (Retsch, Haan, Germany). Samples were subsequently incubated for 30 min at 50 °C. This procedure was performed two more times with the exception of not repeating the addition of the $^{13}$C-labeled internal standard. A volume consisting of 500 μL of the extract containing soluble sugars was filtrated with 3 kDa Amicon Ultra 0.5 mL centrifugal device (Millipore, Burlington, MA) for 30 min at 14 000*g* and at room temperature. Then, 300 μL of the eluent was diluted with 1200 μL of acetonitrile/water (60:40, v/v), and 5 μL was injected into a 2.0 × 150 mm Shodex Asahipak NH2P-50 2D column with a Shodex Asahipak NH2P-50G 2A guard column (Showa Denko America, New York, NY). The analysis was carried out using an UHPLC 1290 Infinity II (Agilent, Santa Clara, CA) for liquid chromatography (LC) separation and a QTRAP 6500+ linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer (AB Sciex Instruments, Framingham, MA) for detection. A gradient with acetonitrile started at 85% for 8.5 min, and then it was decreased to 78% for 6.5 min. Finally, the level was increased again to 85% and equilibrated at this concentration for 5 min. The remaining pellets were defatted by the addition of 1.5 mL of chloroform/methanol (1:1, v/v). After agitation for 5 min at 30 Hz, the samples were centrifuged for 10 min at 17 000*g* at room temperature and the supernatants discarded. The remaining pellets were washed with 1 mL of water three times, centrifuged for 10 min at 17 000*g* at room temperature, and then saved for starch extraction. Starch was extracted and quantified as described by Cocuron et al.,[19] and the remaining pellets were washed with 1.5 mL of water three times after centrifugation for 10 min at 17 000*g* at room temperature between each wash. Acetone (500 μL) was added to each pellet, vortexed, and dried under a stream of $N_2$. Hemicellulose and crystalline cellulose were extracted and quantified following the procedure published elsewhere.[21]

**Intracellular Metabolite Extraction.** Metabolites were extracted from 15 mg of dried coffee seeds obtained from a set of three coffee seeds. Boiling water was used to extract intracellular metabolites (amino acids, sugars, sugar alcohols, and organic acids) as described by Cocuron et al.[19] and Casas et al.[22] At the time of extraction, 200 nmol of [U-$^{13}$C]-glucose, 200 nmol of [U-$^{13}$C]-glycine, and 50 nmol of [U-$^{13}$C]-fumarate were added as internal standards. A Hypercarb column (100 × 2.1 mm, 5 μm pore; Thermo Fisher Scientific, Waltham, MA) was used for amino acids; a Shodex Asahipak NH2P-50 2D column (2.0 × 150 mm) with a Shodex Asahipak NH2P-50G 2A guard column was used for sugars and sugar alcohols, and an IonPac AS11 column (250 × 2 mm) with a Guard column AG11 (50 × 2 mm; Dionex, Sunnyvale, CA) was used for the organic acids and phosphorylated compounds as described previously.[19]

Phenolics were extracted using 1 mL of methanol/water (40:60, v/v) and 100 nmol [U-$^{13}$C]-benzoic acid was added as an internal standard to each sample during the extraction. The samples were shaken in a bead beater for 5 min at 30 Hz and then sonicated for 10 min. After centrifugation of the samples for 15 min at 17 000*g* at room temperature, 0.5 mL of the supernatant was cleaned using a 3 kDa Amicon filtering device for 45 min at 14 000*g* at room temperature. The extract (200 μL) was added to a vial containing 800 μL of water/methanol (60:40, v/v) and 10 μL was injected into the LC/MS/MS using a reversed-phase C18 Symmetry column (4.6 × 75 mm; 3.5 mm) associated with a Symmetry C18 precolumn (3.9 × 20 mm; 5 mm; Waters, Milford, MA). The analyses were carried as described by Cocuron et al.[23]

**LC/MS/MS Quantification of Intracellular Metabolites.** Data acquisition and processing were performed with Analyst v. 1.7 software (AB Sciex, Framingham, MA). Metabolite quantification was performed by correlating the resulting peak area of each metabolite with its corresponding standard as described previously.[19]

**Statistical Analysis.** To determine statistical differences for each component and their three biological replicates, we performed a Student *t*-test ($p < 0.05$) on RStudio.[24] Data were normalized using log-transformation and were mean-centered and divided by the standard deviation of each variable for principal component analysis (PCA), supervised partial least-squares discriminate analysis (PLS-DA), and heat mapping, which were performed using MetaboAnalyst 4.0.[25]

## RESULTS

**Limited Impact of Coffee Berry Borer Infestation on the Biomass Composition of Green Coffee Seeds.** Various seed composition parameters were assessed in arabica coffee from Colombia (Colombia Supremo) and robusta coffee from Vietnam (Table 1). No significant differences were found in total biomass (hemicellulose, cellulose, fatty acids, and proteins) when uninfested and infested seeds were compared, except that total proteins were significantly lower in infested Vietnam robusta seeds (Table 1; $p < 0.05$). Although the insect had no impact on the total fatty acid

**Table 1. Total Biomass Composition in Arabica (Colombia Supremo, CS) and Vietnam Robusta (VR) Seeds[a]**

| | % of total biomass (w/w) | | | |
|---|---|---|---|---|
| treatment | hemicellulose | cellulose | fatty acids | proteins |
| CS-U | 20.3 ± 2.5 | 5.4 ± 3.2 | 11.5 ± 1.0 | 9.3 ± 1.1 |
| CS-I | 20.3 ± 2.8 | 6.9 ± 0.7 | 12.8 ± 0.6 | 9.1 ± 0.4 |
| VR-U | 21.6 ± 3.4 | 6.7 ± 2.1 | 9.5 ± 0.7 | 18.3* ± 0.8 |
| VR-I | 19.4 ± 1.8 | 4.6 ± 2.3 | 9.1 ± 0.4 | 16.3* ± 0.5 |

[a]Numbers are averages (w/w) ± standard deviation of three biological replicates ($n = 3$). An asterisk (*) denotes a statistically significant difference ($p < 0.05$) between uninfested (U) and coffee berry borer infested (I) seeds.


https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

**Table 2. Six Most Abundant Intracellular Metabolites in Arabica (Colombia Supremo, CS) and Vietnam Robusta (VR) Seeds**[a]

| | quantity (pmol·mg$^{-1}$ DW) | | | |
|---|---|---|---|---|
| metabolite | CS-U | CS-I | VR-U | VR-I |
| 3-CQA | 5356.8 ± 498.9 | 5561.5 ± 257.0 | 10149.2* ± 1037.4 | 7478.7* ± 680.4 |
| 5-CQA | 51731.3* ± 585.0 | 54414.3* ± 1192.2 | 69759.9* ± 2389.0 | 60626.7* ± 757.3 |
| 4-CQA | 7807.4* ± 220.9 | 8715.2* ± 351.9 | 13326.9 ± 815.8 | 11513.1 ± 868.5 |
| 3,5-di-CaQA | 16224.6 ± 1690.7 | 19136.9 ± 965.0 | 28303.5 ± 7818.5 | 28031.1 ± 4157.7 |
| caffeine | 83557.7* ± 4250.3 | 72474.8* ± 1002.2 | 153231.1 ± 4685.0 | 160449.3 ± 12088.3 |
| sucrose | 54968.9 ± 7071.7 | 54519.3 ± 1571.4 | 43510.4 ± 4909.1 | 34493.0 ± 4126.5 |

[a]Numbers are average quantities in pmol·mg$^{-1}$ dry weight (DW) ± standard deviation of three biological replicates ($n$ = 3). An asterisk (*) denotes a statistically significant difference ($p$ < 0.05) between uninfested (U) and coffee berry borer infested (I) beans within each taxa. Abbreviations (in alphabetical order): 3,5-di-CaQA, 3,5-dicaffeoylquinic acid; 3-CQA, 3-caffeoylquinic acid; 4-CQA, 4-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid.

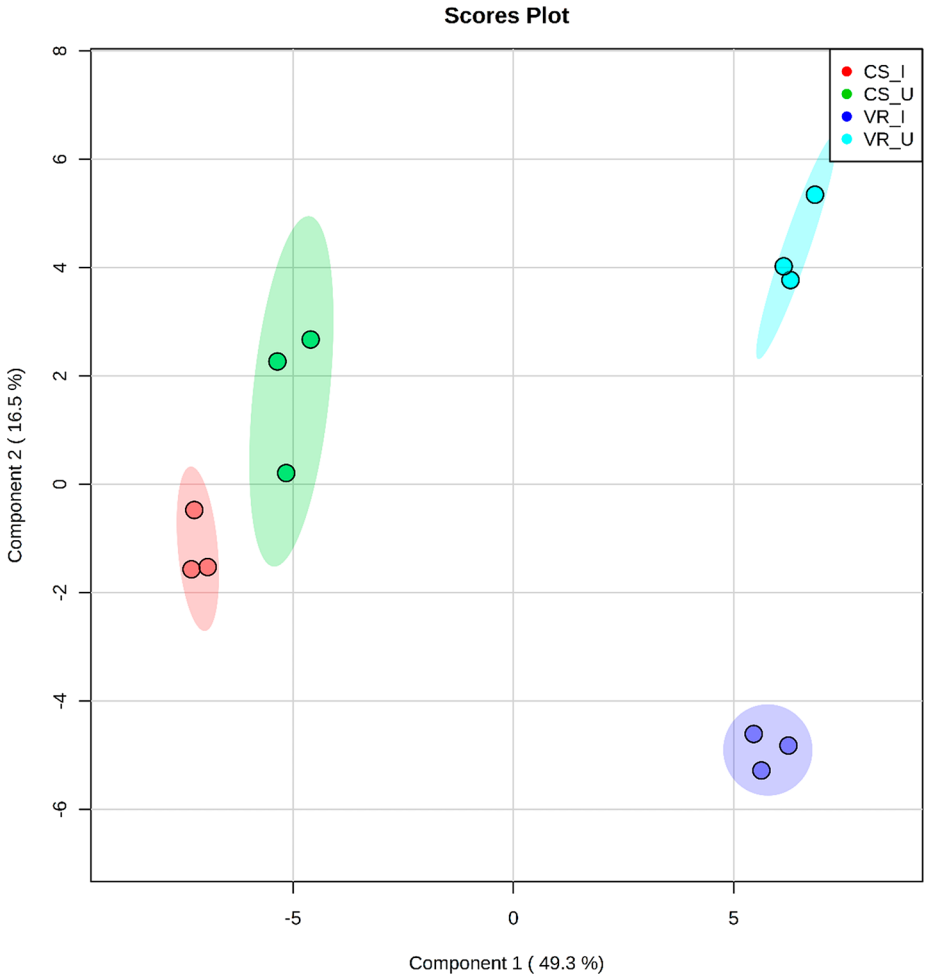


**Figure 1.** Partial least-squares discriminant analysis of the metabolites and biomass components in arabica (Colombia Supremo, CS) and Vietnam robusta (VR) seeds. Metabolites and biomass components were extracted from arabica Colombia Supremo uninfested (CS-U), coffee berry borer infested (CS-I), Vietnam robusta uninfested (VR-U), and coffee berry borer infested (VR-I) seeds and quantified as described in Materials and Methods. Shaded red, green, cyan, and blue regions in this plot represent 95% confidence interval between the taxa and treatments.

content, infested Vietnam robusta seeds exhibited significant reductions in palmitic acid (Table S1; $p$ < 0.05). There were no other significant changes in total hemicellulose or cellulose contents (Table 1) nor hemicellulose composition (Table S1, Supporting Information). Lastly, starch was consistently found to be below 1% of the total biomass (data not shown). These results demonstrate that coffee berry borer infestation has only a limited impact on final biomass composition of the seed.

**Effects of Coffee Berry Borer Infestation on the Levels of Major Intracellular Metabolites.** Four main chemical classes of intracellular metabolites were analyzed and quantified by targeted metabolomics using liquid chromatography tandem mass spectrometry (LC/MS/MS): (1) amino acids and associated derivatives; (2) sugars and sugar alcohols; (3) anionic compounds (organic acids and phosphorylated metabolites); and (4) phenolic compounds. Caffeine and chlorogenic acids (3-, 4-, and 5-caffeoylquinic acids; 3-CQA, 4-


https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

CQA, and 5-CQA, respectively) were found to be the most abundant metabolites in green coffee beans, followed by sucrose (Table 2). Infested arabica contained 10 times less caffeine and 10 times more caffeoylquinic acids than uninfested arabica (significantly different, $p < 0.05$), whereas the infestation of Vietnam robusta caused a significant decrease in caffeoylquinic acids (Table 2; $p < 0.05$). Vietnam robusta exhibited a significant increase in total soluble sugars upon infestation (Table S2; $p < 0.05$).

Twenty-five amino acids and their derivatives were quantified, with the most abundant being glutamate, asparagine, and aspartate (Table S2). The levels of arginine, glycine, and glutamine were significantly lower in infested arabica seeds than in uninfested ones (Table S2; $p < 0.05$). Methionine was significantly higher in infested arabica seeds than in uninfested seeds, whereas the opposite was found for Vietnam robusta; that is, levels were significantly higher in uninfested seeds than in infested seeds (Table S2; $p < 0.05$). Additionally, levels of glutamate, valine, threonine, isoleucine, and leucine were significantly lower in infested Vietnam robusta seeds than in uninfested seeds ($p < 0.05$).

Ten sugars and sugar alcohols were quantified, and sucrose levels were the highest in seeds from the two taxa (Table S2). Infested seeds had significantly lower levels of fructose, glucose, sorbitol, and pentitols in arabica seeds by a factor of 4.5, 3.5, 1.6, and 4.2, respectively. Infested Vietnam robusta seeds had significantly higher ($p < 0.05$) levels of inositol (1.2×), pentitols (3.4×), and erythritol/threitol (2.9×).

Of the 22 organic acids and phosphorylated metabolites that were quantified, malate, citrate, and quinic acid were the most abundant (Table S2). Infested arabica seeds had significantly higher $\alpha$-ketoglutarate, quinic, and adipic acids, and lower glycerol phosphate, glutarate, galactose 1-phosphate, *trans*-aconitate, and sebacic and maleic acids than uninfested seeds (Table S2; $p < 0.05$). Fumarate, suberic acid, *trans*-aconitate, sebacic acid, azelaic acid, maleic acid, malonic acid, tartaric acid, adipic acid, glucose/mannose 1-phosphate, and *cis*-aconitate were found at significantly higher levels in infested Vietnam robusta seeds than in uninfested seeds, whereas levels of $\alpha$-ketoglutarate were significantly lower in infested seeds than in uninfested seeds ($p < 0.05$).

Finally, 11 phenolic compounds and alkaloids were quantified. There were significantly lower levels of 5-CQA and 4-CQA in uninfested arabica seeds than in infested seeds, whereas caffeine levels were significantly higher in uninfested than in infested seeds. Levels of 5-CQA and 3-CQA were significantly higher in uninfested Vietnam robusta seeds than in infested seeds (Table 2; $p < 0.05$).

**Coffee Seed Metabolism Shaped by Genotype and Treatment.** Biomass analysis (Table S1) and metabolomics (Table S2) resulted in four separated groups by partial least-squares discriminant analysis (PLS-DA) when uninfested and infested arabica and Vietnam robusta seeds were compared. Coffee taxa (arabica or robusta) was divided by component 1 while the treatment (infested or uninfested) was separated by component 2 (Figure 1). This division underlines metabolic differences caused by taxa and infestation or noninfestation. Some arabica and robusta metabolites cluster together (Figure 2). For instance, cluster A grouped compounds that are higher in arabica than in robusta: (i) biomass components such as total fatty acids, linoleic acid, linolenic acid, and mannose from hemicellulose; (ii) amino acids and their derivatives (proline, methionine, glutamine, ornithine, and nicotinamide); (iii) total

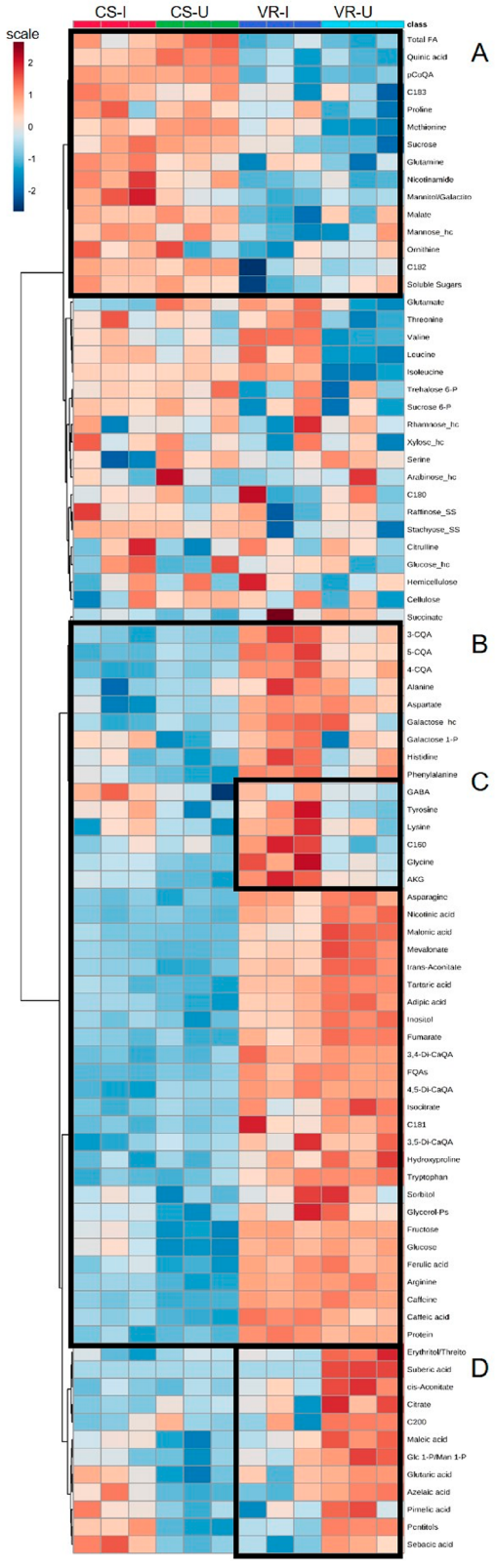


**Figure 2.** Heat map analysis of intracellular metabolites and biomass components in arabica (Colombia Supremo, CS) and Vietnam robusta (VR) seeds. Metabolites and biomass components were extracted from arabica Colombia Supremo uninfested (CS-U), coffee berry borer infested (CS-I), Vietnam robusta uninfested (VR-U), and coffee berry borer-infested (VR-I) seeds, and quantified as described in Materials and Methods. Colors represent metabolite relative intensity with red and blue symbolizing higher and lower values,


https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

**Figure** 2. continued

respectively. Fold-change values are shown by the color scale. Highlighted sections represent metabolites more abundant in CS (A), VR (B), VR-U (C), and VR-I (D). Abbreviations (in alphabetical order): 3,4-Di-CaQA, 3,4-dicaffeoylquinic acid; 3,5-Di-CaQA, 3,5-dicaffeoylquinic acid; 3-CQA, 3-caffeoylquinic acid; 4,5-Di-CaQA, 4,5-dicaffeoylquinic acid; 4-CQA, 4-caffeoylquinic acid; 5-CQA, 5-caffeoylquinic acid; AKG, $\alpha$-ketoglutaric acid; Arabinose_hc, hemicellulosic arabinose; C160, C16:0 (palmitic acid); C180, C:18:0 (stearic acid); C181, C18:1 (oleic acid); C182, C18:2 (linoleic acid); C183, C18:3 (linolenic acid); C200, C20:0 (arachidic acid); Erythritol/Threito, erythritol/threitol; FQAs, feruloylquinic acid isomers; GABA, $\gamma$-aminobutyric acid; Galactose 1-P, galactose-1-phosphate; Galactose_hc, hemicellulosic galactose; Glc 1-P/Man 1-P, glucose-1-phosphate/mannose-1-phosphate; Glucose_hc, hemicellulosic glucose; Glycerol-Ps, glycerol phosphates; Mannose_hc, hemicellulosic mannose; pCoQA, *p*-coumaroylquinic acid; Raffinose_SS, sugar-soluble raffinose; Rhamnose_hc, hemicellulosic rhamnose; Stachyose_SS, sugar-soluble stachyose; Sucrose-6P, sucrose-6-phosphate; Total FA, total fatty acids; Trehalose 6-P, trehalose-6-phosphate; Xylose_hc, hemicellulosic xylose.

soluble sugars, sucrose, and mannitol/galactitol; (iv) the organic acid malate; and (v) phenolic compounds quinic acid and *p*-coumaroylquinic acid (Figure 2A). Cluster B showed lower metabolite abundances in arabica than in robusta: (i) biomass components, such as oleic acid, and hemicellulosic galactose; (ii) amino acids and their derivatives (alanine, aspartate, histidine, phenylalanine, asparagine, hydroxyproline, tryptophan, and arginine); (iii) sugars and sugar alcohols (inositol, sorbitol, fructose, and glucose); (iv) organic acids and phosphorylated compounds (galactose-1-phosphate, nicotinic acid, malonic acid, mevalonate, *trans*-aconitate, tartaric acid, adipic acid, fumarate, isocitrate, and glycerol phosphates); and (v) phenolic compounds: 3-, 4-, and 5-CQA, feruloylquinic acids, 4,5- and 3,5-dicaffeoylquinic acid, ferulic acid, caffeine, and caffeic acid (Figure 2B). Within cluster B it was possible to identify a group of metabolites (box C) that accumulated at higher levels in uninfested Vietnam robusta: tyrosine, glycine, $\gamma$-aminobutyric acid (GABA), $\alpha$-ketoglutarate, and palmitic acid (Figure 2C). In contrast, cluster D highlighted metabolites that were lower in uninfested Vietnam robusta. It grouped mainly sugar alcohols, organic acids, and phosphorylated compounds such as erythritol/threitol and pentitols, suberic acid, *cis*-aconitate, citrate, maleic acid, azelaic acid, pimelic acid, sebacic acid, and glucose and mannose 1-phosphate (Figure 2D).

**Different Responses from Arabica and Robusta to Coffee Berry Borer Infestation.** PLS-DA analysis and the most important features, variable importance in projection (VIPs), revealed metabolic differences between arabica and robusta in response to coffee berry borer infestation (Figure 3). For instance, PLS-DA graphs showed a clear separation between uninfested and infested samples for each coffee taxa (Figure 3A,B). The VIPs, which are a weighted sum of squares of the PLS loadings for a given component,[25] are represented by the most important features (metabolites) responsible for the separation between infested and uninfested beans (Figure 3C,D). Figure 3 illustrates that arabica and robusta accumulate distinct metabolites because of the damage caused by the insect. Glucose, pentitols, and 4,5-dicaffeoylquinic acid were the top three differentially accumulated metabolites in infested arabica in contrast to suberic acid, valine, and isoleucine in robusta (Figure 3C,D). In contrast to robusta, the VIP scores highlighted numerous metabolites that were more abundant in uninfested arabica, such as glucose, pentitols, arginine, glycine, and fructose (Figure 3C). In robusta, suberic acid, pentitols, malonic acid, erythritol, and threitol were found to be significantly higher in infested seeds, whereas valine, isoleucine, methionine, leucine, and 5-CQA were significantly lower (Figure 3D).

**India Robusta Response Validation of Metabolic Markers for Coffee Berry Borer Infestation.** For validation of the metabolites that are characteristic of infested robusta coffee, a principal component analysis (PCA) was performed on infested and uninfested India robusta seeds, using only the top 20 most important features highlighted by PSL-DA from Vietnam robusta (Figure 3D). There was a clear separation between treatments (Figure 4), confirming the specific metabolic difference in infested India robusta coffee. The results from biomass analysis (total and component composition) revealed a similar trend to those in Vietnam robusta seeds (Table S3). Nonetheless, there was no significant difference in total soluble sugars and in the levels of fatty acid species between infested and uninfested seeds in India robusta, in contrast to those in Vietnam robusta. Many similarities to Vietnam robusta were found when intracellular metabolites from infested and uninfested India robusta seeds were compared. For instance, glutamate, isoleucine, nicotinic acid, inositol, pentitols, erythritol, threitol, fumarate, suberic, sebacic, azelaic, maleic, malonic, and tartaric acids differed significantly between uninfested and infested India robusta and Vietnam robusta ($p < 0.05$). However, some compounds, such as ferulic, feruloylquinic, *p*-coumaroylquinic, and 3,5-dicaffeoylquinic acids, were significantly different only in India robusta seeds but not in Vietnam robusta seeds (Tables S2 and S3).

## DISCUSSION

Even though the coffee berry borer is the most important insect pest of coffee worldwide, not much is known about the metabolic response of seeds infested with the insect. The results indicate the coffee berry borer induces different metabolic changes in arabica and robusta seeds. It is important to recognize that the seeds were not collected in the same region; therefore, other factors might have influenced the results. However, Souard et al.[26] reported distinct metabolic profiles in arabica and robusta leaves collected during the same period and at the same location, showing that the *Coffea* taxa is also an important driver for the observed metabolic profile divergence. The purpose of our study was not to compare arabica and robusta because they are different genotypes, but rather to assess whether they display distinct metabolic responses to the coffee berry borer infestation.

**Arabica and Robusta Showing Different Levels of Defense-Related Compounds.** In comparison to infested Vietnam robusta seeds, the uninfested ones had high levels of phenolics, compounds associated with plant defense.[27] This finding contrasts previous studies showing the usual higher accumulation of chlorogenic acids (3-, 4-, and 5-CQA) upon herbivory attack on various plants.[28] This higher level of phenolics in uninfested seeds was also inconsistent with a previous study on rice infested by several insects that had an induced accumulation of phenolic compounds.[29] Levels of caffeic acid were significantly higher in infested India robusta, indicating a defense response toward the coffee berry borer


https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

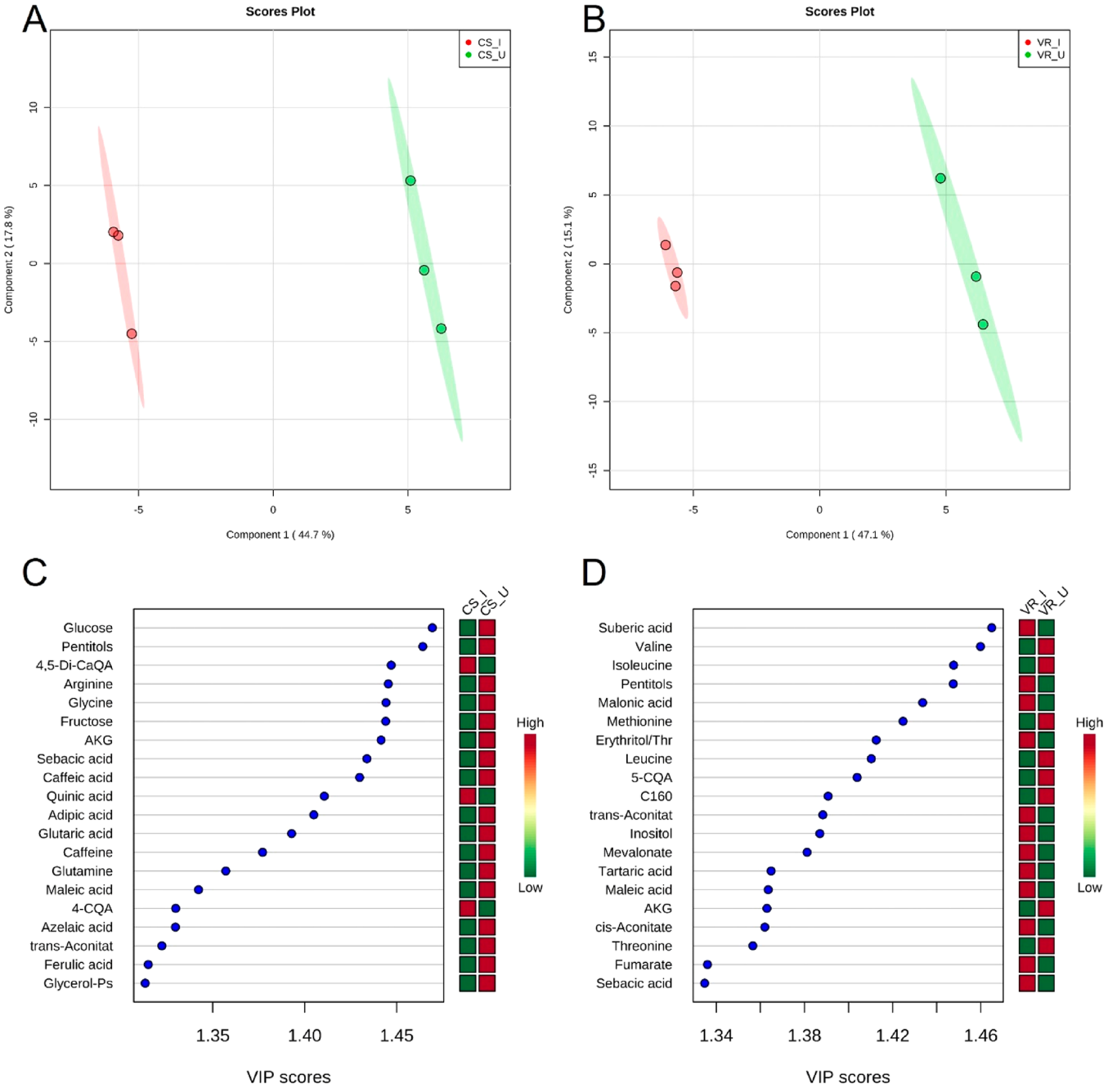


**Figure 3.** Partial least-squares discriminant analysis of metabolites and biomass components in arabica (Colombia Supremo, CS; A) and Vietnam robusta (VR; B) seeds; and the top 20 most important features resulting from the PLS-DA (C, D). Metabolites and biomass components were extracted from arabica Colombia Supremo uninfested (CS-U), coffee berry borer infested (CS-I), Vietnam robusta uninfested (VR-U), and coffee berry borer infested (VR-I) seeds, and quantified as described in Materials and Methods. Shaded red and green regions in the PLS-DA plots represent 95% confidence interval between the two treatments, uninfested and infested by the coffee berry borer. Colors in the variable importance in projection plot represents relative intensity, where red symbolizes higher values and green symbolizes lower values.

infestation. Indeed, upregulation of the genes associated with this metabolite accumulation was related to a higher resistance of potato tubers against the Lepidoptera *Phthorimaea operculella*.[30] Ferulic acid, a substrate that enhances the activity of ferulate-5-hydroxylase (F5H), also exhibited a significant increase in response to coffee berry borer infestation in India robusta. Overexpression of F5H induced an upregulation of genes associated with defense and stress response in *Arabidopsis* infested with aphids and did result in higher accumulation of lignin.[31] The differences in metabolites induced by infestation, such as arginine and glutamate in India and Vietnam robusta, indicate upregulation of polyamines and phenolamide pathways. These compounds are usually abundant in reproductive plant tissues and are directly related to lignin production and plant defense against herbivory.[32]

**Increased Chlorogenic Acids in Infested Arabica Indicating Targeted Host Defense.** The defense-related compounds 4,5-dicaffeoylquinic acid, 5-CQA, and 4-CQA were detected at significantly higher levels in infested than in


https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

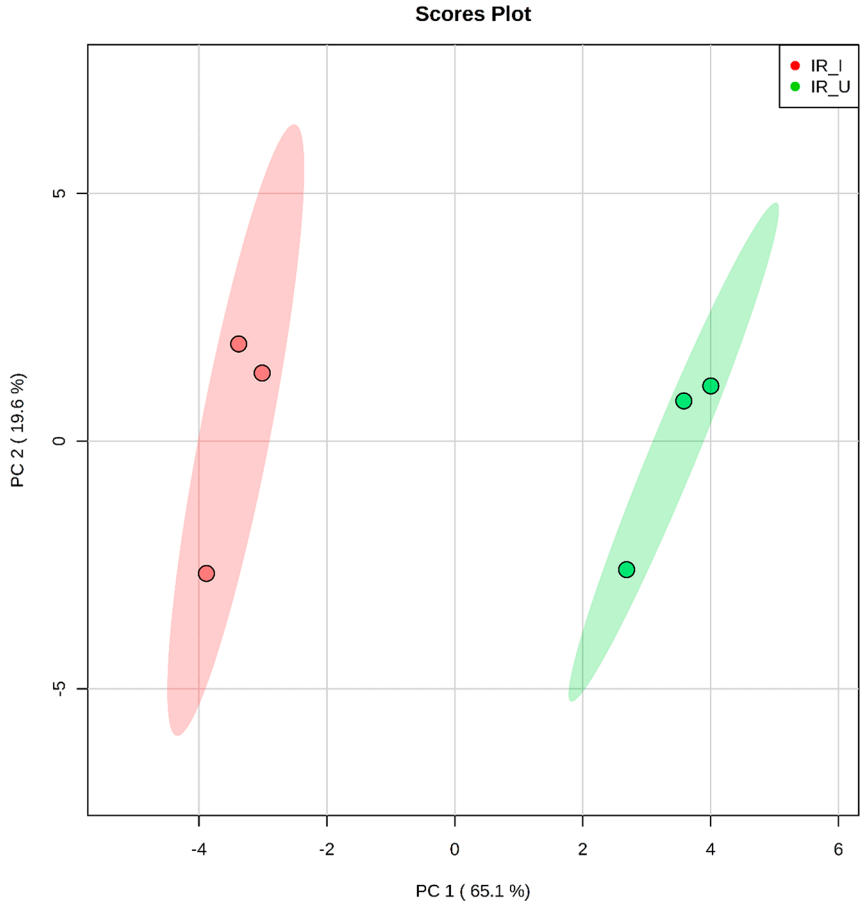


**Figure 4.** Principal component analysis of the metabolites found in seed extracts from India robusta. Metabolites and biomass components were extracted from India robusta uninfested (IR-U) and infested by the coffee berry borer (IR-I) seeds and quantified as described in the Materials and Methods. The top 20 features from Vietnam robusta PLS-DA were used for this analysis. Shaded red and green regions in this plot represent 95% confidence intervals between the two treatments.

uninfested arabica seeds. Increased chlorogenic acids during herbivory attack have been related to lignin biosynthesis and, hence, plant defense.[33] Also, plant extracts containing phenolics can be toxic for insects. For instance, the essential oils of *Ocimum gratissimum* (Lamiaceae), rich in di-*O*-caffeoylquinic acid, showed chronic toxicity to adult insects and larvae upon exposition.[34] Additional research is needed to determine the following: (i) how coffee berry borer infestation might influence changes in these defense-related compounds and (ii) the impact of the plant defense compounds on insect behavior. For instance, transcriptomics analyses have been previously performed on *C. arabica* var. Caturra and on *C. liberica* Bull. ex Hiern infested by the coffee berry borer.[35] Both species showed overexpression of genes related to ethylene production and pathogen recognition when infested by the coffee berry borer. However, differential expression comparisons revealed that there were also changes dependent on the genotype, demonstrating that the response to the coffee berry borer infestation is genotype-dependent.[35] Further transcriptomics studies on arabica Colombia Supremo targeting genes from the phenylpropanoid pathway could validate the results presented in this study.

**Higher Levels of Sugar Alcohols and Organic Acids in Infested Robusta Suggesting Host Priming.** Sugar alcohols (inositol, erythritol/threitol, and pentitols) and organic acids (azelaic acid, fumarate, and maleic acid) were significantly higher in infested Vietnam and India robusta seeds. Sugar alcohol accumulation has been correlated with osmotic stress and plant defense activity against pathogen attack.[36−38] Organic acid accumulation in plants have been related to biotic and abiotic stresses.[39] Additionally, azelaic acid has been reported to act on plant immunity and priming,[40,41] which could mean that the accumulation of this organic acid in coffee seeds infested by the coffee berry borer was a response to insect attack. Combining metabolomics with other omics, such as transcriptomics and proteomics, is a promising strategy for disclosing the complex relationship between plants and biotic stress. In fact, hormone and terpene production was validated as a response to herbivory by *Chilo suppressalis* (Lepidoptera) in rice using a combination of transcriptomic and metabolomic approaches.[42] Also, gene−metabolite associations performed in *Nicotiana attenuata* under herbivore attack uncovered systemic and local responses that were mainly driven by changes in genes and metabolites in the phenylpropanoid pathway.[32] Another study that combined transcriptomics and metabolomics reviewed the effects of aphids in maize and revealed important correlations with hormone synthesis, shikimate pathway metabolism changes, terpene production, and plant defense.[43] Complementing the data of the present study with transcriptomics analysis would validate and enlighten many aspects of the relationship coffee−insect that still need clarification. Additionally, evaluating the metabolomic profiles and gene expression in different tissues, not only seeds but also leaves and shoots, would integrate key


https://dx.doi.org/10.1021/acs.jafc.9b07363
J. Agric. Food Chem. 2020, 68, 2597−2605

information for understanding the coffee berry borer systemic effect on its host.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.9b07363.

Percentage of hemicellulose and fatty acid composition from the biomass of arabica Colombia Supremo or Vietnam robusta uninfested or coffee berry borer infested seeds; intracellular metabolites of arabica Colombia Supremo or Vietnam robusta uninfested or coffee berry borer infested seeds; total biomass: hemicellulose and fatty acid composition from the biomass and intracellular metabolites of India robusta uninfested or coffee berry borer infested seeds (PDF)

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### Funding

This project was partially supported by a National Science Foundation Grant NSF-DoB 1638999 to A.P.A.

### Notes

The authors declare no competing financial interest.


## ■ ACKNOWLEDGMENTS

We thank Gabriella Kratzer for her technical support during biomass and intracellular metabolite extractions and the BioAnalytical Facility at the University of North Texas where GC/MS and LC/MS/MS for hemicellulose and soluble sugars analysis were performed. We also thank Christopher Johnston for his careful reading of the manuscript.

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