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Short Communication

Electronically Monitored Feeding Behavior of *Phorodon humuli* (Homoptera: Aphididae) on Resistant and Susceptible Hop Genotypes

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Dorschner and Baird (1989) screened numerous accessions of the United States Department of Agriculture (USDA) World Hop (*Humulus lupulus* L.) Germ Plasm Collection for antibiosis to the hop aphid, *Phorodon humuli* (Schrank). A high level of antibiosis was found in USDA accession 58016, a native North American genotype. A native Yugoslavian hop, USDA accession 21090M, was also found to be antibiotic, but to a lesser degree than 58016. These findings suggest the possibility of incorporating aphid resistance into an established commercial cultivar to produce an aphid-resistant hop while maintaining acceptable brewing qualities and desirable agronomic features.

Hop breeding programs in the United States have not traditionally been concerned with breeding for aphid resistance despite the fact that hop aphids are a perennial pest and require constant vigilance to prevent severe quality-lowering infestations. Questions also arise as to the long-term effectiveness, safety, and continued availability of aphicides for controlling this pest. A hop strongly antibiotic to *P. humuli* would clearly be advantageous. But the mechanisms by which genotypes such as 21090M and 58016 resist the hop aphid are not known, and because of this, the process of incorporating resistance into an acceptable cultivar would require the time-consuming task of performing hybridizations and evaluating hundreds of progeny with bioassays for aphid resistance. This prospect and the fact that high levels of hop aphid antibiosis

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have only recently been identified have discouraged breeders from seeking an aphid-resistant cultivar.

The objective of this study was to determine where the mechanisms involved in the antibiosis of 58016 and 21090M to hop aphids are located and to speculate as to their nature. Future research efforts can then be focused on the relevant plant tissues. This was done by contrasting the electronically monitored feeding behavior of hop aphids on two resistant accessions to hop aphids on the highly susceptible USDA accession 60038.

McLean and Kinsey (1964) were the first to introduce a system for electronic monitoring of aphid feeding behavior. Histological examinations later confirmed that certain waveforms generated by voltage fluctuations through the aphid/plant system are associated with salivation and ingestion activities in specific plant tissues (McLean and Kinsey, 1967). Several investigators have successfully used this technique to monitor the behavior of aphid species feeding on dicotyledonous plants (Kennedy *et al.*, 1978; Adams and Wade, 1976; Nielson and Don, 1974; Nault and Styer, 1972) as well as monocotyledonous plants (Niassy *et al.*, 1987; Ryan *et al.*, 1987; Shukle *et al.*, 1987; Scheller and Shukle, 1986; Montllor *et al.*, 1983; Campbell *et al.*, 1982).

In all cases, the waveforms observed from the aphid/plant combinations have been nearly identical. Essentially, only three separate waveforms have been observed (cf. Fig. 1, Shukle *et al.*, 1987; Fig. 1, Campbell *et al.*, 1982; Fig. 1, this study). The "S"-waveform corresponds to salivation, stylet sheath formation, and movement of the stylets within plant tissues. The "I"-waveform is generated when an aphid ingests for a continuous period of time. Finally, the "X"-waveform indicates stylet penetration of phloem sieve elements.

Various sequential combinations of these waveforms can occur. When an I-wave is preceded by the S-wave (S–I sequence), ingestion from nonphloem tissues is occurring. Ingestion from phloem is indicated when an I-wave immediately follows an X-wave (S–X–I sequence). Aphid stylets have never been observed to be within a phloem sieve element during an ingestion waveform which was not immediately preceded by at least one X-wave. Additional evidence of the diagnostic power of the X-wave is provided by studies involving barley yellow dwarf virus (BYDV). BYDV is phloem limited and obligatorily transmitted by several aphid species. Viruliferous aphids do not transmit BYDV unless an X-wave is formed just prior to an I-wave when feeding on healthy plants (Scheller and Shukle, 1986; Shukle *et al.*, 1987). This provides additional evidence that the X-wave is a definitive indicator of sieve element penetration by aphids.

The aphids used in this study were the descendants of a single individual field collected in the fall of 1986. They had been reared continuously in a controlled-environment room (18L:6D photoperiod, 23–26°C, 50–79% RH). Young, apterous adults were used for monitoring and were obtained by infesting

hop (cultivars L-8 or Cascade) with 25 to 50 adult aphids, usually from a crowded stock colony. All adults were removed the next day, leaving only nymphs less than 24 h old. These aphids were used for experimentation when between 10 and 15 days of age. Due to low-density rearing conditions, our test aphids were usually much larger than their parents and this greatly facilitated the monitoring procedure.

The hop plants were mist-propagated softwood cuttings planted in 10-cm plastic pots filled with a 3:1:1 mixture of peat moss, sand, and vermiculite. They were watered daily to prevent wilting, fertilized weekly with a balanced water-soluble fertilizer, and grown in the same conditions under which the aphids were reared. After climbing a 60-cm section of bamboo cane, the plants were judged to be sufficiently large for monitoring.

Hop aphid feeding behavior was monitored using 25-Hz battery-powered feeding monitors adapted from Brown and Holbrook (1976) and manufactured by Kendow Technologies, Perry, Oklahoma. A 200-mV AC current was introduced to the potting medium via a 7-cm piece of stiff copper wire. The aphid was affixed to the second electrode with approximately 4 cm of 10-μm gold wire. A drop of colloidal silver paint (Ted Pella, Redding, Calif.) was placed on a glass slide and the free end of the wire dipped into it repeatedly until a very small ball of silver had accumulated. The aphids were affixed by touching their dorsum to the sticky ball and holding it there until the paint had sufficiently dried (approx. 10 to 15 s). Monitoring began immediately upon aphid contact with the test plants.

The youngest fully expanded leaf on the plant was selected for monitoring with the aphid initially placed on the abaxial surface. All aphid monitoring began at approximately 0900 and continued for 24 h. Aphids which fell from the plant before 24 h of data could be collected were eliminated from analysis. If an aphid broke or disconnected her tether, a different aphid was attached the following day using a different plant of the same genotype.

Waveforms were recorded on a strip-chart recorder with a chart speed of 0.5 cm/min and manually transcribed into the various behavioral events. In addition, a penetration-spike waveform was identified (Niassy *et al.*, 1987). This was seen as a very sharp voltage increase rapidly followed by a sharp voltage decrease and described the initial penetration of an aphid's stylets into plant tissues. This waveform served as a convenient marker for the total number of probes performed during the monitoring session. A probe was defined as that period of time when the aphid's stylets were in constant electrical contact with the host. Probes are separated by baseline events (nonprobing) and are always initiated with a penetration-spike. Some probes consisted of a penetration-spike only.

The frequency, mean, and total duration of all behavioral events (i.e., nonprobing, penetration-spikes, salivation, nonphloem ingestion, X-waves, and

phloem ingestion) were subjected to an analysis of variance with multiple comparisons using the SAS general linear models procedure (SAS Institute, 1982, pp. 139–199). Other data analyzed included the number of test probes (probes made before the first probe containing an ingestion event), time to the first X-wave (first phloem contact), time to first phloem ingestion event longer than 60 min, percentage of probes that were successful (with phloem contact), and duration of salivation before the first phloem contact within a successful probe. Also, the duration of phloem ingestion over time was examined by dividing the monitoring session into four 6-h intervals and analyzing each separately. Of the 60 aphids monitored on the three hop genotypes, 52 could be used for data analysis (18 on 60038, 18 on 58016, and 16 on 21090M).

P. humuli feeding on hop produce waveforms identical to those of other aphid species which have been monitored on their hosts using McLean and Kinsey's AC system. Typically shaped S-waves, I-waves, and X-waves were all clearly discernible and easily transcribed into the various feeding behaviors (Fig. 1).

On all hop genotypes, probing began shortly after the aphids were placed on the leaf surface and was immediately followed by salivation and ingestion

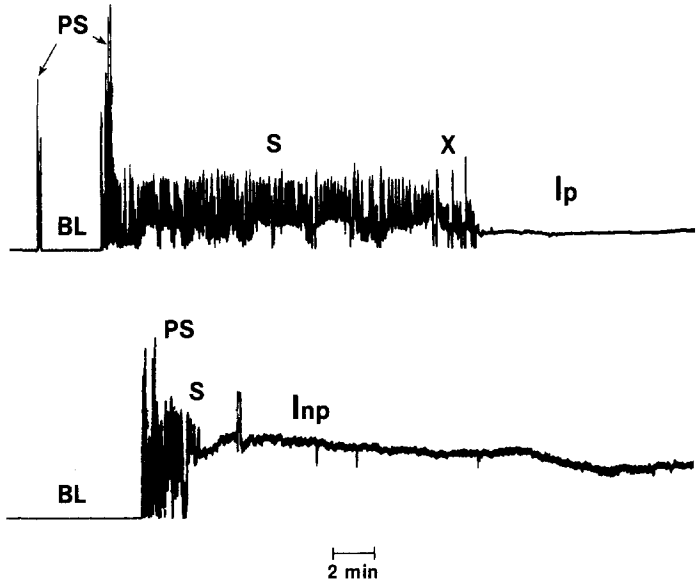

Fig. 1. Typical waveforms generated by *Phorondon humuli* feeding on hop. BL, baseline (nonprobing); PS, penetration-spike; S, salivation waveform; X, penetration of phloem sieve element; I, ingestion waveform. Ingestion from phloem is indicated by the sequence S–X–Ip, and the S–Inp sequence indicates ingestion from nonphloem tissues.

activities. There were no significant differences among hop genotypes for the number of test probes produced or for the time required to contact the phloem for the first time (first X-wave) (Table I). However, the time required to initiate a phloem ingestion event >60 min was significantly longer on the resistant accessions (Table I).

Resistance significantly increased the frequencies of probing, X-wave formation, phloem ingestion, and salivation but did not alter the frequency of nonphloem ingestion (Tables I and II). The mean durations of behavioral events were not as affected by aphid antibiosis. The mean durations of salivation and nonphloem ingestion events were not significantly altered (Table II). However, the mean duration of a phloem ingestion event was significantly reduced on the resistant accessions (Table II). The total durations (summed over the 24-h recording session) of all behaviors were significantly altered by host resistance with the exception of nonphloem ingestion (Table II). Resistance increased nonprobing (Table I) and salivation but decreased phloem ingestion (Table II). The ranking of hop genotypes with regard to the duration of phloem ingestion was found to be consistent over time (Table III). Aphids on all accessions increased the amount of phloem ingestion after the first 6 h on the plant, but from that point on the phloem ingestion time remained relatively constant.

Although the frequency of probing was dramatically increased on the resistant hops, the percentage of probes leading to phloem contact (successful probes) was not altered (Table I). Nor was the amount of salivation before the first phloem contact within the successful probes (Table I). Regardless of hop genotype, *P. humuli* produced at least 2 X-waves prior to phloem ingestion and occasionally produced as many as 10.

Plants resist aphid feeding attempts in many ways. Most plants are exploited by few, if any, aphid species, thus testifying to the effectiveness of plant barriers for the prevention of successful aphid colonization. Once on the plant surface the aphid can be deterred by leaf pubescence, certain volatile substances, and even the chemical composition or amount of epicuticular waxes. As probing begins, the internal physical and chemical features of the plant become important. The aphid may encounter tissues which resist stylet penetration either by mechanical means or by resisting depolymerization of plant tissues by aphid salivary enzymes. The presence of chemical antifeedants or the lack of feeding stimulants may also inhibit aphid feeding attempts. Other mechanisms of resistance may alter the aphids path to the phloem, making that cell type difficult to locate. Resistance may also involve features of the phloem itself which inhibit penetration and/or ingestion by the aphid. The phloem sap of resistant plants may also be nutritionally inadequate for proper aphid growth, development, and reproduction.

Electronic monitoring systems provide a unique opportunity to follow, moment by moment, the activities of an aphid as it probes and attempts to feed

Table I. Means[a] of Various Electronically Recorded Events for *P. humuli* on Different Hop Genotypes During a 24-h Monitoring Session

Hop genotype (resistance rating)[b]	*N*	Duration nonprobing (min)	Test probes (number)	Probes (number)	Time to first X-wave (min)	X-waves (number)	Time to first prolonged Pi[c] (min)	% successful probes[d]	Duration of salivation before phloem ingestion (min)[e]
21090M (R)	16	102.1b	2.2a	26.8a	130.2a	27.7b	241.5a	24.4a	33.2a
58016 (R)	18	166.8a	3.3a	32.2a	119.5a	45.5a	306.5a	26.7a	35.8a
60038 (S)	18	16.6c	2.7a	7.8b	94.1a	13.8c	122.1b	28.0a	28.7a

[a]Means followed by the same letter within a column are not significantly different at the $\alpha = 0.05$ level (LSD).
[b]R, resistant; S, susceptible.
[c]First phloem ingestion event with a duration of at least 60 min.
[d]Probes with phloem contact/total probes × 100.
[e]Time spent in salivation before first phloem ingestion within a probe.

Table II. Means[a] of Ingestion and Salivation Waveforms for *P. humuli* Electronically Monitored on Different Hop Genotypes[b]

Hop genotype (resistance rating)[c]	*N*	Salivation			Nonphloem ingestion			Phloem ingestion		
		Frequency	Mean	Sum	Frequency	Mean	Sum	Frequency	Mean	Sum
21090M (R)	16	22.0b	17.2a	378.8b	3.5a	26.6a	92.8a	7.5b	112.3b	842.0b
58016 (R)	18	34.0a	16.2a	550.8a	3.1a	30.7a	95.1a	12.5a	46.6b	583.0c
60038 (S)	18	5.3c	17.8a	94.3c	2.0a	36.5a	72.4a	3.1c	413.4a	1281.5a

[a] Means followed by the same letter within a column are not significantly different at the $\alpha = 0.05$ level (LSD).
[b] Frequency, total number of events during 24 h; mean, average duration of an event; sum, duration of individual behaviors summed over 24 h.
[c] R, resistant; S, susceptible.

Table III. Mean[a] Total Duration (Minutes) of Phloem Ingestion for *P. humuli* Feeding on Different Hop Genotypes[b]

Hop genotype (resistance rating)[c]	*N*	Time interval (h)			
		0–6	6–12	12–18	18–24
21090M (R)	16	143.9b	239.1b	212.9b	246.0b
58016 (R)	18	102.5b	158.6c	156.7b	165.2c
60038 (S)	18	237.6a	334.7a	339.2a	335.1a

[a]Means followed by the same letter within a column are not significantly different at the $\alpha = 0.05$ level (LSD).
[b]The 24-h recording session was divided into four time intervals and each was analyzed separately.
[c]R, resistant; S, susceptible.

upon an intact plant. By contrasting feeding behavior on susceptible and resistant hosts, it should be possible to identify the stage of plant penetration where resistance is encountered. Thus, the nature of various resistance mechanisms may be inferred. This approach has been utilized by Nielson and Don (1974), Kennedy *et al.* (1978), Campbell *et al.* (1982), and Ryan *et al.* (1987).

The resistance to hop aphids of USDA accessions 21090M and 58016 apparently does not involve volatiles or surface features common to these genotypes. Aphids commenced probing almost immediately regardless of hop genotype even though they were not intentionally starved prior to the recording session. The numbers of test probes (probes before the first feeding probe) were also similar between hop genotypes (Table I). The test probes were very short in duration and consisted of a penetration-spike and very little, if any, salivation. Resistance, therefore, probably does not involve the plants cuticular or epidermal layers.

The hop aphids were commonly observed to ingest from nonphloem tissues shortly after beginning to probe. These events were highly variable in both frequency and duration between individual aphids and were not significantly altered by the host plant resistance (Table II). Apparently, significant quantities of feeding deterrents are not encountered in nonphloem tissues and the resistance does not incline the aphids to spend more time ingesting from this nutritionally inferior food source.

Although the frequency of probing was significantly increased on the resistant accessions, the percentage of those probes which were successful (with phloem contact and ingestion) was not significantly altered (Table I). An aphid beginning a probe on the resistant hops had about the same probability of locating the phloem during that probe as aphids on the susceptible hop. The amount

of salivation before the first phloem contact within successful probes was also similar for each hop genotype, as was the time required to find the phloem for the first time (time to first X-wave) (Table I). The host-plant resistance did not seem to inhibit stylet penetration of plant tissues or misdirect the aphids as they attempted to locate the phloem. The saliva of apterous hop aphids feeding on hop contains little or no pectinase (McAllan and Adams, 1961). This observation and our findings indicate that aphid resistance in hops may not adhere to the general scheme for aphid resistance proposed by Dreyer and Campbell (1987).

The mean duration of a salivation event was very similar among hop genotypes, therefore, the total amount of salivation observed was directly proportional to the frequency of this behavior. However, the mean durations of phloem ingestions were greatly reduced on 21090M and 58016 and this resulted in a significant reduction in phloem ingestion over the entire 24-h monitoring period, despite an increase in phloem ingestion frequency (Table II). The time required to begin uninterrupted phloem ingestion lasting at least 60 min was much shorter on the susceptible hop and, on average, quickly followed the first overall phloem contact (Table I). Because the frequencies of probing, salivation, X-waves, and phloem ingestion all displayed similar trends between hop genotypes, the main effect of plant resistance is to force hop aphids to perform repeatedly behavioral sequences which quickly lead to successful phloem location and penetration but not to allow the aphids to commit routinely to phloem ingestion for long periods of time. The resistance of hop genotypes 21090M and 58016 is associated with the phloem. The lack of a feeding stimulant, the presence of a feeding deterrent, or some response of the phloem to sap removal and/or aphid saliva may be the mechanism involved.

The resistance of the phloem to prolonged ingestion by the hop aphids was found to be constant over time (Table III). This is fundamentally different from the findings of Montllor *et al.* (1983) and Ryan *et al.* (1987) for *Schizaphis graminum* (the greenbug) on resistant and susceptible hosts. With greenbugs, prolonged phloem ingestion was delayed on the resistant genotypes, similar to the findings of this study. But after several hours of feeding, even greenbugs on the resistant plants ingested phloem sap for long periods of time. There is evidence that this may be due to an enhancement of the susceptible host's nutritional quality that is induced by the phytotoxic greenbug (Dorschner *et al.*, 1987) and that the greenbug may become conditioned to a lack of feeding stimulants when unable to induce damage symptoms on resistant plants (Montllor *et al.*, 1983). Montllor and Gildow (1986) have also shown that greenbugs commit to phloem ingestion much sooner on barley yellow dwarf virus-infected oats than on healthy plants. The increased nutritional quality of the diseased plants may explain this observation. Because hop aphids did not appear to become condi-

tioned to the resistant host plants, the hypothesis of insufficient nutrients in the phloem sap of the resistant accessions responsible for the aphid resistance becomes less attractive.

The levels of reproductive performance for hop aphids on 60038, 21090M, and 58016 (Dorschner and Baird, 1989) closely follow the total duration of phloem ingestion observed in this study. The antibiosis previously described may not be antibiosis per se; rather, the resistance may actually be of the non-preference (antixenosis) type. Accessions 58016 and 21090M may appear antibiotic because hop aphids partially starve when they are forced to feed from these genotypes. Partial starvation and host-plant resistance can have a similar effect on aphid performance (Auclair and Cartier, 1960). Aphids on the susceptible 60038 spent 87% of the recording session in phloem ingestion, compared to 58% on 21090M and only 40% on 58016. The reluctance of aphids to feed from 21090M and 58016 remained evident over the entire recording session. However, it is also possible that the phloem of resistant plants responds in some manner effectively to diminish sap flow into the aphid's food canal or through the penetrated sieve element. The results of this experiment suggest that future efforts to elucidate the nature of hop aphid resistance mechanisms should center on phloem physiology and biochemistry.

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