FEEDING SITE AND SPIITTLE OF CLASTOPTERA ARBORINA BALL (HOMOPTERA: CERCOPIDAE)

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Abstract

Feeding punctures of Clastoptera arborina Ball stained bright red with safranin 0 dye in cross sections of juniper twigs. The punctures were primarily intracellular, passed through all tissue layers, and ended in the outer layers of xylem tracheids. Xylem feeding is consistent with this and other aspects of C. arborina's biology, and with the habits of other cercopids. Most feeding sites were established midway between the resin canal and the scale edge, and a disproportionate number entered through the stomata. The stylet sheath bore diameter increased with age, and matched the maxillary stylet diameter. The mandibular styles do not penetrate beyond the outer mesenchyme and/or epidermis.

In the field, spittle is a white frothy mass, but this becomes more fluid in later instars. Many kinds of insects are found in spittle masses, but these are probably accidental entrapments, and not indicative of natural enemies. Under 100% humidity, the spittle production rate of a fifth instar nymph is 109.6 mg/day.

INTRODUCTION

Juniperus virginiana L., the host plant of Clastoptera arborina Ball, usually grows in the well drained soil of limestone outcroppings, bluffs, and glades. These more arid environments present special problems of water retention for developing insects, especially during the cuticle tanning period immediately following ecdysis. Spittle insects are well adapted for coping with these problems, as they are able to utilize the plant's own sap to create a virtually aquatic microhabitat in a semiarid environment. The following study establishes the source and acquisition of nutrients and water for C. arborina, and describes the spittle and its rate of production.

MATERIALS AND METHODS

1. Feeding Site Description

Twigs with spittle masses were removed from the plant and trimmed as close as possible to the feeding nymph. The nymph was usually removed from the twig before the twig sample was fixed in formalin, acetic acid, and 50% ethanol (18:1:1) for one or more days. The specimens were dehydrated in an ethanol series with 2 changes of 100% ethanol, cleared in xylene, and infiltrated and embedded in Paraplast.

Young juniper twigs consist of a fibrous xylem core surrounded by phloem and mesenchyme. The difference in hardness between the former and the latter two often resulted in the fracture of the vascular cambium during sectioning. This artifact was eliminated in later work by trimming one end of the Paraplast block down to the specimen and soaking the block in water for one or more weeks. The embedded specimens were then mounted on wood blocks and sectioned at 10μm. The resulting ribbon of serial sections was mounted on glass slides, stained with safranin 0 and fast green according to Johansan (1940), and covered with a cover slip. Measurements were made with a calibrated ocular micrometer.
2. Rate of Spittle Production

A humidity chamber was constructed of a 25 x 25 cm square polyethylene sheet. A hole approximating the mouth aperture of a polyethylene funnel (about 5.5 cm in diameter) was cut in the center, and the funnel taped in place, being fastened around its entire circumference. Two opposing edges were taped together to form a cylinder, with the funnel nozzle directed outward.

The host plant was a small, potted *J. virginiana* kept in an incubator under a photoperiod of 18 hours light and 6 hours dark. A trial began by placing a fifth instar nymph on an appropriate section of twig in the humidity chamber with the funnel directly below the nymph. The chamber was closed tightly around the twig proximal to the trunk with a twist tie, and similarly with the distal end, except that a moistened dental wick was included in the closure so that one end projected to the outside. The wick was kept moist throughout the trial. A preweighed vial was then fastened with tape or clay to the funnel nozzle. Similar units not containing nymphs served as zero controls. After three days the twig and apparatus were removed from the plant and examined. Only those setups with healthy nymphs remaining at the end were used for spittle production data. The vials and collected spittle were weighed on an analytical balance, and the volume estimated by drawing the contents into a 1 ml pipet graduated in 0.01 ml divisions.

RESULTS AND DISCUSSION

1. Feeding Site Description

The histological study showed the feeding punctures of *C. arborina* in the tissues of its host, *Juniperus* spp., as indicated by the following evidence. Generally one feeding puncture was located in each twig specimen. Because *C. arborina* remains in the same spittle mass through several stadia however, two feeding punctures were occasionally found in the same specimen. Also, as nymphs sometimes withdraw their mouthparts and move when disturbed, they may move far enough away from the feeding site such that the sheath is excised during trimming. Thus, some samples inevitably contained no feeding sheaths, but in general the number found was as expected. When two nymphs were fixed with the twig specimens, their mouthparts were observed inserted in the feeding punctures after sectioning. Finally, no other type of damage was found consistently that would befit the mouthpart morphology and feeding behavior of *C. arborina*. The feeding punctures are very distinctive (Fig. 1).

As is the case with most Cicadoidea, the feeding puncture is primarily intracellular, and is lined with a sheath staining bright red with safranin 0 dye (Wiegerzt, 1964; Pollard, 1967; Cheung and Marshall, 1973). This is thought to be a salivary secretion, although some basophilic staining may be due to autolysis of the ruptured cells (Pollard, 1967). From observations on a cicadellid feeding through an artificial membrane, Bennett (1934) reported the secretion of a colorless fluid from the mouthparts that quickly coagulated to form a hyaline sheath around the stylets. This corresponds with the nature of the sheath reported here. Aphids produce a similar structure, but the feeding track is primarily intercellular (Balch, 1952).
The bore of the sheath is smooth, with the diameter increasing with the insect's age (see below), and the outer surface is irregular, being constricted by the plant cell walls, and expanding slightly into the cytoplasm, although not replacing it. The sheath continues through the air spaces between mesophyll cells, where its surface exhibits helical ridges, like twisted wrought iron.

The feeding tracks passed through many tissue types before ending in the xylem. A fibrous hypodermis was present near most feeding sites (Fig. 1), although it was absent directly below the stomata and other areas where punctures were predominantly located. Other tissues always encountered were the mesophyll, phloem sieve cells, ray parenchyma, and concentric bands of thick walled fiber cells. The styles were usually worked between the latter. Except for the cells broken by the styles, there was no other sign of damage to the tissues. Seventy-seven percent of all sheaths terminated in the xylem tracheids. These were generally in the outer layer of vessels, and usually only one cell showed signs of damage. The deepest tracks ended in the seventh layer of xylem from the outside. Sixteen percent ended in the xylem ray parenchyma, and here there was no evidence of xylem feeding. Pollard (1967) noted however, that in the mesophyll feeding cica
dellid, Eupteryx melissae Curtis, maxillary extrusion was commonly far beyond the end of the sheath, in which instance, xylem actually may have been contacted. The thickness of our sections however, made this difficult to establish. In contrast to that of the phloem, xylem sap is a very dilute solution of inorganic ions, amino acids, and sugars. Thus xylem feeding is in keeping with the large amounts of fluid excreta produced by C. arborina nymphs and other spittlebugs, as large volumes of sap must be processed to extract the necessary nutrients.

The angle of stylet penetration was usually radial, although in 37% of the cases it was skewed from 20° to 45° from the radial. Also, the course of the stylets was generally straight, but in some specimens it curved slightly in either the transverse or longitudinal plane (29% of the cases), showed a slight elbow (14%), or curved in an “S” shape (2%). Branching was very infrequent, and often occurred at the level of the fiber cell bands. Usually only one branch was patent.

Clastoptera arborina nymphs and adults prefer the outer twigs and foliage branchlets of Juniperus, with the younger individuals predominantly on the more succulent tissue (Kuenzi and Coppel, 1985). Histological location of the actual punctures provides more specific circumstantial evidence characterizing feeding site choice and establishment. Examination of 42 sites revealed penetrations at virtually any point around the twig’s circumference, including: between the resin canal and the scale edge (fig. 1) (88.0%), between scales (2.4%), at the edge of a scale (4.8%), into the resin canal (2.4%), and through the periderm (2.4%). Usually the point midway between the resin canal and the edge of the scale coincided with the lateral edge of an overlying scale (47.6% of the total), which is in keeping with the nymph’s preference for sheltered locations. Where a first instar attempted penetration directly into a resin canal, sheath material was deposited a few microns inside the canal lumen, but there was no sign of continuation through to the other side. Interestingly, 54% of all penetrations were directly between guard cells of the stomata, and in two additional cases they were within one cell of a stoma. Brandes (1923) also noted that the stylets of Aphis maidis Fitch frequently entered the thin cuticle of the guard cells in corn, and Putman (1941) found over 10% of the stylets either entering the stomata, or passing between a guard cell and an adjacent epidermal cell in the mesophyll feeding cicadellid, Typhlocyba pomaria McAttee. As the stomata rarely occupy a large fraction of epidermal surface (7.8% of 218 mm² in
these observations may reflect a tendency of the spittlebugs either to use the vapor leaking through open stomata as a cue to initiate a feeding puncture, or simply to use the stoma as a path of low mechanical resistance.

The bore diameter, measured midway between the epidermis and xylem ranged from 4.2 to 25.2 µm (Table 1). A one way analysis of variance showed a highly significant instar effect ($P < 0.005$), but a least significant difference test failed to detect differences between successive instars at the 90% confidence level. A regression of the bore diameter against instar (the adult considered as the sixth instar) gave a linear trend with a slope of 2.7 µm ($P < 0.001$) and $R^2$ of 69.8%. It should be noted however, that the ranges and 95% confidence intervals of the first and second instar measurements overlapped completely (Table 1). Excluding size, the stylet sheaths of the nymphs were all similar in morphology. The thickness of the sheath was from 2.9 to 11.6 µm in the second instar, and those of the other immature instars fell within this range. The sheaths of the adults and some fifth instar nymphs however, were very thin or not visible, and did not appear to traverse the air spaces as did those of the younger nymphs. The older individuals also damaged the xylem tracheids more, with several vessels being ruptured.

Pollard (1967) suggested that the mandibular stylets penetrated very shallowly, with the maxillae being extruded much farther. Thus the presence of such long sheaths in *C. arborina* feeding punctures may indicate extensive mandibular penetration into the plant tissue. As *C. arborina* feeds with its body aligned with the long axis of the stem, and the mandibular stylets are positioned lateral to the maxillae, the sheath bore diameter should correspond to either the maxillary or the combined stylet width. Transverse sections of second and fifth instar nymphs allowed direct measurement of the stylets. Allowing for some shrinkage of the plant tissue, the combined mouthparts were clearly too large to account for the bore diameter, but the maxillae were only slightly larger than the bore (Table 1). Thus the mandibles of *C. arborina* are apparently inserted only a short distance, and in contrast to the mesophyll feeding *E. melissae*, sheath material is deposited along the entire length of the maxillary track.

Pollard (1967) also found that the depth of penetration increased with each instar in *E. melissae*, but this would not be expected in *C. arborina* due to the uniform thickness of mesophyll in the photosynthetic twigs. Indeed, a one way analysis of variance showed no difference in average penetration (all measurements were trigonometrically corrected for nonparallel sectioning) with an

<table>
<thead>
<tr>
<th>Instar</th>
<th>Sheath Bore Diameter ($\bar{x} \pm SD$)</th>
<th>Sheath Thickness (range)</th>
<th>Length ($\bar{x} \pm SD$)</th>
<th>Mouthparts Maxillae (Rep.)</th>
<th>Max. and Mand. (Rep.)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>6.13 ± 1.69 (6)</td>
<td>2.3 - 11.6</td>
<td>519 ± 64 (5)</td>
<td>8.7</td>
<td>17.4</td>
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<tr>
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<td>6.18 ± 1.18 (8)</td>
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<td>489 ± 118 (8)</td>
<td>8.7</td>
<td>17.4</td>
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<td>10.09 ± 3.65 (8)</td>
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<td>482 ± 90 (8)</td>
<td>8.7</td>
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<td>12.78 ± 5.16 (6)</td>
<td>4.4 - 8.7</td>
<td>533 ± 97 (6)</td>
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<td>49.2</td>
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<tr>
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<td>15.75 ± 3.48 (5)</td>
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<td>507 ± 99 (5)</td>
<td>29.5</td>
<td>49.2</td>
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<tr>
<td>Adult</td>
<td>19.43 ± 4.66 (4)</td>
<td></td>
<td>551 ± 61 (4)</td>
<td>29.5</td>
<td>49.2</td>
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overall mean of 513 μm and pooled standard deviation of 94.7 (30 degrees of freedom; \( P > 0.25 \)). This is demonstrated by the values in Table I.

2. Description of Native Spittle

Early in the season the spittle produced by a first instar nymph is a small, white mass of tiny bubbles deposited in the fork of foliage branchlets or outer twigs. The size of this mass and of the bubbles increases with the growth of the insect, and the whitish appearance persists. Even in the first instar, the spittle often includes silken strands of unknown origin, plant hairs and other detritus, and the bodies of dead insects. All these components, with the addition of crystalline material, are found on the twig surface in abandoned spittle masses. In later instars, especially the fifth, the spittle becomes less frothy, and large globules of clear to yellowish, gelatinous spittle are found, sometimes with correspondingly colored patches of crust on the surface. Other than this, there is no change in the spittle of the pharate adult, as opposed to the condition of Philaenus spumarius (L.) and Lepyroa quadrangularis (Say), where, at this stage, the spittle is allowed to dry, and a cavity formed inside to accommodate the ecdysing insect (Doering, 1922; Weaver and King, 1954).

The following insects were found in various spittle masses in the summer of 1983: in the order Hemiptera: Plagiognathus ilicis Knight, P. annulatus v. cuneatus Knight (Mirididae); in the Hymenoptera: Euderomphale, and Omphale (Eulophidae), Aphanogmus (Ceraphororinae), Copidosoma (Encyrtidae), Laelius pedatus (Say) (Bethylidae), Chelonus (Braconidae), Polynema (Mymaridae), Metaclisis, Platygaster (Platygasteridae); in the Psocoptera: Psocus (Psocidae), and Lachesilla pedicularis (L.) (Pseudocaeciliidae); and a moribund unidentified dipterous larva. None of these have been reported as predators or parasitoids of cercopids, and we believe that their occurrence is either entirely accidental, or that they became trapped while utilizing the spittle as a water source.

3. Rate of Spittle Production

The spittle collected under near 100% humid conditions in the laboratory was slightly different from the spittle in the field. There were almost no bubbles in the mass around the insect, and usually none in the spittle collected in the vial. The spittle was very fluid and colorless, and never mucoid or of yellow tint. Most of it flowed from the insect into the vial, leaving only a film and a few bubbles covering the nymph and surrounding twig.

The liquid volume of spittle collected in the field was impossible to determine due to the difficulty in disassociating the entrapped bubbles (these bubbles were very persistent, even in alcohol-preserved spittle masses). Thus, without invoking more elaborate instrumentation, the density of native spittle could not be determined.

The density of spittle collected in the humidity chamber was 1.04 ± 0.02. The daily spittle production was 194.6 ± 73.7 mg (n = 9, range 101.6 – 291.3) for the three days. For comparison, Horsfield (1978) found that the fifth instar of P. spumarius produced 1310 ± 80 mg/day under similar conditions, or 6.7 times that of C. arborina. P. spumarius is a larger animal feeding on herbaceous plants, and this may account for some of the discrepancy. Upon desiccation, a thin residue consisting of a mat of very fine interconnected fibers and small white crystals was deposited on the vial walls. None of the silken strands or detritus associated with the field spittle was observed. The residue thus probably corresponds to the filmy varnish seen coating the crystals and foreign inclusions in abandoned spittle masses in the field, and this soluble component, being concentrated by evaporation from the surface, could act as a thickening agent to increase the viscosity and structural integrity of the spittle mass. No further tests were performed, but studies have demon-
strated the presence of amino acids (Wiegert, 1964; Hagley, 1969), sugars, and microorganisms (Wilson and Dorsey, 1957) in spittle of other cecropids.

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LITERATURE CITED


