

# ASSIMILATION EFFICIENCY OF FREE AND PROTEIN AMINO ACIDS BY *HOMALODISCA VITRIPENNIS* (HEMIPTERA: CICADELLIDAE: CICADELLINAE) FEEDING ON *CITRUS SINENSIS* AND *VITIS VINIFERA*

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## ABSTRACT

The feeding of *Homalodisca vitripennis*, also known as the glassy-winged sharpshooter (GWSS), on *Citrus sinensis* cv. Navel and *Vitis vinifera* cv. Chardonnay was monitored in relation to free and protein bound amino acids in xylem fluid. Mean consumption rate of GWSS confined to feeding assemblies was significantly higher on Chardonnay (2.0 cm<sup>3</sup>/d) than on Navel (0.4 cm<sup>3</sup>/d). The concentration of free amino acids in xylem fluid was substantially higher than amino acids in protein form for both host species. For Navel, the major amino acids were asparagine/aspartic acid and proline, which represented 75% of the free amino acids. Glutamine/glutamic acid accounted for 75% of the free amino acids in Chardonnay. To test whether GWSS feeding induced changes in xylem fluid chemistry, GWSS were caged on shoots of Navel and Chardonnay for 10 d, at which time feeding rate was determined and amino acids in xylem fluid and in insect excreta were quantified. There was no significant effect of prior feeding on GWSS feeding rate. For Navel, GWSS feeding induced a significant increase in serine, arginine, alanine, methionine, isoleucine, leucine and lysine in protein form; however, the concentrations of most protein amino acids in xylem fluid of Chardonnay were not significantly increased. The assimilation efficiency (AE) of total free amino acids exceeded 99%, whereas total amino acids in protein form were assimilated with 90 to 98% efficiency. The AE was significantly increased for GWSS feeding on GWSS-caged shoots compared to that of control shoots.

**Key Words:** amino acids, *Citrus sinensis*, glassy-winged sharpshooter, *Homalodisca vitripennis*, proteins, *Vitis vinifera*, xylem fluid

## RESUMEN

Se realizó un monitoreo de la alimentación de *Homalodisca vitripennis*, también conocido como el salta hojas (Cicadellidae) de alas vidriosas (GWSS), sobre *Citrus sinensis* cv. Navel y *Vitis vinifera* cv. Chardonnay, en su relación a los aminoácidos libres y ligados por proteínas en fluido de xilema. El promedio de la tasa del consumo de GWSS confinadas por sus patrones de alimentación fue significativamente más alto en Chardonnay (2.0 cm<sup>3</sup>/d) que en Navel (0.4 cm<sup>3</sup>/d). La concentración de los aminoácidos libres en el fluido de xilema fue substancialmente más alta que los aminoácidos en la forma de proteína para las dos especies de hospederos. Para la Navel, los aminoácidos principales fueron ácido asparragina/aspártico y prolina, que representaron 75% de los aminoácidos libres. El ácido glutamina/glutámico conto de 75% de los aminoácidos libres en Chardonnay. Para probar si la alimentación por las GWSS indujo cambios en la química del fluido de xilema, las GWSS fueron enjauladas sobre brotes de Navel y Chardonnay por 10 días, durante la cual la tasa de alimentación fue determinada y los aminoácidos en el fluido de xilema y en el excremento de los insectos fueron cuantificados. No hubo un efecto significativo de la alimentación hecha previamente sobre la tasa de alimentación de GWSS. Para Navel, la alimentación de GWSS indujeron un aumento significativo en serina, arginina, alanina, metionina, isoleucina, leucina y lisina en forma de proteína; sin embargo, las concentraciones de la mayoría de los aminoácidos de los proteínas en el fluido de xilema de Chardonnay no se aumentaron significativamente. La eficiencia de asimilación (EA) de los aminoácidos libres totales fue en exceso de 99%, mientras que los aminoácidos totales en la forma de proteína fueron asimiladas con una eficiencia de 90 a 98%. La EA fue aumentada significativamente para la alimentación de GWSS sobre brotes en las jaulas con GWSS en comparación con los brotes de control.

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis*, is endemic to the southeastern United States and northeastern Mexico (Turner & Pollard 1959; Redak et al. 2004; Takiya et al. 2006). The GWSS may feed on the xylem

fluid from hundreds of plant species from 37 families (Turner & Pollard 1959; Redak et al. 2004). It is an important vector of many diseases caused by *Xylella fastidiosa* (Wells et al. 1987), including Pierce's disease of grapevine, phony peach dis-

ease, plum leaf scald, and citrus variegated chlorosis (Hopkins & Purcell 2002). The GWSS was first detected in California in 1989 (Sorensen & Gill 1996). Pierce's disease precludes the culture of *Vitis vinifera* in the southeastern US and is threatening the 33 billion dollar grape industry in California ([www.wineinstitute.org](http://www.wineinstitute.org)).

The determinant of host plant selection and the performance of polyphagous insects is often organic nitrogen (Slansky & Feeny 1977; Mattson 1980; Slansky & Scriber 1985). Nitrogen form may be more important than total nitrogen (Taylor & Medici 1966; Brodbeck & Strong 1987). Polyphagy may be more critical to the success of GWSS and other xylem feeders than insects of other feeding guilds because xylem fluid contains the lowest concentration of nutrients and organic carbon of any plant tissue (Raven 1983; Andersen & Brodbeck 1989; Andersen et al. 1989, 1992). Xylem fluid is 95 to 99% water and total osmolality is usually 10 to 20 mM (Andersen & Brodbeck 1989; Andersen et al. 1989, 1995). The primary organic compounds in xylem fluid are amino acids, organic acids, and sugars; inorganic ions account for most of the remaining solutes (Andersen & Brodbeck 1989; Andersen et al. 1989, 1992, 1995). The concentrations of secondary compounds in xylem fluid are exceedingly low (Raven 1983), al-

though proteins occur in low concentration (Biles et al. 1989; Biles & Abeles 1991; Buhtz et al. 2004).

Physiological and behavioral adaptations of GWSS that allow subsistence on xylem fluid include (1) an extremely high assimilation efficiency (AE) (>99%) of organic compounds in monomeric form; (2) extremely high feeding rates (10 to 100 times body weight per hour); (3) conservation of organic carbon by ammonotelism, and; (4) host plant switching to obtain an appropriate mix of nutrients (Andersen et al. 1989, 1992; Brodbeck et al. 1990, 1993). In xylem fluid of *V. vinifera* Chardonnay, the predominant amino acid is glutamine (Andersen et al. 2005), whereas asparagine and proline predominate in orange (*Citrus sinensis* L.) xylem fluid (Bi et al. 2005). Wu et al. (2006) isolated the bacterial symbionts *Baumannia cicadellinicola* and *Sulcia muelleri* from GWSS and suggested that they function to synthesize vitamins and essential amino acids, respectively. The abundance (Brodbeck et al. 1990; Andersen et al. 2005) and consumption rate (Andersen et al. 1992, 2005; Brodbeck et al. 1993) of adult GWSS have been correlated with the concentration of the amides in xylem fluid. It is unknown how GWSS utilizes host plant proteins. Bi et al. (2005), studying GWSS feeding on orange

TABLE 1. TOTAL PROTEIN AND FREE AMINO ACIDS IN XYLEM FLUID OF *CITRUS SINENSIS* CV. NAVEL FOR GLASSY-WINGED SHARPSHOOTER (GWSS)-CAGED AND CONTROL SHOOTS. VALUES PRESENTED ARE MEANS  $\pm$  1 STANDARD ERROR,  $N = 4$ .

	Concentration ( $\mu$ M)					
	Total Amino Acids		Protein Amino Acids		Free Amino Acids	
	GWSS	Control	GWSS	Control	GWSS	Control
ASP/ASN	1278 $\pm$ 123	1847 $\pm$ 213	351 $\pm$ 39	394 $\pm$ 44	928 $\pm$ 89	1453 $\pm$ 203
GLU/GLN	309 $\pm$ 26	380 $\pm$ 43	214 $\pm$ 18	196 $\pm$ 32	95 $\pm$ 12	184 $\pm$ 33
SER	211 $\pm$ 25	212 $\pm$ 36	54 $\pm$ 2*	15 $\pm$ 9	157 $\pm$ 27	197 $\pm$ 34
GLY	173 $\pm$ 11	152 $\pm$ 34	144 $\pm$ 6	104 $\pm$ 20	30 $\pm$ 5	48 $\pm$ 15
HIS	30 $\pm$ 2	23 $\pm$ 5	16 $\pm$ 3	10 $\pm$ 2	14 $\pm$ 4	13 $\pm$ 5
ARG	250 $\pm$ 60	165 $\pm$ 66	54 $\pm$ 7*	31 $\pm$ 5	196 $\pm$ 56	134 $\pm$ 64
THR	68 $\pm$ 6	63 $\pm$ 12	47 $\pm$ 3	32 $\pm$ 9	20 $\pm$ 2	31 $\pm$ 7
ALA	146 $\pm$ 10	112 $\pm$ 20	101 $\pm$ 2*	69 $\pm$ 10	46 $\pm$ 10	43 $\pm$ 11
PRO	1118 $\pm$ 231	1173 $\pm$ 138	122 $\pm$ 7	167 $\pm$ 35	995 $\pm$ 225	1006 $\pm$ 152
TYR	25 $\pm$ 4	13 $\pm$ 5	11 $\pm$ 4	2 $\pm$ 3	13 $\pm$ 3	11 $\pm$ 3
VAL	89 $\pm$ 6	87 $\pm$ 15	62 $\pm$ 6	47 $\pm$ 10	28 $\pm$ 4	41 $\pm$ 8
MET	8 $\pm$ 1	4 $\pm$ 1	2 $\pm$ 1*	0 $\pm$ 0	6 $\pm$ 1	5 $\pm$ 1
CYS	1 $\pm$ 0	0 $\pm$ 0	1 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
ILE	60 $\pm$ 3	50 $\pm$ 8	50 $\pm$ 2*	35 $\pm$ 5	10 $\pm$ 1	15 $\pm$ 4
LEU	97 $\pm$ 6	70 $\pm$ 12	84 $\pm$ 4*	57 $\pm$ 9	13 $\pm$ 2	13 $\pm$ 4
PHE	65 $\pm$ 7	48 $\pm$ 12	53 $\pm$ 5	38 $\pm$ 11	12 $\pm$ 2	10 $\pm$ 1
LYS	83 $\pm$ 8	55 $\pm$ 10	56 $\pm$ 5*	33 $\pm$ 5	27 $\pm$ 7	22 $\pm$ 7
Total	4007 $\pm$ 507	4453 $\pm$ 439	1420 $\pm$ 87	1228 $\pm$ 131	2587 $\pm$ 432	3225 $\pm$ 383

\*Signifies that GWSS shoots are significantly different from controls,  $t$ -test, 95% level.

ND = not detectable.

TABLE 2. TOTAL PROTEIN AND FREE AMINO ACIDS IN XYLEM FLUID OF *VITIS VINIFERA* CV. CHARDONNAY FOR GLASSY-WINGED SHARPSHOOTER (GWSS)-CAGED AND CONTROL SHOOTS. VALUES PRESENTED ARE MEANS  $\pm$  1 STANDARD ERROR,  $N = 4$ .

	Concentration ( $\mu$ M)					
	Total Amino Acids		Protein Amino Acids		Free Amino Acids	
	GWSS	Control	GWSS	Control	GWSS	Control
ASP/ASN	99 $\pm$ 15	97 $\pm$ 29	47 $\pm$ 10	36 $\pm$ 9	52 $\pm$ 6	61 $\pm$ 21
GLU/GLN	1089 $\pm$ 158	1356 $\pm$ 575	244 $\pm$ 42	272 $\pm$ 135	846 $\pm$ 117	1084 $\pm$ 441
SER	48 $\pm$ 9	37 $\pm$ 10	28 $\pm$ 6	15 $\pm$ 1	20 $\pm$ 3	22 $\pm$ 10
GLY	52 $\pm$ 14	31 $\pm$ 5	30 $\pm$ 8*	10 $\pm$ 4	22 $\pm$ 5	21 $\pm$ 10
HIS	12 $\pm$ 2	13 $\pm$ 5	2 $\pm$ 0	1 $\pm$ 0	10 $\pm$ 2	12 $\pm$ 5
ARG	96 $\pm$ 15	99 $\pm$ 14	8 $\pm$ 3	1 $\pm$ 0	88 $\pm$ 17	97 $\pm$ 14
THR	30 $\pm$ 7	19 $\pm$ 7	13 $\pm$ 6	3 $\pm$ 2	17 $\pm$ 2	16 $\pm$ 7
ALA	39 $\pm$ 7	25 $\pm$ 5	18 $\pm$ 6	8 $\pm$ 2	21 $\pm$ 3	17 $\pm$ 3
PRO	29 $\pm$ 6	25 $\pm$ 8	12 $\pm$ 6	6 $\pm$ 4	17 $\pm$ 3	18 $\pm$ 5
TYR	14 $\pm$ 3	9 $\pm$ 2	6 $\pm$ 2*	2 $\pm$ 0	8 $\pm$ 2	7 $\pm$ 2
VAL	30 $\pm$ 6	27 $\pm$ 10	11 $\pm$ 4	3 $\pm$ 2	20 $\pm$ 3	24 $\pm$ 11
MET	4 $\pm$ 1	3 $\pm$ 2	0 $\pm$ 0	0 $\pm$ 0	4 $\pm$ 0	5 $\pm$ 2
CYS	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	1 $\pm$ 0
ILE	20 $\pm$ 5	18 $\pm$ 7	9 $\pm$ 4	3 $\pm$ 1	11 $\pm$ 2	15 $\pm$ 8
LEU	21 $\pm$ 6	19 $\pm$ 7	16 $\pm$ 5	8 $\pm$ 2	5 $\pm$ 1	11 $\pm$ 6
PHE	22 $\pm$ 5	19 $\pm$ 6	6 $\pm$ 3	1 $\pm$ 0	16 $\pm$ 2	17 $\pm$ 6
LYS	8 $\pm$ 2	6 $\pm$ 1	5 $\pm$ 2	3 $\pm$ 0	3 $\pm$ 0	3 $\pm$ 1
Total	1611 $\pm$ 222	1803 $\pm$ 685	451 $\pm$ 98	369 $\pm$ 143	1160 $\pm$ 127	1433 $\pm$ 532

\*Signifies that GWSS shoots are significantly different from controls, *t*-test, 95% level.

ND = not detectable.

and lemon (*Citrus limon* L.) trees, found that free amino acids, but not proteins, in xylem fluid were correlated with seasonal leafhopper abundance.

The quantity of assimilates removed by high populations of xylem feeding leafhoppers can be substantial (Andersen et al. 2003). In California, the density of GWSS on a lemon tree can be 2,000 to 5,000 per tree (Luck & Hoddle 2002). Insect feeding may induce defensive responses by host plants (Stout & Duffey 1996; Karban & Baldwin 1997; Stout et al. 1998). The concentrations of primary metabolites, tissue N concentration and secondary compounds (alkaloids, oxidative enzymes, etc.) can be altered by insect feeding (Stout et al. 1998). Not only can insect feeding affect plant physiology, but it also can affect subsequent insect behavior and performance. There have been over 100 reports of a reduced level of insect performance (survival, growth, fecundity, etc.) in response to induced plant resistance (e.g., Broadway et al. 1989; Rausher et al. 1993; Stout & Duffey 1996; Karban & Baldwin 1997; Stout et al. 1998).

The objectives of this study were to (1) determine whether GWSS feeding on *V. vinifera* cv. Chardonnay and *C. sinensis* cv. Navel induces a change in amino acids in xylem fluid; (2) examine whether or not prior GWSS feeding alters subsequent feeding; (3) quantify the relative contribu-

tion of free and protein bound amino acids to the nutrition of GWSS feeding on xylem fluid, and; (4) quantify the efficiency of amino acid assimilation, both free and protein bound, by GWSS.

## MATERIALS AND METHODS

### Experimental Material

We used 4-year-old, field-grown *C. sinensis* cv. Navel on Carizzo rootstocks and 1-year-old container-grown *V. vinifera* cv. Chardonnay grapevines located at the Univ. of Florida North Florida Research and Education Center-Quincy, FL. No leafhoppers were observed on Navel during daily observation in the field. Chardonnay grapevines were grown in a leafhopper-proof greenhouse. GWSS were collected on site and mainly from crape myrtle (*Lagerstroemia indica* L.). Twenty and 10 GWSS were caged in a fine mesh sleeve on a shoot of Navel (on 28 Aug 2006) and Chardonnay (on 1 Sep 2006), respectively, for a period of 10 d. The sleeve on Navel trees was positioned over a major shoot, whereas the sleeve for Chardonnay enclosed the main shoot that was to become the trunk. The rationale for enclosing 20 leafhoppers on Navel and 10 on Chardonnay was based on the fact that Navel was a medium-sized tree, and Chardonnay was a small container-grown plant.

TABLE 3. ASSIMILATION EFFICIENCIES (%) OF THE GLASSY-WINGED SHARPSHOOTER (GWSS) FEEDING ON XYLEM FLUID OF *CITRUS SINENSIS* CV. NAVEL GWSS-CAGED AND CONTROL SHOOTS. VALUES PRESENTED ARE MEANS  $\pm$  1 STANDARD ERROR,  $N = 4$ .

	Assimilation Efficiency (%)					
	Total Amino Acids		Protein Amino Acids		Free Amino Acids	
	GWSS	Control	GWSS	Control	GWSS	Control
ASP/ASN	99.9 $\pm$ 0.0	99.7 $\pm$ 0.1	99.5 $\pm$ 0.2	98.6 $\pm$ 0.4	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
GLU/GLN	98.8 $\pm$ 0.3	96.8 $\pm$ 0.9	98.7 $\pm$ 0.4*	95.0 $\pm$ 0.6	98.9 $\pm$ 0.8	98.6 $\pm$ 1.1
SER	98.6 $\pm$ 0.5*	96.4 $\pm$ 0.4	95.8 $\pm$ 0.9*	86.1 $\pm$ 22.4	99.7 $\pm$ 0.2	99.4 $\pm$ 0.0
GLY	95.7 $\pm$ 1.4*	88.7 $\pm$ 1.2	95.2 $\pm$ 1.6*	84.6 $\pm$ 1.9	98.5 $\pm$ 0.9	98.0 $\pm$ 0.7
HIS	97.3 $\pm$ 0.9*	88.4 $\pm$ 0.9	97.4 $\pm$ 1.5*	69.8 $\pm$ 8.8	98.9 $\pm$ 1.1	100.0 $\pm$ 0.0
ARG	98.8 $\pm$ 0.5	96.5 $\pm$ 0.3	96.2 $\pm$ 0.8*	84.7 $\pm$ 4.0	99.9 $\pm$ 0.0	99.2 $\pm$ 0.6
THR	98.8 $\pm$ 0.4*	92.0 $\pm$ 1.0	98.8 $\pm$ 1.1*	82.8 $\pm$ 2.8	98.9 $\pm$ 1.1	99.4 $\pm$ 0.6
ALA	96.6 $\pm$ 0.5	89.5 $\pm$ 1.2	95.3 $\pm$ 0.7*	85.1 $\pm$ 2.4	99.7 $\pm$ 0.3*	96.3 $\pm$ 0.5
PRO	99.7 $\pm$ 0.1	99.5 $\pm$ 0.1	97.4 $\pm$ 0.7	95.1 $\pm$ 2.7	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
TYR	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 1.3	98.1 $\pm$ 9.9	98.4 $\pm$ 1.6	93.1 $\pm$ 2.7
VAL	96.9 $\pm$ 0.4*	92.1 $\pm$ 1.3	96.5 $\pm$ 0.8	87.4 $\pm$ 3.1	98.3 $\pm$ 1.0	96.6 $\pm$ 1.7
MET	76.1 $\pm$ 16.1	70.0 $\pm$ 7.6	ND	88.9 $\pm$ 67.6	100.0 $\pm$ 0.0	71.4 $\pm$ 24.0
CYS	100.0 $\pm$ 0.0	ND	ND	ND	100.0 $\pm$ 0.0	ND
ILE	97.1 $\pm$ 0.7*	89.6 $\pm$ 1.2	96.6 $\pm$ 0.9*	91.0 $\pm$ 2.4	100.0 $\pm$ 0.0*	83.4 $\pm$ 4.3
LEU	96.4 $\pm$ 0.4*	86.6 $\pm$ 1.9	96.4 $\pm$ 0.4*	83.8 $\pm$ 2.6	96.8 $\pm$ 1.9	98.9 $\pm$ 1.1
PHE	98.0 $\pm$ 0.4*	91.9 $\pm$ 1.3	98.1 $\pm$ 0.7*	89.7 $\pm$ 2.2	98.5 $\pm$ 1.5	98.1 $\pm$ 1.9
LYS	98.2 $\pm$ 0.5*	91.8 $\pm$ 1.0	97.4 $\pm$ 0.4	94.1 $\pm$ 4.2	100.0 $\pm$ 0.0*	85.1 $\pm$ 3.5
Total	99.0 $\pm$ 0.2*	97.7 $\pm$ 0.5	97.5 $\pm$ 0.6*	92.8 $\pm$ 1.7	99.8 $\pm$ 0.1*	99.6 $\pm$ 0.1

\*Signifies that GWSS shoots are significantly different from controls,  $t$ -test, 95% level.

ND = not detectable.

There were 4 plants (replications) and 4 plants were retained as control plants (a sleeve with no leafhoppers). For the last 3 d of the experiment, feeding assemblies (Andersen et al. 1992) were installed at the end of each shoot in a region that was formerly enclosed by the sleeve. In brief, the feeding assembly consisted of a 50-mL centrifuge tube with sponge rubber inserted on one end and a 3-mm hole drilled through the center of the other end to accommodate the shoot. A snap cap was glued to the bottom of the centrifuge tube at an angle, and insect excreta were collected daily in a removable collection tube that hung vertically. After feeding rate was quantified the shoot was excised and xylem fluid was extracted with a pressure chamber apparatus (Scholander et al. 1965). The tissue exterior to the vascular cambium was removed from the stem protruding from the chamber and a 0.25 MPa overpressure was used for a period of 60 seconds (Andersen et al. 1989, 1992; Berger et al. 1994).

#### Chemical Analyses

Each sample of xylem fluid and of leafhopper excreta was divided for quantification of free and protein bound amino acids. One subsample was filtered through a 10,000-MW filter (Millipore Corp., Bedford, MA, USA) for analysis of free

amino acids. The other subsample was not filtered, and was retained for analysis of total amino acids (protein bound plus free). We analyzed amino acids at the Florida State University Analytical Laboratory. Samples were lyophilized and then hydrolyzed with constant boiling (110°C) in 6 M hydrochloric acid under  $N_2$  atmosphere. Samples were derivitized by adding 2:2:1 ethanol: triethanolamine (TEA): water. Next, 7:1:1:1 ethanol: TEA: water: phenylisothiocyanate were added and reactions proceeded for 20 min under  $N_2$  atmosphere. Samples were analyzed in 5 mM sodium phosphate buffer with 6% acetonitrile on a Waters HPLC gradient system with a Pico Tag column (Waters Division, Millipore Corp., Milliford, MA USA) (Heinrickson & Meredith 1984).

#### Calculations and Statistics

The concentration of total amino acids was obtained from hydrolyzed samples. Free amino acids were determined from filtered samples that were not hydrolyzed. Amino acids bound in protein form were calculated as the difference in concentration between hydrolyzed samples minus nonhydrolyzed samples. The concentrations of total, free, and protein amino acids in xylem fluid and in insect excreta were determined from in-

TABLE 4. ASSIMILATION EFFICIENCIES (%) OF THE GLASSY-WINGED SHARPSHOOTER (GWSS) FEEDING ON XYLEM FLUID OF *VITIS VINIFERA* CV. CHARDONNAY GWSS-CAGED AND CONTROL SHOOTS. VALUES PRESENTED ARE MEANS  $\pm$  1 STANDARD ERROR,  $N = 4$ .

	Assimilation Efficiency (%)					
	Total Amino Acids		Protein Amino Acids		Free Amino Acids	
	GWSS	Control	GWSS	Control	GWSS	Control
ASP/ASN	99.2 $\pm$ 0.3*	95.7 $\pm$ 0.9	98.3 $\pm$ 0.7*	89.1 $\pm$ 3.2	100.0 $\pm$ 0.0	99.7 $\pm$ 0.3
GLU/GLN	99.9 $\pm$ 0.0*	99.2 $\pm$ 0.2	99.3 $\pm$ 0.1*	96.2 $\pm$ 0.9	100.0 $\pm$ 0.0	99.9 $\pm$ 0.1
SER	96.1 $\pm$ 1.0	91.5 $\pm$ 2.5	94.4 $\pm$ 1.8	88.4 $\pm$ 6.7	98.1 $\pm$ 1.9	94.7 $\pm$ 3.2
GLY	95.0 $\pm$ 1.5*	85.2 $\pm$ 3.0	91.8 $\pm$ 2.9	48.3 $\pm$ 24.1	98.9 $\pm$ 1.1	95.0 $\pm$ 2.9
HIS	97.2 $\pm$ 2.8	90.0 $\pm$ 2.9	75.0 $\pm$ 2.5	33.3 $\pm$ 33.3	100.0 $\pm$ 0.0	98.4 $\pm$ 1.6
ARG	99.0 $\pm$ 0.4	98.5 $\pm$ 0.6	91.5 $\pm$ 3.5	ND	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
THR	97.9 $\pm$ 1.3	87.4 $\pm$ 5.5	92.9 $\pm$ 4.7	ND	100.0 $\pm$ 0.0	98.9 $\pm$ 1.1
ALA	96.7 $\pm$ 1.5	87.8 $\pm$ 1.4	92.6 $\pm$ 3.7	70.0 $\pm$ 11.5	100.0 $\pm$ 0.0	95.1 $\pm$ 2.6
PRO	97.4 $\pm$ 1.0	90.3 $\pm$ 2.4	89.3 $\pm$ 5.7	47.6 $\pm$ 26.8	100.0 $\pm$ 0.0	96.7 $\pm$ 1.8
TYR	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	100.0 $\pm$ 2.5	100.0 $\pm$ 0.0	97.7 $\pm$ 2.3	100.0 $\pm$ 0.0
VAL	97.6 $\pm$ 0.9	91.2 $\pm$ 3.6	95.5 $\pm$ 3.0	94.4 $\pm$ 5.5	98.8 $\pm$ 1.3	91.4 $\pm$ 4.3
MET	87.5 $\pm$ 12.5	61.1 $\pm$ 3.1	50.0 $\pm$ 5.0	ND	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
CYS	ND	100.0 $\pm$ 0.0	ND	100.0 $\pm$ 0.0	ND	100.0 $\pm$ 0.0
ILE	98.2 $\pm$ 1.8	90.2 $\pm$ 3.6	95.0 $\pm$ 5.0	93.3 $\pm$ 6.7	100.0 $\pm$ 0.0*	90.0 $\pm$ 3.4
LEU	96.5 $\pm$ 1.5	86.4 $\pm$ 6.9	95.2 $\pm$ 2.1	75.5 $\pm$ 10.5	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
PHE	98.3 $\pm$ 1.7	91.6 $\pm$ 2.5	87.5 $\pm$ 12.5	ND	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0
LYS	90.5 $\pm$ 3.8	75.8 $\pm$ 5.5	85.2 $\pm$ 6.2	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0*	52.8 $\pm$ 12.0
Total	99.2 $\pm$ 0.2*	97.4 $\pm$ 0.6	97.3 $\pm$ 0.9*	89.9 $\pm$ 2.8	99.9 $\pm$ 0.1*	99.3 $\pm$ 0.00

\*Signifies that GWSS shoots are significantly different from controls,  $t$ -test, 95% level.

ND = not detectable.

sect-caged shoots and control shoots. Assimilation efficiency (AE) of amino acids by GWSS was calculated as  $AE = (\text{amino acid in xylem fluid} - \text{amino acid in insect excreta}) / (\text{amino acid in xylem fluid})$ . Means  $\pm$  1 standard error ( $n = 4$ ) were calculated. Amino acid data were also subjected to analysis of variance with SAS general linear models (SAS 1999). Significant differences between GWSS-caged and control shoots were determined by a  $t$ -test ( $P < 0.05$ ). AE data were statistically analyzed with an arcsin square root conversion because most of the AE values were 90 to 100% (SAS 1999).

## RESULTS AND DISCUSSION

Consumption rates were higher for GWSS feeding on Chardonnay ( $2.0 \text{ cm}^3/\text{d} \pm 0.7$ ; mean  $\pm$  1 standard error) compared to Navel ( $0.4 \text{ cm}^3/\text{d} \pm 0.2$ ; mean  $\pm$  1 standard error). Feeding rate of GWSS on GWSS-caged and control shoots was not significantly different. The amino acid profile of Navel xylem fluid is presented in Table 1. The concentrations of total (free plus protein) amino acids were 4.0 to 4.4 mM. Free amino acids represented 65 and 72% of total amino acids in xylem fluid from GWSS and control treatments, respectively. Aspartic acid/asparagine and proline predominated in the free amino acid pool, and conse-

quently for total amino acids as well. These amino acids accounted for at least 75% of the total free amino acids, but only 33 to 46% of the amino acids in protein form. Thus, the profile of amino acids bound in protein form was far more "balanced" (Taylor & Medici 1966; Brodbeck & Strong 1987) than the profile of free amino acids. In the free amino acid pool, the concentrations of aspartic acid/asparagine and glutamic acid/glutamine were reduced by 36 and 48%, respectively, as a result of GWSS feeding; however, these differences were not significant at the 95% level. By contrast, GWSS feeding induced a significant increase in the concentrations of many amino acids in protein form (serine, arginine, alanine, methionine, isoleucine, leucine, and lysine). The reason for the increase in protein is not clear. Buhtz et al. (2004) reported that the most abundant proteins (peroxidases, chitinases, and serine proteases) are present in the xylem of many plant species and they function to maintain cell wall metabolism, lignification, and insect-host, and pathogen-host responses. Thus, it appeared possible that GWSS feeding induced an increase in proteins that function in plant defense.

The concentration of glutamic acid/glutamine in the xylem fluid of Chardonnay accounted for 75% of the total free amino acids and between 68 and 76% of the total amino acids (Table 2). Free

amino acids comprised between 72 and 79% of the total amino acids. There was a trend for free amino acids to be reduced in concentration in xylem fluid of GWSS-caged shoots, but significant differences did not occur. By contrast, the concentration of many amino acids in protein form (serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, isoleucine, leucine, and phenylalanine) were increased about 2-fold or higher in xylem of GWSS-caged shoots, although differences were significant for only glycine and tyrosine.

The assimilation efficiencies (AE) of GWSS feeding on free, protein, and total amino acids in Navel xylem fluid are presented in Table 3. The AE of free, protein bound, and total amino acids were higher for GWSS-caged shoots than control shoots. Free amino acids were assimilated with at least 99.6% efficiency, which agrees with the literature (Andersen et al. 1989; Brodbeck et al. 1993). Many free amino acids were assimilated with 100% efficiency. The AE of alanine, isoleucine, and lysine in free amino acid form were higher for insects feeding on GWSS-caged shoots than control shoots. The AE of total amino acids in protein form was 97.5 and 92.8% for GWSS-caged and control shoots, respectively. This is the first work to show that proteins are assimilated by GWSS with high efficiency, but not as high as free amino acids. The feeding of GWSS on GWSS-caged shoots was associated with a significantly higher AE of many bound protein amino acids (glutamic acid/glutamine, serine, glycine, histidine, arginine, threonine, alanine, valine, isoleucine, leucine, and phenylalanine) and total amino acids (serine, glycine, histidine, threonine, valine, isoleucine, leucine, phenylalanine, and lysine) compared to control shoots. These data are not consistent with the hypothesis that GWSS feeding induces an increase in xylem proteins that are inhibitory to GWSS metabolism.

For Chardonnay the AE of total free, protein, and total (free plus protein) amino acids was significantly higher on GWSS-caged shoots than for the control (Table 4). Total free amino acids were assimilated with greater than 99% efficiency. The AE of total protein amino acids was approximately 97 and 90% for GWSS-caged and control shoots, respectively. GWSS assimilated total amino acids with 99 and 97% efficiency for GWSS-caged and control shoots, respectively. For free amino acids, the AE for isoleucine and lysine were higher for GWSS-caged shoots. The AE for leafhoppers feeding on GWSS-caged shoots was higher for aspartic acid/asparagine and glutamic acid/glutamine in protein form and total protein amino acids than for control shoots.

There is a wealth of literature showing that insect herbivory induces the synthesis and/or release of chemical defenses (Broadway et al. 1989; Stout & Duffey 1996; Karban & Baldwin 1997;

Stout et al. 1998). However, there are few secondary compounds in xylem fluid (Raven 1983), and to the best of our knowledge an antifeedant in xylem fluid has not been reported. Feeding by GWSS on xylem fluid of Navel and Chardonnay induced a 20% decrease in free amino acids, yet there was an increase in many essential amino acids in protein form. Despite this plant response to herbivory, there is no evidence that GWSS feeding is deleterious to their subsequent feeding. In Florida, GWSS often aggregate, and the presence of GWSS on a sticky card or GWSS cadavers placed on a shoot often attracts more GWSS than the controls without GWSS (R. F. Mizell, III, personal observation). In California, the number of GWSS on orange and lemon trees can exceed 4,000 per tree (Luck & Hoddle 2002). Perhaps the induction of protein bound amino acids by GWSS feeding, and the increased efficiency in assimilating these amino acids, can provide a nutritional basis for GWSS aggregation on a plant.

#### SUMMARY

The majority of amino acids in xylem fluid of Navel and Chardonnay are present in monomeric form. The profile of free amino acids is very "unbalanced" with 1 or 2 amino acids accounting for the great majority of the total amino acids. Proteins also occur in xylem, and the amino acid profiles of proteins were far more balanced than the free amino acid pool. GWSS feeding on xylem fluid of Navel induced a significant increase in amino acids in bound in protein form, and for both Navel and Chardonnay there was an increased efficiency in assimilating amino acids when GWSS fed on GWSS-caged shoots compared to control shoots. The induction of proteins and the consequent increase in AE by GWSS may, at least partially, explain a nutritional basis for GWSS aggregation.

#### ACKNOWLEDGMENTS

We thank D. Fiore and the Analytical Laboratory at Florida State University for assistance in chemical analysis. We thank J. H. Aldrich and J. Herbet for their helpful reviews. The authors gratefully acknowledge the financial support of the California Department of Food and Agriculture and the University of California-Davis.

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