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Title: Plant architectural barriers to feeding site selection by the meadow spittlebug, Philaenus spumarius (L.)

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While current theories describing the insect-plant interaction have emphasized the biochemical aspects of the relationship, morphological components can also play a significant role in determining which plants or tissues are susceptible to insect attack. Xylem sap on which spittlebugs feed may lack many of the plant compounds responsible for host selection and preference, and for this species architectural barriers may be more significant in restricting host plant utilization. This investigation examined the role of plant anatomical structures as barriers to the selection of feeding sites by the meadow spittlebug, Philaenus spumarius (L.).

The distribution pattern of P. spumarius on Anaphalis margaritaceae (D.C.) suggested that trichomes on the stem may restrict the first through third instar nymphs to feeding on the leaves, while tissue hardness may prevent nymphs from feeding on the lower stem.

Fifth instar nymphs feeding on Medicago sativa (L.) may also be confronting a tissue hardness barrier on the lower stem.

Caging experiments on hirsute vs. shaven stems confirmed that trichomes were a barrier to the first three instar nymphs at the apex of the plant. Depth of xylem elements and tissue hardness were not significant barriers to feeding. The mechanism of resistance appeared to be that trichome height exceeds the length of the nymphs' beak, and thereby interferes with the initiation of stylet penetration.

Fewer nymphs were able to feed when caged at increasing distance below the terminal bud (DBTB). For A. margaritaceae, tissue hardness and the trichome layer were the barriers to feeding; for M. sativa, tissue hardness and decreased availability of xylem vessels reduced feeding.

Stem segments within the cages were sectioned to determine which tissues were impeding stylet penetration. In A. margaritaceae the progressive lignification of the bundle cap and interfascicular region with increasing DBTB were the main tissues preventing stylets from reaching the xylem. In M. sativa on the other hand, the bundle cap and the interfascicular parenchyma were penetrable at maturity, but the increasing number of lignified fibers in the xylem prevented the stylets from reaching a xylem element.

The predictive capability of a needle penetrometer was assessed by correlating feeding ability of fifth instar nymphs with tissue hardness measurements on the two hosts. Penetrometer measurements on the lower stem lacked sensitivity to tissues impeding stylet penetration, and were a poor indication of feeding potential in this region.

Preference tests in the absence of trichome and tissue hardness barriers showed that the nymphs fed on normally restricted areas of the plant. Gradients in two parameters of the spittlebugs' food niche, xylem sap tension and the concentration of amino acids in the sap, indicated that the preferred stem was more favorable in terms of

xylem sap tension. While tissue hardness restricted nymphs from a portion of their preferred range of feeding sites, the uniform distribution of nymphs suggested that they either did not respond to variation in these parameters, or responded to a combination of parameters with opposite gradients.

Plant architectural barriers to feeding site selection by the meadow spittlebug, *Philaenus spumarius* (L.)

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Chapter 1

Introduction

The meadow spittlebug, *Philaenus spumarius* (L.), (Homoptera: Cercopidae) is common in Europe and North America, and has been reported from Asia, Africa, Japan, and South America. *P. spumarius* is a univoltine species. In North America the overwintering eggs hatch in the early spring, nymphs develop through five juvenile instars, molting to the adult in the early summer. Mating occurs in the early fall and eggs are laid on senescing plant tissue, particularly grass stubble.

P. spumarius, feeds solely on the xylem sap of its host plant (Wiegart, 1964, Horsfield, 1978); penetrating intracellularly to the xylem vessels. The concentration of nitrogenous compounds and sugar in the xylem sap is extremely low (Pate, 1973, 1976, 1980); and necessitates a high feeding rate and the excretion of excess water. In the nymphal stage this water contains a glycoprotein which decreases the surface tension of the excreta and the nymph pumps air into it forming the characteristic spittlemass (Weaver and King, 1954).

The host range of *P. spumarius* includes several hundred species of plants, mostly herbaceous dicotyledons. The only woody species utilized as hosts are those on which the nymphs can find adventitious shoots or the current year's tender growth (Weaver and King, 1954). What probably allows this taxonomic diversity is that the xylem sap is a homogeneous food source. Nitrogen compounds frequently comprise the major dry matter component in xylem sap. The nitrogen is transported mainly as NO_2 and reduced forms, usually amino acids. In most dicotyledons studied two amino acids make up 60 to 70% of the reduced nitrogen usually glutamine, and/or asparagine predominate. Three or four other amino acids comprise the remainder of the reduced nitrogen.

Occasionally nitrogen is transported in compounds such as alkaloids (Pate, 1971, 1973). The limited diversity of amino acids ingested is probably compensated for by the intracellular gut flora found throughout the Homoptera (Houk and Griffiths, 1980). Xylem sap may be lacking in many of the plant metabolites which influence herbivore feeding, either because the large molecular weight compounds such as tannins are absent from the sap, or are like alkaloids, transported in dilute and easily excreted forms (McKey, 1979).

Insects discriminate among plants on the basis of nutrition, secondary plant metabolites, phenology, and anatomical features, yet the emphasis in the theory of insect-plant interactions has been on the biochemical aspects of the relationship (van Emden, 1973, Gilbert, 1979). This is because the high degree of chemical distinctiveness of plant species is thought to be the principle factor leading to host specialization (Feeny, 1975, Ehrlich and Raven, 1964, Janzen, 1978). Because xylem sap is more homogeneous between species, factors which determine host plant specialization may be different for cercopids McEvoy (1983a) has proposed that architectural structures on plants are the major basis for host plant specialization in P. spumarius . The range of stem utilized by P. spumarius was correlated with the tissue hardness of the stem, and appeared to prevent the nymphs from feeding lower on the stem in some species, and eliminate other species as potential hosts. The availability of wide leaf axils, which prevent the spittlemass from running down the stem, further restricts host suitability.

This thesis adressed the role of architectural barriers in restricting the feeding sites of P. spumarius. Objectives were to determine whether tissue hardness prevented the nymphs from feeding below their normal feeding range. If the spittlebug was restricted to the apex of the plants because of tissue hardness, what were the specific tissue barriers causing the restriction.

The resistance of the stem to penetration by the stylets was estimated by a needle penetrometer, which measures the pressure required to force a needle through the stem. The predictive capabilities of the penetrometer were assessed in view of the possibility that the variation in stem anatomy affects stylet penetration and penetrometer readings differently.

The degree to which anatomical structures other than tissue hardness affect the selection of feeding sites was investigated. In addition to extending the list of anatomical structures influencing spittlebug feeding behavior, this study determined if the barriers interact with tissue hardness to enhance the resistance of the plant. The individual role of tissue hardness in plant resistance can not be determined unless the effect of other barriers has been removed or taken into account. The meadow spittlebug did not utilize stems which were within its range of penetrable hardness if the leaf axils were not wide enough to prevent the spittlemass from running down the stem (McEvoy 1983a). A failure to take this into account would have obscurred the relationship between tissue hardness and feeding site selection.

Architectural barriers may be restricting the spittlebug from feeding on otherwise preferred regions of the stem. Parameters of the spittlebug food niche may vary between the different areas on the host plant. If the nymphs are responding to this variation, tissue hardness and other barriers may prevent them from feeding on the most favorable tissues. The two parameters investigated were the nitrogen concentration of the xylem sap, and xylem sap tension. The low nitrogen content of plant tissues, which limits growth and reproduction, is a "hurdle" to the colonization of plants by insects (Southwood, 1973; Lawton and McNeill, 1978; Mattson, 1980). The diet of spittlebugs is very low in nitrogen and a preference for feeding at sites high in nitrogen is expected under these circumstances. Xylem sap tension is a parameter which is unique to xylem-sap feeders, and their response to variation in this parameter is largely unknown.

The study of spittlebugs offers an opportunity to expand and refine the existing theories on the animal-plant relationship. The theories of Feeny (1975, 1976) and Rhodes and Cates (1976) were developed from studies of insects whose primary barriers to the utilization of the apparent resources base were secondary plant compounds. Doubts have been raised about whether they apply to other herbivore guilds (Janzen, 1978). Recent studies on the feeding ecology of spittlebugs (McEvoy, 1983a, b) have begun to elucidate these differences. This study further defines the role of plant architecture in the feeding ecology of the xylem-feeding insects.

Chapter 2

Anatomical barriers to feeding site selection in the meadow spittlebug (*Philaenus spumarius* L.)

Abstract

The distribution of *P. spumarius* (L.) on pearly everlasting (*Anaphalis margaritaceae* D.C.) and alfalfa (*Medicago sativa* L.) suggested several potential anatomical barriers which may restrict feeding site selection on these hosts. Caging the five instars on the upper stem with the trichomes present or absent confirmed the hypothesis that the trichomes were a barrier to the younger instars feeding on the stem. The other proposed barriers, the depth of the xylem elements and tissue hardness, were not significant.

Caging fourth and fifth instar nymphs at increasing distances below the terminal bud defined the ability of the fourth and fifth instar nymphs to feed along the stem of *A. margaritaceae*, with or without trichomes, and the fifth instar to feed along the stem of *M. sativa*. Fifth instar *Aphrophora spp.* were included in the caging experiments on *A. margaritaceae* in order to determine whether larger body size conferred greater feeding ability. On both host plants there was a significant decrease in the ability to feed the further the nymphs were caged below the apex. On *A. margaritaceae* this restriction was due to two barriers, tissue hardness of the stem, and the presence of trichomes in regions of hard tissue. On *M. sativa* the barriers to feeding lower on the stem were a combination of tissue hardness and the reduction in the availability of xylem vessels in the

penetrable region of the stem. The larger nymphs could penetrate the anatomical barriers on these plants more readily than the smaller nymphs.

The stem segments within the cages were sectioned to determine the specific tissues impeding stylet penetration. In A. margaritaceae the consecutive lignification of the bundle cap and interfascicular region with the increase in the distance below the apex were the main tissues blocking the stylets. In M. sativa the lignified xylem fibers prevented the stylets from reaching xylem vessels; the bundle cap and interfascicular parenchyma were penetrable at maturity.

The predictive capabilities of a needle penetrometer was assessed through the correlation of the feeding ability of the fifth instar and the tissue hardness measurements on the two hosts. The difference in the ability to feed on hard tissues indicated that the penetrometer was not sensitive to the tissues which impede stylet penetration in hard regions of the stem. The penetrometer can be used to predict the range of available feeding sites for those insects which feed on soft tissue because of a limited ability to penetrate hard tissues.

Introduction

Hairston et al. (1960) hypothesized that because large scale defoliations rarely occur, herbivores are not limited by food resources. Yet evidence indicates that much of the seemingly apparent resource base is unavailable to herbivores because of its deterrent or antibiotic properties. Barriers to feeding may be of three types: 1) biochemical, related to nutrition or secondary plant compounds (House, 1962; Fraenkel, 1969; McNeill and Southwood, 1976; Feeny, 1976; Rhoades and Cates, 1976; Rosenthal and Janzen, 1979; Mattson, 1980), 2) anatomical (Painter, 1951; Levin, 1973; Norris and Kogan, 1980), or 3) ecological (Janzen, 1966, 1972; Feeny, 1976; Atsatt and O'Dowd, 1976; Bently, 1977).

The theory of insect-plant interaction has emphasized the biochemical aspects of the relationship (van Emden, 1973; Gilbert, 1979). The anatomical component, although perhaps not as widely significant, does play a major role in determining which plants or tissues are susceptible to insect attack. The meadow spittlebug (Philaenus spumarius L.) feeds on the xylem sap and may not be confronted with the lethal array of secondary plant compounds which influence herbivore feeding, either because the compounds are absent from the sap e.g., tannis, or, are transported in dilute quantities and in forms easy to excrete, e.g., alkaloids (McKey, 1979). For xylem feeders the anatomical component of the insect-plant relationship may be a critical factor in host-plant specificity. This study examines the role of anatomical structures in the selection of feeding sites by P. spumarius.

The meadow spittlebug feeds on hundreds of species in North America, mostly herbacious dicotyledons (Weaver and King, 1954). Yet this taxonomic generalist does not utilize plants in proportion to their occurrence in the habitat (Halkka et al., 1977; McEvoy, 1983a). Host restriction may be related to variation in xylem sap nutrients,

(Bollard, 1960; Pate, 1976, 1980), or physical barriers to feeding (McEvoy, 1983). The distribution of feeding nymphs on individual plants (Weaver and King, 1954; Halkka, et al. 1977; McEvoy, 1983a), indicates restriction to, or preference for, particular regions of the plant, perhaps in response to external physical gradients (Whittaker, 1970), nutritional gradients (Horsfield, 1977), or anatomical barriers (McEvoy, 1983a).

McEvoy (1983a) has proposed that plant architecture is the main factor accounting for variation in plant resistance to P. spumarius. He found a strong correlation between gradients in tissue hardness along the stem, and the distribution of P. spumarius nymphs. Tissue hardness appeared to restrict the nymphs from feeding lower on the stem of some species, and eliminated other species as potential hosts. The availability of wide leaf axils, which prevent the spittlemass from running down the stem, further restricted host suitability.

The objective of this study was to investigate the role of anatomical barriers in restricting the within plant distribution of P. spumarius feeding sites. Does tissue hardness prevent the nymphs of P. spumarius from feeding on the stem below their preferred range of feeding sites as the correlations of McEvoy (1983) suggest? If the spittlebug is restricted from feeding lower on the stem because of tissue hardness, what are the specific anatomical barriers causing the restriction? Plant support tissues such as sclerenchyma and collenchyma confer hardness to the stem and can block the stylets of Homoptera (Staniland, 1924; Entwistle and Longsworth, 1963; and Knight and Alston, 1974; Horsfield, 1977). These tissues may prevent P. spumarius from reaching the xylem vessels in the lower stem.

The depth of the xylem elements is an alternative explanation for restriction in feeding sites. Leafhoppers (Houston et al., 1947; Day et al., 1951) and aphids (Quiras et al., 1977; Chatters and Schelehuber, 1951) are prevented from feeding on certain plant parts by the depth of the vascular tissues. The allometric ratio of stylet length to head capsule width is greater in younger compared to older nymphs (Appendix 1) indicating that the depth of the xylem elements

may influence feeding behavior. Secondary stem growth from the cambium layer will increase the depth of the xylem elements in the lower stem and may account for restriction in feeding sites.

Anatomical structures other than tissue hardness may influence the feeding site selection of P. spumarius on the host plants studied. Trichomes are a factor in plant resistance to many stylet feeding insects (Painter, 1951; Levin, 1973; Webster, 1975), and may be a barrier to feeding by P. spumarius. In addition to extending the list of anatomical barriers to spittlebug feeding these barriers may be additive or interactive with tissue hardness in limiting the feeding range of P. spumarius.

The last question is whether resistance to stylet penetration can be adequately measured by a needle penetrometer. A penetrometer measures the pressure required to force a needle through plant tissue and is thus a measure of tissue hardness rather than toughness (Pollard, 1971). Clearly penetration by a needle is not a precise analog of stylet penetration. However, if penetrometer measurements of hardness are correlated with the ability to feed they could be used to predict the fraction of host tissue available for feeding.

Species studied

P. spumarius has been reported from Europe, Asia, Africa, Japan, and North and South America. The overwintering eggs hatch in the early spring. The nymphs develop through five instars, molting to the adult in early summer. Eggs are laid in the early fall (Weaver and King, 1954).

P. spumarius feeds by piercing intracellularly to the xylem vessels and ingesting the sap (Wiegart, 1964; Horsfield, 1978). The low concentration of nutrients in the xylem sap (Pate, 1975, 1976,

1980) necessitates a high feeding rate and the excess water is excreted. In the nymphal stage this excreted water contains a glycoprotein which decreases the surface tension of the water, and the nymphs pump air into the fluid forming the characteristic spittlemass (Weaver and King, 1954), This mass creates a moist, cool microclimate (Whittaker, 1970) without which the nymphs dehydrate and die (personal observation). The presence of the mass is evidence of the nymphs having feed at that location on the plant.

The study concentrated on two host plants, pearly everlasting (Anaphalis margaritacea), and alfalfa (Medicago sativa var. Du Puits). The within plant distribution of P. spumarius on A. margaritaceae was studied on Mary's Peak, Benton Co., Oregon, (elevation 700 m). Nymphs and pearly everlasting were gathered from the site for use in laboratory experiments. The study site was a northeast slope which had been clearcut approximately ten years earlier. The current vegetation consisted of young Pseudotsuga menziesii, small shrubs, and a mixture of grasses and herbacious plants in which A. margaritaceae was common. A. margaritaceae grows from a perennial running root stock, with stems commonly 2-9 dm high. Although several stems may emerge from the older roots, stems were considered individual plants. Meadow spittlebug eggs hatched in late April and the nymphs molted to adult in mid to late July.

The within plant distribution of nymphs on M. sativa was studied at a three-year-old alfalfa field in the Willamette Valley, Benton Co., Oregon. Nymphs and plants were gathered from the site for caging experiments. In plotting the distribution of nymphs, each stem was treated as an individual. In the caging experiments, the individuals were root crowns, and one of the emerging stems was selected for treatment. The period of nymphal development was from late March to early June.

Materials and Methods

Identification of potential barriers

Anatomical structures on the utilized and unutilized portion of the hosts were examined to determine if tissue hardness and other anatomical structures might be influencing feeding site selection. The feeding sites of the five instars were recorded approximately weekly on A. margaritaceae at the Mary's Peak study site during the spring of 1979; additional data on the first instar were collected in 1980. Points were located at random and all plants within a 1 m radius were examined for spittlemasses. The nymphs were collected from each mass separately and returned to the laboratory for instar determination according to head capsule widths given by Weaver and King (1954). The location of each mass was described by measuring the height of the plant, the distance of the mass from the terminal bud, and whether it was on the stem or leaf. The distance below the terminal bud (DBTB) was chosen as a descriptive variable because tissue hardness increases as stem tissues mature. Maturation begins after stem elongation has finished, and this occurs at a characteristic distance below the terminal bud (Esau, 1977). The host plants were returned to the lab and representative segments preserved in formalin-acetic acid-alcohol (FAA) for later analysis.

The distribution of the fifth instar on M. sativa was recorded in 1980 to determine whether the response of P. spumarius to the hypothesized tissue hardness barrier varied between host species. Internal stem anatomy varies widely within the dicotelydons (Esau, 1977) and may affect the spittlebugs' ability to penetrate hard tissue. The method of data collection was identical to that on A. margaritaceae except that spittlemasses on the main stem were distinquished from those on the side branches.

The preserved segments of the host plants were examined for differences in external and internal stem anatomy between the used and unused portion of the plant. The preserved segments were dehydrated in a series of tertiary-butyl-alcohol solutions, embedded in paraffin, and sectioned at 10 μm. The sections were mounted on glass slides, stained with safranin and fast green (Johansen, 1940),and examined microscopically.

Barriers to feeding

Experiment 1: Caging experiments were conducted to determine the extent to which the nymphs of P. spumarius are restricted to the leaves of A. margaritaceae, and to distinguish among the potential barriers to feeding on the stem. At the time each instar was active in the field, thirty host plants and nymphs were collected from the Mary's Peak study site. Clumps of 1-3 plants were selected at random, were dug up, placed in pots, and returned to the laboratory. Plants which had not recovered from transplanting shock by the following morning were discarded. Nymphs were collected from a variety of hosts and placed on A. margaritaceae until used in the experiments.

The thirty host plants were randomly divided into two groups, trichomes present, which had an intact trichome layer, and trichomes removed, which had the trichomes shaven off in the area being caged. The cages were half cylinders, 2 cm in diameter and .3 to .6 cm high. The tops and bottoms were plastic and the sides nylon mesh. Slots for the stem were punched in the center of the flat side and lined with nylon mesh. This allowed the cage to fit snugly on the stem with a minimum of compression of the trichome layer. Approximately two-thirds of the stem circumference was exposed inside the cage.

All caging experiments in the study were conducted using the following procedures. The nymphs were placed singly inside the cages which were attached to the stems at the specific DBTB. The plants were placed at 20-25°C and 30-50% RH and left until the nymphs had formed a mass or dehydrated and died. The feeding numphs were frozen on the stem with a stream of cold CO_2. The cages were removed, the approximate location of the stylet marked on those sections where the nymphs fed, and all stem segments preserved in FAA for later sectioning. In this experiment the nymphs were caged at 1.5 to 2.5 cm below the terminal bud (BTB).

Controls were set up to determine if the cage inhibited feeding. Fifteen of the plants on which the first instars were caged also had nymphs caged on the normal feeding site, the leaves. All these nymphs quickly formed masses, indicating the cage did not interfere with feeding.

An assumption of the caging experiments was that the nymphs would attempt to feed in order to obtain sap to form a spittlemass. If the behavioral response to avoid dehydration and death overrides other stimuli affecting feeding behavior, the death of a caged individual could attribute to the presence of the physical barrier to feeding.

Nymphs failing to reach the xylem sap may possibly obtain fluid from other tissues. To test this possibility, eight of the stem segments on which the first instars fed were sectioned and searched for stylet sheaths. The segments were sectioned and stained using the standard procedures. Approximately 250 sections were searched at a magnification of 40 to 400x for stylet sheaths. Safranin stains the stylet sheaths produced by P. spumarius and many other Homoptera and Hemiptera, as well as the lignified and suberized tissue. The stylet sheath remains in the plant after stylet withdrawal, and is therefore a permanent record of stylet "behavior" during the feeding process (Pollard, 1971). This technique was used by Wiegart (1964) and Horsfield (1977) to identify the xylem vessels as the feeding site of P. spumarius.

In addition to confirming the feeding site of the caged nymphs, the sectioning allowed me to infer whether nymphs experienced difficulty because of the depth of the xylem elements or tissue hardness. To determine the potential difficulty an instar may have in reaching the xylem vessels at varying depths, the maximum penetrable distance (MPD) for each instar was calculated and compared to the depth of the closest average xylem vessel at the apex of the plant. Since the first instar must penetrate deeper in relation to its stylet length than the older nymphs, the longest stylet sheaths found in the eight sections were used in calculating the percent of the external stylet length which can be utilized by all instars. The four longest stylet sheaths out of the sixteen found were averaged and divided by the average external stylet length of the first instar (Appendix 1) to obtain the percent of the stylet length utilized. The percent of the stylet used, multiplied by the average stylet length of the first through fifth instar, gives the MPD of these nymphs. The depth of the closest average xylem vessel was measured by choosing a section from each of fifteen stem segments on which the first instar was caged, and measuring the depth of the closest xylem vessel in a randomly chosen vascular bundle in each quarter of the section. The mean of these four values was used to calculate the average for the fifteen plants.

Experiment 2a: Caging experiments were performed to determine if anatomical barriers were restricting the nymphs of P. spumarius to feeding at the apex of A. margaritaceae and M. sativa. Accordingly, confined nymphs can not choose between sites based on physiological preferences, and it was assumed they would attempt to feed in order to prevent dehydration and death. Size dependent differences in the ability to feed at a range of DBTB would support the hypothesis that reduction in the ability to feed is due to an anatomical barrier. In order to make an additional size comparison, nymphs of the larger Cercopidae genus, Aphrophora, were included in the caging experiments on A. margaritaceae. Nymphs of similar size, A. permutata (Uhler) and

A. maculosa (Doering), were pooled because they could not be distinguished and their densities were low.

The cages used in these experiments were full cylinders, 2 cm in diameter and .5 to .8 cm in height. The cages opened into two 1/2 cylinders, which clamped around the stem. The hole in the middle of the cage was lined with mesh to prevent compression of the trichome layer.

The following experiment was performed on fourth instar nymphs of P. spumarius in June, 1980, and the fifth instar nymphs of P. spumarius and Aphrophora in June and July, 1982. At the time each of the two instars were active in the field, thirty A. margaritaceae were collected from the Mary's Peak study site in the previously described manner. The healthy plants were placed in pairs according to height and randomly divided into the trichomes present group, for which the trichome layer remained intact, and the trichomes removed group, in which 1/4 of the stem circumference was shaved of trichomes at each caging site. Fourth instar nymphs were caged at 5, 10, 15, 20, and 25 cm BTB (Fig. 2.1); and the fifth instar nymphs were caged at 5, 10, 20, 30, 35, 40, and 45 cm BTB. Variation in plant height resulted in a decrease in the number of nymphs caged at the lower sites. The fifth instar of the Aphrophora spp. was caged at 30 and 35 cm BTB just below the cages containing the fifth instar of P. spumarius. The experiments on the fourth and fifth instars were conducted under the prescribed conditions with the exception that the location of the feeding nymphs in the trichome absent group was recorded as being either on the shaven spot, or on the trichomes.

In July, 1980, the fifth instar of P. spumarius was caged only on lanate stems of A. margaritaceae. Experimental procedures were similar to the previous tests except that P. spumarius nymphs were caged on sixteen plants. The plants and nymphs also were collected later in the season so the plants were taller; accordingly the nymphs

Figure 2.1 Fourth instar nymphs caged on a pair of A. margaritacae.

also were caged at 50 cm BTB. To document the nymphs' difficulty in producing spittlemasses lower on the stem, the time it took for the nymphs to initiate a mass was recorded. Observation periods were at 20 minute intervals for the first two hours, at three hours, and at twelve hours. A spittlemass was considered to be initiated when fluid with bubbles was seen, or when the fluid drop at the anus of the nymph at one observation period had become a mass by the next period.

In June, 1980, the fifth instar nymphs of P. spumarius and fifteen M. sativa plants were collected from the Willamette Valley study site. The caging experiments were performed under the standard conditions. The caging heights were at 10, 30, 50, 70, 80, 90, and 100 cm BTB. Variation in plant height resulted in a decrease in the number of nymphs caged at the lower locations.

In the experiments, the caging site at 5 cm BTB was within the normal feeding range of P. spumarius and served as the control. In the one instance where the control nymphs died, the pair of A. margaritaceae was replaced.

Nymphs were caged at specific DBTBs rather than at specific tissue hardness locations because this allowed easily replicable caging sites (with a similar degree of tissue development). Using tissue hardness would require measurements damaging to the plant. Also, the accuracy of the penetrometer in measuring tissues which impede stylet penetration had not been established.

Experiment 2b: The potential barriers to feeding identified on the lower stem were tissue hardness, the depth of the xylem vessels, the availability of xylem vessels, and the trichomes. The parameters of these barriers were measured on the stem segments on which the nymphs were caged.

Tissues blocking stylet penetration in the two hosts were identified by sectioning and staining the segments and noting the pathway of the stylet sheaths in relation to specific stem tissues. Fifteen A. margaritaceae were sectioned, ten stems on which the fourth

instar was caged, and five stems from the 1980 fifth instar test. Five M. sativa stems were sectioned. Two hundred sequential sections were cut from each segment at the location of the marked feeding site, or if the nymph had died, from one end of the segment.

To determine if the depth of the xylem elements was a barrier to feeding, the depth of the closest and median xylem vessel were measured and compared to the maximum penetrable distance of the fourth and fifth instars. On A. margaritaceae the two measurements were made on a random section taken at 5, 10, 20, 30, and 40 cm BTB on the five sectioned stems on which the fifth instar was caged. The depth measurements on M. sativa were made at 10, 30, 50, 70, and 90 cm BTB on the five section stems. On each section a vascular bundle in each quarter of the stem circumference was randomly chosen for measurement. The mean of these four values was used to calculate the mean and standard error for the five plants.

The availability of xylem elements in the stem of M. sativa was determined by measuring the number of xylem elements, and the percent of xylem circumference at the outer edge of the xylem. The criterion used to determine availability was that the xylem vessel had to be within four xylem fibers of the edge of the xylem tissue. Observations of sections cut from 70 to 90 cm BTB showed that stylet sheaths terminated after penetrating a maximum of four lignified xylem vessels. This criterion biases the estimates of availability for the upper stem, where the amount of lignification was small or absent and stylets could probably penetrate further into the xylem tissue. The estimates of availability were measured at 10, 30, 50, 70, and 90 cm for the five sectioned stems. A random section from each segment was chosen and the measurements made at a random point within each quarter of the stem circumference. At each point the number of xylem vessels along an arc .808 cm long were counted. Xylem vessels within four xylem fibers of the edge, but lying directly behind another vessel were not considered available. The count was then expressed as the number per 1 mm. To account for differences in the size of the xylem

vessels the percent of the .808 cm arc containing available xylem vessels was recorded. The four values for each stem segment were averaged and means averaged to obtain the mean and standard error at each height.

Variation in the trichome layer which may influence feeding was measured on the five sectioned stems of A. margaritaceae on which the fifth instar was caged. Three characteristics which may influence feeding were chosen based on the structure of the trichome layer. Density per mm^2 may affect the ability of the nymphs to grasp the stem and exert the pressure necessary for stylet penetration. The vertical density is an estimate of the difficulty the nymph may have in pushing its beak through the trichome layer down to the epidermis. The height of the trichome layer restricts the young instars of P. spumarius from feeding on the stem (Chapter 3). While the fourth and fifth instars could feed at the apex of the stem, excessive height of the trichome layer lower on the stem may be important.

The three characteristics were measured at a random point in each quarter of the stem circumference at 5, 10, 20, 30, and 40 cm BTB The characteristics were measured using the methods of described in Chapter 3. The mean of the four values for each paramenter were averaged to obtain the mean and standard deviation at each height.

Adequacy of penetrometer measurements

The predictive capabilities of the needle penetromenter were determined by comparing the correlation between the ability of the fifth instar to feed vs. tissue hardness on the two host plant species. A needle pentromenter (Cherrett, 1968) was used to make hardness measurements on the fifteen A. margaritaceae plants which had been shaven of trichomes, and ten of the fifteen M. sativa plants on which the fifth instar nymphs were caged. A standard needle was used

since the diameter and taper of a needle can affect resistance (Pollard, 1971). If the penetrometer is sensitive to the tissues which impede stylet penetration the percent of nymphs able to feed at a given tissue hardness should be similar on both host plants.

Data analysis

When the dependent variable is binary, theoretical and empirical considerations suggest that the slope of the response function will be curvilinear (Neter and Wasserman, 1974). The data from the caging experiment fit this pattern, and a logistic regression package, Biomedical Programs, P. Series, 1981 (Dixon, 1981) was used to determine the significant variables in the model. The decision to enter or remove a variable was based on the log of the ratios of the maximized likelihood functions, X^2=2 log L(B current)/ L (B candidate) (Appendix II). The significance values given in the results are for the improvement chi-square when the independant variable in question is added to the model. The significance of the chi-square statistic is evaluated at d.f.=1, which is the difference in the number of degrees of freedom in the current and candidate models. In those models where the predicted probability of feeding at the control site (5 cm BTB) was less the .99 a dummy variable (CONTROL) was used to designate this DBTB as a control. This procedure moved the fitted regression curve to within a .99 probability at the control site.

Results

Identification of potential barriers

The feeding sites of P. spumarius on the two hosts appeared to be restricted. On A. margaritaceae the limitation was greatest in the young instars (1-3), which feed almost exclusively on the leaves of the upper stem. The percent feeding on the stem increased as the nymphs matured to later instars. Nymphs fed only at the apex of the host; the proportion of stem length utilized increased with each instar. The range of feeding sites of the fifth instar extended to 8 cm below the terminal bud (Fig. 2.2a). On M. sativa, P. spumarius nymphs also fed on a small proportion of the stem (Fig. 2.2b). Its feeding range extended to 28 cm BTB on the main stem and 15 cm BTB on the side branches.

Differential development of several anatomical structures occurred between the feeding site of P. spumarius and the nonutilized region of the hosts (personal observation), and therefore were potential barriers to feeding site selection. The stem and underside of the leaves of A. margaritaceae were covered by a dense mat of long tangled woolly hairs, called lanate trichomes (Harrington and Durvell, 1957). The pubescence was more extensive on the stem than on the leaves, and could restrict the young nymphs to leaves and the older nymphs to the apex of the stem.

In both species, tissue hardness was a potential barrier to feeding. In A. margaritaceae the fibers overlying the vascular bundle and the area between the vascular bundles became progressively lignified with the increase in the distance below the terminal bud. This sclerotization of the bundle cap and interfascicular region increases the hardness of the stem and could account for the restriction in feeding sites. In M. sativa the development of the

Figure 2.2 Distribution of P. spumarius nymphs on host plants (a) five instars on A. margaritacae, N for instars (1-5) = 49, 58, 23, 70, 69, (b) fifth instars on M. sativa, N=41.

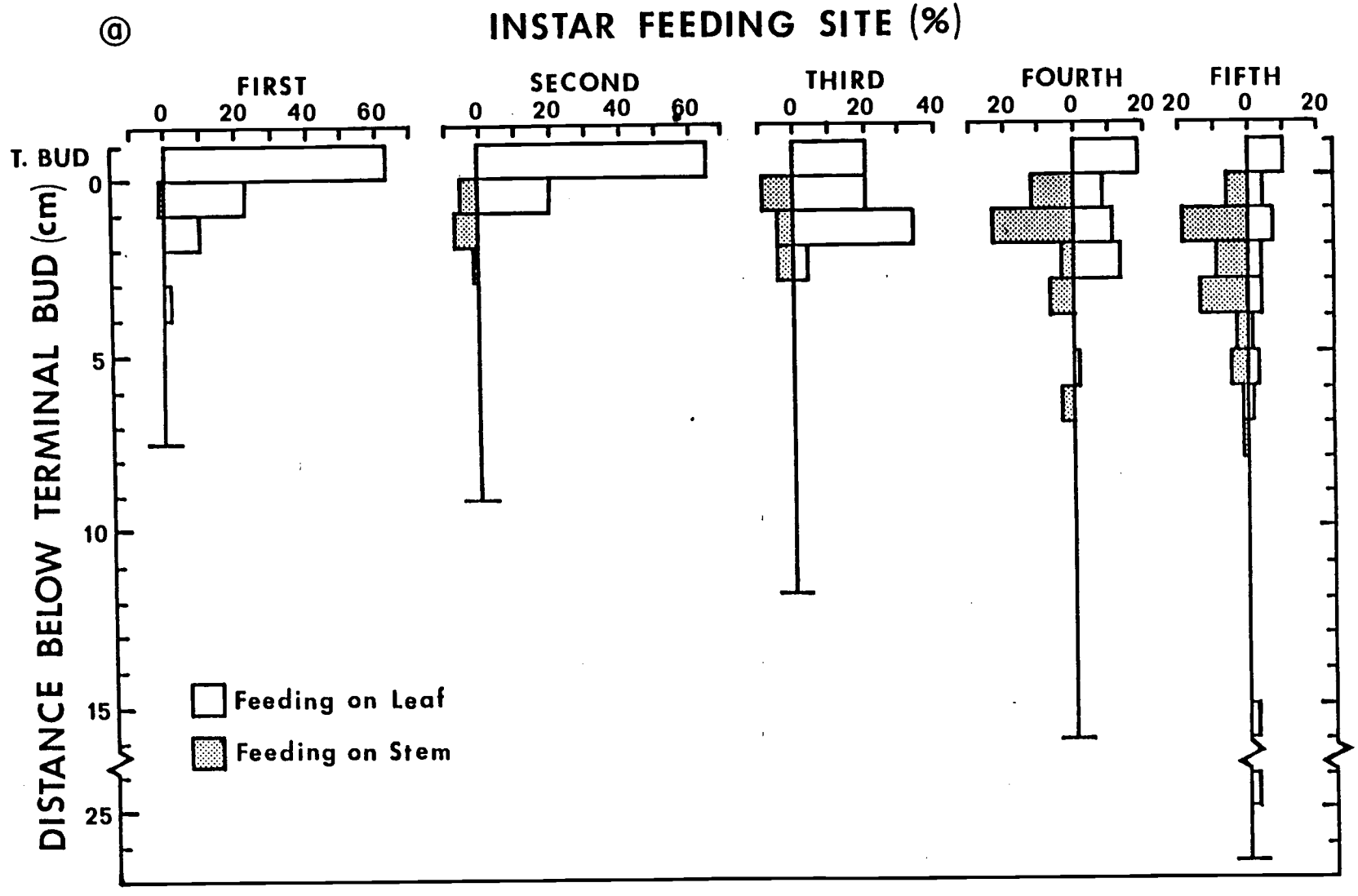

@
INSTAR FEEDING SITE (%)
FIRST
SECOND
THIRD
FOURTH
FIFTH
0 20 40 60
0 20 40 60
0 20 40
20 0 20
20 0 20
T. BUD
0
5
10
15
25
DISTANCE BELOW TERMINAL BUD (cm)
Feeding on Leaf
Feeding on Stem

Figure 2.2

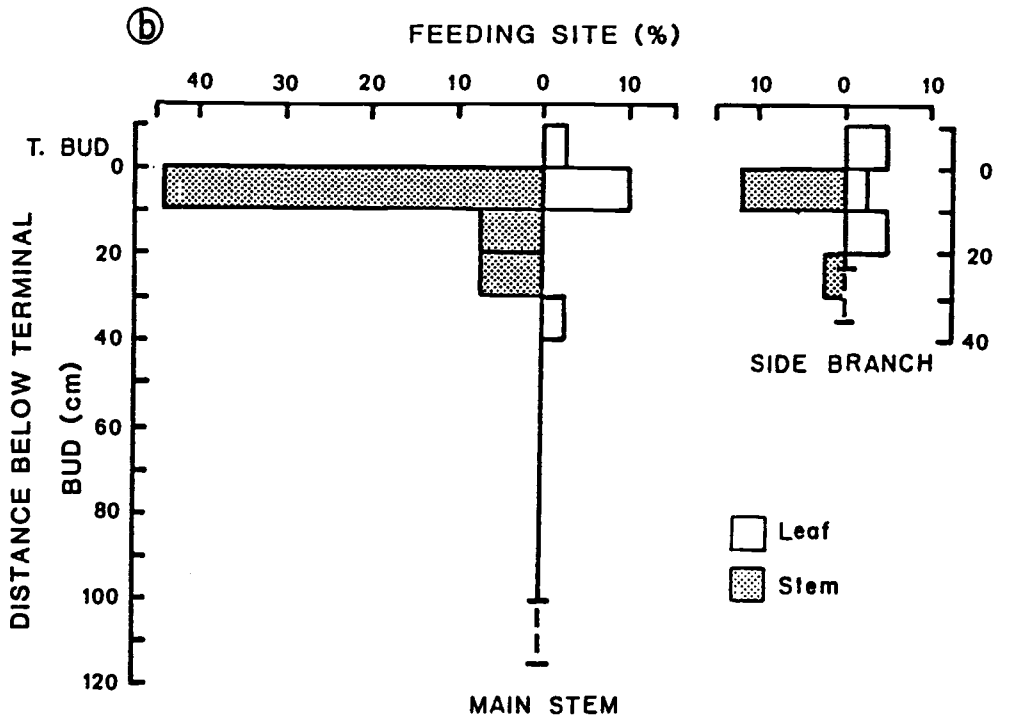

ⓑ
FEEDING SITE (%)
40
30
20
10
0
10
10
0
10
T. BUD
0
20
40
60
80
100
120
DISTANCE BELOW TERMINAL BUD (cm)
0
20
40
SIDE BRANCH
Leaf
Stem
MAIN STEM

Figure 2.2

collenchyma tissue in the bundle cap and the lignification of the xylem fibers in the lower stem increases stem hardness and could restrict the nymphs to the apex.

In both plant species, the depth of the xylem elements increased with an increase in the distance below the terminal bud. Young nymphs may be unable to reach their feeding sites in the stem of A. margaritacae, and older nymphs could be restricted to the apex in both species.

The limited availability of the xylem elements at lower sites on M. sativa may limit the nymphs from feeding in this region. The ratio of xylem vessels to xylem fibers produced in the vascular bundles decreased at increasing DBTB, and as the interfascicular regions increased in diameter. These two developmental patterns decreased the number and area of xylem vessels at the periphery of the xylem tissue.

The distribution of the nymphs may actually be a result of preference based on physiological stimuli rather than a restriction due to anatomical barriers. The spittlebugs could be responding to gradients in xylem sap tension or nutrients, other stem physiological stimuli, or external environmental gradients.

Barriers to feeding

Experiment 1: The young nymphs of P. spumarius were restricted to the leaves of A. margaritaceae by an inability to feed on the stem. In the regression model, the decrease in ability to feed when caged on the stem with the trichomes was significant ($p<.0005$) and associated with the variable TRICHOME (Fig. 2.3). Few first through third instar nymphs were able to feed when caged on the lanate stems. Even though the fourth instar was able to feed on the stem, it experienced difficulty in initiating a spittlemass. Eight of the fifteen nymphs had not formed a mass after 45 minutes, and two took as long as 4 1/2 hours. The fifth instar feed more readily on the lanate stem; all

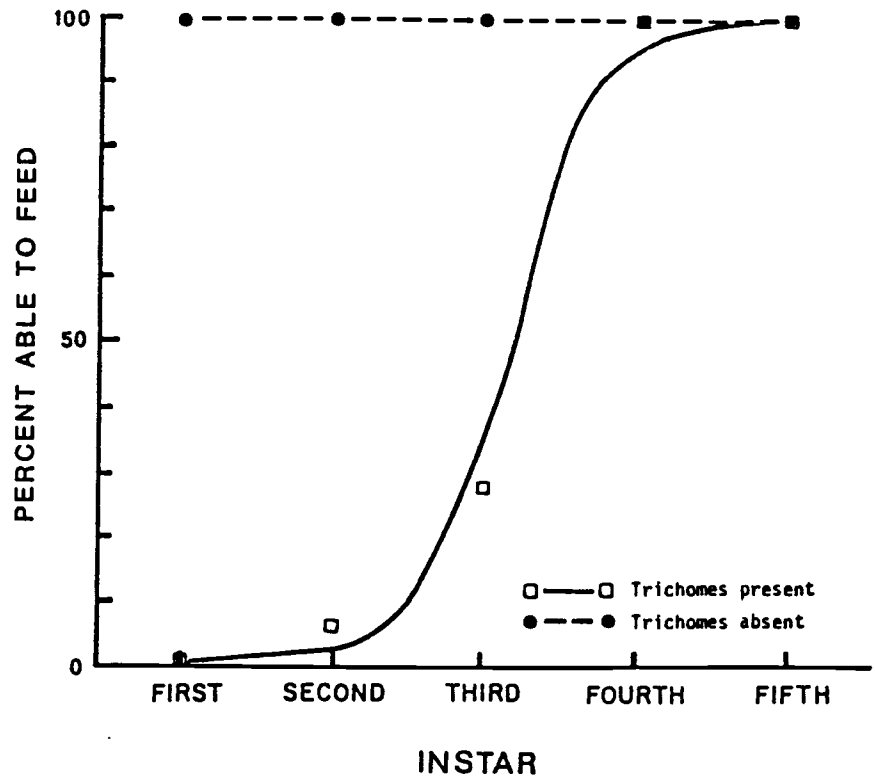


Figure 2.3 Percent of first through fifth instar nymphs able to feed when caged on the stem of A. margaritaceae with trichomes present and absent. Regression curves fitted by hand through the points generated by the model. Regression coefficients are in Appendix II, N=15. Significance value for the improvement in the fit of the regression curve when the variable is added to the model: TRICHOME, $p<.0005$; INSTAR, $p<.0005$. Goodness-of-fit of the model, $p=.897$.

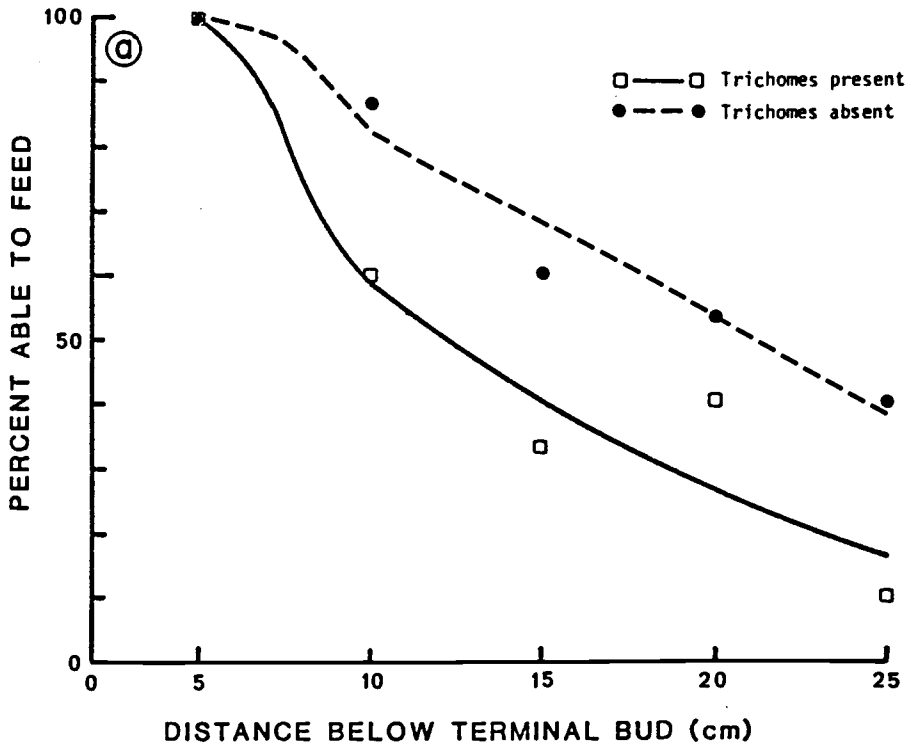
fifteen individuals formed a mass within 45 minutes. The increase in the caged nymphs' ability to feed on the lanate stem during later instars was significant ($p<.0005$) and associated with the variable INSTAR (Fig. 2.3); this increase in feeding ability approximates the increase in the percent feeding on the stem under natural conditions.

The trichome layer was the barrier to the nymphs feeding on the lanate stem; with trichomes absent all the nymphs were able to feed on the stem (Fig. 2.3). Stylet sheaths in the eight sectioned shaven stem segments upon which the first instar fed, indicated that the caged nymphs fed from the xylem elements. A comparison of the maximum penetrable distance of the first instar (.265 mm $\pm$ .002) with the average closest xylem vessel (.196 mm $\pm$ .002) suggests that the depth of the xylem vessels was not a barrier to the nymphs. Since all nymphs fed on the shaven stem, and the sectioned stems showed no tissues interfering with penetration, tissue hardness was not a barrier in the upper stem.

Experiment 2a: Meadow spittlebug nymphs were restricted by architectural barriers to feeding at the apex of the two host plants. When the fourth and fifth instar nymphs were caged along the stem of A. margaritaceae, the percent able to feed decreased with the increase in DBTB (Fig. 4a, b - trichomes present). The relationship between the feeding ability of the fifth instar and DBTB was the same in 1980 and 1982 ($p=.868$ logistic regression), accordingly these data were combined for the analysis. Even through a portion of the nymphs were able to feed on the lower stem, the increase in the time required to initiate a spittlemass as DBTB increased was evidence of the difficulty in forming a spittlemass in this region (Fig. 2.5). The ability of the fifth instar to feed on M. sativa below its normal feeding range also declined with an increase in DBTB (Fig. 2.4c).

The restriction of the nymphs to the apex of A. margaritaceae was due to the presence of several anatomical barriers. One was the barrier within the stem; in the regression model the barrier was associated with the variable DBTB, which was significant in explaining

Figure 2.4 Percent of nymphs able to feed when caged at increasing distances below the terminal bud. Regression curves by hand through the points generated by the model. Regression coefficients are in Appendix II. Significance values are for the improvement in the fit of the regression curve when the variable is added to the model. a) Fourth instar on A. margaritaceae. N at increasing DBTB = 15, 15, 15, 10. Variables added: DBTB, p=.001; TRICHOME, x=.009; DBTB*TRICHOME, p=.971, not entered into model. Goodness-of-fit for model, p=.90 b) Fifth instar on A. margaritaceae. N at increasing DBTB for stems with trichomes present = 31, 31, 31, 31, 26, 18, 12. N at increasing DBTB for stem with trichomes absent = 15, 15, 15, 15, 10, 5. Variable added; DBTB, p<.0005; TRICHOME, p=.001; DBTB*TRICHOME p=.07, entered into model. Goodness-of-fit of the model, p=.883. c) Fifth instar on M. sativa. N at increasing DBTB = 15, 15, 15, 15, 13, 8,7. DBTB p=.007. Goodness-of-fit of the model, p=.608.


100
50
0
PERCENT ABLE TO FEED
ⓐ
Trichomes present
Trichomes absent
0
5
10
15
20
25
DISTANCE BELOW TERMINAL BUD (cm)

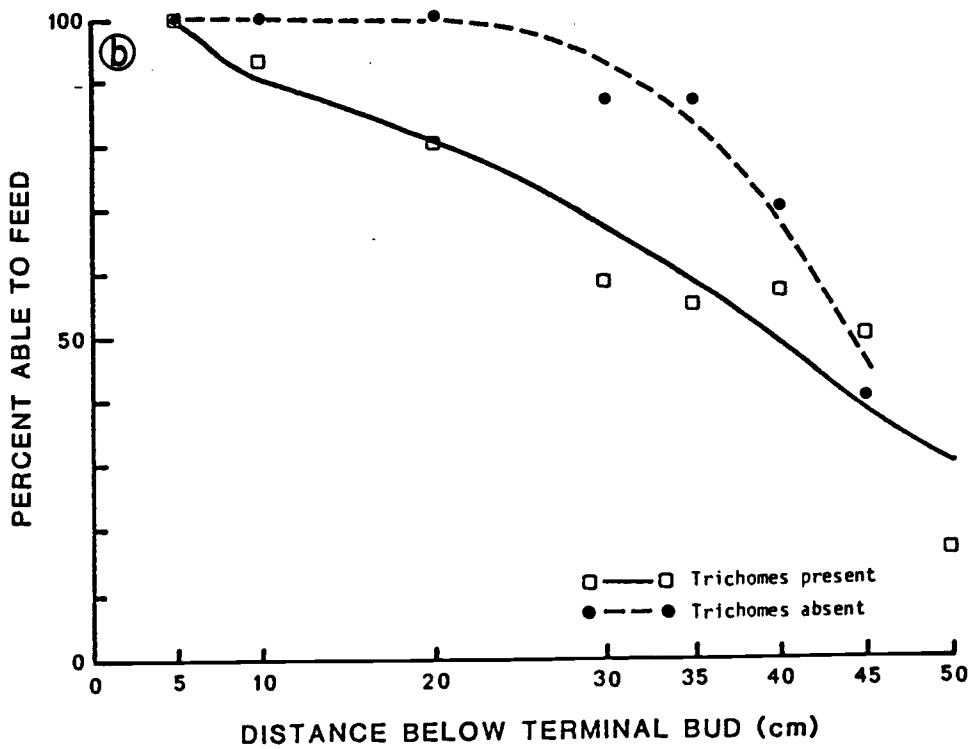

100
50
0
PERCENT ABLE TO FEED
ⓑ
Trichomes present
Trichomes absent
0
5
10
20
30
35
40
45
50
DISTANCE BELOW TERMINAL BUD (cm)

Figure 2.4

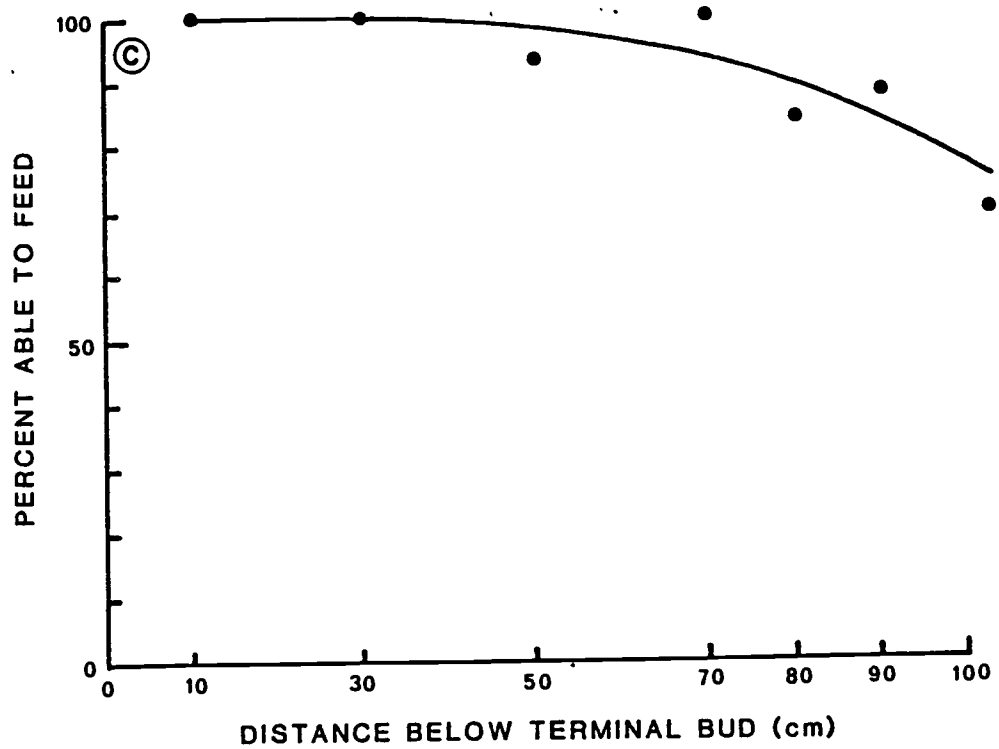

100
50
0
PERCENT ABLE TO FEED
©
0
10
30
50
70
80
90
100
DISTANCE BELOW TERMINAL BUD (cm)

Figure 2.4

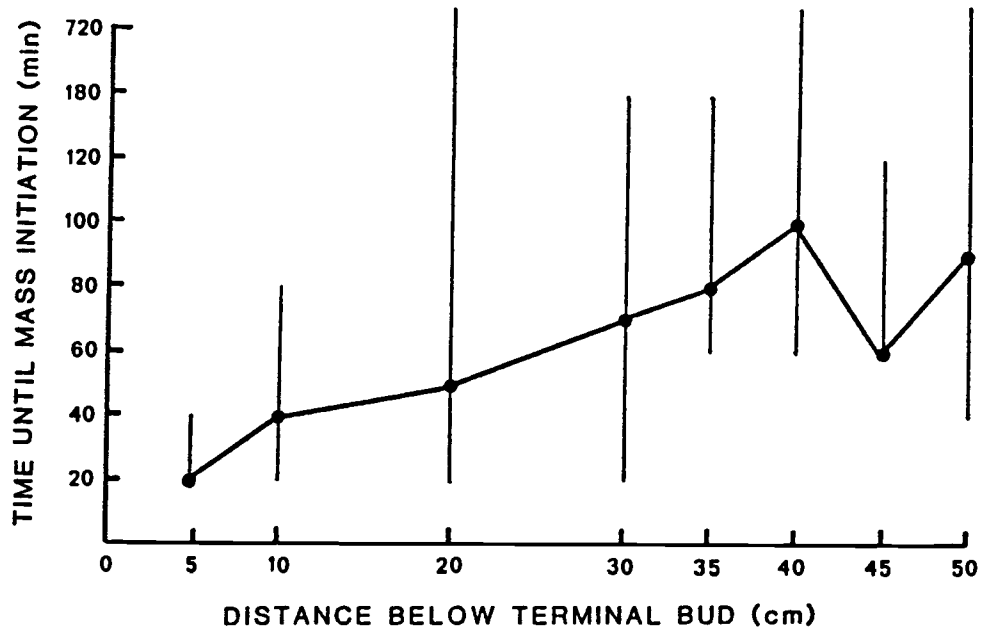


Figure 2.5 Time until mass initiation for nymphs forming masses at increasing distances below the terminal bud (median, range). N at increasing DBTB = 13,12,10, 6, 8, 8, 5, 2. There is a significant positive correlation between the two variables (T=.7143, P=.0071, Kendals rank correlation).

the reduction in feeding, (Figs. 2.4a, b). On M. sativa the restriction was only due to the barrier within the stem; it was associated with the variable DBTB in the regression model (Fig. 2.4c).

For the caging experiment, it was assumed that the barrier within the stem was anatomical rather than physiological. Evidence supporting this assumption was that on both hosts the size of the nymphs had a significant effect on their ability to feed on the lower stem. On A. maragaritaceae stems without trichomes, the rate of decrease in feeding with DBTB was smaller for the fifth instar than for the fourth instar ($p<.0005$, logistic regression). The number of fifth instar nymphs able to feed at 30 and 35 cm BTB on the shaven stem of A. maragaritaceae was larger for the Aphrophora spp. than for P. spumarius ($p=.056$, $n=60$, Fisher Exact Test). This difference would probably have been greater if the nymphs were caged lower on the stem. The presence of an anatomical barrier in the stem of M. sativa is suggested by the differences in the distributions of fifth instar P. spumarius and Aphrophora spp. in a feeding site preference test. P. spumarius nymphs were confined to the apex of the plant as they were under natural conditions, while the Aphrophora nymphs fed along the entire stem length (Chapter 4).

Experiment 2b: In A. margaritaceae there were two anatomical barriers in the stem which may develop with an increase in DBTB:, tissue hardness and the depth of the xylem elements. The depth of the xylem elements (Table 2.1) was well within the maximum penetrable distance of the stylets of fourth and fifth instars (Table 2.2). The longest stylet sheaths found in the sectioned material were .36 and .41 mm for the fourth and fifth instars respectively. While these were shorter than the MPD, they were longer than the depth of the median xylem vessel at the base of the stems.

The presence of specific tissues impeding stylet penetration confirmed tissue hardness as the barrier in the stem of A.

Table 2.1. Depth of xylem element, and characteristics of the trichome layer on A. margaritaceae.

Distance below terminal bud (cm)	Depth of xylem element† (mm)		Characteristics of the Trichome layer†		
	Closest	Median	Density mm^2	Vertical density‡	Height (mm)
5	.191 ± .014	.227 ± .013	265.5 ± 52	34 ± 7	.441 ± .055
10	.202 ± .009	.267 ± .012	204 ± 32	25 ± 5	.321 ± .033
20	.204 ± .01	.272 ± .019	194 ± 18	25 ± 4	.328 ± .044
30	.210 ± .006	.293 ± .019	184 ± 37	21 ± 2	.326 ± .067
40	.222 ± .005	.338 ± .01	175 ± 10	20 ± 2	.372 ± .033

†Mean and standard error of the average of 4 points at each DBTB for 5 plants.

‡Vertical density in total number of intercepts for 5 perpendicular lines.

Table 2.2 The maximum penetrable distance of the five nymphal instars of *P. spumarius*†

Instar	Maximum penetrable Distance (mm)
1	.265 ± .003
2	.359 ± .004
3	.466 ± .004
4	.658 ± .005
5	.870 ± .008

† Values are the mean and standard error of 25 observations.

margaritaceae. The sectioned A. margaritaceae segments upon which the fourth and fifth instar P. spumarius were caged illustrate the tissue barriers to stylet penetration (Table 2.3, 2.4). Within the normal feeding range of P. spumarius nymphs (0-8 cm BTB), the tissues were in the process of elongation and neither the immature fibers of the bundle cap nor the interfascicular parenchyma cells deterred stylet penetration (Fig. 6a). At 10 cm BTB, just below the feeding range of both the fourth and fifth instars, the first traces of lignin appeared in the bundle cap and it started to form a barrier to penetration (Fig. 6b). The fifth instar was still able to penetrate some of the average-sized bundle caps at 10 cm BTB. With the pathway to the xylem through the vascular cap becoming more difficult to penetrate at greater DBTB, the nymphs increasingly reached the xylem by penetrating the interfascicular region (Fig. 6c). If the number of cell layers forming the cap were few,the nymphs were able to penetrate the lignified bundle caps (Fig. 6d); the stylets often travelled intercellularly rather than intracellularly. Starting at 15 to 20 cm BTB the interfascicular region became lignified thus restricting the alternate pathway to the xylem (Fig. 6e). The larger fifth instar nymphs were able to penetrate these hard tissues with greater frequency than the fourth instar nymphs. If the stylet did penetrate to the xylem tissue at the lower caging sites, the lignified xylem fibers were an additional barrier to reaching a xylem vessel (personal observation).

Potential barriers in the stem of M. sativa were tissue hardness, the depth of the xylem elements, and the availability of xylem vessels at the periphery of the xylem. As in A. margaritaceae, the depth of the xylem vessels (Table 2.5) was within the distance penetrable by the fifth instar (Table 2.2). Both the number of xylem vessels and the percent of the xylem circumference containing xylem vessels decreased by approximately one half with an increase in DBTB (Table

Table 2.3. Stylet sheaths of the fourth instar on A. margaritaceae.

	Stylet Sheath Location			
	Number to vascular caps		Number between vascular caps	
DBTB (cm)	Penetrate	Terminate	Penetrate	Terminate
5	7	0	4	0
10	4*	4	1	0
15	2*	8	3	3
20	1*	8	5	13
25	1*	4	1	2

* Went through edge of cap, or thin cap.

Table 2.4. Stylet sheaths of the fifth instar on A. margaritaceae.

	Stylet Sheath Location			
	Number to vascular caps		Number between vascular caps	
DBTB (cm)	Penetrate	Terminate	Penetrate	Terminate
5	2	0	2	0
10	2	1	4	0
20	2*	3	4	1
30-35	1*	8	2	5
40-45	0	15	5	2
50	0	4	0	3

* Went through edge of cap, or thin cap.

Figure 2.6 Stem tissues in A. margaritaceae in relation to stylet penetration by the fourth instar (a) stylet sheath through the unlignified primary cell walls of the bundle cap at 5 cm BTB. (b) stylet sheath terminating in the bundle cap at .10 cm BTB. (c) maxillary stylet penetrating interfascicular region at 15 cm BTB and entering xylem vessel. (d) stylet sheath through thin cap of leaf traced at 25 cm BTB. (e) stylet sheath terminating in interfascicular parenechyma which has developed lignified secondary cell walls.

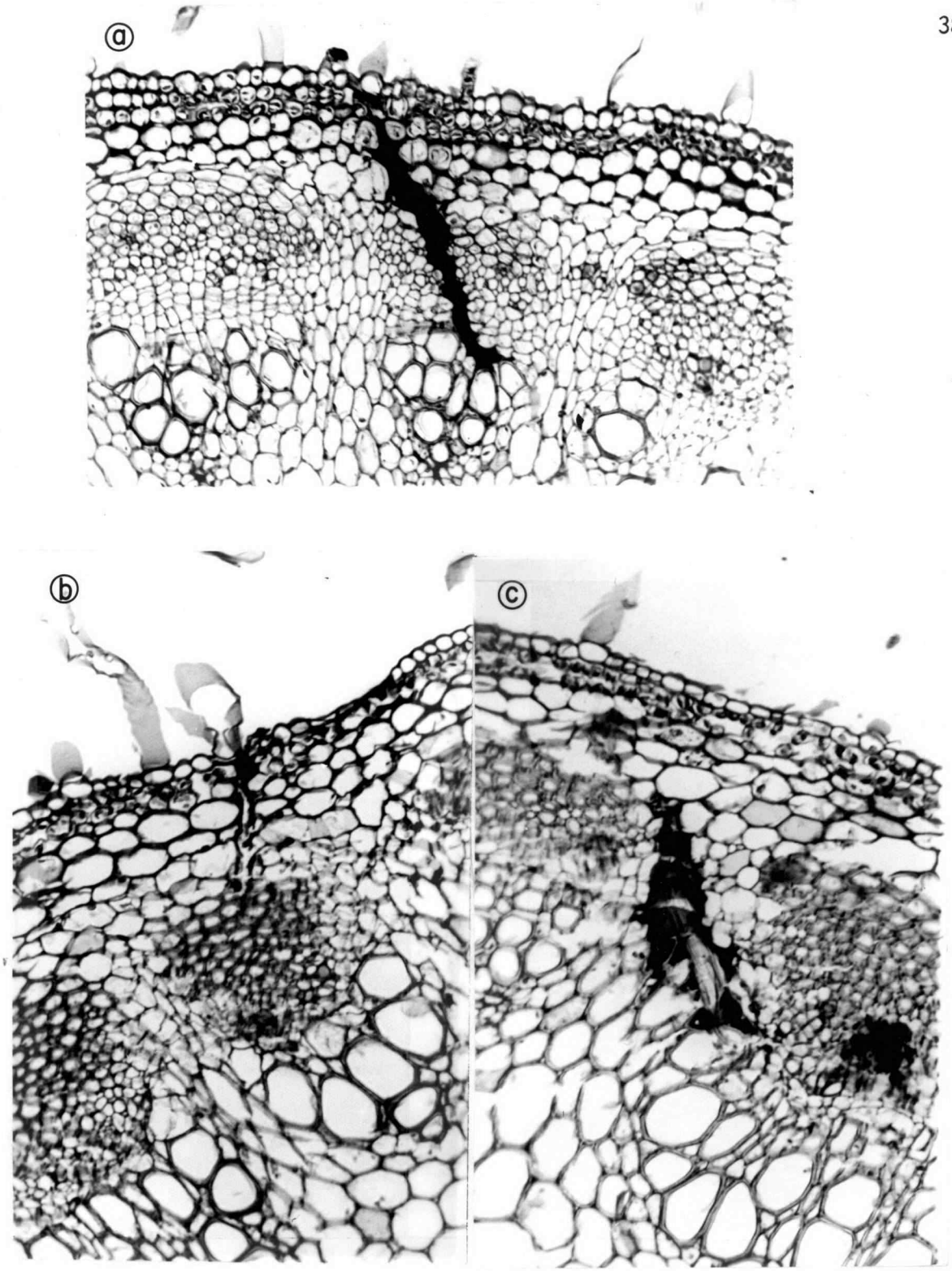
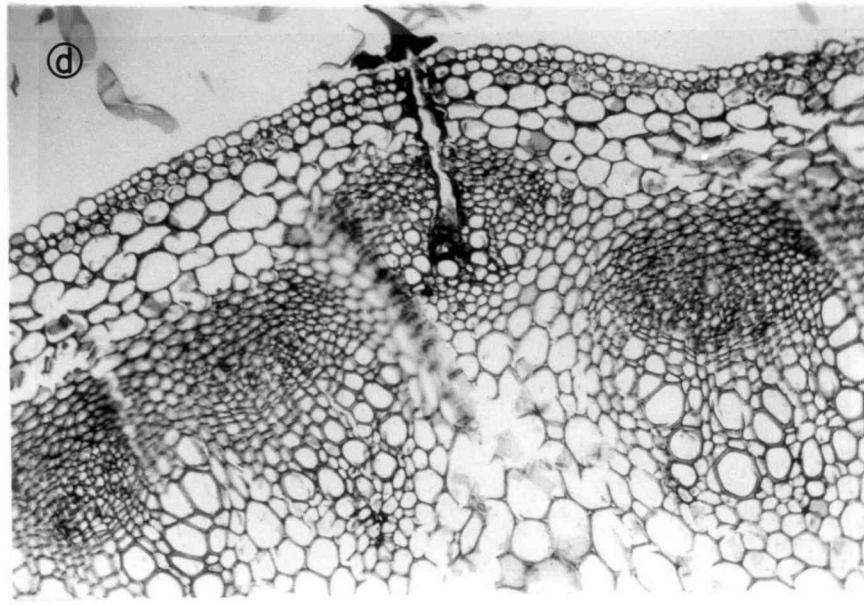


Figure 2.6



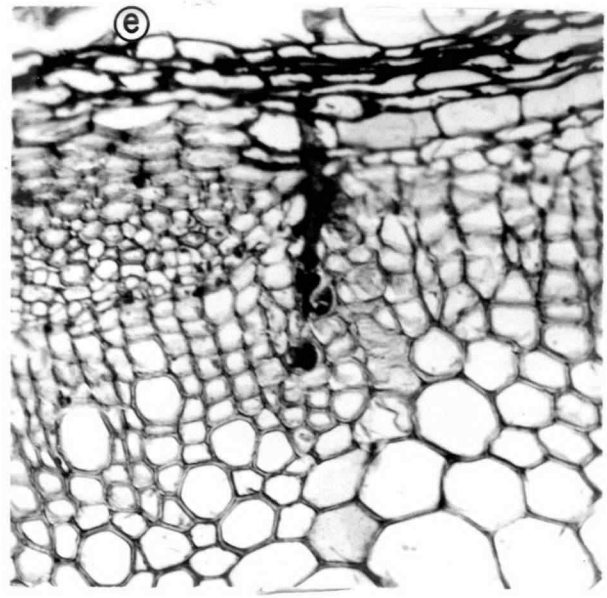


Figure 2.6

2.5). This pattern of development suggested that the decreased availability of xylem vessels in the lower stem may be partly responsible for the decrease in percent feeding. The larger Aphrophora spp. fed on the lower stem of M. sativa (Chapter 4), implying that tissue hardness was an additional barrier.

The stem tissues of M. sativa which impeded stylet penetration were different from those on A. margaritaceae, and offered less resistance to penetration (Table 6). The bundle cap was composed of non-lignified collenchyma cells and was penetrated by 15 of 19 stylet sheaths at 30 to 100 cm BTB (Fig. 7a). The interfascicular region between the bundle caps did not become lignified and was not a barrier to penetration. The main tissue which blocked the stylets were the lignified fibers of the xylem. These fibers became increasingly lignified with the increase in DBTB, and limited the distance the stylets penetrated into the xylem. The fifth instar could penetrate at most four heavily lignified fiber cells, but sometimes terminated penetration upon encountering the first lignified fiber at the periphery of the xylem. The combination of tissue hardness and the low proportion of xylem vessels at the periphery of the xylem in the lower stem resulted in fewer xylem vessels which could be fed upon (Fig. 7b).

Experiment 2a: On A. margaritaceae the trichomes on the stem were a second barrier to feeding, further reducing the number of caged fourth and fifth instar nymphs able to feed. In the regression model, the barrier was associated with the variable TRICHOME and was significant ($p=.009$ and $p=.001$) for the fourth and fifth instars respectively. A positive interaction term, TRICHOME* DBTB, was entered into the model for the fifth instar, indicating that the effect of the trichome barrier decreased at high DBTB (Fig. 2.4b).

The added resistance of the trichome layer is the difference between the regression curves for the percent of nymphs able to feed on the stems with trichomes present and removed. For both species the added resistance in the upper stem increased with the increase in

Table 2.5. Depth and availability of xylem elements in M. sativa.

Distance below terminal bud (cm)	Depth of xylem elements† (mm)		Availability of xylem elements†	
	Closest	Median	Number/mm	% Circumference
5	.204 ± .013	.243 ± .013	19 ± 1	43.6 ± 2.6
10	.242 ± .01	.381 ± .015	12 ± 1	31.1 ± 1.2
20	.253 ± .008	.433 ± .016	10 ± 1	21.2 ± .9
30	.304 ± .023	.561 ± .051	7 ± 1	20.9 ± 1.3
40	.286 ± .004	.522 ± .026	9 ± 1	21.3 ± .9

†Mean and standard error of the average of 4 points of each DBTB for 5 plants.

Table 2.6. Stylet sheaths of fifth instar on M. sativa.

	Number to vascular caps			Number between vascular caps		
	Penetrate		Terminate	Penetrate		Terminate
DBTB (cm)	Reaching vessel	Terminating in fibers		Reaching vessel	Terminating in fibers	
10	2	2	0	1	0	0
30	3	3	2	0	1	0
50	2	2	1	0	2	0
70-80	3	3	1	1	2	0
90-100	1	1	0	0	3	0

Figure 2.7 Stem tissues in M. sativa in relation to stylet penetration by the fifth instar. (a)stylet sheath through bundle cap at 100 cm BTB. (b) stylet sheath through bundle cap and terminating in lignified fiber cells short of xylem vessels, at 50 cm BTB.

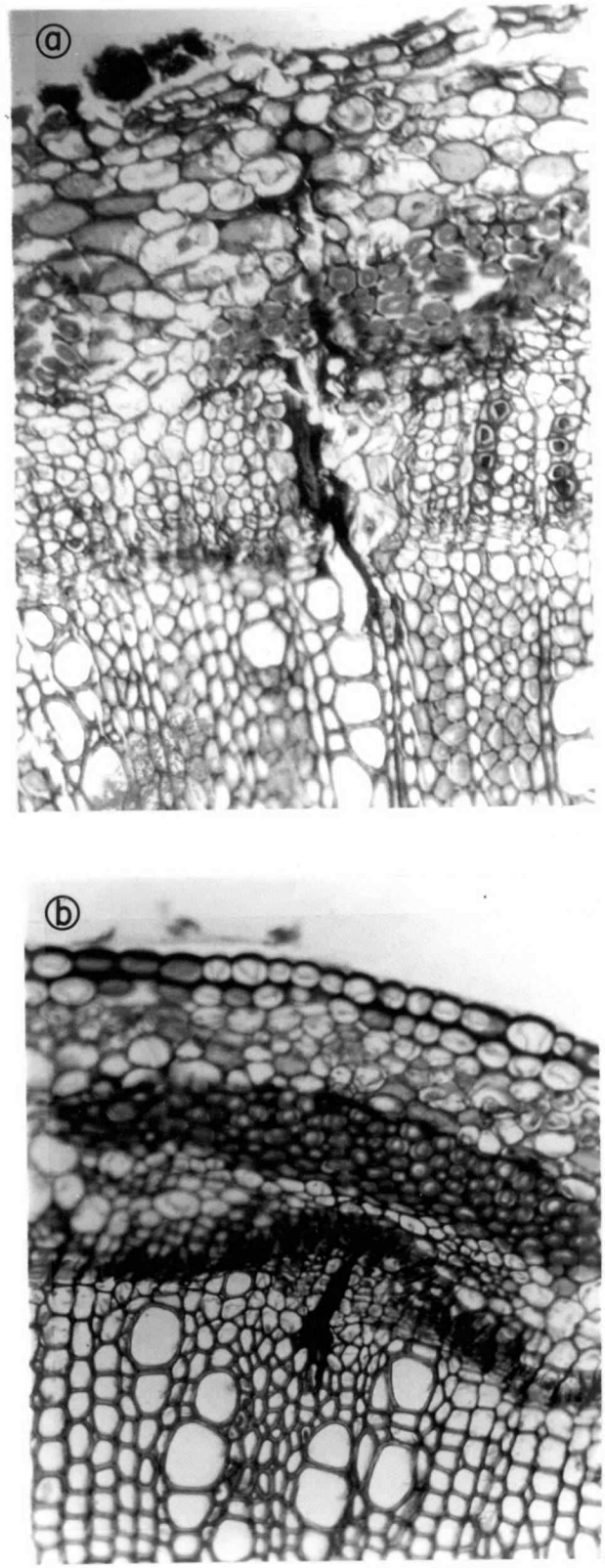


Figure 2.7

DBTB. At a given DBTB, the additional resistance of the trichomes was greater for the fourth instar. At caging sites below 35 cm the additional resistance of the trichome to the fifth instar decreased. There was a similar decrease in trichome resistance to the fourth instar at 20 cm and below; however, it was not large enough to require an interaction term in the model (Fig. 2.4a,b). The characteristics of the trichome layer which may affect feeding were approximately constant below the zone of stem elongation (Table 2.1), indicating that the additional resistance of the trichome layer was due to an interaction between the trichomes and tissue hardness, rather than to the variation in the trichome layer in this region of the stem.

If the difference between the percent able to feed on the stems with and without trichomes was due to the additional resistance of the trichomes in regions of hard tissue, the percent of nymphs which fed on the 1/4 of the stem shaven of trichomes should be greater than 25% and increase as the difference in percent feeding increased. The response of the fifth instar nymphs was greater than 25% ($p<.005$, chi-square) and fit the expected pattern (Fig. 2.8) The response of the fourth instar was greater than 25 % ($p<.001$, chi-square) but did not increase with the increase in the difference between the regression curves (Fig. 2.8).

Adequacy of penetrometer measurements

The variable SPECIES was included in the regression model of percent able to feed versus the average tissue hardness at each DBTB (Fig. 2.9). This indicated that the correlation between total hardness, and the hardness of tissue impeding stylet penetration, was not constant between host species over the range of hardness measured. This difference in feeding response to penetrometer measurements of hardness was not consistent over the full range of tissue hardness.

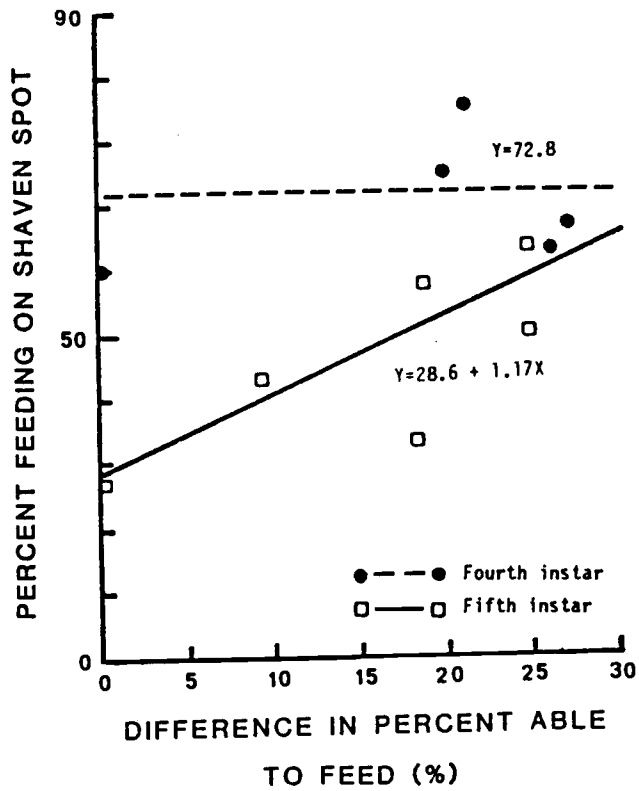


Figure 2.8 Percent of nymphs feeding on shaven spot versus the difference in percent able to feed on stems with trichomes present or absent. Fourth instar, increase in Y with X not significant, p=.3601. Fifth instar, increase in Y with X significant p=.032. Point at 45 cm DBTB dropped because of low N.because of low

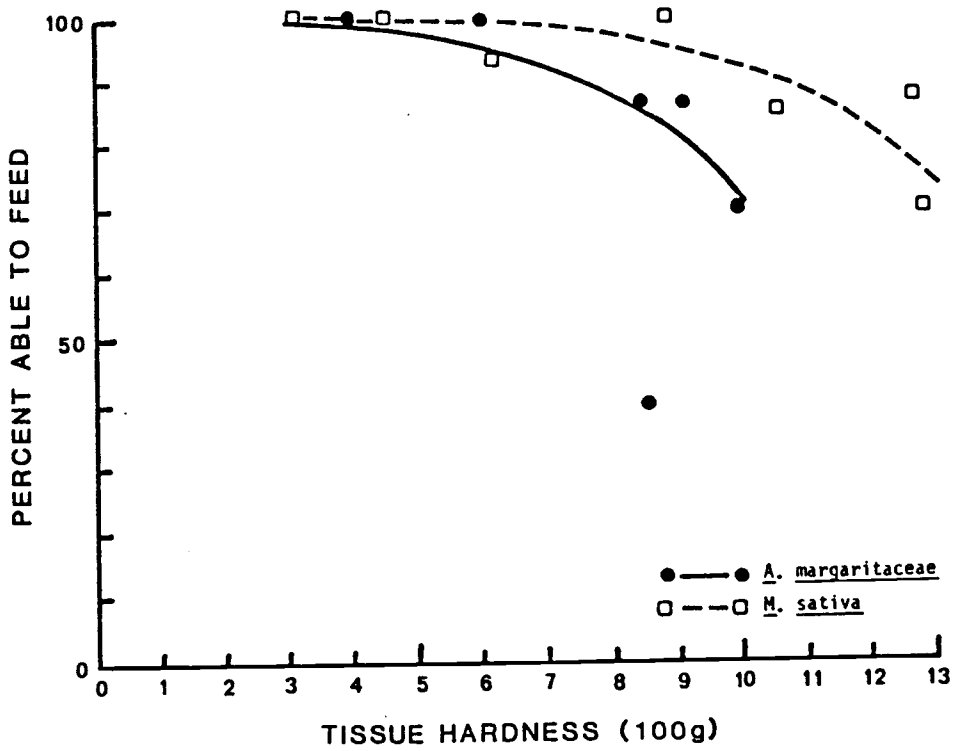


Figure 2.9 Percent of nymphs able to feed on the two hosts at increasing average tissue hardness at each DBTB. Regression curve fitted by eye through points generated by the model. A. margaritaceae, N at increasing tissue hardness 15, 15, 15, 15, 5, 15, 10. M. sativa, N at increasing tissue hardness, 15, 15, 15, 15, 13, 8. Significance of the improvement in the fit of the regression curve when variable added to the model: TISSUE HARDNESS, p=.001; SPECIES, p=.018. Goodness-of-fit of the model, p=.332. Regression coefficients are in Appendix II.

Although the variable CONTROL, which would have distinguished the soft upper stem from the hard lower stem, was not significant, the feeding response of the nymphs to tissue hardness of the two hosts was similar on the upper stem (Fig. 2.9). It was only on the lower portion of the stem, where tissue hardness values were high, that the response of the nymphs began to differ significantly. This suggests that penetrometer measurements of tissue hardness can only be used to predict the range of stem available to species such as P. spumarius which do not penetrate hard tissue barriers.

Discussion

These experiments support the hypothesis of McEvoy (1983a) that P. spumarius is restricted in its use of the host plant by architectural barriers. On A. margaritaceae and M. sativa the percentage of fourth and fifth instar nymphs able to feed declined the further they were caged below the terminal bud. On both hosts, nymphs in the field fed where all caged nymphs were able to feed. At the apex of the plant, the first three instars suffered 89 percent mortality when caged on the stem. The percent of caged nymphs able to feed increased with the age of the instar, as did the proportion of nymphs feeding on the stems in the field.

Many of the nymphs were able to feed when caged below their natural range of feeding sites on the two hosts. The failure to extend the range of stem utilized in the field may be due to the greater time and effort required to penetrate to the xylem and initiate a spittlemass in this region. Prior to forming a spittlemass, nymphs are exposed to desiccation and natural enemies. Under experimental conditions the larger nymphs were able to live for approximately three hours, however, when exposed in a desiccator the fourth instar began to lose coordination after 20-30 minutes (McEvoy, unpublished data). The first instar can survive only minutes in direct sun (Wiegart, 1964). The lack of a mass may increase the risk of predation (Whittaker, 1970) or parasitism (Coe, 1966). Given the danger of being without a mass, even a small resistance to penetration may result in the nymph moving to a region of the stem devoid of architectural barriers.

The nymphs of P. spumarius were restricted in feeding by a combination of architectural barriers on the two host plants studied. Tissue hardness was a major barrier to stylet penetration in both A. margaritaceae and M. sativa. The increase in hardness which restricted the nymphs to the apex of the plant was due to the

maturation and development of stem tissues below the region of stem elongation. The specific tissues which impeded stylet penetration differed in the two hosts; however, the critical factor in both species was the degree of lignification of these tissues. The lignified bundle cap and interfascicular parenchyma were the tissue barriers in A. margaritaceae, while the lignified xylem fibers were the source of resistance in M. sativa. Developmental patterns in other stem tissues, such as the collenchyma in the bundle cap of M. sativa, and the lignified xylem fibers of A. margaraitaceae, were additional sources of resistance.

Homoptera with thin flexible stylets which penetrate using digestive enzymes, Aphidae (Staniland, 1924; Knight and Alston, 1974) and Pseudococcidae (Entwistle and Longsworth, 1963), have difficulty penetrating lignified and other hard tissues. These hard tissues are responsible for a portion of the resistance in the host to these pests. This study showed that the Cercopidae, which have more robust stylets and penetrate intracellularly without using enzymes, were also prevented from reaching feeding sites by lignified tissue.

In M. sativa, the reduced availability of xylem vessels at the periphery of the xylem acted in conjunction with tissue hardness to decrease the percent of nymphs able to feed. Tissue hardness alone would offer little resistance to feeding if there were large numbers of xylem vessels within four lignified fibers of the edge of the xylem. Conversely, in the absence of tissue hardness the nymphs could penetrate deep into the xylem tissue and would have an abundance of vessels available. The decrease in percent feeding from 50 to 90 cm BTB was due to an increase in tissue hardness, since the availability of xylem vessels was approximately constant over this region of the stem.

The tissue hardness barrier in A. margaritaceae, particularly the lignified fibers of the bundle cap, was a formidable barrier to stylet penetration and was the primary barrier to feeding on the lower stem. An additional plant developmental pattern which may increase the

resistance of the lower stem is the production of small xylem vessels in the lower stem late in the growing season. In response to water stress in the early summer, the secondary xylem, formed by the cambium below the region of stem elongation, produces small xylem vessels which are better able to conduct water under negative pressure (Carlquist, 1975). Many of the spittlebugs caged lower on the stem produced small spittlemasses, and stylet sheaths were found to penetrate these small vessels. Because the flow rate of sap is proportional to the fourth power of the radius of the vessel (Diamond, 1966) the rate at which sap can be obtained from these small vessels may be much less than the rate from the larger vessels in the primary xylem at the apex of the plant.

The trichome layer of A. margaritaceae was a significant barrier to spittlebug feeding. This was not surprising given the literature on trichome resistance to stylet feeding insects (Painter, 1951; Levin, 1973; Webster, 1975). What is interesting is that the mechanism of trichome resistance was apparently different on the hard and soft areas on the stem. At the apex of the stem the mechanism of resistance was independent of tissue hardness. The height of the suitably dense trichome layer prevented the beak of the nymph from coming in contact with the epidermis and receiving the stimuli necessary to initiate stylet penetration (Chapter 3). The fourth and fifth instars were able to overcome this resistance mechanism because of their longer beaks.

The trichome layer decreased in density and height in the zone of stem elongation, and if the above resistance mechanism were the only one present, trichomes should not be a barrier to the larger nymphs lower on the stem. The additional resistance of the trichomes to the larger instar nymphs caged lower on the stem suggested a mechanism of resistance in this region which was dependent upon tissue hardness. In the Cicidellidae, the mandibulate stylets anchor the mouth parts in the plant and serve as the base for the extension of the maxillary stylets to the feeding site; the method of penetration of the

Cercopidae is probably the same (Pollard, 1969). When attempting to force the maxillary stylets through hard tissue the spittlebug may have to apply pressure to the mandibulate stylets to keep them anchored in the plant; the trichome layer may prevent the nymphs from grasping the stem tightly enough to apply this pressure. Spittlebugs sectioned on the stem had their legs above the dense mat of trichomes and appeared unable to grasp the stem directly. An inability to firmly grasp the substrate can interfere with stylet penetration (Carter, 1945). This proposed mechanism of resistance is consistent with the finding that, as tissue hardness increased, the degree to which the trichomes reduced the feeding ability of the fifth instar also increased.

Below 35 cm the trichome resistance to the fifth instar nymphs decreased. It may be that at very high tissue hardnesses the stem was so difficult to penetrate that the ability to feed was related more to the probability of hitting a "soft spot" in the stem, e.g., the small cap of a bundle trace, than to the additional resistance of the trichome layer. The decrease in trichome resistance to the fourth instar occurred at 20 cm. This difference between the two instars was probably because the fourth instar nymphs had greater difficulty in penetrating hard tissue.

The percent of fifth instar nymphs feeding on the shaven portion of the stem increased with the difference between the regression curves for the stems with trichomes present and absent. This relationship was additional evidence that the difference between the two curves was due to the added resistance of the trichomes on hard tissue. The percent of fourth instars feeding on the shaven spot did not increase with the difference between the regression curves, probably because both mechanisms of trichome resistance mentioned above were affecting feeding. If the height of the trichome layer was a barrier to the nymphs in this region, it would obscure the relationship between the number of nymphs feeding on the shaven spot in relation to the difference between the two regression curves.

The ability of nymphs to penetrate the tissue hardness and trichome barriers was strongly size dependent. The larger nymphs were less affected by the trichomes at the apex of the stem, and the additional resistance of the trichome layer on a given region of hard tissue was greater for the fourth instar. The tissue hardness barrier offered less resistance to the larger of the two groups when the percent able to feed versus DBTB was compared between the fifth instars of Aphrophora species and P. spumarius, and the fourth and fifth instars of P. spumarius. The size dependent differences in the ability to penetrate architectural barriers can result in a larger proportion of the plant being available to larger nymphs (Halkka et al., 1977; McEvoy, 1983; Chapter 4).

A potential barrier to feeding is the depth of the xylem elements, but it was not a barrier in the hosts studied. However, the proportionally longer stylet length in the younger instars of P. spumarius (Appendix 1), and a similar allometric relationship for leafhoppers (Pollard, 1968) and aphids (Mittler, 1954) suggests that evolutionary pressure has been placed on the feeding apparatus of stylet feeders by the depth of the tissues from which they feed.

Penetrometer measurements of tissue hardness can be used as an index of the hardness barrier with the following reservations. In the region of the stem devoid of large amounts of lignified xylem tissue internal to the feeding site, the penetrometer appeared to be sensitive to the development of tissue hardness barriers, e.g., the lignification of the bundle cap at 10 to 15 cm BTB in A. margaritaceae and the beginning of the lignification of the xylem fibers in M. sativa. The penetrometer appeared to lose sensitivity to the development of tissue barriers as the hardness due to these barriers decreased in proportion to the hardness of tissues in the center of the stem. In this region most of the resistance to needle penetration was the extensively lignified xylem internal to the feeding sites.

The main constraint to the use of penetrometer measurements as a predictive tool is that the resistance of the stem tissues which

impede stylet penetration must be a significant proportion of the total resistance. Penetrometer measurements have predictive value for stylet feeding insects which are sensitive to hardness barriers and thus feed on areas of the stem where little xylem lignification has occurred. The distribution of P. spumarius, which is sensitive to hard tissues, can be predicted on a range of hosts using penetrometer measurements (McEvoy, 1983a). The larger Aphrophora spp. penetrated the hard tissue barriers found on regions of the stem where the xylem internal to the feeding site had become extensively lignified, and the distribution of these nymphs did not conform to predictions based on penetrometer measurements. The feeding range of these nymphs ended at 24 cm BTB on A. margaritaceae and 100 cm BTB on M. sativa (Chapter 4). Data in this study indicated that these regions of the stem had an average hardness of 725 and 1200 cm, respectively. Thus, the distribution of Aphrophora spp. in relation to tissue hardness on one host cannot predict the distribution on the other. To improve the sensitivity of the penetrometer, measurements should only be made on tissues which the stylets must penetrate to reach the feeding site.

The second constraint is that when the stem contains anatomical barriers in addition to tissue hardness, such as the limited availability of the xylem vessels, penetrometer measurements may fail to predict the ability of the insect to feed at different locations on the stem.

Architectural barriers to feeding site selection may prevent the spittlebug from feeding on areas of the host more favorable in terms of other parameters of the spittlebug niche. Spittlebug masses formed lower in the canopy are larger and may provide better protection forthe nymphs from high temperatures and natural enemies (Whittaker, 1970).

The gradient in xylem sap tension on A. margaritaceae and M. sativa was lowest at the base of the plant, becoming more negative as the sap ascended the stem and entered the leaves. Differences between the bottom and top of the stem of A. margaritaceae and M. sativa on a warm day ranged from -2.2 to -7.0 bars and -5 to -10 bars,

respectively. At the apex of A. margaritaceae the sap tension was .25 to 2.75 bars more negative in the leaves than in the adjacent stem. There was a 1 to 4.9 bar difference between the stem of M. sativa and an adjacent branch (Chapter 4). Though Mittler (1967) found that a 1 bar pressure difference did not affect the feeding rate of a leafhopper, it may increase the energy required to extract a given amount of sap.

The nitrogen concentration in the diet of herbivores is often limiting (McNeill and Southwood, 1978; Mattson, 1980). In many dicotelydons, such as most legumes, the Rosaceae, Hordeum, and Raphanus (Brennan et al., 1964; Cooper et al., 1972; Pate, 1973) the concentration of reduced nitrogenous compounds is greatest at the base of the plant and decreases as the sap ascends the stem.

Architectual barriers which prevent a spittlebug from feeding at the base of the plant or forces them to feed on the leaves rather than the stem, may restrict the insect to feeding on suboptimal areas of the plant. This could be particularly critical for spittlebugs, which feed on a food source low in nitrogen, and almost devoid of carbohydrates. The energy required to pump against the high xylem sap tension of the apex of the plant may consume a significant fraction of the injested nutrients. The degree to which the barriers restrict the insect to feeding on unfavorable regions of the plant may influence the between plant distribution of P. spumarius.

Chapter 3

The mechanism of trichome resistance in *Anaphalis margaritaceae* (D.C.) to the meadow spittlebug, (*Philaenus spumarius* L.)

Abstract

The lanate trichomes on the stem of *Anaphalis margaritaceae* (D.C.) inhibited the feeding of the young nymphs of *Philaenus spumarius* (L.). Sectioned stems on which the nymphs were caged showed an absence of stylet punctures on those stems where the nymphs had been unable to feed. This indicated that the trichomes prevented the initiation of stylet penetration, rather than interfering with feeding once it had commenced. Analysis of the trichome density per mm^2, vertical density, and trichome height on the sections fed and not fed upon by third instar nymphs showed that only the height of the trichome layer was significant in determining whether or not nymphs fed at a site. The proposed mechanism of resistance is that on a suitably dense trichome layer a height exceeding nymph beak length could prevent the initiation of stylet penetration, probably because the necessary stimuli are not received from the sensilla on the tip of the beak.

Introduction

Plant trichomes interfere with the oviposition, attachment, feeding and digestion of insects (Levin, 1973; Webster, 1975; Norris and Kogan, 1980). The within plant distribution of feeding sites of the meadow spittlebug, Philaenus spumarius (L.), a stylet feeding insect, is strongly influenced by the 'lanate' trichomes (long tangled woolly hairs) on the stem of Anaphalis margaritaceae (D.C.) (Chapter 2). The trichomes inhibited the feeding of first through fourth instar nymphs, but did not appear to affect the fifth instar. The degree of trichome resistance declined steadily with the increase in instar number. This increase in the ability of the larger nymphs to feed when caged on the lanate stems was similar to the increase in the proportion of nymphs feeding on the stem under field conditions. The present study investigated the mechanism by which the lanate trichomes interfered with feeding.

Trichomes are a factor in the resistance of many crop plants to stylet feeding insects, and are used in breeding programs for a variety of crops including cotton, soybean, and forage crops (Painter, 1951; Maxwell and Jennings, 1980). In most cases the mechanism of resistance is unknown, which may be why variable results are obtained when the resistance due to particular trichome characteristics is evaluated. For instance, in breeding for resistance to leafhoppers, a high correlation between the development of the trichome layer and resistance to the pests was often found (Parnell et al., 1949; Turnipseed, 1972; Turnipseed and Sullivan, 1976). This correlation was not found in other studies (Poos and Smith, 1931; Taylor 1956; Wolfenbarger and Sleesman, 1961, 1963; Broersma et al. 1972); these authors concluded that resistance was due to some trichome characteristic not measured in the study, or to some other plant factor associated with pubescence. The reason for some of this discrepancy in results was elucidated when Pillemer and Tingey (1976,

1978) determined that the mechanism of trichome resistance in Phaseolus vulgarus (L.) to Empoasca fabae (Harris) was capture of the nymphs and adults on hooked trichomes, and that resistance in this crop was related to the number of hooked trichomes rather than the total density of all four trichome types.

The purpose of this paper is to examine the mechanism of resistance of the trichome layer on A. margaritaceae to the young nymphs of P. spumarius. The analysis of this insect-plant interaction at a fine level of resolution should provide a greater understanding of the role of plant architecture in defining the feeding sites of P. spumarius. It may also provide an insight into the mechanism of trichome resistance to the important crop pests in a related family, the Cicadellidae. The specific objectives were to determine how the lanate trichomes inhibited the feeding behavior of P. spumarius nymphs and to identify which characteristics of the trichome layer caused resistance.

Materials and Methods

P. spumarius is a univoltine species, hatching in the early spring, developing through five instars, and molting to the adult in the early summer. It feeds exclusively on the xylem sap (Weigart, 1964; Horsfield, 1977), and has a host range of several hundred plant species, primarily dicotelydons (Weaver and King, 1954).

The material used in this study was obtained from experiments which determined the ability of the five instars of P. spumarius to feed when caged on the lanate stems of A. margaritaceae (Chapter 2). At the time each instar was active in the field, thirty host plants and thirty nymphs were collected from the Mary's Peak study site. The plants were randomly divided into two groups: trichomes absent (trichomes removed from the caged stem section), or trichomes present (trichomes remained intact). The cages allowed access to two-thirds of the stem circumference. The nymphs were caged individually on hosts at 1.5 to 2.5 cm below the terminal bud. The plants were placed at 20 to 25°C and 30 to 50% RH, and left until the nymphs had formed a spittlemass or had dehydrated and died. The feeding nymphs were frozen on the stem with a stream of cold CO_2 prior to removing the cages. The approximate location of the stylets was marked, and the stem segment within the cage was preserved in formalin-acetic acid-alcohol.

Hypothetical mechanisms of trichome resistance were evaluated by observations on the stylet "behavior" of the caged nymphs. P. spumarius, along with many other stylet feeding insects, produces a stylet sheath when it penetrates into the plant. This sheath remains in the plant when the stylets are withdrawn and can be differentiated from plant material by tissue stains. Thus, the stylet sheaths in sectioned stem segments are a permanent record of the response of the insect to the anatomical barriers to feeding.

There are three possible explanations for the nymphs' inability to feed on the lanate stem. First, the trichomes may limit the distance the nymphs can penetrate into the stem by holding the nymphs away from the epidermis. Second, the trichome layer may limit the nymphs' ability to grasp the stem and apply the necessary pressure to penetrate to xylem tissue. Third, the trichome layer may prevent the proper orientation of the beak necessary for the initiation of stylet penetration.

The absence of stylet sheaths in the stem segments on which the nymphs were unable to form spittlemasses would indicate that they failed to penetrate the stem. This evidence would allow exclusion of the first two proposed resistance mechanisms which operate after stylet penetration has been initiated. The presence of 'short' stylet sheaths (those that do not reach the xylem tissue), would exclude the third proposed mechanism, which suggests that the pubescence prevents initiation of stylet penetration. The presence of short sheaths would further require the discrimination between mechanism one and two. Mechanism one is dependent upon the height of the trichome layer and due to variation in the height of the trichome layer, the nymphs would produce short stylet sheaths variable in length. Stylet sheaths which are fairly consistent in length and which may terminate in tissue known to impede stylet penetration would exclude mechanism one. Short sheaths of variable length would not necessarily allow exclusion of mechanism two.

The stylet behavior of nymphs caged on the lanate stems was determined by sectioning eight random segments out of the fifteen stems on which the first, second, fourth and fifth instar nymphs were caged. Because the third instar can begin to overcome the trichome resistance that inhibits the younger nymphs, all fifteen stem segments were sectioned for the third instar. Stem segments selected for sectioning were dehydrated in a series of tertiary-butyl-alcohol solutions and embedded in parafin. The segments were sequentially

sectioned at 10 μm. The entire stem segment within the cage was sectioned for those stems on which the first three instars were caged, producing approximately 250 sections. The fourth and fifth instars were enclosed in larger cages; on these stems 250 sections were cut from the area containing the spot marking the location of the feeding nymph. The sectioned material was mounted on glass slides and stained with safranin and fast green (Johansen, 1940). Safranin stains the stylet sheath material as well as lignified and suberized plant tissue. The sections were searched at 40 to 400x magnification for evidence of feeding. The number of short and full length stylet sheaths was recorded for each instar. A stylet sheath entering the xylem tissue, though not necessarily entering a xylem vessel, was considered to be a 'full length' sheath. A 'short' sheath terminated short of the xylem tissue.

To further elucidate the specific characteristics of the trichome layer which might interfere with beak orientation, vertical density, height of the trichome layer, and trichome density per mm^2 were examined. These characteristics were compared among sites fed upon and sites not fed upon by third instar nymphs. Since trichome variability around the stem cirmumference was unknown, the trichome layer was measured at two points per section. One points was in the center of the caged area and the second was taken at a point 90° to either side of the first point. At each point the closest vascular bundle was designated the "marker bundle" and in all measurements the sampling grid was positioned over the marker bundle (Fig. 1).

It was assumed that except in the four cases where the nymphs had fed, the probabilities of feeding at the two sites sampled within each cage were independent. The nymphs which died had wandered on the stem for 1 to 2 hours and had the opportunity to feed at both sampling points. It was assumed that the nymphs attempted to feed at both points and were unable to form a mass at either point; therefore, both points were therefore included in the analysis. In the four cases where the nymphs formed masses it could not be assumed that the nymphs

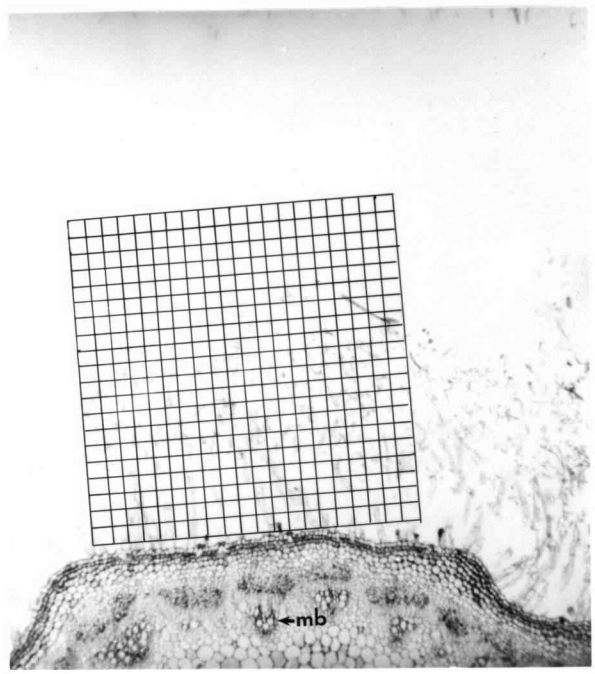


Figure 3.1 Sampling grid centered over marker bundle (mb). Grid is .808mm x .808mm at 100x.

were unable to feed at the other point, because the point at which they fed may have been the only site sampled. Accordingly the points where masses were not formed were excluded from the analysis. On two of the stems stylet sheaths were found at one of the sampling points but the nymphs had abandoned the site and died without forming a mass. In these two cases it was assumed that both points were sampled by the nymph and they were included in the analysis as points fed and not fed upon.

Density per mm^2 was measured by counting the number of trichomes emerging from the epidermis along .808 mm of stem surface on a sequence of 12 randomly chosen sections. The width of a trichome determined the probability of it being counted on more than one section, therefore the total number of trichomes for the 12 sections was corrected for the average width of the trichomes at that sampling point. The average trichome width was determined by measuring the first bisected trichome base on either side of the center of the marker bundle on five randomly chosen stem sections of the twelve being measured for trichome density. This corrected density was then expressed on a mm^2 basis.

Vertical density was measured using a modification of the "point quadrat" method of plant ecologists (Greig-Smith, 1964), which records the presence of a species vertically above a number of points in the community. On each of five random stem sections, one of the 20 vertical lines of the sampling grid was randomly selected and oriented perpendicular to the stem surface. The number of trichome segments intersecting this line per two horizontal grid units was counted. The counts for the five sections were summed to obtain vertical density.

The height of the trichome layer was measured using criteria which weighted the measurement in favor of the height of the region of dense interlocking hairs, and de-emphasized the few trichomes which protruded above this mat. The sampling grid was centered on the marker bundle and the height was measured as the highest horizontal grid band, 1 by 20 units, which contained at least three trichome segments in each half, or that band with twenty trichome segments

within and above it, whichever was highest. The height of the trichome layer at a single point was the average of the heights for the five random sections measured.

The significance of the height of the trichome layer in the resistance of the lanate stems to each of the five instars was further examined by comparing average beak length to the average trichome height at sites where each instar was caged. Beak length was measured on twenty-five field collected individuals of each instar (Appendix 2). Trichome height was measured on the stem segments which had been sectioned to obtain data on stylet and sheath behavior. All measurements were made in the center of the caged portion of the stem segment.

Results

Trichomes prevented the proper orientation of the beak necessary for stylet penetration (Table 3.1). Only one short stylet sheath was found out of the 46 located and this was not a definite short sheath. In all but two cases, if the nymphs had not produced a spittlemass, they produced no stylet sheaths at all. Stylet punctures without sheath material would have been seen, so the failure to produce sheath material when producing short sheaths would not have biased the results. Two of the third instar nymphs which died had produced full length stylet sheaths at one site on the stem within the cage. The nymphs abandoned these sites after many probes, and subsequently died. The absence of short stylet sheaths indicated that the trichome layer prevented the initiation of the feeding process, rather than interfering with stylet penetration once it had commenced.

Of the three trichome characteristics measured, only the height of the trichome layer was significant ($p<.01$) in discriminating between the points fed and not fed upon (Fig. 3.2). The trichomes in areas where third instars produced stylet sheaths were much shorter ($\bar{x}=.582$) than average ($\bar{x}=.701$). Trichome density per mm^2 and vertical density at these points ($\bar{x}=558/mm^2$; $\bar{x}=62/5$ points) fell in the middle of the distribution for all sites ($\bar{x}=548/mm^2$; $\bar{x}=60/5$ points) (Fig. 3.3).

For the first through fifth instar, the progressive increase in beak length relative to trichome height paralleled the increase in the percent of nymphs able to feed when caged on the lanate stem (Fig. 3.4). Beak length of the first two instars was much shorter than the height of the trichomes and only one of the thirty nymphs was able to feed. The stem on which this second instar nymph fed had a trichome height much shorter than average at the point of feeding (.364mm) which, was less than the average beak length of this instar (.438mm).

Table 3.1. Number of full length and short stylet sheaths found in the sectioned stems on which the five instars were caged.

Instar	No. of segments sectioned	No. of masses	No. of segments containing stylet sheaths	No. of 'short' stylet sheaths	No. of 'full' length stylet sheaths
1	8	0	0	0	0
2	8	1	1	0	1
3	15	4	6	1	21
4	8	8	8	0	13
5	8	8	8	0	11

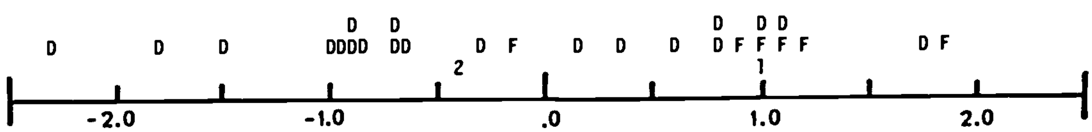


Figure 3.2 Histogram of canonical variable from Stepwise Discriminant Analysis. Trichomie height was the one significant variable in discriminating among the locations where the nymphs fed (F) and did not feed (D), p<.01. Mean of F locations (1), mean of D locations (2).

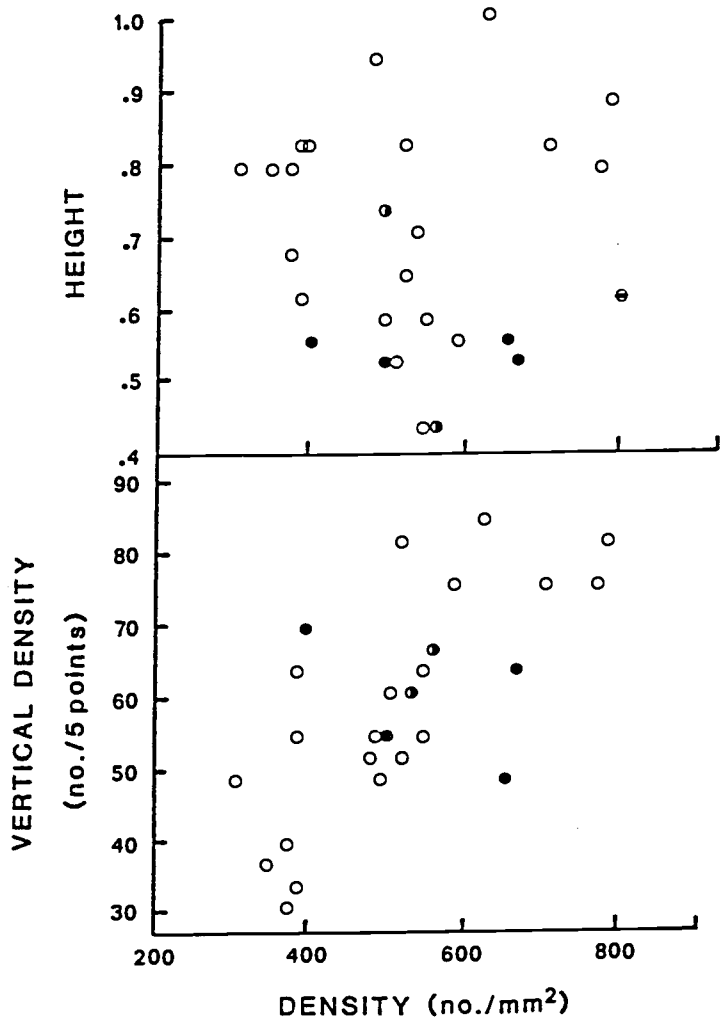


Figure 3.3 Distribution of trichome height, vertical density and density per mm^2 for sampled points within the cages. ● Stylet sheath produced, nymphs formed mass; ◑ stylet sheath produced, nymphs abandoned site; ○ no stylet sheaths found.

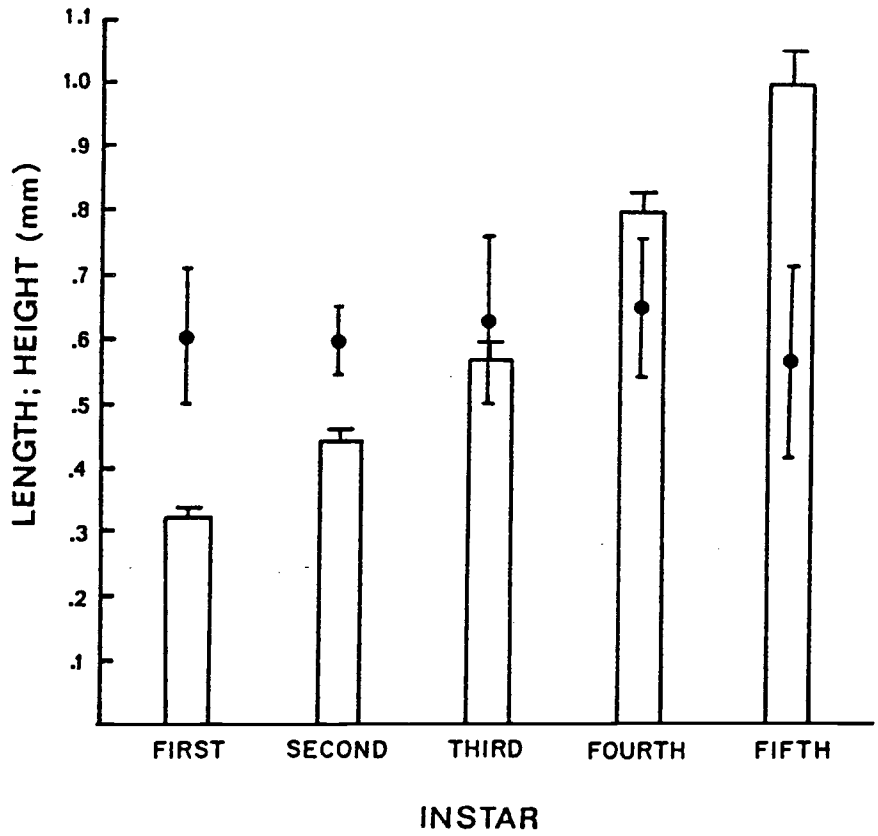


Figure 3.4 Length of beak compared to height of the trichome layer for the five instars of P. spumarius. ⊓ Beak length, n=25, x, SD; ⊕ trichome height, n=8, x, S.D.

The beak lengths vs. trichome heights began to overlap in the third instar, and four of fifteen nymphs were able to form masses. If the single nymph which formed a stylet sheath at a trichome height of .7mm is excluded (there may have been damage to the trichome layer during the embedding procedure), the cutoff point in height beyond which the trichomes conferred resistance was .575mm. This was close to the average beak length for the third instar (.568mm). All fourth and fifth instars were able to feed, and the average beak length of these nymphs was longer than the average height of the trichome layer.

Discussion

The young instars of P. spumarius are unable to feed on the lanate stems of A. margaritaceae due to the presence of tricomes (Chapter 2). The mechanism of host resistance appeared to be that trichome height exceeded the length of the nymphs' beak, and thereby interfered with initiation of stylet penetration. Evidence for this was a lack of short stylet sheaths on stems with a trichome layer higher than the length of the nymphs' beak. The failure to initiate stylet penetration may have been due to failure of the sensilla on the tip of the beak to contact the epidermis which prevented reception of the stimuli required to initiate feeding (Fig. 3.5). This mechanism of resistance explains the plant's loss of resistance with the progressive increase in nymph size.

The density of the trichomes was not a factor in resistance in this study. This was probably because even the minimum density of trichomes on the stem of A. margaritaceae was sufficient to hold the nymph away from the stem surface. Longer hairs may compensate for inadequate density, in which case there could be an interaction between trichome height and density. Resistance to leafhoppers in cotton is in some cases dependent both on trichome length and density (Tidke and Sane, 1962; Sikka et al., 1966).

Variable results have been obtained when making correlations between the density of simple trichomes and resistance in crops such as cotton, soybeans, and alfalfa to leafhoppers in the genus Empoasca. The problem may be that since the mechanism of trichome resistance was unknown, the specific characteristics of the pubescence which were responsible for resistance were not always analyzed. Pillmer and Tingey (1978), in their work on leafhopper resistance in beans,

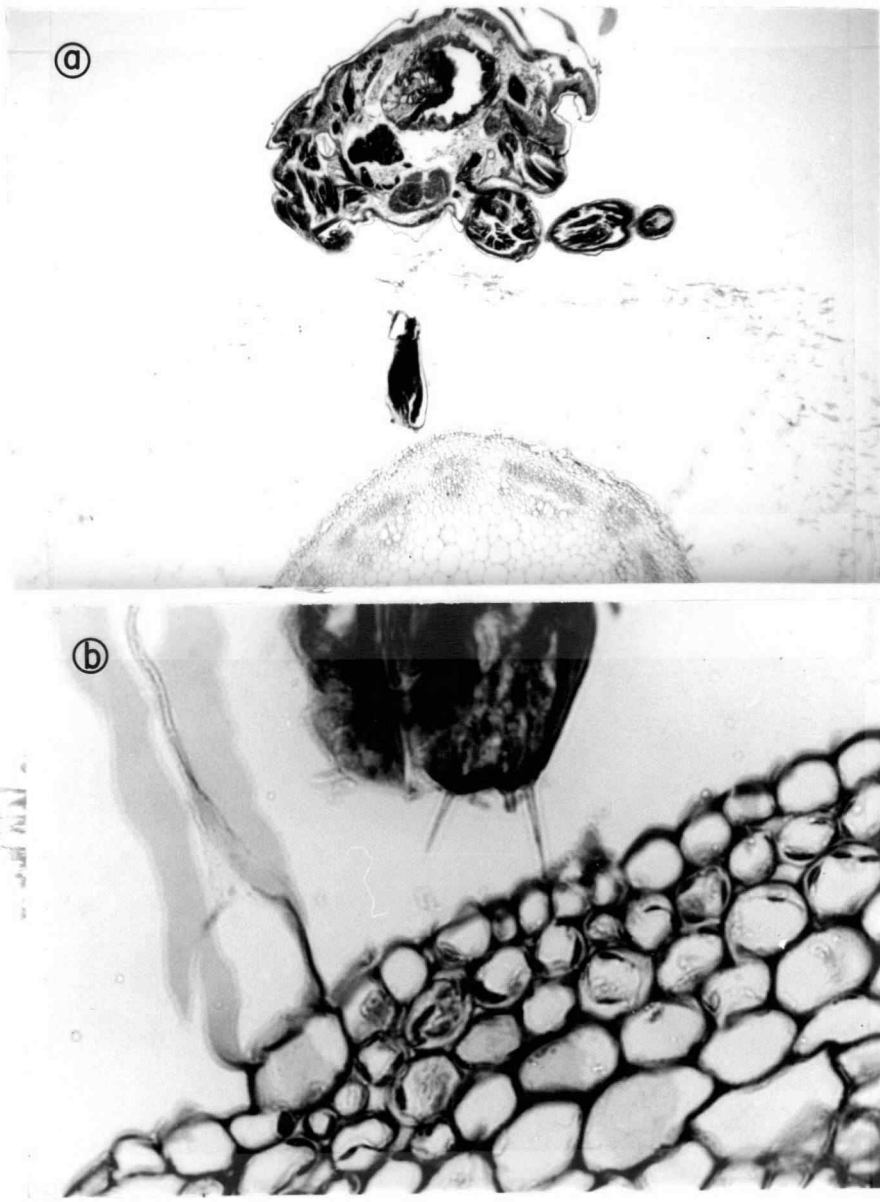


Figure 3.5 Section through third instar feeding on the stem, a) the nymph is prevented from lying closer to the epidermis by the dense mat of trichomes at the periphery of the trichome layer. In this instance the beak was able to reach the epidermis. b) The beak of the same individual. The sensilla on the tip of the beak touch the epidermis.

demonstrated the advantage of knowing the specific mechanism of resistance when assessing the correlation between resistance to a pest and the development of the trichome layer.

My results suggest that the height of the trichome layer, when combined with adequate density, may be the critical characteristic responsible for host resistance to Empoasca. It is interesting to note that in studies by other researchers in which an approximate measure of height was included such as percent erectness, the number of large erect hairs, and hair length, good correlations were found between host resistance and characteristics of the trichome layer (Parnell et al., 1949; Turnipseed, 1972; Turnipseed and Sullivan, 1976; Lyman and Cardona, 1982). In studies measuring density alone, or density and hair length, resistance was often poorly correlated with trichome development (Poos and Smith, 1931; Taylor, 1956; Wolfenbarger and Sleesman, 1961, 1963; Robbins and Daugherty, 1969; Broersma et al. 1972).

Whether host resistance to Empoasca spp. is directly related to feeding or to another factor such as differential oviposition is unclear. A negative correlation was found between trichome density and oviposition rates (Robbins and Daugherty, 1969; Ramahlo and Ramos, 1980). However, Poos and Smith (1931) found no correlation between trichome density or type and oviposition rates. It may be that the stimuli required for oviposition are received during stylet probing and the feeding process (Carlson and Hibbs, 1962).

When the mechanism of trichome defense is unknown, correlations between the development of pubescence and host resistance are used to evaluate the affect of the trichomes on the ecology of the insect and their role in host plant resistance. These correlations are often not consistent because of the failure to study important components of the relationship. Determining the mechanism of resistance provides a more complete understanding of the insect-plant relationship, and allows a precise analysis of the correlation between the development of specific trichome characteristics and plant resistance.

Chapter 4

The restriction of Philaenus spumarius (L.) from its preferred feeding sites by architectural barriers on the host plant

Abstract

Anatomical barriers restrict the nymphs of Philaenus spumarius L. from feeding on certain regions of the host plant. These restricted regions of the plant may be more favorable than the normal feeding sites in terms of two parameters of the spittlebugs food niche, xylem sap tension and concentration of amino acids in the sap.

In the absence of the trichome barrier on the stem of Anaphalis margaritaceae (D.C.), the second instar nymphs preferred to feed on the stem. The fourth instar, which can with difficulty penetrate the trichome barrier, preferred to feed on the stem, but did not expand its utilization of this area when the trichomes were removed. Sap tension in the stem of A. margaritaceae was less negative than that in the leaves and may explain why the stem was the preferred feeding site.

The distribution of the larger spittlebugs (Aphrophora spp.) on A. margaritaceae and Medicago sativa L. suggested that P. spumarius was restricted from a portion of the preferred feeding sites by tissue hardness. In A. margaritaceae the gradients in sap tension and amino acid concentration were opposite. The uniform distribution of feeding sites along the stem suggests that the nymphs were responding to a combination of these two factors. Although the gradient in amino acid concentration could not be determined for M. sativa, the uniform distribution of nymphs in the preferred region implies that they also were responding to a complex of factors.

The insect-plant interaction literature suggests the P. spumarius should respond to variation in nitrogen concentration and tension of the xylem sap of its host; however, it may be restricted by its need to remain protected by a spittlemass.

Introduction

Southwood (1973) proposed that the nutritional 'hurdle' is one of the major difficulties in the colonization of plants by herbivores. The composition of plant and animal tissues is very different, and acquiring an adequate diet solely from plant tissues may be difficult. In particular, the low proportion of nitrogen in plant tissues requires special adaptations to obtain sufficient quantities for growth and reproduction. Behavioral mechanisms which enhance the quantity of nitrogen in the diet include switching from tissue to tissue within a single host, and among hosts (McNeil and Southwood, 1978, Mattson, 1980). Physiological mechanisms include increasing the rate of feeding on diets low in nitrogen (Slansky and Feeny, 1977) and regulating plant chemistry by galling plant tissues (Osborne, 1973). Secondary plant metabolites are a major barrier to the utilization of plant tissues because of their toxic or nutrient-binding properties (Levin, 1971; Rhoades and Cates, 1976; Reese, 1977; Rosenthal and Janzen, 1979). Anatomical characteristics of plants have also been found to confer resistance to herbivore attack, interfering with locomotion, feeding, mating, and oviposition (Painter, 1951; Levin, 1973; Norris and Kogan, 1980). Xylem-feeding insects may not be deterred by secondary plant metabolites, either because they are not present in xylem sap or because they are sufficiently dilute and in forms easily excreted by the insect (McKey, 1979). This study investigated the role of architectural barriers in restricting Philaenus spumarius to areas of the host plant which may be suboptimal in terms of the nitrogen concentration and tension of xylem sap.

The meadow spittlebug, P. spumarius, is a polyphagous herbivore, feeding on the xylem sap of hundreds of plant species in North America (Weaver and King, 1954). Spittlebugs feed by penetrating

intracellularly to xylem vessels and drawing up sap by means of the cibarial pump. What probably allows this taxonomic diversity is that xylem sap is a very homogeneous food source. Nitrogenous compounds frequently comprise the major dry matter component in xylem sap. The nitrogen is transported as NO_2 and in reduced forms, usually amino acids. In most dicotyledons studied, two amino acids comprise approximately 60 to 70 percent of the reduced nitrogen, with glutamine, asparagine or both usually predominating. Another four or five amino acids make up the difference. Occasionally, the majority of nitrogen is transported in compounds such as alkaloids (Pate, 1971, 1973). The limited diversity of amino acids in the diet is probably compensated for by the activity of intracellular gut flora (Houk and Griffiths, 1980). The quantity of nitrogen in xylem sap is very low (.01-.21% w/v), and is approximately 10 to 20 times lower than the concentration in phloem (Pate, 1980). More extreme variation is found in some situations (Mattson, 1980). Gradients in the concentration of reduced nitrogen can either increase or decrease as the sap ascends the stem; the particular pattern found depends on whether the major site of nitrogen reduction occurs in the leaves or stems, (Wallace and Pate, 1967). The low quantity of reduced nitrogen is particularly disadvantagous to xylem feeders because the sap is practically devoid of carbohydrates (Bollard, 1960; Pate, 1976), and a portion of the reduced nitrogen is probably used as an energy source.

The low water content of plant tissue can limit its food value (Mattson, 1980). Xylem sap is 99% water (w/v), yet because it is usually under negative pressure (Scholander, 1965; Slayter, 1967), obtaining this water, and the nutrients dissolved in it may be a process requiring large amounts of energy. Tension up to -15 bars is often found in herbaceous plants on sunny days (Scholander et al., 1965; Boyer, 1967; Hickman, 1970). Gradients in xylem sap tension in a transpiring plant increase as the sap ascends the stem and enters the leaves (Scholander, 1965; and Begg and Turner, 1970). A spittlebug must pump against this negative pressure to obtain nutrients and to replenish the evaporating spittlemass. Even though

a 1 bar pressure difference did not change the feeding rate of a leafhopper species (Mittler, 1967), it probably increased the energy required to obtain a given amount of sap.

The meadow spittlebug is restricted in its choice of feeding sites by anatomical barriers on the host plant (McEvoy 1983a, Chapter 2). On A. margaritaceae the trichomes prevented the younger nymphs from feeding on the stem and tissue hardness restricted the nymphs to feeding at the apex of both A. margaritaceae and M. sativa (Chapter 2). These barriers may restrict the nymphs from feeding on preferred regions of the plant, regions which may be more favorable in xylem sap tension and the concentration of amino acids. The objectives of this study were to determine feeding site preference on A. margaritaceae and M. sativa in the absence of anatomical barriers. Gradients in xylem sap tension and amino acid concentration were measured to determine if nymphs preferred parts of the plant most favorable in terms of these parameters. Differences in the preference of nymphs with and without anatomical barriers were used to determine the degree to which the nymphs were restricted to suboptimal plant parts.

Materials and Methods

To determine if trichomes on the stem of A. margaritaceae were preventing the nymphs from feeding on an otherwise preferred site, the distribution of the second and fourth instar nymphs were compared on control stems and stems denuded of trichomes. Preference tests on the second instar were conducted in the laboratory. The preference test on the fourth instar was made in the field because the larger plants were prone to transplanting shock. Plants and nymphs used in the preference tests were obtained from the Mary's Peak study site, Benton Co., Oregon. At the time each instar was active in the field, 12 host plants were picked at random; eight had the upper 10 cm of stem was denuded of trichomes in eight plants, and the other four plants were controls. Four or five nymphs were placed at the apex of the plant at the start of each experiment. To determine if the placement site (leaf or stem) affected preference, half the plants from each treatment had nymphs placed on the leaves and the others received nymphs on the stem. The location of the second instar was recorded every hour for the first 4 hours and at the end of seven hours. The location of the fourth instar was recorded only after seven hours. A preference was recorded if the nymphs were in a mass or remained in a feeding position during the time the position of the nymphs was being recorded. Preference was not recorded for wandering nymphs and those that were not probing the plant tissue. Two days following the first test another set of nymphs were tested on the same plants but the site of placement was reversed on the two groups of plants.

The effects of tissue hardness on the feeding site preference of feeding P. spumarius on A. margaritaceae and Medicago sativa could not be determined by eliminating the tissues impeding stylet penetration. Assuming that the larger nymphs of the genus Aphrophora would respond

to gradients in nitrogen, xylem sap tension, and the physical environments in a manner similar to P. spumarius, the distribution of the fifth instar of Aphrophora spp., (mostly A. permutata Uhler), and A. maculosa Doering)), was compared to that of the fifth instar of P. spumarius. The larger spittlebugs were able to penetrate harder tissue (Chapter 2), and thus should have had a greater area of stem available.

The Aphrophora nymphs were found at the Mary's Peak study site and the location of their feeding sites was recorded using the procedures outlined in Chapter 2. Aphrophora nymphs were not found at the Willamette Valley study site, Benton Co., Oregon, probably because this site lacked conifer hosts used by adults. Accordingly, a preference test was performed. Ten pairs of stems were randomly picked and separated from the surrounding plants. The pairs were assigned to have nymphs placed at the apex or in the middle of the stem. Nymphs of both genera were collected from other hosts, and allowed to feed on potted alfalfa one day prior to the experiments. Nymphs were placed on the plants in the morning and checked 24 hours later. Location of the feeding sites was described as in Chapter 2. Two days later the same plants were used in a second test, but the placement of the nymphs was reversed.

Xylem sap tension measurements on A. margaritaceae were made at the Mary's Peak study site on June 17 and 24, 1980. Six plants were measured each day, two each at 7:00 am, 12:00 pm, and 5:00 pm. Sap tension measurements on M. sativa were made at the Willamette Valley study site on July 7, 9, and 12, 1980. Six plants were measured July 7 and 12 and four additional plants were measured at 12:00 pm on July 9.

The vertical gradient in xylem sap tension in A. margaritaceae and M. sativa was measured using a pressure bomb (Waring and Clary, 1966) and the procedures of Begg and Turner (1970). Stem and leaf, and stem and branch water potentials were measured on A. margaritaceae and M. sativa, respectively. Stem sap tensions were determined by

measuring the xylem sap pressure of a leaf or branch which had been enclosed the previous afternoon in aluminum foil and a polyethylene bag. This prevented evaportation from the enclosed leaf, and, since vascular bundles are assumed to be connected at the nodes (Esau, 1977), this enabled the leaf to come to equilibrium with the potential in the stem. Leaf sap tensions were measured on adjacent exposed leaves.

The vertical gradient was established from observations on three or four enclosed and exposed leaves at different heights on the plant. Measurements were taken on the lowest pair of green leaves or branches, one or two intermediate pairs, and the highest half-to fully-expanded leaf or branch 3 cm long.

M. sativa var. Du Puits, used in the analysis of the gradient in the concentration of amino acids, was grown from seed in soil obtained from the Willamette Valley study site. A. margaritaceae seeds did not germinate, therefore young plants were obtained from the Mary's Peak study site and grown in a greenhouse. Plants were analyzed when flower buds began to form, the time at which amino acid concentration of the xylem sap has been shown to be highest in many plants (Pate, 1971).

Plant sap for the analysis of the concentration of amino acids was collected using the method of sap bleeding (Brennan et al., 1964). The 20 μl of sap needed for the analysis could not be collected at one site, so plants were placed in groups of five according to height and the number of leaves. The five plants were placed at 100% RH for eight hours to allow positive root pressure to develop. The lowest green leaf or branch, two intermediate leaves or branches, and the highest one half expanded leaf or branch 3 cm long were excised and the cut surface blotted with a tissue after ten minutes to remove the sap from damaged phloem and parenchyma cells. The plants were left for two hours to allow xylem sap to exude from the cut surfaces. Sap was collected from sites at the same height with a micropipet, placed in a small glass vial covered with paraffin, and frozen until the analysis.

The quantity of amino acids in the collected samples was analyzed using a modification of the ninhydrin colorimetric analysis method (Rosen, 1957). Ammonia reacts with the ninhydrin, but because it is found in trace amounts in xylem sap (Pate, 1973) it does not significantly affect the results significantly. The 20 µl of sap was diluted with distilled water to 100 µl and used in the reaction with appropriately adjusted quantities of reagents. The samples were read in a Beckman 2000 spectrophotometer.

Results

In the absence of the trichome barrier second instar nymphs preferred to feed on the stems rather than the leaves of A. margaritaceae (Table 4.1). Placement of the nymphs on stems vs. leaves did not affect feeding site selection. Although most nymphs did not move once they had selected a feeding site, those that did move between observation periods usually had been feeding on a leaf (Table 4.2). All fourth instar nymphs caged on the lanate stem were able to feed, but a proportion of them appeared to have difficulty in doing so (Chapter 2). This difficulty did not restrict them from feeding on their preferred region of the plant. In the absence of the trichomes, the proportion of nymphs feeding on the leaves and stems was similar to that for the controls (Table 4.1). On both A. margaritaceae and M. sativa the larger Aphrophora species fed lower on the stem than P. spumarius (Figure 4.1a, b).

Xylem sap tension became more negative as the sap ascended the stem and entered the leaves (Fig. 4.2). The gradient of sap tension in the stem was lowest in the morning, increased with the increase in transpiration to a high in the early afternoon, and started to decrease by the late afternoon. In both plant species, the gradient increased by a factor of approximately 3.5 from the morning to the afternoon (Table 4.3). There was a similar trend in the increase in sap tension between the stem and the leaves or branches. This difference was lowest in the morning and evening and highest at mid-day (Table 4.4). These differences were significant in terms of the actual xylem sap tension found in the plants. Approximately one half of the xylem tension at the top of the plant was due to the increase in tension as the sap ascended the stem (Table 4.3, 4.5). As the sap was moved from the stem to the leaves or branch the tension increased by a factor of one fourth to one third (Table 4.4, 4.5).

Table 4.1. Preference of second and fourth instar nymphs on A. margaritaceae stem with or without trichomes.†

	Second instar‡		Fourth instar*	
Feeding location	Trichomes present	Trichomes absent	Trichomes present	Trichomes absent
Leaf	25	14	14	12
Stem	7	48	24	49

† Number of individuals at each location at end of test.

‡ Difference significant (X^2=24.58, d.f.=1, p<.001).

* Difference not significant (X^2= 2.73, d.f.=1, .05<p<.1).

Table 4.2. Total number of nymphs moving from, or remaining at, feeding site on A. margaritaceae during four observation periods.†

Previous feeding location	Moved	Remained
Leaf	11	52
Stem	7	180

† Difference significant (X^2= 11.297 d.f.= 1, p<.001).

Figure 4.1 Distribution of fifth instar nymphs of P. spumarius and Aphrophora spp. a) Natural distribution on A. margaritaceae. Aphrophora spp. fed lower on the stem than P. spumarius ($p<.001$, Median Test). b) Preference test on M. sativa, Aphrophora spp. fed lower on the stem than P. spumarius ($p<.001$, Median Test).

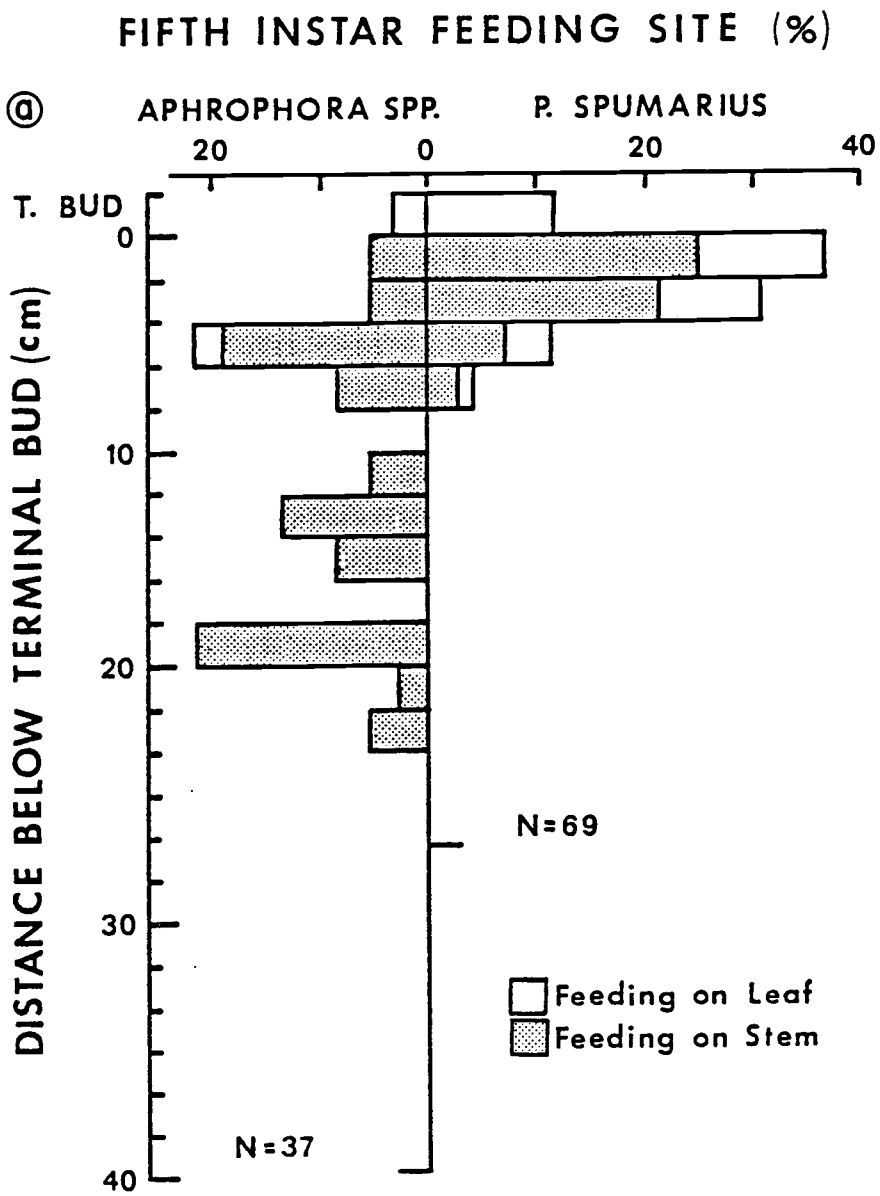

FIFTH INSTAR FEEDING SITE (%)
@
APHROPHORA SPP.
P. SPUMARIUS
20
0
20
40
T. BUD
0
10
20
30
40
DISTANCE BELOW TERMINAL BUD (cm)
N=69
N=37
Feeding on Leaf
Feeding on Stem

Figure 4.1

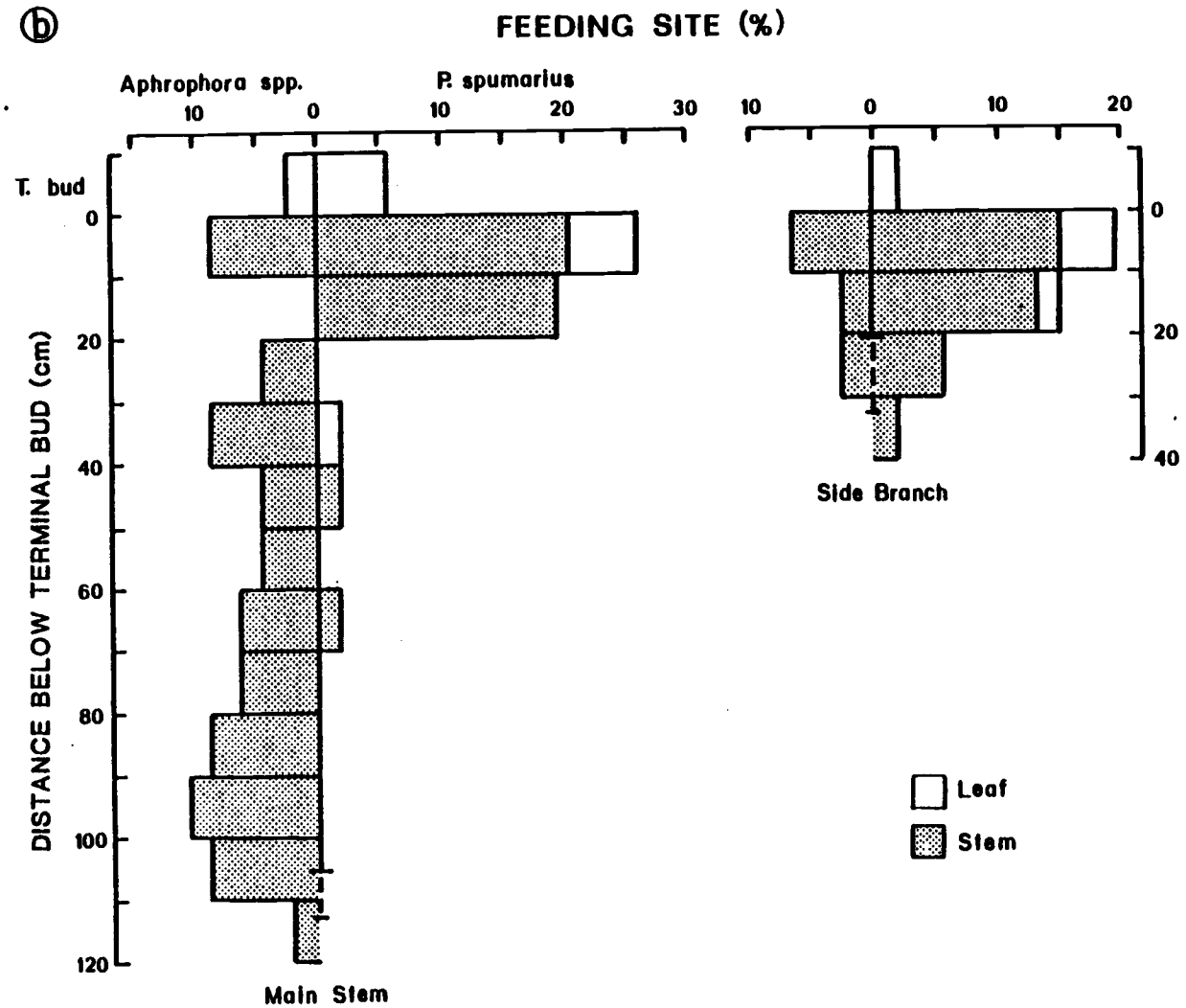

(b)
FEEDING SITE (%)
Aphrophora spp.
P. spumarius
10
0
10
20
30
10
0
10
20
T. bud
0
20
40
60
80
100
120
DISTANCE BELOW TERMINAL BUD (cm)
Main Stem
0
20
40
Side Branch
Leaf
Stem

Figure 4.1

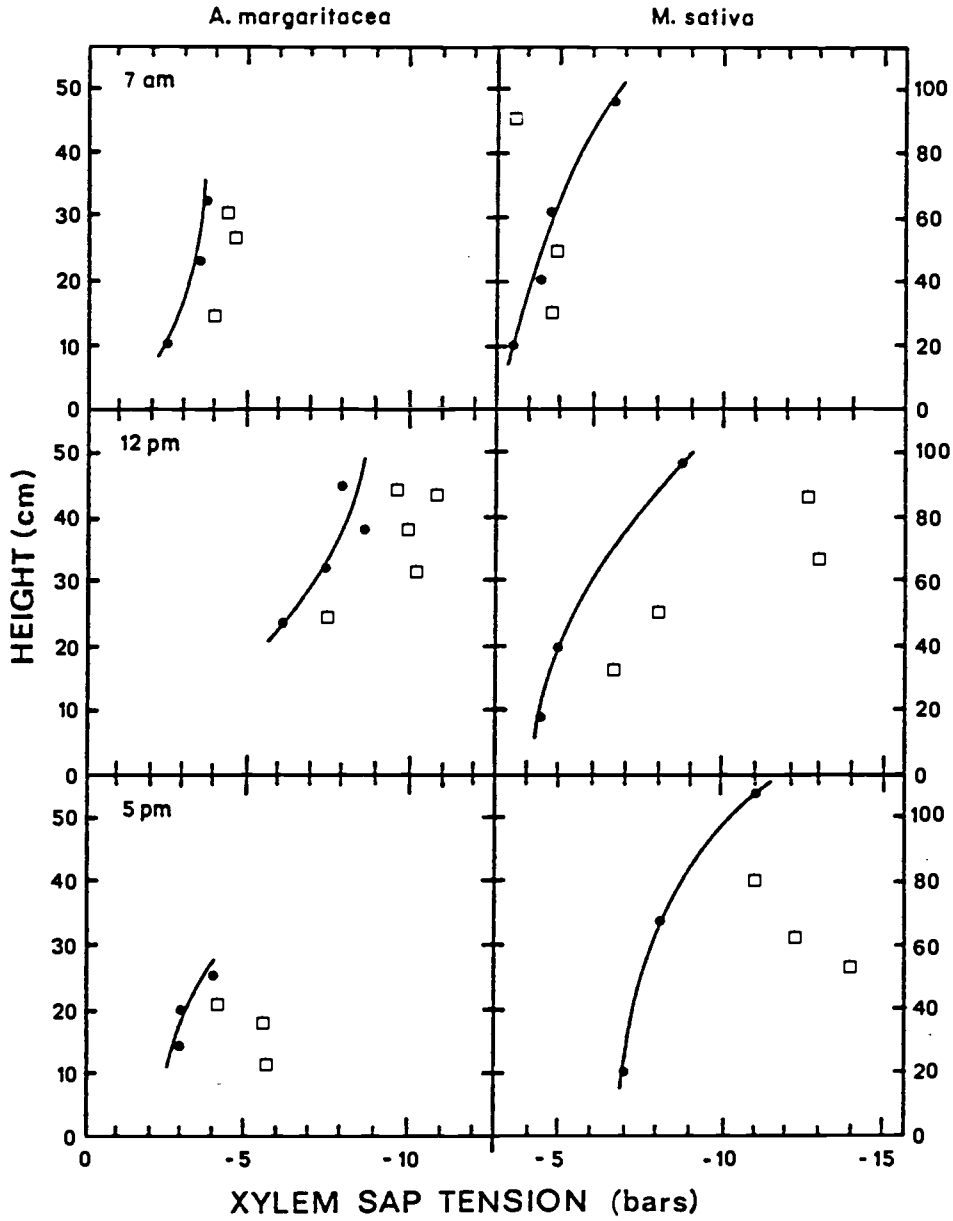


Figure 4.2 Gradients in xylem sap tension in representative A. margaritaceae and M. sativa plants at three times during the day. ● Sap tension in the stem, □ sap tension in leaves. Gradient fitted to points by hand.

Table 4.3. Difference between apex and ground level xylem sap tensions in the stem.†

Time of day	A. margaritaceae		M. sativa	
	Median	Range	Median	Range
7:00 am.	1.25	+ .25 - 2.2	1.9	.75 - 3.5
12:00 pm.	4.5	2.2 - 7.0	7.0‡	5.0 - 10.0
5:00 pm.	2.3	0. - 4.25	3.2	2.2 - 6.0

† Data from gradients drawn by eye through the data points and extrapolated to the apex and ground levels.

All values in -bars except where indicated as + bars.

‡ Values based on 8 plants rather than 4.

Table 4.4. Difference between stem and leaf/branch xylem sap tensions at the apex of the plant.†

Time of day	A. margaritaceae		M. sativa	
	Median	Range	Median	Range
7:00 am.	1.25	+ .25 - 3.0	2.9	+ 2.5 - 10.0
12:00 pm.	2.3	.25 - 2.75	3.5‡	1.0 - 4.9
5:00 pm.	.5	.25 - .75	3.0	1.5 - 6.75

† All values in -bars unless indicated as + bars.

‡ Values based on 8 plants rather than 4.

Table 4.5. Xylem sap tensions in the stem at the highest spot measured. †

Time of day	A. margaritaceae		M. sativa	
	Median	Range	Median	Range
7:00 am.	3.5	2.0 - 5.5	4.5	4.25 - 6.5
12:00 pm.	9.2	9.0 - 11.0	12.0*	10.0 - 14.5
5:00 pm.	5.7	4.3 - 7.50	10.3	6.0 - 12.0

† All values in - bars.

* Values based on 8 plants rather than 4.

Table 4.6. Gradient in amino acid concentration in A. margaritaceae.†

Relative Height	Amino Acid concentration (μM/ml)	
	Median	Range
Bottom	2.52	.75 - 6.75
Med-low	4.5	1.0 - 7.25
Med-high	6.25	2.5 - 8.75
Apex	6.6	3.15 - 10.15

† Values based on 4 groups of 5 plants.

Concentration of amino acids in A. margaritaceae was lowest at the base of the plant and increased as the sap ascended the stem (Fig. 4.3). Amino acid gradients among plants could not be assumed to be normally distributed, therefore, the median and range of the concentrations were determined at the bottom green leaf, lower and upper middle leaves, and top leaf (Table 4.6). Four of the five groups of plants had a gradient which increased with leaf height. The concentration of sap in the fifth group of plants was highest at the two middle leaves and lowest at the top and bottom leaf (this group was not included in the calculations). The fact that amino acid concentration did not increase steadily with stem height in all groups was probably due to the fact that if one of the plants had a higher than average concentration of amino acids, and exuded a large amount of sap at one collection point, it would bias the concentration at this point. The nitrogen concentration gradient in M. sativa could not be determined because this species failed to bleed xylem sap at 100% RH.

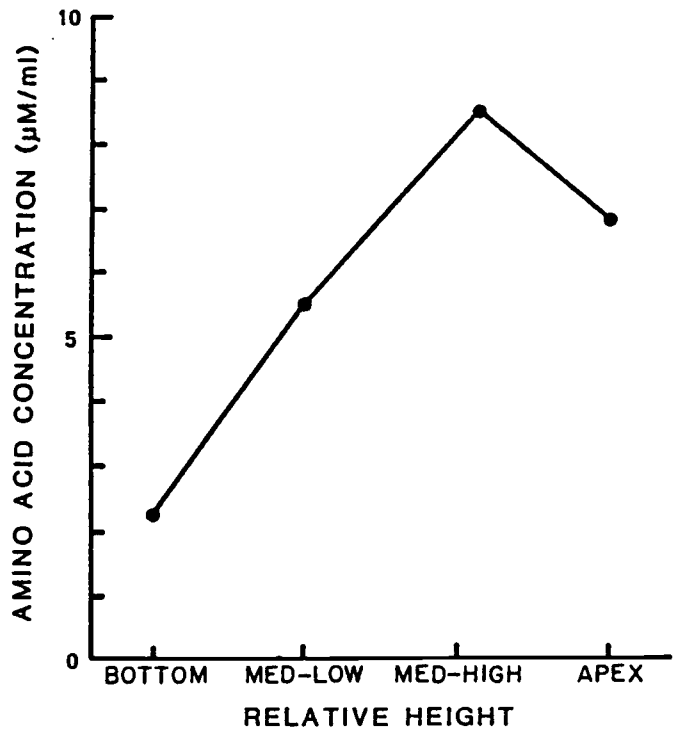


Figure 4.3 Gradient in amino acid concentration in the xylem sap of a representative group of five A. margaritaceae plants.

Discussion

Xylem sap tension was lower in the stems than the leaves and should be the preferred feeding site in the absence of the trichome barrier if nymphs are sensitive to these differences in sap tension. The second instar was restricted from their preferred site by the trichomes; the percent of nymphs feeding on the stem increased by a factor of 3.6 when the trichome layer was removed. With some difficulty the fourth instar was able to penetrate the trichome barrier (Chapter 2). However, they did not increase their utilization of the stem when the trichomes were removed. The proportion of nymphs feeding on the stem with the trichomes removed was approximately the same for second and fourth instars, suggesting that the variation in response of these two instars to the physiological status of the host plant may be similar. Not all nymphs chose to feed on the stem indicating that either the gradients in sap tension were not large enough to affect preference in these cases, or the sap tension in the leaves was not high enough to cause the nymphs to sample alternate sites. This preference for the stem by the second and fourth instars suggests that they responded to the differences in xylem sap tension.

The nymphs of P. spumarius appeared to be restricted from feeding lower on the stem of A. margaritaceae and M. sativa by the tissue hardness of the stem. Aphrophora nymphs were able to feed lower on the stem on both hosts because of their ability to penetrate harder tissue. The distribution of the Aphrophora spp. was fairly uniform over the entire range of feeding sites on both hosts. This indicated that the nymphs were either insensitive to the variation in food quality and environmental gradients over this range, or they were responding to a complex of stimuli which varied independently among the plants on which the nymphs fed.

On A. margaritaceae the preferred site for feeding in terms of xylem sap tension was low on the stem; for the concentration of amino acids it was at the stem apex. The distribution pattern indicated that nymphs did not respond primarily to either one of these parameters. Within an individual plant the amino acid concentration will vary inversely with the xylem sap tension because of the effect of transpiration on the concentration of sap nutrients (Pitman, 1975). The daily fluctuation in the excretion of nitrogen into the transpiration stream could moderate this decline in concentration as sap gradients increase (Brennan et al., 1964). As the xylem sap tension increases, making the base of the plant a more favorable feeding site, the amino acid concentration would fall, forcing the nymph to feed at the highest nitrogen concentration. The optimal feeding site in terms of both parameters might be expected to be somewhere between the two extremes and could vary among plants depending upon the absolute values and gradients for these parameters. This may be why the nymphs were uniformly distributed over the stem. Factors such as the evaporation rate of the mass in relation to canopy height (Whittaker, 1970) might be an additional variable in the regime of parameters affecting feeding preference.

The Aphrophora nymphs on M. sativa also may be responding to gradients for a combination of parameters. Because of the height of M. sativa, the differences in xylem increase in sap tension and evaporation rate between the base and apex could be greater than that in A. margaritaceae. The uniform distribution of the nymphs on this host suggested they were either insensitive to these parameters, were unable to respond during the 24 hour course of the experiment, or were responding to an additional unknown parameter. The concentration of amino acids in some legumes decreases as the sap ascends the stem (Brennan et al., 1964; Wallace and Pate, 1967; Pate, 1973). However, the fact that some legumes such as Trifolium (Pate, 1973) have a

limited nitrogen reductase system in the roots indicates that in some legumes the concentration increases as it ascends the stem. The parameter responsible for nymphs feeding at the apex of M. sativa, a region unfavorable in xylem sap tension and evaporative rate, may or may not be the increasing concentration of amino acids in the sap.

It may be that parameters of the food niche are not used in selecting feeding sites. This seems unlikely in light of what is known about the ability of insects to distinguish and feed on tissues high in nitrogen (McNeill and Southwood, 1978, Mattson, 1980). P. spumarius will abandon feeding sites low in nitrogen and settle of those higher in nitrogen (Horsfield, 1977). The preference of insect feeding in response to variation in xylem sap tension is unknown. Leafhoppers prefer to feed on shaded plants during the hot part of the summer (DeLong, 1965; Purcell, 1976), implying that they may be influenced by the water status of their host plants. There was no difference in feeding rate of a leafhopper between plant sites with a 1 bar difference in sap tension (Mittler, 1967); this is not surprising considering the high sap tensions found in the field. However, the laws of fluid dynamics (Slatyer, 1967) suggest that insects would have to expend more energy to pump against the additional pressure. This loss in efficiency,i.e., the amount of nutrients or energy injested per energy expended, is probably significant for insects feeding on a diet low in nutrients. The quantity of nitrogen injested could be increased by increasing the consumption rate (Slansky and Feeny, 1977), but this may not be possible at high xylem sap tensions.

Even though it appeared likely that the P. spumarius was sensitive to parameters of the food niche when selecting feeding sites, the fact that nymphs remained at one site for periods of 24 hours (personal observation) indicated they are not extremely sensitive to these parameters. Even if the nymphs choose a feeding

site optimal in terms of these parameters prior to forming a mass, it is likely that the optimal site will shift as gradients in xylem sap and nitrogen change during the day . Part of the reason for failing to track these changes might be because it would require leaving their spittlemass. The mass prevents the desiccation and death of the nymphs (Wiegart 1964; personal observation) and may protect them from predators (Whittaker,1970) and parasites (Coe, 1966). Abandoning a spittlemass would be particularly critical during the day when danger of desiccation is high.

Architectural barriers restrict the feeding site selection of P. spumarius. In some situations the barriers prevent nymphs from feeding on preferred regions of the host. While some of the parameters of the feeding niche have been identified, the response of the spittlebug to variation in the individual parameters is largely unknown. Until the effects of these parameters on the behavior and fitness of P. spumarius are known, the extent to which architectural barriers restrict the nymph to suboptimal sites will remain unclear. The degree to which P. spumarius is restricted to suboptimal feeding sites may influence its distribution among plant species.

Chapter 5

Summary and Conclusions

Architectural barriers restricted the feeding site selection by P. spumarius nymphs. Tissue hardness was the major barrier to the nymphs feeding lower on the stem of both A. margaritaceae and M. sativa. When fourth and fifth instar nymphs were caged at increasing distances below the terminal bud (DBTB) the percent able to feed decreased. These findings provide experimental support to the correlation between the range in feeding sites of P. spumarius and the tissue hardness of the stem (McEvoy 1983a). A portion of the nymphs caged below their normal feeding range were able to feed. The reason for not doing so in nature maybe that the time it takes to form a spittlemass lower on the stem exposes nymphs to desiccation and natural enemies.

The tissues responsible for impeding stylet penetration in the two hosts were different. In A. margaritaceae, the progressive lignification of the bundle cap and interfascicular parenchyma increased the resistance of the stem as DBTB increased. In M. sativa, these areas were penetrable when the tissues were mature. In this species the lignified xylem fibers were the most important tissue blocking access to the xylem elements.

Tissue hardness was not the only anatomical barrier associated with the stem in the two plants studied. In M. sativa, the decrease in the availability of xylem elements lower on the stem acted in conjunction with tissue hardness to increase the resistance of the stem. In A. margaritaceae, the decrease in the size of the xylem elements at the periphery of the xylem may increase the stem resistance to feeding. These small vessels transport less water and may limit the rate at which the spittlebugs can feed.

The trichome layer on A. margaritaceae was an additional barrier to feeding. In regions of the stem not protected by tissue hardness the trichome layer prevented access to the stem. The mechanism of resistance was that a trichome layer higher than the length of the beak prevented the initiation of stylet penetration. This was probably due to a lack of stimuli from the sensilla on the tip of the beak. On areas of the stem where tissue hardness became significant, the trichome layer provided additional resistance. The mechanism of trichome resistance on hard tissue was probably that the trichomes were limiting the ability of the nymphs to grasp the stem and apply the pressure necessary for penetration.

In the absence of the tissue hardness and trichome barriers, the nymphs utilized more areas on the plant. The trichomes prevented the young nymphs from feeding on their preferred site, the stem. In the absence of the tissue hardness barrier, the range of stem utilized increased but the nymphs did not show a marked preference for this normally restricted region.

Two parameters of the spittlebug food niche are the xylem sap tension and the amino acid concentration of the xylem sap. The stem had a lower xylem sap tension than the leaves and this may be why it is the preferred feeding site for all nymphs in the absence of the trichome layer. Gradients in xylem sap tension and amino acid concentration opposed and interacted with each other along the length of the stem. The height of the optimum stem site may vary between plants in the habitat which may be why the distribution of the Aphrophora spp. was fairly uniform within in the range of feeding sites on the two hosts. It may also be that nymphs are not responding to variation in the parameters over the range experienced on the available region of the stem.

The resistance of a plant to its potential herbivores can rarely be attributed to a single resistance mechanism. The suite of defenses may come from different classes of resistance mechanisms (biochemical, anatomical, and ecological), or it may be composed of variable defense mechanisms within a single class. These defensive compounds or structures usually form a defense hierarchy. For insects feeding on plant tissue containing secondary plant metabolites, compounds characteristic of the taxon will form the first line of defense, screening out non-adapted insects, e.g., the cyanogenic glycosides in crucifers. Compounds more specific to individual species could interfere with the attack of herbivores which have become adapted to the first or second line of defense. Anatomical defense mechanisms usually are found in the upper levels of the hierarchy because they usually interfere with the behavior of specific or closely related herbivores which are only signficiant once an animal has circumvented the lower levels of defense, e.g., callosites of the leaves which mimic the eggs of visual egg-assessing lepidoptera (Shapiro, 1981a, 1981b).

Spittlebugs differ from most other herbivores in that they feed on a diet which is probably devoid of most of the plant secondary metabolites influencing the selection and preference of host plants and tissues. Therefore, the level of the defensive hierarchy at which the different classes of resistance mechanisms are found will be different. For xylem-feeding insects anatomical barriers probably form the first line of defense. It is only after they penetrate, or circumvent these barriers that other mechanisms may come into play. Once fed upon, a host plant may be rejected because of nutritional inadequacy, e.g., amino acid composition of the plant. It may also be rejected if the anatomical barriers restrict the herbivore from regions of the plant which are adequate in the parameters of the food niche, forcing it to feed on parts of the plant which are outside the tolerance limits for the parameters. In this instance anatomical barriers may influence the distribution of the herbivore among plant species.

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APPENDICES

Appendix 1

Head capsule width, stylet length, and the allometric relationship between head capsule width and stylet length.†

Instar	Head capsule width	External stylet lenght‡	Stylet length / Head capsule width
1	.478 ± .003	.322 ± .004	.674 ± .007
2	.672 ± .004	.438 ± .004	.654 ± .006
3	.950 ± .004	.568 ± .005	.598 ± .005
4	1.321 ± .008	.802 ± .006	.612 ± .004
5	1.891 ± .013	1.061 ± .009	.562 ± .004

† Mean and standard error, N=25.

‡ Approximate length of beak.

Head capsule width measured across head from outside of each eye. Stylet length of external stylet measured from end of clypeus to tip of maxillary stylets. This approximates the length of the beak when it is positioned for feeding.

Appendix II

The BMDP 81 stepwise logistic regression estimates the parameters (Bi) of the linear logistic model:

$$E(y) = \frac{e^{Bx}}{1+e^{Bx}}$$

The parameters Bi are estimated as the values which maximize the likelihood function:

$$L(B) = \pi \frac{e^{yBx}}{1+e^{Bx}}$$

Model		Term	Coefficient	Standard error
Fig. 2.3	Instars (1-5) on stem of *A. margaritaceae*	INSTAR	3.564	1.015
		TRICHOME	-10.255	3.187
		CONSTANT	-.99	.000
Fig. 2.4a	Fourth instar on *A. margaritaceae*	CONTROL	-4.924	.000
		DBTB	-.130	.041
		TRICHOME	-.538	.21
		CONSTANT	7.11	.713
Fig. 2.4b	Fifth instar on *A. margaritaceae*	CONTROL	-4.296	.000
		DBTB	-.129	.033
		TRICHOME	-2.471	1.248
		D*T	.052	.033
		CONSTANT	9.775	1.248
Fig. 2.4c	Fifth instar on *M. sativa*	DBTB	-.053	.026
		CONSTANT	6.404	2.136
Fig. 2.9	Fifth instar versus tissue hardness	HARDNESS	-.005	.002
		SPECIES	.784	.359
		CONSTANT	6.91	1.617