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The probing behaviour of nymphs of *Vanduzeea arquata* and *Enchenopa binotata* (Homoptera: Membracidae) on host and non-host plants

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ABSTRACT. 1. Nymphs of *Vanduzeea arquata* Say have been found to be more host-specific in nature and to show a higher degree of selectivity in host discrimination experiments than nymphs of *Enchenopa binotata* (Say). It was hypothesized that this differential selectivity would be reflected in the probing behaviour of individuals placed on twigs of host and non-host plants. Probing behaviour was examined by direct observation of nymphs and by sectioning and staining the probed plant tissues.

2. All nymphs probed readily and for extended periods on both host and non-host twigs. *E.binotata* nymphs showed no consistent differences in probing behaviour on hosts versus non-hosts, but *V.arquata* nymphs were more likely to withdraw their stylets within 60 s when on non-host twigs and produced honeydew only when on their host species. *V.arquata* nymphs reached the phloem sieve elements only when on host twigs and broke many cells in peripheral plant tissue layers while probing. *E.binotata* nymphs broke few cells and often reached the phloem of non-host as well as host plants.

3. Nymphs of *V.arquata* always reject non-host plants, apparently in the course of probing and prior to encountering the phloem sap. Chemical compounds released from ruptured parenchyma cells may act as probing stimulants or inhibitors. *E.binotata* nymphs often feed on non-host plants in a non-choice situation; their preferential settling on host twigs in discrimination experiments may reflect a tendency to abandon non-host twigs more readily than host twigs.

Key words. Homoptera, Membracidae, *Enchenopa binotata, Vanduzeea arquata,* host-selection, probing behaviour.

Introduction

The distinction between (pre-ingestive) host-recognition and (post-ingestive) host-acceptance as elements of host-selection behaviour (Panda, 1979) is particularly useful when applied to the feeding behaviour of phloem-feeding homopterans. Acceptance of a particular host plant requires first a positive probing response, in which the insect passes its stylets through peripheral tissue layers of the plant, and then a positive feeding response to the phloem sap itself. Host-discrimination based on

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probing behaviour is more efficient than discrimination based on feeding behaviour because of the amount of time required for stylet penetration to the phloem. Thus, host-specific phloem-feeders may be expected to show differential probing responses when placed upon host versus non-host plants.

The membracid *Vanduzeea arquata* Say is found throughout its range only on the black locust tree, *Robinia pseudoacacia* L. (Funkhouser, 1915). *Enchenopa binotata* (Say) is associated with at least six species of woody plants in the northeastern United States but has been shown to comprise a complex of sympatric, genetically isolated host-races or cryptic species. Each of these host-races appears to be restricted to a single genus or species of plants (Funkhouser, 1915, 1917; Wood, 1980; Kiss, 1983).

Nymphs of both membracids differentiate between twigs of host and non-host plants in feeding experiments but show different degrees of selectivity (Kiss, 1983). In pairwise choice experiments individuals of *E.binotata* settled on twigs of their respective host species approximately 75% of the time, while *V.arquata* nymphs settled exclusively on twigs of the black locust. Similarly, in a non-choice situation, *E.binotata* nymphs could be reared on one anothers' hosts (with the exception of the hop-tree), while *V.arquata* nymphs survived only on the black locust. The present study was undertaken to determine whether these differences in selectivity could be related to differences in the probing behaviour of the nymphs.

The path of the stylets of a phloem-feeding homopteran may be intercellular, passing between parenchyma cells en route to the phloem, or intracellular, breaking through those cells. The mode of penetration is important because of the different physical and chemical features which such insects are likely to encounter at each tissue level. Compounds (including many secondary metabolites) which are sequestered within parenchyma cells are not released unless the cells are broken (McKey, 1979). Thus, only phloem-feeders which penetrate intracellularly should encounter these materials directly. Conversely, those which penetrate intercellularly are more likely to be influenced by the nature of the intercellular matrix.

The stylets of most aphids penetrate intercellularly (e.g. Nault & Styer, 1972) while those of many cicadellids and fulgoroids penetrate intracellularly (e.g. Fisk, 1980). To the best of our knowledge, no previous study has examined probing behaviour in membracids.

Materials and Methods

Third, fourth and fifth instar nymphs of *V.arquata* and of *E.binotata* were collected in the field near Ann Arbor, in Washtenaw Co., Michigan. The *E.binotata* nymphs were taken from populations occurring on three different host plant species. These populations are here referred to as the 'hop-tree *E.binotata,*' from the hop-tree, *Ptelea trifoliata* L., the 'locust *E.binotata,*' from the black locust, *Robinia pseudoacacia* L., and the 'bittersweet *E.binotata,*' from the bittersweets, *Celastrus scandens* L. and *C.orbiculatus* Thunb. Twigs were clipped from field-growing plants and maintained indoors for up to 24 h with their severed ends in tap water.

Nymphs were placed consecutively on the twigs by means of a small paint brush and observed directly through a dissecting microscope or a 10× hand-lens. Eight different individuals were used in each membracid/plant species combination. The insertion and the removal of the stylet tips from the plant tissues were easily visible, and a nymph was said to be probing as long as any portion of its paired stylets was inserted. The interval between the initial contact with the twig and the beginning of probing was measured; the nymph was then observed continuously for 10 min during which the durations of all probes were recorded. Following this 10 min observation period the nymph was left undisturbed for up to 1 h or until it either produced a drop of honeydew or withdrew its stylets from the plant. Production of honeydew was taken as an indication that feeding had begun.

To examine probing behaviour in deeper tissue layers, five to ten individuals of each membracid were caged on separate twigs of each plant species for 2 days. The twigs were then collected, dehydrated in a standard alcohol series, embedded in paraffin, and sectioned transversely (15 μm). The plant tissues were stained with safranin and counterstained with fast green to reveal the salivary sheaths left by the membracids' stylets (Chang, 1978). The path and the termination point of each stylet was determined by following the sheath through serial transverse sections.

TABLE 1. Parameters of the probing behaviour of nymphs of *Vanduzeea arquata* and *Enchenopa binotata* placed on twigs of host and non-host plants. Values represent means from eight nymphs in each combination, ± SE.

	Seconds to first probe	Percentage of time with stylets inserted	No. producing honeydew	
			Within 30 min	In 30–60 min
Hop-tree *E. binotata*				
On hop-tree	39 ± 5.8	84 ± 1.5	1	2
On bittersweets	39 ± 3.8	88 ± 1.2	0	1
On black locust	12 ± 0.8*	96 ± 0.4**	4	2
Bittersweet *E. binotata*				
On hop-tree	45 ± 2.9	84 ± 3.2	0	1
On bittersweets	37 ± 2.9	84 ± 1.0	0	4
On black locust	51 ± 6.4	84 ± 2.1	1	1
Locust *E. bionotata*				
On hop-tree	37 ± 5.9	80 ± 3.0	0	0
On bittersweets	21 ± 2.8*	92 ± 0.6	1	1
On black locust	55 ± 11.6	89 ± 1.2	5	1
V. arquata				
On hop-tree	39 ± 3.8	63 ± 1.3	0	0
On bittersweets	77 ± 20.9†	62 ± 4.0	0	0
On black locust	40 ± 10.6	70 ± 5.0	2	0

Significantly different from the corresponding value on the host species: $*P<0.05$; $**P<0.01$ (Student's *t*-test).
† Contains one value >400.

Results

In all membracid species/plant species combinations most nymphs spent the majority of their time with stylets embedded in the plant tissues. Neither the interval to the first probe nor the proportion of time spent probing as opposed to walking was consistently different on host versus non-host twigs, although in two cases *E. binotata* nymphs appeared to probe more readily on a non-host species than on their respective hosts (Table 1).

The nymphs showed considerable individual variation with respect to the durations of probes on all three plant species (Fig. 1). The *V. arquata* nymphs differed from the *E. binotata* nymphs in that a larger proportion of their probes on non-host twigs were short (under 60 s); however, some *V. arquata* individuals did probe continuously for over an hour on both of the non-host species. There was no consistent variation in probe durations on host versus non-host twigs among *E. binotata* nymphs (Fig. 1).

While many nymphs did not produce honeydew even after 60 min of continuous probing some differentiation between host and non-host was apparent. *Vanduzeea arquata* nymphs expelled droplets only when on the black locust, and overall the proportion of *E. binotata* nymphs which did so was slightly higher on host than on non-host twigs.

Unexpectedly, the hop-tree *E. binotata* nymphs began to probe most readily, engaged in the greatest number of very long probes, spent the largest percentage of their time probing, as opposed to walking, and produced honeydew most frequently when on the black locust rather than their own host.

Fig. 2 summarizes data on the numbers of stylet tracks ending in the cortical parenchyma (C), in the phloem sieve elements (P), at the boundary between the phloem and the xylem (B) and in the xylem (X), in each membracid/plant species combination. The stylets of *V. arquata* nymphs reached the phloem sieve cells of the black locust in almost every case, but always ended in the cortex, before reaching the phloem, both on the hop-tree and the bittersweets. *Enchenopa binotata* nymphs were less consistent: on twigs of their respective host species they ended a relatively large proportion of

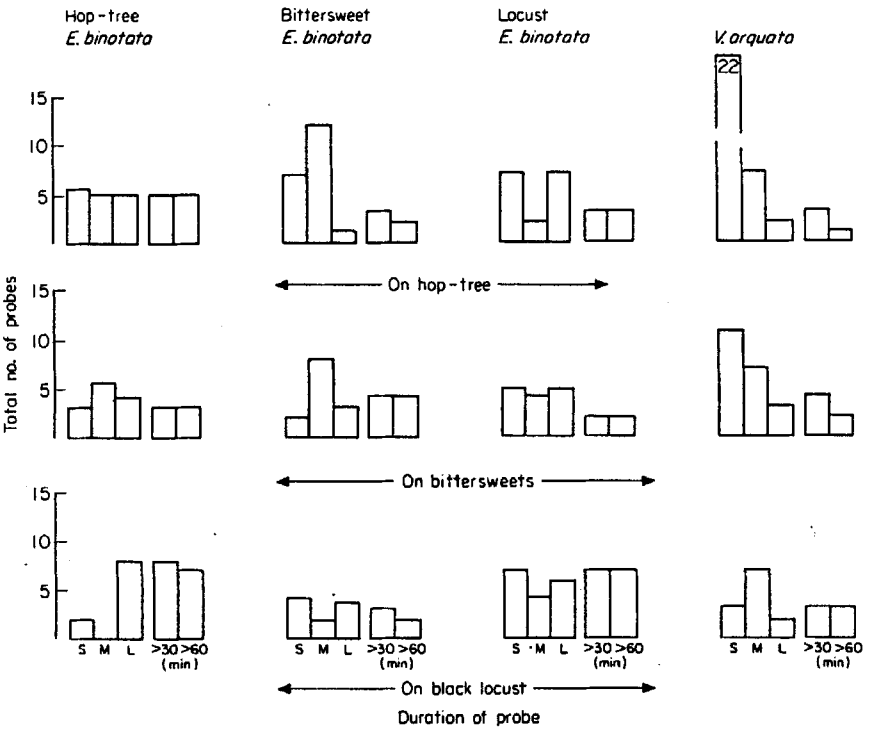

FIG. 1. Probing behaviour of nymphs of *Vanduzeea arquata* and three host-specific populations of *Enchenopa binotata*: durations of probes on twigs of host and non-host plants. Nymphs were placed individually on twigs and observed continuously for 10 min, then checked after intervals of 30 and 60 min. S = short probe (under 1 min); M = medium probe (1–9 min); L = long probe (9–10 min, i.e. uninterrupted from first insertion of stylets into plant to the end of the observation period). Hop-tree = *Ptelea trifoliata*, host of the hop-tree *E. binotata*; bittersweets = *Celastrus orbiculatus* or *C. scandens*, hosts of the bittersweet *E. binotata*, black locust = *Robinia pseudoacacia*, host of the Locust *E. binotata* and of *V. arquata*. Frequencies indicate all probes by a total of eight nymphs in each membracid/plant species combination.

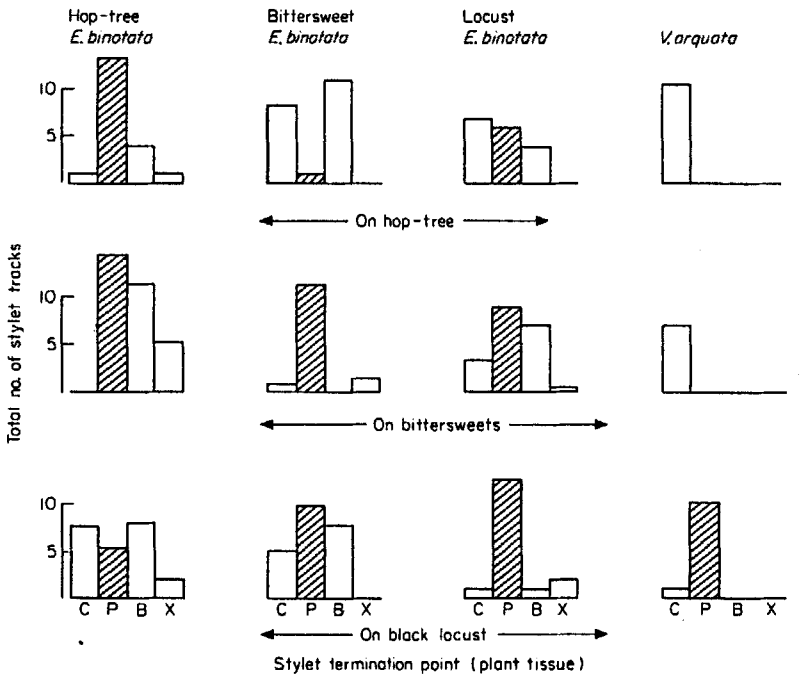

FIG. 2. Probing behaviour of nymphs of *Vanduzeea arquata* and three host-specific populations of *Enchenopa binotata* species: proportions of stylet tracks terminating in different tissue layers in twigs of host and non-host plants. Plant and insect designations as Fig. 1. Tissue layers: C = cortex; P = phloem (hatched bars); B = boundary between phloem and xylem; X = xylem. Results represent probes by five to ten nymphs of each membracid type caged on twigs for 48 h.

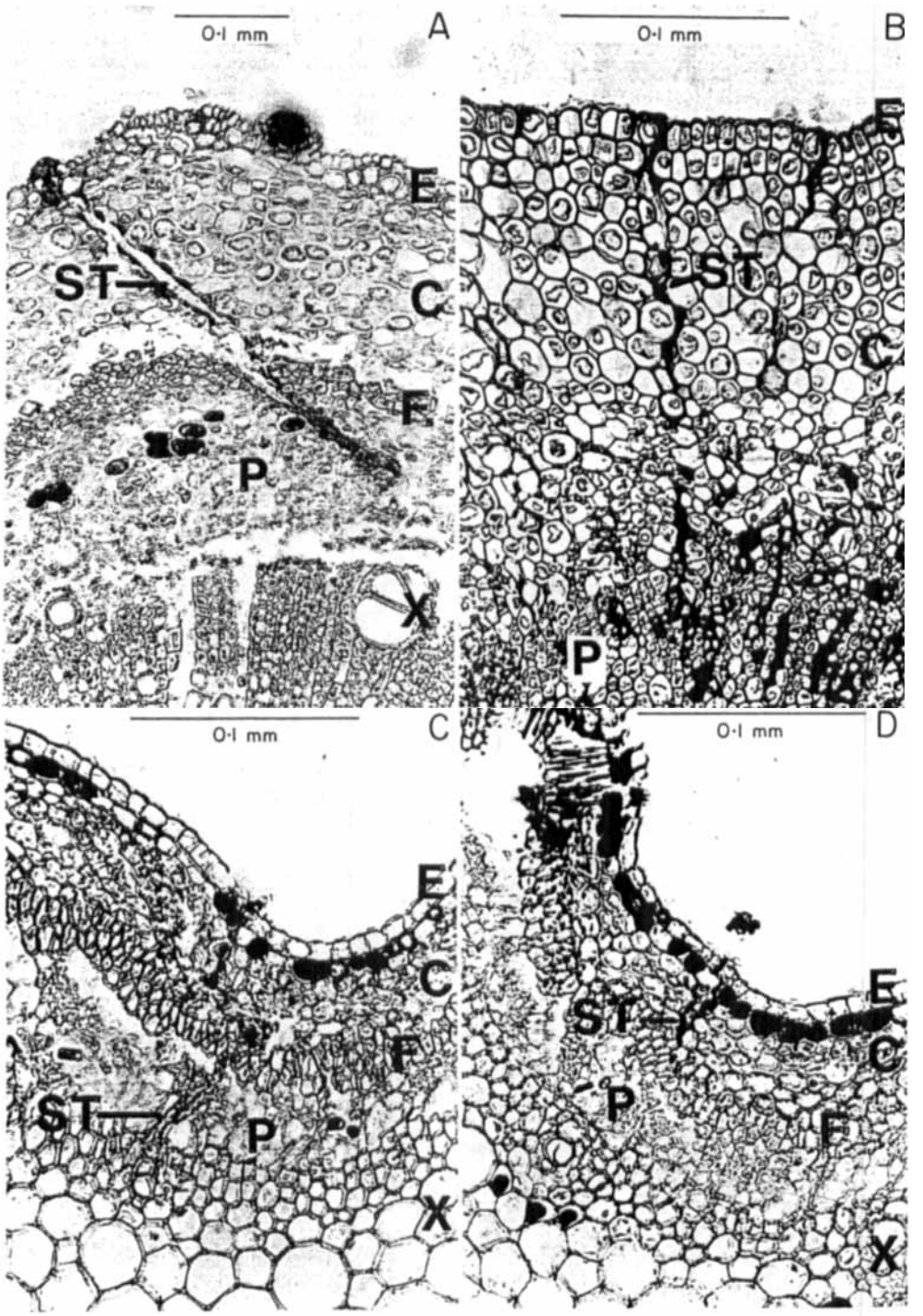

FIG. 3. Cross sections from host plant twigs showing salivary sheaths deposited by the stylets of probing membracid nymphs. Entrance point of the stylet track is at top or right, termination point at bottom or left, in each photograph. (A) *Vanduzeea arquata* probing on a petiole of its host species, the black locust (*Robinia pseudoacacia*); (B) bittersweet *E. binotata* probing on a green stem of a host species, the oriental bittersweet (*Celastrus orbiculatus*); (C, D) hop-tree *E. binotata* probing on leaf petioles of a non-host species, the black locust. E = epidermis; C = cortex; F = fibre (lignified, old phloem sieve cells); P = phloem; X = xylem, ST = stylet salivary sheath.

probes in the phloem, but they occasionally withdrew their stylets while still in the cortex or penetrated through the phloem layer to the xylem.

With the exception of the hop-tree *E.binotata* nymphs on the bittersweets, the proportion of probes ending in the cortex was always higher on non-hosts than on hosts. Many of the probes by hop-tree *E.binotata* or bittersweet *E.binotata* nymphs which ended in the cortex of the black locust twigs stopped at the layer of fibres (lignified, old phloem sieve cells) lying just peripheral to the functional phloem sieve elements in the mature leaf petioles and stems of this species (Fig. 3D). The proportion of *E.binotata* probes which passed through the phloem to the xylem or to the phloem/xylem boundary was also much larger on non-hosts than on hosts.

Despite the large number of hop-tree *E.binotata* nymphs which produced honeydew when on the black locust, a relatively small proportion of the stylet tracks in that membracid/plant species combination terminated in the phloem sieve elements.

The stylets of *V.arquata* nymphs left tracks which were broad and straight, breaking many of the cortical parenchyma cells (Fig. 3A). The tracks of the *E.binotata* stylets were narrower and usually passed between cortical cells, although occasionally these cells did appear to be broken, as indicated by the presence of saffranin-stained salivary sheath material within the cell walls (Fig. 3B).

Discussion

Host-discrimination behaviour in these two membracids appears to differ in a way which is consistent with their degree of host-specificity in nature. *Vanduzeea arquata* is strictly monophagous. Nymphs of this species probed readily when placed on non-host twigs but invariably rejected them prior to beginning to feed, possibly in response to chemical cues released by the parenchyma cells which were broken in the course of probing. These may include probing inhibitors present in the cells of non-hosts and/or needed stimulants present only in the black locust. *Enchenopa binotata* may be considered less specialized, both because it represents a complex of host-races or closely related cryptic species, which collectively utilize a diverse array of hosts, and because some of these races or species are known to utilize several congeneric host species. Nymphs of *E.binotata* do not always reject non-host twigs, but will often feed upon them in non-choice situations. Their stylets frequently reach the phloem of non-host as well as host twigs, so that their host-discrimination does not appear to be based entirely on probing responses. In this regard, it may be significant that their stylets rarely rupture peripheral parenchyma cells en route to the phloem.

Host-discrimination could also occur at the level of feeding. The data on feeding behaviour of *E.binotata* nymphs are inconclusive for several reasons: first because honeydew production is an indirect means of assessing ingestion, second because many nymphs did not produce honeydew even on their host species and third because the durations of individual feeding bouts were not measured. The results do show that (with the exception of the locust *E.binotata* on the hop-tree) at least one nymph of each *E.binotata* host-race initiated feeding on twigs of each plant species.

The question remains as to the means by which *E.binotata* nymphs discriminate between host and non-host twigs. In the feeding discrimination experiments cited above, preference was determined by the proportion of nymphs found on each of the two twigs after 24 h. In these experiments approximately 75% of the *E.binotata* nymphs were consistently found on the twig of their host species (Kiss, 1983). Such preferential settling might result if nymphs tended to abandon non-host twigs more readily than host twigs. For example, if feeding bouts were longer on the host, nymphs should spend a larger proportion of time on host twigs. This possibility was not investigated in the present study, but reduced ingestion or shorter feeding bouts on non-hosts or on resistant varieties of host species have been demonstrated for several aphid species (Campbell *et al.*, 1982, and references therein). Another factor which might lead to early abandonment of non-host twigs is a low rate of success in locating the phloem tissue. Fisk (1980) found that a smaller proportion of probes by the planthopper *Peregrinus maidis* reached the phloem when individuals probed on younger than on older sorghum plants. Fisk suggested that higher levels of phenolic com-

pounds in the younger plants may make their phloem tissues more difficult to locate. The relatively large proportion of *E. binotata* stylet tracks which did not reach the phloem, or passed through to the phloem/xylem boundary, suggests that these nymphs also have greater difficulty in reaching and recognizing the phloem tissue of non-hosts. However, the factors which guide them to the phloem in their host plants remain unknown.

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