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Research Note

Effects of infestation by grape phylloxera on sugars, free amino acids, and starch of grapevine roots

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K e y w o r d s : amino acids, fructose, glucose, grape phylloxera, grapevine roots, nutrients, starch, storage compounds, sucrose.

Introduction: Despite the long history of devastating effects of grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), on the European grape, *Vitis vinifera* L., the limitations on population growth are not well understood (OMER *et al*. 1997). Performance of grape phylloxera increases with increasing population load on susceptible and weakly resistant, but not on strongly resistant rootstocks (OMER *et al*. 1999). We hypothesize that phylloxera root galls serve to mobilize nutrients stored in grape roots. We therefore determined the effect phylloxera have on the root nutrients, starch, free amino acids and soluble sugars. Phylloxera survival and growth were measured simultaneously to control for root quality. Since we know that excised roots support grape phylloxera populations, we chose to do these initial studies using this simplified plant insect system.

Material and Methods: Excised roots from greenhouse grown Merlot and AXR#1 plants were maintained in Petri dishes in the dark at 24 °C. For infestation, 30 eggs (4-5 d old) from California biotype A or B phylloxera (DE BENEDICTIS and GRANETT 1993) were used per root piece. Survival was the percentage of the original number of insects on each root piece living after 3, 4, or 5 weeks. A population age structure index (PASI) was determined as described (OMER *et al*. 1999). Experiment 1 focused on effect of infestation on root chemistry during weeks 3, 4, and 5. Experiment 2 focused on differences between treatment effects at week 0 and 5. Each tissue sample chemically analyzed was about 30 mg fresh weight taken from the bark and parenchyma of feeding and non-feeding sites on roots. Sugars were quantified in ethanolic extracts by HPLC (AUNG *et al.* 1998). Free amino acids in extracts were determined using ninhydrin (MOORE and STEIN 1948) with leucine as the standard. Starch was measured as glucose after digestion of the pellet with α-amylase and amyloglucosidase with correction for residual free glucose. Two- or 3-way factorial ANOVAs were used to analyze all data. Data were transformed prior to ANOVA as necessary to equalize variance. Means were separated by least significant differences (LSD) at $\alpha = 0.05$.

Results and Discussion: In expt. 1, phylloxera survival ranged from 48 to 53% and was similar for biotypes A and B ($F = 2.00$; $P = 0.166$). Developmental rate of biotype B was significantly faster ($F = 44.07$; $P = 0.0001$) than biotype A on AXR#1 (Figure). By week 5, all biotype B phylloxera had attained the adult stage but only 38 % of biotype A had. Both phylloxera biotypes developed at similar rates on Merlot roots. In expt. 2, survival of biotype A was 40 % and 41 % on AXR#1 and Merlot, respectively, significantly lower than in the first expt. ($F = 25.19$; $P = 0.0001$) possibly indicating lesser root quality. In expt. 2, the PASI for biotype A was 4.84 on Merlot roots and 3.49 on AXR#1, significantly lower than in expt. 1 ($F = 29.10$; $P = 0.0001$). In both expts. phylloxera formed galls, survived, developed to the adult stage and reproduced on the excised roots, suggesting dependence on stored nutrients.

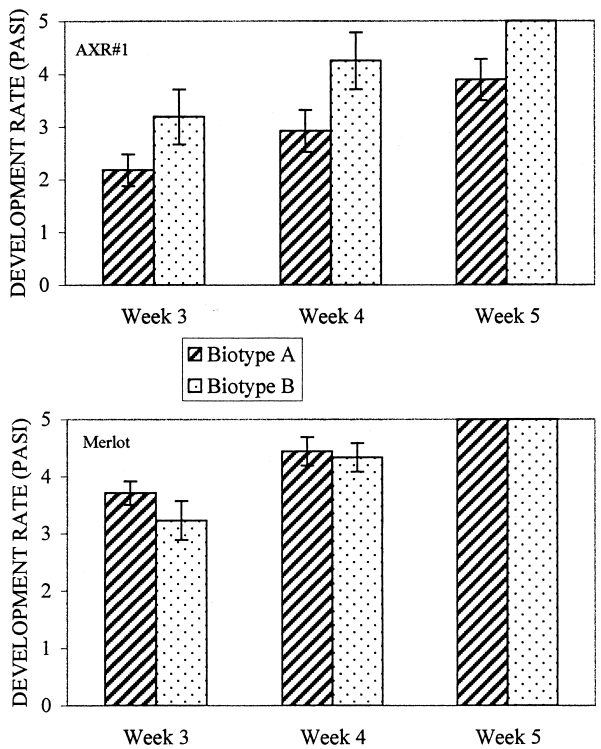

Figure: Development rate of grape phylloxera biotypes A and B on AXR#1 and Merlot. See Materials and Methods for details on PASI. Bars are means and standard deviations.

In expt. 1, starch level was higher in Merlot than AXR#1 in all comparisons with one exception, biotype A at week 4 (Table). Free amino acids were higher in the Merlot roots with one exception, week 4 with biotype B. Differences between cultivars for the soluble sugars were smaller than for the above compounds. Infestation did not influence starch or free amino acid levels in either root type.

Sucrose levels were generally higher in the presence of phylloxera than in uninfested root samples. The effect apparently increased with time, supporting the idea that insect activity stimulates mobilization. Results for glucose and fructose were mixed. Raffinose, stachyose, and sorbitol were not detected.

In expt. 2, for both *Vitis* cultivars, the concentration of soluble sucrose was lowest at the site of the phylloxera infestation and there was a decline in sucrose at the uninfested site as well in comparison to uninfested roots of the same age. Free amino acid content of roots at week 0 was significantly lower than for any treatment at week 5. Starch content of Merlot roots declined significantly between the be-

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T a b l e

Effects of phylloxera infestation on root compounds in AXR#1 and Merlot grape cultivars

Time	Cultivar	Treatment	Glucose[a]	Fructose[a]	Sucrose[a]	Free amino acids[b]	Starch[c]
Experiment 1: Biotype A (N=5 for each determination)							
Week 3	AXR#1	Uninfested	12 ± 3 a*	8 ± 2 a	12 ± 4 a	47 ± 19 a	62 ± 52 a
		Infested	26 ± 10 b	11 ± 3 a	13 ± 5 a	55 ± 14 a	62 ± 47 a
	Merlot	Uninfested	10 ± 4 a	10 ± 3 a	17 ± 7 a	109 ± 41 b	81 ± 32 b
		Infested	13 ± 4 a	12 ± 1 a	37 ± 15 b	110 ± 15 b	120 ± 28 c
Week 4	AXR#1	Uninfested	6 ± 1 a	13 ± 3 a	15 ± 3 a	83 ± 31 a	107 ± 83 a
		Infested	8 ± 2 a	21 ± 5 b	22 ± 5 b	61 ± 18 b	143 ± 63 b
	Merlot	Uninfested	9 ± 4 a	10 ± 3 a	14 ± 2 a	149 ± 43 c	69 ± 18 c
		Infested	15 ± 5 b	14 ± 7 a	22 ± 9 b	122 ± 30 c	88 ± 22 d
	Biotype B						
Week 3	AXR#1	Uninfested	20 ± 9 a	21 ±15 a	24 ± 20 a	23 ± 18 a	43 ± 20 a
		Infested	34 ± 10 b	34 ±10 a	27 ± 18 a	88 ± 77 b	27 ± 15 b
	Merlot	Uninfested	13 ± 7 a	13 ± 4 b	26 ± 2 a	146 ± 74 c	80 ± 23 c
		Infested	12 ± 7 a	12 ± 4 b	47 ± 15 b	142 ± 64 c	47 ± 11 a
Week 4	AXR#1	Uninfested	20 ± 8 a	25 ± 9 a	23 ± 12 a	142 ± 64 a	75 ± 19 a
		Infested	27 ± 6 a	22 ± 4 a	35 ± 9 b	16 ± 9 d	82 ± 32 a
	Merlot	Uninfested	23 ± 21 a	12 ± 6 b	26 ± 9 a	53 ± 40 b	238 ± 49 b
		Infested	16 ± 10 a	7 ± 2 c	48 ± 15 b	38 ± 20 c	206 ± 17 b
Week 5	AXR#1	Uninfested	20 ± 8 a	21 ± 6 a	15 ± 4 a	20 ± 16 a	37 ± 24 a
		Infested	24 ± 6 a	19 ± 4 a	31 ± 5 b	36 ± 13 b	39 ± 15 a
	Merlot	Uninfested	31 ± 18 a	19 ± 4 a	26 ± 8 a	67 ± 97 c	103 ± 62 b
		Infested	23 ± 10 a	13 ± 6 b	55 ± 28 b	100 ± 108 d	107 ± 62 b
Experiment 2: Biotype A phylloxera only (N=6 for each determination)							
AXR#1							
Week 0	Freshly excised uninfested roots		10 ± 3 a*	10 ± 3 a	30 ± 12 a	55 ± 14 a	73 ± 10 a
Week 5	Uninfested sites on uninfested roots		10 ± 8 a	10 ± 8 a	26 ± 12 a	113 ± 57 b	59 ± 46 a
Week 5	Uninfested sites on infested roots		7 ± 7 a	14 ±12 a	16 ± 6 b	172 ± 44 bc	52 ± 48 a
Week 5	Infested sites on infested roots		10 ± 7 a	18 ±10 b	14 ± 6 b	161 ± 50 c	50 ± 42 a
Merlot							
Week 0	Freshly excised uninfested roots		3 ± 1 a	4 ± 0 a	30 ± 11 a	97 ± 22 a	129 ± 12 a
Week 5	Uninfested sites on uninfested roots		6 ± 3 b	7 ± 4 b	26 ± 6 ab	144 ± 27 b	76 ± 25 b
Week 5	Uninfested sites on infested roots		4 ± 2 a	7 ± 3 b	25 ± 7 b	159 ± 17 b	101 ± 28 b
Week 5	Infested sites on infested roots		7 ± 3 b	14 ± 3 c	12 ± 3 c	154 ± 46 b	81 ± 15 b

[a] mg sugar g^{-1} dry weight; [b] µmoles leucine g^{-1} dry weight; [c] mg glucose equivalents g^{-1} dry weight.

* For each phylloxera biotype and week assay, values of compounds (mean ± SD) within column followed by same letters are not significantly different using LSD ($P < 0.05$).

ginning of the experiment and week 5, but infestation did not influence starch at week 5. These results differ from expt. 1 and may be due to differences in root quality and depletion of starch.

The nitrogen composition of the insect, approximately 1.6 µg N per insect (calculated from data in RILLING *et al.* 1974) in comparison to the nitrogen available as free amino acids suggests that the nutrient resources of the plant do not limit phylloxera population size. This suggests that the deleterious effect of the insect on the whole plant is caused by means other than usurpation of resources.

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