# HOST PLANT RELATIONS OF APHIDS WITH SPECIAL REFERENCE TO WATER STATUS

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## Abstract

The work described was done with larvae and adults of virginoparae of the green peach aphid, Myzus persicae Sulz. and the cabbage aphid, Brevicoryne brassicae L. on Brussels sprouts, Brassica oleracea L. var. bullata gemmifera.

The responses of the aphids to three leaf ages of the plants subjected to differing levels of intermittent water strain are described and compared for each aphid species in terms of 1) the selection of the leaves and plants by settling alatae, 2) the preferences of the apterae for leaves of different age and water status, and 3) the fecundity, reproductive rate, rate of development, longevity, reproductive life, post-reproductive life and proportion of alatae in the progeny of apterae on leaves of different age and water status. The suitability of the different leaves and plants as hosts for the two aphid species is assessed.

The responses of the aphids to three leaf ages of plants subjected to no water strain or to a single period of increasing water strain are described and compared for each aphid species in terms of the survival, reproductive rate and restlessness of apterae. A relationship is demonstrated between the water content of the growing medium of the plants and the larviposition of aphids feeding on the plants.

The methods of penetration and the destinations of the stylets and salivary sheaths of the two aphid species in the leaves of the plants are described.

Preliminary experiments are described in which aphids were fed on artificial liquid diets under controlled pressures.

The possible causes of the responses of the aphids to leaves of different age on plants of different water status are examined particularly by comparing the two aphid species. The possible economic importance of the responses of aphids to water strain in plants is discussed with reference to aphid control.

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# CHAPTER I

## INTRODUCTION

In natural vegetation, aphids, like many phytophagous insects, are dependent for their survival on successful associations with their host plants. Complex interactions between the aphids and plants enable the aphid populations to move from one temporary host plant to another in a continuous cycle of arrival, exploitation and departure. This association between an aphid and its host plant is, of necessity under natural conditions, so close that almost any change in the condition of the plant may be expected, in turn, to affect the aphid and this has been shown. To the agricultural entomologist , this association therefore suggests that crop plants may perhaps be deliberately manipulated to assist in the 'integrated control' of the aphids which attack crops.

Manipulation of the water status of crop plants may be one way in which plant resistance to aphids could be enhanced. Kennedy (1958) concluded that "we should eventually be able to obtain aphid resistance through altering the water economy of the plants, by cultural or plant breeding methods". However, little is known of the responses of aphids, particularly individual aphids, to variations in the water status of their host plants. The only detailed studies of this problem yet undertaken, by Kennedy and co-workers and Weismann

and co-workers, have been with Aphis fabae Scop. responding (primarily in the short-term) to largely continuous, severe water strain in several species of host plants.

The present study was undertaken to measure some of the long- and short-term responses of Myzus persicae Sulz. and Brevicoryne brassicae L. to intermittent mild and severe water strain in three leaf ages of Brussels sprouts grown in pots in a constant temperature growth room. For each aphid species, its arrival on, exploitation of, and departure from the plants were studied. To obtain a clearer understanding of these aphid responses to intermittent water strain in the plants, the responses of the aphids to continuous water strain were measured and the methods of feeding of the aphids were studied, first by examining the sites of feeding of the mouthparts of the aphids within the plants, and secondly by feeding the aphids on artificial diets.

# CHAPTER II

## REVIEW OF THE LITERATURE

The literature is vast concerning the host plant relationsof aphids. This reflects 1) the agricultural importance of the damage to plants caused by aphids and the viruses which they transmit, 2) the importance of the host plant in the biology of aphids and 3) the complexity and diversity of the associations between aphids and their host plants. This review, like the experimental work, is primarily concerned with the influence of the host plant on its aphid population, though some reference is made to the reverse relationship. A brief addendum summarises the reported effects of water stress on plants, with emphasis on those effects most likely to influence aphids.

The papers by Kennedy (1951c, 1958), Painter (1958a), Kennedy and Stroyan (1959), Auclair (1963) and Banks (1965) reviewing various aspects of the biology of aphids refer to many papers on their host plant relations. In the present review the influence of the host plant on aphid populations will be considered in stages corresponding to the cycle of movement of aphids from one host plant to another (p. 72 ).

a) Arrival and Establishment on the Host Plant

General Theories of Host Plant Selection and Adaptation

The chemical constitution of the host plant is generally considered to be the prime factor governing the selection of host plants by phytophagous insects such as aphids. Other properties of the plant, such as colour and structure, are usually accorded a secondary role except in initial location of suitable vegetation and in eliciting the settling response. The insects are thought to respond to two sets of chemicals, acting as gustatory/olfactory attractants and/or repellents: 1) "odd" or "token" plant chemicals (e.g. alkaloids), and 2) "nutrient" chemicals (e.g. sugars). Fraenkel (1953) considered that "odd" chemicals, acting against a general background of nutritional suitability, were wholly responsible for host plant selection; Dethier (1953) recognised the importance of "odd" chemicals but reserved judgement on the role of "nutrients"; Thorsteinson (1960) accorded an important role to both "odd" chemicals and "sapid nutrients"; Jermy (1965) (also Wardle, 1929) stressed the significance of feeding inhibitors in plant avoidance. With particular reference to aphids, Kennedy and Booth (1951) and Kennedy (1951b) proposed a 'dual discrimination theory' of host plant selection in which the insects were thought to respond to both "flavour" ("odd") and "nutrient" stimuli in the sap of plants. Both sets of chemicals were thought to take part in every act of selection but "flavour" was considered to be primarily significant in selection of the species of plant, particularly for host alternating aphids, while "nutrients" were primarily significant in the selection of different aged leaves on the same plant.

Kennedy, Ibbotson and Booth (1950) suggested that the degree of adaptation of an aphid to a host plant increased with

the ability of the aphid to colonise the mature leaves of the plant as well as the young and old leaves. A similar theory has since been conceived by Mosbacher (1964). Kennedy and Booth (1951) equated adaptation with acquired preference for the specific "flavour" chemical of the plant, which, they suggested, was strongest in the mature leaves. Thus, Aphis fabae Scop. was seen to be more adapted to sugar beet and spindle than was Myzus persicae Sulz. On the same basis, Hull (1964) found Acyrthosiphon pisum (Harris) to be more adapted to sweet pea than was A. fabae, and Heathcote (1962) found M. persicae to be less well adapted to sugar beet and spinach than to Brassica sp. (see also Kennedy and Booth, 1954; Tambs-Lyche and Kennedy, 1958; Müller, 1966; Wyatt, 1965).

In accordance with the above theories of host plant selection, the range of host plants colonised by an aphid should depend on the number of plant species in which the correct chemical signal pattern for selection occurs. This correct pattern is very frequent for a polyphagous aphid, such as M. persicae, and unusual for an oligophagous aphid, such as Brevicoryne brassicae L. which occurs on Cruciferae and Resedaceae (see Markkula, 1953).

a) i) Selection of the Host Plant by Alate Aphids Before Alightment

Flying aphids alight on plants primarily in response to visual attraction. Kennedy, Booth and Kershaw (1959a,b) were unable to demonstrate olfactory attraction of flying aphids to plants, a finding which supports the view of Kennedy (1950b)

that, in the location of host plants by aphids in the air, the odour of plants probably contributes less than subjective observation sometimes suggests (Müller, 1951; Müller and Unger, 1951).

The visual response of flying aphids to plants has two components, a colour and an optomotor reaction (Kennedy, Booth and Kershaw, 1961). Kennedy et al (1961) concluded that the type of colour vision shown by alighting aphids mainly distinguishes between sky and ground, the latter being more attractive by reflecting a greater proportion of long-wave visible radiation (>500mμ) (cf. Moericke, 1950). Kennedy et al further suggested that the known attraction of flying aphids (particularly host-alternating species) to yellow surfaces (Moericke, 1952, 1953, 1955a,b; Eastop, 1955; Heathcote, 1957; Lamb, 1958; also Pospíšil, 1963) was a further specialised manifestation of the attraction of aphids to surfaces reflecting the greatest proportion of long-wave visible radiation. This "yellow-sensitivity" was thought to assist the selection of leaves and plants in the correct physiological state for colonisation e.g. those suffering mild water strain, infected with viruses or young and senescent (cf. Moericke, 1955a), and hence to assist host alternation by heteroecious aphids in which alatae arrive at growing and senescing herbaceous plants in spring and senescing woody plants in the autumn. Cartier (1963) found that greater numbers of alate pea aphids alighted on those varieties of peas with the yellowest foliage (e.g. "Melting Sugar"). In contrast, a dramatic reduction in the numbers of aphids trapped

in a crop has been obtained by placing aluminium reflectors (reflectors of short-wave visible radiation) between the plants (Smith, Johnson, Kahn and Bing, 1964).

Kennedy et al (1961) suggested that the optomotor response of flying aphids may serve in distinguishing one kind of plant from another by structure. Such a mechanism may have contributed to the findings of Cartier (1963) that taller pea plants "intercepted" more flying pea aphids than shorter plants and of Pimentel (1961) that more alatae (except M. persicae) settled on cabbages which were widely spaced than on cabbages grown close together.

However, it would seem that flying aphids are hardly influenced by differences between plant species. This is shown by the large numbers of alatae which alight on non-host as well as host plants (Kennedy et al, 1959a,b; Müller, 1962; see also Painter, 1963).

After alightment

The selection of host plants by alate aphids occurs primarily after the aphids have alighted on plants. Similar numbers of alatae alight on host and non-host plants but fewer take off again from the former (Kennedy, 1950a,b; Kennedy et al, 1959a,b; Müller, 1962). Müller (1953,1958) found a similar mechanism operating among A. fabae alighting on susceptible and resistant varieties of beans. Johnson (1954,1958) made a comprehensive study of the settling responses of alatae. He found that alate A. fabae flown on pins for a fixed period of time (30 sec.) and released on host and non-host leaves stayed

longer on the host leaves before again taking off (cf. Müller, 1953; Orlob, 1961). This was confirmed with free-flying aphids by Kennedy and Booth (1964) and Kennedy (1965) who also found that, compared with non-host leaves and green cards, the period of settlement on host leaves "boosted subsequent flight the least and depressed it the most".

By this process of "differential dispersal", which diminishes with the length of time the aphids have flown (Johnson, 1958; Kennedy and Booth, 1964; Kennedy, 1965) and the age of the aphids (Itô, 1960b), alatae gradually settle on their preferred host plants. Gregariousness of the aphids may assist this process (Ibbotson and Kennedy, 1951).

After alightment on plants, alatae briefly insert their stylets (probe) a short distance into the tissues of the plant, apparently testing its suitability. Complementing the visual response of flying aphids, alatae probe more frequently on surfaces reflecting a greater proportion of long-wave visible radiation (Moericke, 1950), such as yellow green leaves. Furthermore, alatae generally probe the leaf of a host plant (or susceptible host plant) more times than a non-host plant (or resistant host plant) before again taking off or settling (Müller, 1953, 1962; Johnson, 1958). It is during this test-probing, and subsequent deeper probing, that a major part of host plant selection is thought to occur by aphids responding to the "flavour" and "nutrient" chemicals in the plant sap.

The method(s) whereby alatae "detect" differences between plants is imperfectly understood. The odour of plants

may be important in selection by alighted and probing alatae (Kennedy, 1950b), particularly since Hennig (1959a,b, 1963) and Ehrhardt (1963) have shown that there is little or no uptake of $P^{32}$ during short probes by aphids on radioactive plants. Müller (1958a) and Hennig (1963) found that such probes are often intercellular and suggested that the purpose of test-probing by aphids was primarily to select a plant (or plant part) of suitable mechanical structure for feeding (see also Hennig, 1959b). Ehrhardt (1963) found that the stylets of Megoura viciae Buckt. penetrated to the phloem of non-host plants (e.g. Poa pratensis L.) before the aphids rejected the plants (cf. Tate, 1937). However, Hennig and Ehrhardt studied apterous virginoparae which Kennedy and Booth (1954) found relatively less sensitive to plant kind ("flavour") than alate virginoparae and gynoparae. Alatae and particularly gynoparae may test-probe intracellularly or may be capable of olfactory discrimination between plants which would preclude the need for intracellular probing. Furthermore, measurement of $P^{32}$ in the aphids may be too insensitive to detect the minute 'uptake' of sap which may perhaps be required for host selection if chemoreceptors are present in the tips of the mandibles of aphids as suggested by the work of Bradley (1962). Wensler (1962) showed that sinigrin was perceived by B. brassicae while making short probes.

The importance of "odd" chemicals in host selection by oligophagous aphids is shown clearly by the work of Wensler (1962) who found that the mustard oil glucoside, sinigrin, is a specific stimulus for host selection by B. brassicae. Simila

ly, the alkaloid sparteine produces a positive feeding response in Acyrthosiphon spartii Koch. (Smith, 1957) while, in contrast, Brusse (1962) found an inverse relationship between the alkaloid content of lupins and their infestation by the polyphagous aphid Macrosiphum euphorbiae (Thos.). The importance of the nutrient content of the plant sap in host plant selection must be regarded as unproven. Kennedy and Booth (1951, 1954) found that, in preference tests between spindle and sugar beet, alate A. fabae exercised a preference for spindle which overshadowed the normal leaf age preferences of the aphids. The parallelism between the direction of preference and the "nutritive value" of the preferred leaf (as measured by aphid reproduction) was less exact in comparisons between plants than between leaves on the same plant. "Nutrient" (leaf age) discrimination thus appeared to be secondary to "flavour" (plant kind) discrimination in selection of the host plant.

Other plant properties may influence host plant selection by alatae. Way and Murdie (1965) found that alate M. persicae settled preferentially on a glossy- rather than a waxy-leaved variety of Brussels sprout. Kennedy, Lamb and Booth (1958) found heavier infestations of A. fabae on watered spindle than on unwatered spindle. Counts of the motherless groups of young on the leaves suggested that more alatae had taken off again from the unwatered plants. The preference for watered over wilting plants was confirmed in later preference tests in 2-leaf cages on greenhouse plants. The preference was reversed by watering the previously unwatered plants. Kennedy

and Booth (1959) obtained similar results with A. fabae on spindle in the field except that after sunset the alatae preferred the leaves of unwatered plants. When both spindle and sugar beet had received ample water, the alatae settled on the mature leaves of spindle in preference to any leaf age of beet ("flavour" preference > "nutrient" preference, see above). However, in hot dry weather, when the beet were wilting, the alatae settled preferentially on the leaves of beet rather than spindle. Kennedy et al (1958) and Kennedy and Booth (1959) (also Kennedy, 1958) related the non-preference of the alatae for unwatered plants to their reduced turgor and/or increased sap viscosity impeding sap uptake by the aphids (see p. 34 ). A similar mechanism was thought to govern the preference for beet over spindle in hot weather, since the more rigid leaves of spindle suffered greater turgor deficits (see Kennedy and Booth, 1958). When previously unwatered plants were watered (or after sunset), the recovery of turgor was thought to initiate a translocatory "surge" of the products of hydrolysis (e.g. amino-acids), which had occurred in the leaves during water strain. This "surge" of soluble nitrogen compounds was suggested to contribute to the observed reversal of the preference responses.

The stage of growth of plants influences the proportion of alighting aphids which take off again. Johnson (1958) found that of 50 alate A. fabae, flown for 30 secs. and released on the mature leaves of beans, all took off again within one hour. Of 61 aphids similarly treated and released on seedling

beans, 55 settled overnight. This difference in settling response may be related to differences in the nutrient content of the two ages of plant and/or to the more sheltered feeding sites offered by the curled leaves of seedling plants (see Painter, 1953). Higher nutrient levels, particularly soluble nitrogen compounds, are also thought to govern the preference of alatae for more actively growing or senescing plant organs (Cutright, 1930; Taylor, 1955; Tambs-Lyche and Kennedy, 1958).

The ability of some plants to cause a very high proportion of alighting alatae to take off again is a most valuable source of plant resistance to aphid attack (e.g. Taylor, 1955; Cartier and Painter, 1956; Howitt and Painter, 1956; Müller, 1956, 1958; Tambs-Lyche and Kennedy, 1958; Cartier, 1963). Plants incur very little direct damage from the feeding of aphids during "differential dispersal" though some larvae may be deposited (Johnson, 1954, 1958; Taylor, 1955) and the transmission of non-persistent viruses may cause long-term damage (Kennedy, 1950a; Orlob, 1961). For aphid species which feed on similar plants in the same ecosystem, host plant selection by the alatae is an important mechanism in segregating the aphid species and reducing interspecific competition (Itô, 1955).

Selection of the part of the host plant

Alatae frequently move from their alighting positions to preferred feeding sites on the plants. Some "yellow-sensitive" aphids may be attracted before alightment to a particular part of the plant, e.g. senescent leaves (Kennedy *et al*, 1961). Other aphids may fly at a level above the ground which brings them

directly to their preferred feeding site on the plant (Nietzel and Müller, 1959; Broadbent, 1954). However, it would appear that most aphids are restless during final settlement on the host plant. Johnson (1958) described the movements of A. fabae (after flight) placed on different plants and suggested that the structure of plants may assist or hinder aphids in finding their preferred feeding sites (see also Taylor, 1955). Table 1 summarises some preferred feeding sites of alatae settling on plants.

Table 1 Preferred feeding sites of settling alatae

| Aphid Species | Plant Species | Preferred Feeding Site | Reference |
|---|---|---|---|
| Acyrthosiphon pisum (Harris) | Sweet pea | Mature leaves | Hull (1964) |
| Aphis fabae Scop. | 1) Bean | Young leaves, shoots and inflorescences | Banks (1958) Müller (1958) Tambs-Lyche & Kennedy (1958) |
| (incl. gynoparae) | 2) Spindle & sugar beet | Young and old leaves | Kennedy & Booth, (1951, 1954) Kennedy, Ibbotson & Booth, (1950) |
| Aphis nasturtii Kltb. | Potato | Old leaves | Taylor (1955) |
| Brevicoryne brassicae L. | 1) Cabbage | Young leaves | Broadbent (1954) Miyashita (1953) Otake (1966) |
| | 2) Hirschfeldia | Flower stem | Hughes (1963) |
| Myzus persicae Sulz. | 1) Potato | Old leaves | Taylor (1955) Nietzel & Müller (1959) |
| | 2) Cabbage | Old leaves | Miyashita (1953) Otake (1966) |

Table 1 (cont.)

| Aphid Species | Plant Species | Preferred Feeding Site | Reference |
|---|---|---|---|
| Toxoptera aurantii Boy. | Citrus | Young twigs | Rivnay (1937) |

Kennedy and Booth (1951, 1954) found that alate A. fabae usually expressed preferences for those leaf ages on which they reproduced most rapidly (see Table 1). Such preferences are thus frequently regarded as "nutrient" preferences and are discussed more fully with reference to apterae (p. 23 ). The large number of alatae which select the plant apex for feeding may also indicate a preference for a sheltered feeding site. Taylor (1955) suggested that alatae may gather on the lower leaves of plants to escape disturbance by wind and rain.

Changes in the physiology of the host plant may alter the leaf age preferences of aphids. During the flowering of sugar beet, alate A. fabae settle preferentially on the flower spikes and middle leaves of the plants (Kennedy et al, 1950). Water strain in the host plant may obliterate the normal leaf age preferences of settling alatae. Kennedy et al (1958) found that the mature leaves of a watered spindle plant were preferred to the young and senescent leaves of an unwatered plant for feeding by A. fabae in cages (cf. Table 1). Low turgor and/or high sap viscosity in the unwatered plant were thought to impede sap uptake by the aphids (see p. 34 ). Kennedy (1950b) accorded considerable importance to the leaf age/plant age preferences of host-alternating alatae which migrate from a maturing woody plant

to a growing and senescing herbaceous plant in spring, and from a dying herbaceous plant to a senescing woody plant in the autumn.

a) ii) Selection of the Host Plant by Apterous Aphids

Compared with alatae, lack of mobility in apterae severely limits host plant selection activities, which are largely confined to a single plant or a group of plants within a small area (see Ribbands, 1965). Nevertheless, apterae exercise preferences for different species and varieties of plants as well as their different parts.

As in alatae (see p. 15 ), apterae test-probe plants and test-probe more frequently on any surface reflecting a greater proportion of long-wave visible radiation (Moericke, 1950; see also van Hoof, 1958; Kloft, 1960a,b; Schmidt, 1960; Marek, 1961; Mittler and Dadd, 1965a). The possible functions of test-probing in host plant selection have already been discussed with reference to alatae (see p. 15 ). Probing by apterae into solutions behind parafilm membranes differs from test-probing on plants which is largely intercellular. Nevertheless, Mittler and Dadd (1965a,c) have shown that apterous M. persicae probing through parafilm membranes respond very rapidly to the chemical composition of the solution behind the membrane, supporting the view expressed earlier that test-probing may contribute to chemical discrimination between plants without the need for uptake of detectable quantities of plant sap by the aphids. Mittler and Dadd (1965a,c) also found that the duration of the initial probes through parafilm varied with

the composition of the substrate. Thus, variation in the length of test-probes by aphids on plants may be a useful indicator of the relative suitability of the plants to act as hosts for aphids. Day and Irzikiewicz (1954) found that the length of pre-feeding probes by B. brassicae on swede turnips were much greater than those of M. persicae.

The chemical composition of the host plant is thought to influence selection by apterae as already reported for alatae (see p. 16 ). But Kennedy and Booth (1954) found that in preference tests with A. fabae between spindle and sugar beet, the "relative fecundity" of the aphids on the two kind of leaf paralleled their feeding preferences less in the apterae than in the alatae. The reverse order (apterae≥alatae) occurred in preference tests between different aged leaves on the same plant. Kennedy and Booth suggested, therefore, that the apterae responded primarily to "nutrient" stimuli in host plant selection, thereby largely governing the distribution of a population of aphids on the host plant, as deduced from previous work (Kennedy et al, 1950). Soluble nitrogen compounds (amino-acids and amides) and sugars are often regarded as important "nutrient" stimuli. Mosbacher (1963b,c) and Alikhan (1960a) found that Dactynotus jaceae L. and A. fabae, respectively, settled preferentially on plants containing higher quantities of amino-acids (lower sugar/nitrogen ratio). Similar preferences are thought to underly the preferences shown by apterae for plants in particular physiological conditions including growing rather than mature and senescing plants (Paschke, 1959;

Heathcote,1962; Mosbacher, 1963c, 1964), and diseased or damaged rather than healthy plants (Kennedy 1951a, 1958; Baker, 1960; Mackinnon, 1961; see also Pennah, (on thrips) 1963, 1965).

The proposed importance of the sugar and nitrogen content of plants in host plant selection by apterae has received support from artificial feeding experiments. Mittler and Dadd (1964b, 1965c) found that apterous M. persicae offered a variety of solutions behind parafilm membranes settled to feed on a mixture of sucrose and six amino-acids in preference to a more complex diet, the six amino-acids alone, sucrose alone or water (see also Auclair, 1965). While feeding through parafilm may more closely resemble intracellular feeding than intercellular test-probing by aphids (see above), the results nevertheless suggest that the sugar/nitrogen ratio and soluble nitrogen components of the plant sap may influence host plant selection by apterae. Likewise, the pH values of plant sap may contribute to host plant selection by apterae. Auclair (1965) found that A. pisum fed on a complex diet with a pH of 7.3 and 7.6 in preference to other pH values (cf. Marek, 1961).

While many aphids are known to exercise preferences for different species and varieties of plants (Jones, 1942; Itô, 1955; Taylor, 1959; Müller and Hennig, 1960; Ehrhardt, 1963; Mosbacher, 1963a,1964; Wyatt, 1965), few such preferences are understood. In addition to the chemical composition of plants, their colour, internal structure (Hennig, 1959b, 1963), external structure, such as the pilosity of leaves (see

Pollard and Saunders, 1956), and microclimate may influence host plant selection by apterae. The increased restlessness of apterae on wilting plants compared with turgid plants (Kennedy et al, 1950; Kennedy et al, 1958; Marek, 1961) suggests that apterae may prefer turgid plants for feeding, as do alatae (p. 21 ).

Selection of the part of the host plant

The distribution of an aphid population on a host plant results primarily from the feeding preferences exercised by the apterae (Ibbotson and Kennedy, 1950; Kennedy et al, 1950; Kennedy and Booth, 1951,1954). The feeding preferences of apterae of several species of aphids, as indicated by their distribution on plants, are shown in Table 2.

Table 2 Preferred feeding sites of apterae

| Aphid species | Plant species | Preferred feeding site | Reference |
|---|---|---|---|
| Acyrthosiphon pisum Harris. | 1) Sweet pea | Mature leaves | Hull (1964) |
| | 2) Bean | (as 1) | Lowe & Taylor (1964) |
| | | All ages of leaf | Tambs-Lyche & Kennedy (1958) |
| Acyrthosiphon sp. | wheat | Mature leaves | Chu, Han and Wang (1961) |
| Aphis abbreviata Patch. | potato | Old leaves | Bradley (1952) |
| Aphis fabae Scop. | 1) bean | Young leaves and shoots | Tambs-Lyche & Kennedy (1958) Banks (1958) |
| | 2) Various | (as 1) | Alikhan (1960a) |

Table 2 (cont.)

| Aphid species | Plant species | Preferred feeding site | Reference |
|---|---|---|---|
| Aphis fabae Scop. | 3) Sugar beet & spindle | (as 1) & late mature lvs. & flower shoots. | Kennedy et al (1950), Ibbotson & Kennedy (1950). |
| Aphis gossypii Glover | Cotton & bamia | Old leaves | Khalifa & Sharaf El-Din (1964) |
| Aphis maidis Fitch | Barley | Young leaves | Noda (1960) |
| Aphis nasturtii Kltb. | Potato | All ages of leaf | Taylor (1955) |
| Aphis pomi Deg. | Apple | Young lvs. & shoots | Sokolov & Sokolova (1952) |
| Brevicoryne brassicae L. | 1) Cabbage | Young & mature lvs. Flower & seed-heads | Markkula (1953) Miyashita (1953) Hughes (1963) Otake (1966) |
| | 2) Kale | Flower shoots | Hughes (1963) |
| Macrosiphum granarium Kirby. | Wheat | Young leaves | Chu, Han & Wang (1961) |
| Macrosiphon koehleri L. | Hydrangea | Young leaves | Lindemann (1948) |
| Macrosiphum solanifolii Ashm. | Potato | Young leaves | Bradley (1952) |
| Myzus persicae Sulz. | 1) Sugar beet | Young and old lvs. | Kennedy et al (1950) Ibbotson & Kennedy (1950) |
| | 2) Sugar beet & spinach | Young lvs. | Heathcote (1962) |
| | 3) Lettuce | All ages of lf. | Heathcote (1962) |
| | 4) Potato | Old lvs. | Bradley (1952) Taylor (1955) |

Table 2 (cont.)

| Aphid species | Plant species | Preferred feeding site | Reference |
|---|---|---|---|
| Myzus persicae Sulz. (red form) | 5) Cabbage | Old lvs. | Miyashita (1953)<br>Ôtake (1966)<br>Tanaka (1957) |
| (green form) | | Young lvs. | Tanaka (1957) |
| | 6) Chrysanthemum | Young lvs. & flower shoots | Wyatt (1965) |
| | 7) Tobacco Nicotiana sp. | Young and old lvs. | Michel & Chouteau (1963a,b) |
| | N.gossei & N.repanda | Old lvs. | Thurston (1961) |
| | 8) Radish | All ages of lf. | Ôtake (1966) |
| Neomyzus circumflexus Buckt. | Cyclamen | Young lvs. | Lindemann (1948) |
| Rhopalosiphum padi L. | Rice | Young lvs. | Ôtake (1966) |
| Rhopalosiphum prunifoliae Fitch. | Wheat | Old lvs. | Chu, Han & Wang (1961) |
| Therioaphis maculata Buckt. | Alfalfa seedlings | Cotyledons & lvs. (susc)<br>Petioles (res) | Kindler & Howe (1961) |
| Toxoptera aurantii Boy. | Citrus | Young lvs. & shoots.<br>Flower shoots. | Rivnay (1938) |

Changes in the distribution of aphids on a host plant may accompany changes in the physiology of the plant, such as flowering. Wyatt (1965) found that M. persicae moved from the old leaves to the flowering shoots of chrysanthemums which had completed their vegetative growth (cf. Kennedy et al, 1950).

In addition, increasing density of aphids on a plant is associated with the dispersal of aphids and colonisation of parts of the plant normally devoid of aphids (Wyatt, 1965).

Although the distribution of aphids on plants may indicate the feeding preferences of the aphids, direct measurement of such preferences are few. Kennedy and Booth (1951,1954) confirmed the leaf age preferences of A. fabae on spindle and sugar beet expected from the distribution of the aphids on the plants, (Table 2). Differences between the leaf age preferences of several aphid species on the same host plant facilitate segregation of the species on the plant and reduce interspecific competition (Miyashita, 1953; Itô, 1954, 1960a; Chu, Han and Wang, 1961; Ôtake, 1966).

Colour preferences, such as that shown by A. pisum for orange-coloured diet (Cartier and Auclair, 1964), may have contributed to the leaf age preferences in Table 2. However, the frequent concentration of aphids on the young and/or old leaves rather than on mature leaves is normally attributed to differences in the chemical composition of the sap of different leaves and particularly to the higher levels of soluble nitrogen known to occur in growing and senescing plant tissues (Burr, 1957; Mittler, 1958c; Weatherley, Peel and Hill, 1959). Similar mechanisms are thought to govern the preference of some aphids for flowering shoots (Table 2) and for excised leaves (MacKinnon, 1961) in which the concentration of free amino-acids increases after detachment from the plant (Fennah, 1965). Further evidence to support the existence of nutrient prefer-

ences in aphids is given on p. 24 . Occasionally, "toxins" are thought to occur in the non-preferred parts of the plants (Thurston, 1961) or "odd" chemicals, acting as repellents to all aphids but those highly adapted to the plant, are thought to occur in the mature leaves (Kennedy and Booth, 1951; Wyatt, 1965).

Some authors have found a correlation between the osmotic pressure of the sap of plant tissues and the distribution of aphids on the plant (Sokolov and Sokolova, 1952; Pintera and Ulrychová, 1959; Weismann, 1960; Kloft, 1960a,b). All these authors found that the aphids settled to feed preferentially on plant parts of lower osmotic pressure. Weismann (1960) related the preference of the aphids for tissues of low osmotic pressure to their high turgor pressure, assisting aphid feeding (see p.34 ), and their lower sugar/nitrogen ratio. Sugar may be repellent to aphids at high concentrations (Thorsteinson in Kennedy, 1958; cf. Mittler and Dadd, 1963b). The leaf age preferences shown by aphids on turgid plants may be altered by loss of turgor. On herbaceous plants suffering water strain, apterae either move to the apices of the plants (Dickson and Laird, 1962; Hull, 1964) or become more evenly distributed over the plants, including the mature leaves in which premature senescence may occur (Kennedy et al, 1950).

Apterae and alatae select not only different aged leaves but also different parts of leaves for feeding. Most aphids, probably in response to light intensity, feed on the shaded abaxial surface of leaves (Kennedy, 1951a) though adaxial

feeding has been recorded on both young (Wyatt, 1965) and old (Khalifa and Sharaf El-Din, 1964) leaves. Ibbotson and Kennedy (1951) found that apterous A. fabae walked preferentially along prominent leaf veins rather than across the smooth areas of leaves; Ibbotson and Kennedy (1959) showed that apterous A. fabae probed more times when astride an artificial semi-circular wax ridge, which may be likened to a prominent leaf vein, than when astride a flat wax ridge of equal cross section. Both these phenomena may contribute to the often observed concentration of feeding aphids along the veins of leaves.

Thus, by a complex series of preferences, only partially understood, aphids arriving on a host plant reach a position on the plant where they remain long enough to feed and reproduce. Continued exercise of these preferences by the subsequent growing population of aphids usually maintains the population on the preferred plant parts, which may change with changes in the physiology of the host plant. Some species of aphid, however, (e.g. B. brassicae, Hughes, 1963), may not redistribute in this way and may perish on deteriorating plant parts.

b) Development of Established Populations on the Host Plant

Feeding and reproduction by an aphid (and autolysis of the flight muscles of alatae, -Johnson, 1958) are normally regarded as indicators of the establishment of an aphid on a plant and the termination of behaviour connected with host plant selection. Yet further acts of selection accompany the deep insertion of aphid stylets into the host plant before (and

during) feeding (see below). The literature on feeding by aphids was reviewed by Auclair (1963,1964) (see also Banks, 1965).

The Penetration of plants and selection of a feeding site within the host plant

Little is known about the effects of host plants on stylet penetration by aphids. Many workers have found that the penetration of plants by the stylets (and salivary sheaths) of aphids is normally by a combination of inter- and intra-cellular routes, the destination usually being the phloem (Zweigelt, 1914; Brandes, 1923; Davidson, 1923; Horsfall, 1923; Smith, 1926; Rawitscher, 1933; Heriot, 1934; Tate, 1937 (15 sp. of aphid); Dykstra and Whitaker, 1938; Leonhardt, 1940; Roberts, 1940; Michel, 1942; Emery, 1946; Lindemann, 1948; Chatters and Schlehuber, 1951; Mittler, 1954, 1957; Skotland and Hagedorn, 1955; Diehl and Chatters, 1956; Pollard, 1958; McMurtry and Stanford, 1960; Sorin, 1960 (34 sp. of aphid), 1961, 1962a,b,c; Esau, Namba and Rasa, 1961; Zimmermann, 1961 1963; Guthrie, Campbell and Baron, 1962; Ehrhardt, 1963; Hennig, 1966). Much less frequently the stylets have been shown to end in individual sieve tubes (Mittler, 1954; Zimmermann, 1961, 1963) or a variety of individual phloem cells (Hennig, 1966). Hennig (1966) found that the salivary sheath and mandibular stylets of A. fabae ended at the wall of the phloem cell, the maxillary stylets only entering the cell lumen (see also Mittler, 1957; Miles, McLean and Kinsey, 1964).

The distance from the (abaxial) surface of leaves to the phloem may be critical in relation to the lengths of the stylets of aphids and may thereby influence the selection of a feeding site on the plant (Pollard, 1958; Sorin, 1962c). Aphids sometimes penetrate, apparently without difficulty, to the phloem of non-host plants before withdrawing their stylets (Ehrhardt, 1963). However, a number of the structural properties of plants, hosts and non-hosts, may hinder stylet penetration. These include the cuticle (Zweigelt, 1914; Leonhardt, 1940 Bobinskaya, 1953) and the internal supporting tissues, such as sclerenchyma (Emery, 1946; Sorin, 1961; Entwistle and Longworth, 1963). Emery (1946) suggested that the deposition of lignin in the walls of the pericycle cells of alfalfa may have contributed to the resistance of the plants to pea aphids under certain environmental conditions, including water shortage. On the other hand, Chatters and Schlehuber (1951) were largely unsuccessful in relating the leaf structure and the resistance to Toxoptera graminum Kond. of plants. Aphids penetrate different plant tissues at different rates (Pollard, 1958) perhaps owing to the greater mechanical resistance of certain tissues to stylet entry or as a result of their chemical resistance to the action of salivary enzymes. These may include pectinase, to hydrolyse the middle lamellae of the cell walls (Adams and McAllan, 1956, 1958; McAllan and Adams, 1961), and a "cellulose hydrolysing factor" (Adams and Drew, 1965).

The host plant may influence the ability of aphids to locate the phloem for feeding: McMurtry and Stanford (1960)

found that the stylets of spotted alfalfa aphids penetrated the phloem less frequently on resistant than on susceptible alfalfa plants. However, this type of host plant influence on aphids can be identified with certainty only when the mechanism(s) governing phloem location is known. This may be 1) an increasing pH gradient from the epidermis to the phloem (Fife and Frampton, 1936; see also Emery, 1946; Marek, 1961; Auclair, 1965), or a similar rising gradient of 2) cell turgor or 3) sugars and amino-acids in the cell sap (see Roeckl, 1949); 4) Phloem location may be a process of trial and error penetration (Horsfall, 1923; Ehrhardt, 1961, 1963), the contents of the phloem sap acting as an arrestant when reached (Dadd and Mittler, 1965b; Mittler and Dadd, 1965a). The pH of the plant sap may also affect phloem location by disturbing the action of salivary enzymes during stylet penetration (Emery, 1946; cf. Harter and Weimer, 1923). The form of the salivary sheath, one of the two types of saliva produced by aphids (see Miles, 1959), varies with the host plant penetrated (Day and Irzikiewicz, 1954).

Feeding

Several authors have found a delay after stylet insertion of from several minutes to several hours in the uptake of radioactive phosphorous from plants by aphids (Day and Irzikiewicz, 1953; Watson and Nixon, 1953; Banks and Nixon, 1959; Hennig, 1959a, 1966; Ehrhardt, 1961, 1963; see also Martini, 1958). This delay is thought to represent, and was

shown by Hennig (1966) to be similar to, the time taken for the stylets to reach the phloem, which, therefore, may be the sole source of food for some aphids (Hennig, 1959a).

The histological evidence that aphids feed in the sieve tubes is scanty (see above). However, the continuous exudation of sap from the cut stylets of feeding aphids, first demonstrated by Kennedy and Mittler (1953) with Tuberolachnus salignus Gmelin on willow, is evidence that aphids probably feed in sieve tubes, whose contents can be rapidly replaced to maintain sap flow (Mittler, 1957). This has been supported by further studies of stylet sap (van Soest and de Meester-Manger Cats, 1956; Mittler, 1958a,c; Weatherley, et al, 1959; Peel and Weatherley, 1959, 1962, 1963; Alikhan, 1960a; Canny, 1961, 1962; von Dehn, 1961a,b; Ehrhardt, 1962; Hill, 1962, 1963; Peel, 1963, 1964, 1965; see also Tammes, 1957; Zimmermann, 1961, 1963; Weatherley, 1962; Hennig, 1966). It would seem that lateral flow between phloem cells is much slower than the longitudinal flow within sieve tubes (Weatherley et al, 1959; Hill, 1963).

The rate of exudation of stylet sap is thought to represent the "normal" rate of feeding of aphids (Mittler, 1957) which must, in view of the high pressures (estimates up to 40 atmosph.) required to maintain this rate, depend to some extent on the turgor pressure in the sieve tubes for "normal" feeding. Water strain and dormancy in the plant, by reducing the rate of flow in the sieve tubes, reduce the rate of sap exudation and may increase sap viscosity (sucrose concentration) (Mittler,

1957; Weatherley et al, 1959; Hill, 1962; Peel and Weatherley 1962, 1963). Water strain may therefore impede sap uptake by feeding aphids (Lamb, 1955; Kennedy et al, 1958; Kennedy and Booth, 1959) and may in turn reduce their rate of excretion (Maxwell and Painter, 1959); nevertheless this impediment is by no means as great as may be anticipated in other plant cells. The maintenance of flow of stylet sap indicates that turgor is maintained in the sieve tube even under extreme water strain (Weatherley et al, 1959; Crafts, 1961). The rate of sap exudation also falls when plants are moved from dark to light (Peel and Weatherley, 1962). Turgor pressure in the sieve tube is thought to force sap through the open end of the salivary sheath (see Miles, et al, 1964) and up the minute stylet food canal, the aphids being required only to swallow the sap (Mittler, 1957; Kloft, 1960a,b; cf. Zweigelt, 1914; Leonhardt 1940).

Since feeding and excretion by aphids are not continuous and may, for example, increase in rate when the aphids are attended by ants, Banks and Nixon (1958) suggested that the so-called "sucking-pump", by acting as a valve which opens periodically, may control the flow of sap under pressure into the gut of the aphid (see also Auclair and Cartier, 1960b; Ehrhardt, 1963; Banks, 1965). This same pump may act as a true "sucking-pump" when aphids feed on plant sap or an artificial diet without pressure (see Schmidt, 1960; Mittler and Dadd, 1962 et seq; Forbes, 1964).

Auclair (1959) (also Auclair and Cartier, 1960b; Auclair and Maltais, 1961) found that the rate of feeding by A. pisum was greater on susceptible than on resistant varieties of peas (cf. Pathak and Painter, 1958). The rate of excretion by aphids may be used to estimate their rate of feeding so long as the external environment does not fluctuate (Schaefer, 1938; Broadbent, 1951; Mittler, 1958a). Thus, the rate (frequency×honeydew droplet volume) of excretion (≡ feeding) of aphids on plants has been found to increase with temperature (Smith, 1937; Maxwell and Painter, 1959; Auclair, 1963; Ehrhardt, 1963), with the susceptibility of the host plant (Auclair, 1958b; Maxwell and Painter, 1959; Taylor, 1959), with light intensity (Maxwell and Painter, 1959) and with water supply to the host plant (l.c.). Auclair (1959) found no effect of photoperiod on excretion by pea aphids on peas but the rate of excretion decreased with the age of the plants and the frequency of excretion was greater when the aphids fed at leaf veins than when they fed elsewhere on the leaf (cf. Maxwell and Painter, 1959). Dixon (1963) found that Drepanosiphum platanoides (Schr.) produced honeydew droplets of similar volume much more frequently on mature than on young leaves of sycamore and Maxwell and Painter (1959) found a variety of effects of plant parts on the frequency of excretion by T. graminum and Therioaphis maculata Buckt.

The host plant may also influence aphid feeding by varying the restlessness of the aphids. Aphids are more restless 1) on wilting than on turgid plants (Davidson, 1923;

Emery, 1946; Kennedy et al, 1950; Mittler, 1957; Kennedy, 1958; Kennedy et al, 1958; Marek, 1961), 2) on resistant than on susceptible plants (Auclair, 1959; Cartier, 1959, 1963; McMurtry and Stanford, 1960), and 3) on dormant than on growing plants (Mittler, 1957; Hill, 1962).

There is abundant evidence, therefore, that the host plant exerts a strong influence on the rate, frequency and duration of feeding by aphids. There is little doubt that the turgor and the rate of flow of sap in the sieve tubes are partly responsible for this influence. However, aphid feeding may also be affected by the colour of the host plant (see Searls, 1935; Cartier and Auclair, 1964, 1965; Cartier, 1965), by chemical stimulants and inhibitors in the sieve tube sap, (see below), and perhaps by its pH (see Dunnam and Clark, 1941; Alikhan, 1960b; Marek, 1961; Auclair, 1965). Susceptible varieties of peas, on which aphids feed at higher rates (see above), contain greater quantities of soluble nitrogen compounds and less sugar than resistant varieties (Auclair, Maltais and Cartier, 1957; Maltais and Auclair, 1957). Auclair (1958b) suggested that the levels of nutrients, particularly soluble nitrogen, in the plants may have influenced the rate of aphid feeding (cf. Mosbacher, 1964). This is partly supported by recent evidence from the artificial feeding of M. persicae that sucrose possesses phagostimulant properties which are enhanced by amino-acids (Mittler and Dadd, 1964b). Alikhan (1960b) found that the rate of excretion by A. fabae was increased by treating the plants with sucrose. The importance of sucrose as a

phagostimulant may also have contributed to the observed greater excretion (= feeding) rates of aphids on plant tissues containing lower levels of nitrogen (higher sugar/nitrogen ratio) (Mittler, 1958c; Dixon, 1963). Subtle combinations of nutrients may affect feeding (see Dadd and Mittler, 1965a,b) and "odd" chemicals acting as feeding stimulants and inhibitors may also occur in sieve tube sap (for refs. see p.16 ).

Finally, aphids may affect their own feeding rate by injecting saliva into the host plant. The role of saliva in feeding by aphids was discussed by Nuorteva (1958), Kloft (1960a,b), Ehrhardt (1962), Auclair (1963) and Miles (1964,1965).

Growth, Survival and Reproduction

Any host plant factors which reduce the feeding of aphids may be expected to reduce similarly their growth, survival and reproduction. In addition, even allowing for the possible presence of gut symbionts in aphids (e.g. Toth, 1950, 1952), the phloem sap of plants would seem to provide aphids with their main source of nutrients after birth. Thus, the growth, survival and reproduction of aphids are closely related to the quantity and quality of the sap ingested from the phloem of plants.

The artificial feeding of aphids (apterae), which has made rapid progress in recent years (e.g. Mittler and Dadd, 1962 et seq.), is now providing details about the effects on aphids of the chemical composition of their food. Dadd and Mittler (1965b) found that an optimal diet for the growth, survival and larviposition of M. persicae contained 10-20%

sucrose, 0.3% amino-acids, c.700ug/ml of potassium, c.60ug/ml of magnesium, and 400-800ug/ml of phosphate (cf. Auclair and Cartier, 1963, 1964). Vitamins were apparently passed from mother to larva before birth; sugar was essential for survival, the longevity of the adults being unaffected by the omission of amino-acids from the diet; amino-acids were essential for larval growth and for high rates of larviposition; the salts, containing potassium, magnesium and phosphorus, were essential for larval growth and their absence reduced adult survival and larviposition; omission of vitamins from the diet of the larva and mother (for 1 day+) reduced larval growth. The relative proportions of different amino-acids in the diet of aphids may also affect the growth of some species e.g. A. pisum (Auclair and Cartier, 1963), while the roles of other nutrients, such as lipids, are, as yet, undetermined (Dadd and Mittler, 1965b; see Strong, 1963 a,b, 1965). Strong and Sakamoto (1963) found that basically the same amino-acids are nutritionally essential for M. persicae as for other insects. Bragdon and Mittler (1963) and Auclair (1965) found differential utilisation of dietary amino-acids by M. persicae and A. pisum respectively.

Clearly, any interpretation of the effects of the host plant on the growth, survival and reproduction of aphids must take into account at least the sugar, amino-acid and mineral content of the sieve tube sap. Dadd and Mittler (1965a,b) pointed out that, despite the greater feeding rates of aphids on plants than on artificial diets, sub-optimal concentrations of

nutrients, particularly amino-acids, may be expected to occur in the sieve tube sap of plants - cf. artificial diets (loc.cit.) and sieve tube sap (e.g. Peel and Weatherley, 1959; Zimmermann, 1960, 1961; Ziegler, 1962; Ehrhardt, 1962). The composition of the sap varies considerably with, for example, the plant species (von Dehn, 1961b) and its age (Lindemann, 1948; Mittler, 1953, 1958a, b, c).

Details of aphid nutrition are a recent study. Many early authors, who recorded the effects of different plants and plant parts on the growth, survival and reproduction of aphids (see below), related the effects to the soluble nitrogen content of the plant, with less regard for other sap constituents or the amount of sap uptake by the aphids. This work may require re-appraisal in the light of modern findings from the artificial feeding of aphids and the now known presence of nitrogen compounds in aphid honeydew (e.g. Maltais and Auclair, 1952; Auclair, 1958a; Lamb, 1959).

A large number of species and varieties of plants are known to be resistant to aphid attack, reducing the growth, survival and larviposition of the aphids, but the nutritional mechanisms governing these resistances (antibioses) have not been investigated (e.g. Harrington, 1941; Heathcote, 1962; see the reviews of Painter, 1958b, 1961).

The nitrogen content of plants

Auclair and Maltais (1950), Maltais (1951), Maltais and Auclair (1957) and Auclair, et al (1957) found that the

varieties of peas resistant to the pea aphid (e.g. 'Melting Sugar') contained lower levels of nitrogen (N) and higher levels of sugars (S) (higher S/N ratio) than the susceptible varieties (e.g. 'Perfection'). Auclair et al (1957) and Auclair and Maltais (1961) suggested that the lower concentrations of amino-acids in the resistant varieties (and the reduced rate of feeding of the aphids - see above) reduced the rate of growth and reproduction of the aphids. Aphids on the resistant varieties grow and reproduce at rates comparable to those of semi-starved aphids (Auclair and Cartier 1960a,b). Lindemann (1948), studying Neomyzus circumflexus Buckt. and Macrosiphon koehleri L.,, and Alikhan (1960a,b), studying A. fabae, found similar relationships between the growth and reproduction of the aphids and the soluble nitrogen content (and S/N ratio) of the host plants (see also Evans, 1938; Marble, Meldeen, Murray and Zscheile, 1959; Shaposhnikov, 1959a; Shaposhnikov and Eliseev, 1960; Kloft, 1960a, b; Weismann, 1965). Banks and Macaulay (1964, 1965), in a detailed study of the feeding and nutrition of A. fabae whose fecundity differed on two varieties of beans, found that the nitrogen content of the sap of both varieties seemed more than adequate for aphid growth and reproduction. However, differences in the nitrogen constituents of the sap of the plants were thought to account for the differences in aphid fecundity.

Higher levels of soluble nitrogen compounds (and lower S/N ratio) in the plant sap are usually thought to account in part for the greater growth and/or reproduction of aphids on:

i) young than older plants (Lindemann, 1948+; Sokolov and Sokolova, 1952; Markkula, 1953; Mittler, 1958c+; Weismann, 1965; Auclair, 1966 (alfalfa)).

ii) old than younger plants (Hirano and Itô, 1964++; Auclair, 1966 (pea)).

iii)young and old than mature plants (von Tokmakoglu, 1965)

iv) rapidly growing or senescing than slowly growing or senescing plants or plant parts (Cutright and Huber, 1928; Cutright, 1930; Ibbotson and Kennedy, 1950; Taylor, 1955; Kennedy, 1958; Müller, 1961++; Khalifa and Sharaf El-Din, 1964; see also van Emden, 1964++).

v) young than older leaves (Lindemann, 1948+; Kennedy and Booth, 1954; Itô, 1955; Lamb, 1955; Kennedy et al, 1958++; Alikhan, 1960a; Noda, 1960; Wildbolz, 1965++).

vi) young and old than mature leaves (Kennedy, 1951b; Kennedy and Booth, 1951; Markkula, 1953; Kennedy, 1958; Mittler, 1958c+; Dixon, 1963, 1966+; Mosbacher, 1964; Khalifa and Sharaf El-Din, 1965++).

vii)flowering shoots than vegetative shoots or leaves (Dahms and Painter, 1940; Markkula, 1953; Lamb, 1961; Hughes, 1963).

viii)discs of mature leaves than discs of leaves of other ages (Müller, 1966+).

ix) undamaged than damaged leaves (by aphids) (Barker, 1952; Kloft 1960a,b; Hughes, 1963++; von Tokmakoglu, 1965).

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+ aphid size was measured
++ aphid survival was similarly affected

x) diseased than healthy plants (Kennedy, 1951a,b; Baker, 1960++).

xi) moderately diseased than healthy or severely diseased plants (Markkula and Laurema, 1964).

xii) healthy than diseased plants (Lowe and Strong, 1963++).

xiii) excised then attached leaves (Thomas, Sorenson & Painter, 1966 - aphid survival only affected).

xiv) 2-4D treated than untreated plants (Maxwell and Harwood, 1960).

xv) untreated than caffeine treated plants (Kessler, Swirski and Tahori, 1959).

In the above list, those authors who measured the nitrogen content of the plants (Lindemann, 1948; Lamb, 1955; Kennedy et al, 1958; Mittler, 1958c; Kessler et al, 1959; Maxwell and Harwood, 1960; Dixon, 1963, 1966; Markkula and Laurema, 1964; Weismann, 1965) found a positive correlation with the growth, size or reproduction of the aphids. Carter (1962) suggested that the high carbohydrate content of diseased plants may increase their nutritive value for aphids. However, the variable effects of diseased plants on aphids (see list) correlate positively with the known effects of the diseases on the amino-acid content of the plants (for refs. see Markkula and Laurema, 1964), though aphid species sometimes differ in their responses to the same disease (loc. cit; Laurema, Markkula and Raatikainen, 1966). Different amino-acid requirements of aphid

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+ aphid size was measured
++ aphid survival was similarly affected

species (see Marble et al, 1959; van Emden, 1966) may account for this and other variations in the listed aphid responses to plants, whose amino-acids vary in concentration in, for example, different leaves (Steward, Wetmore, Thompson and Nitsch, 1954; Plaisted, 1956, 1958). Some aphids are thought to receive adequate nitrogen from every part of a host plant in all stages of its development (see McMurtry, 1962; Hirano and Itô, 1964; Mosbacher, 1964).

In view of this probable variability of the nitrogen (quantity and quality) supply (in plants) and demand (by aphids), plus the many other host plant factors known to affect aphid feeding and nutrition, it is not surprising that the experiments investigating the growth and reproduction of aphids on plant parts treated with nitrogen compounds or on plants receiving different levels of nitrogen fertilisers have produced very variable results (see van Emden, 1966); furthermore, the different forms of nitrogen applied to plants have different effects on their amino-acid composition which have rarely been analysed (e.g. Maltais and Auclair, 1962). Some workers suggested from observations that the reproduction of aphids and other Homoptera increased with the amount of nitrogenous fertiliser supplied to the host plants (Dahms and Fenton, 1940; Schoene, 1941). This has been supported by experimental work (McGarr, 1942, 1943; West, 1945; Haseman, 1946; Barker and Tauber, 1951b; Barker, 1952; Bobinskaya, 1953; Goon, 1959; Pennah, 1959, 1960 (thrips); Alikhan, 1960b (aphid weight); Singh and Painter, 1965b; van Emden and Wearing, 1965;

Metcalfe, 1965; Vijverberg, 1965; Waghray and Singh, 1965; Branson and Simpson, 1966; van Emden, 1966), though some authors found a nitrogen supply to the plants which seemed optimal for aphid reproduction (e.g. Singh and Painter, 1965b). Other authors have found a negative correlation between aphid reproduction and nitrogen supply to the host plant; (Barker and Tauber, 1954; Blickenstaff, Morey and Burton, 1954; Daniels, 1957; Hukusima, 1958) or no correlation at all (Lindemann, 1948; Barker and Tauber, 1951a (aphid development); Taylor, Apple and Berger, 1952; McMurtry, 1962). El-Tigani (1962) concluded that nitrogen deficiency in plants normally favours aphid reproduction while nitrogen excess may retard or accelerate reproduction. Reduction in the size of plants receiving low levels of nitrogen may also lower the reproduction of aphids in free infestations by increasing aphid density (Branson and Simpson, 1966; see also Arant and Jones, 1951; Coon, 1959).

Maltais (1959) and Maltais and Auclair (1960, 1962) found that the growth of pea aphids was increased or decreased depending on the nitrogen compounds supplied to and contained in pea cuttings on which the aphids were feeding. Michel (1963) and Michel and Chouteau (1963a,b) found a negative correlation between the reproduction of M. persicae and a) the nitrogen supplied to leaf discs and b) the nitrogen content of leaf discs of tobacco.

The mineral content of plants

Differences in the growth and reproduction of aphids may be associated with differences in a) the application of

mineral fertilisers to host plants, or b) the mineral content of host plants.

Aphids usually grow and reproduce more rapidly when the application of potassium fertilisers to the host plant is reduced (Loew, 1924; Fennah, 1959; El-Tigani, 1962 (contains further refs.); McMurtry 1962; van Emden and Wearing, 1965; Waghray and Singh, 1965; van Emden, 1966), though this may vary with the aphid biotype (Singh and Painter, 1965b) and some authors have found no correlation (e.g. Taylor et al, 1952; Vijverberg, 1965). The increased reproduction of aphids on potassium deficient plants is often attributed to the increased soluble nitrogen content of the plants resulting from hastened senescence or retarded protein synthesis (see Bonner, 1950; Thompson and Morris, 1956; Fennah, 1960; McMurtry, 1962; van Emden, 1966). Michel (1963) and Michel and Chouteau (1963a,b) found that the fecundity and reproductive rate of M. persicae on leaf discs of tobacco increased with the potassium supply to, and content of the leaf discs. Furthermore, the fecundity of the aphids was lower on young than old leaves (cf. Legay and Reggi, 1964), which contained a higher percentage of potassium.

Michel and Chouteau (loc.cit.) found a very small positive effect of the phosphorus supplied to tobacco on the fecundity of M. persicae. A more extensive positive correlation was found by Loew (1924), Dahms and Fenton (1940), Barker and Tauber (1951a), Barker (1952) and McMurtry (1962), a negative correlation was found by El-Tigani (1962), and no correlation

was found by Haseman (1946), Taylor et al (1952), Coon (1959) and Fennah (1959). Variations in the supply of other minerals (e.g. calcium and magnesium) to plants have little effect on their aphid populations (Haseman, 1946; Barker and Tauber, 1951b; Taylor et al, 1952; El-Tigani, 1962.

The Carbohydrate content of plants

Some effects on aphids of the sugar content of the host plant have already been mentioned, notably the fall of aphid growth and reproduction as the S/N ratio in the plant increases (see p.41 ). As with other constituents of artificial diets for aphids, sucrose has an optimum concentration (see p.38 ) and, therefore, an increase in the concentration of sucrose in plants may be expected to be, in different circumstances, either beneficial or detrimental to aphids, particularly to their longevity (see Dadd and Mittler, 1965b). Sucrose is the main carbohydrate constituent of plant sap (see e.g. Zimmermann, 1960; Ehrhardt, 1962).

Evans (1938) found that the reproductive rate of B. brassicae increased with the carbohydrate content of the host plants (cabbages), which were grown under low light intensity (low carbohydrate) or daylight (high carbohydrate). Similar results were obtained by Barker (1952) and Barker and Tauber (1954) with aphids on plants under different light intensities, though Chu, Han and Wang (1961) found the reverse effect with Rhopalosiphum prunifoliae Fitch feeding on wheat. The application of sugar solutions to plants may also increase aphid reproduction (Baker, 1960; Pintera, 1965).

Detrimental effects of high sugar content in plants on aphid reproduction were reported by Alikhan (1960b), Harper (1964) and Hill (1962). Banks (1965) suggested that the carbohydrate of plant sap may particularly influence those aphid species which cease to excrete but feed slightly during post-reproductive life, e.g. A. fabae (Banks and Macaulay, 1965). He proposed that during this period the aphid gut may be gradually blocked by sugar-derived solids comparable to the solids found in the crops of aphids feeding on Chenopodiacae, such as sugar beet (Edwards, 1965a,b, 1966; see also Moericke, 1960). Martini (1958) found solidification of the gut contents of fasted aphids which had ceased to excrete.

The water content of plants

There is evidence that outbreaks or rapid multiplication of aphids and other Homoptera occur during periods of drought, (Lees, 1926 (physiological drought in the plants through lack of soil aeration during waterlogging); Withycombe, 1926; Mumford and Hey, 1930; Leonhardt, 1940; Wilson, 1946; Cottier, 1953; Walker, 1954; Taylor, 1955; Downes, 1956; Fennah, 1960; Ortman and Painter, 1960; Hobbs, Holmes, Swailes and Church, 1961; Nielson and Barnes, 1961; White, 1966). (Garman (1928) related an outbreak of rosy apple aphis to high rainfall conditions and vigorous plant growth). Although high temperature may increase the growth and reproductive rate of aphids (see below), an increased "nutritional suitability" (particularly higher levels of soluble nitrogen compounds) of the plants for the aphids is usually thought to contribute to aphid outbreaks

during drought (Mumford and Hey, 1930; Pennah, 1960; Nielson and Barnes, 1961; White, 1966). Markkula (1953) found that the reproductive rate of B. brassicae on cabbages increased with the water strain in the plants, but the leaves on which the aphids were caged senesced under water strain making interpretation of the results difficult. Markkula (loc.cit.) attributed the increased reproduction to increased concentrations of nitrogen in the water deficient plants, for which there is considerable evidence (p.61 ).

Negative effects of a reduced water content in the host plant on aphid reproduction were reported by Lindemann (1948), Sokolov and Sokolova (1952, =high osmotic pressure (OP), Lamb (1955), Kennedy et al (1958, aphid survival was also reduced), Kennedy and Booth (1959), Shaposhnikov (1959a,b), Shaposhnikov and Eliseev (1960), Weismann (1960,=high OP) and Weismann and Vallo (1963) (see also Carter, 1927, =high OP). McMurtry (1962) found no effect of water strain in alfalfa on the reproduction of T.maculata.

Lamb (1955), Kennedy et al, (1958) and Kennedy and Booth (1959), studying A. fabae on spindle, bean and sugar beet, recognised two processes operating in host plants suffering water strain and affecting aphids (cf. Addendum, p.60 ): 1) lower turgor pressure and/or increased sap viscosity reducing the uptake of food by the aphids (see p.34 ) and 2) higher soluble nitrogen content, from reduced protein synthesis and increased hydrolysis, increasing the quality of the sap for the aphids. The latter was thought to remain unavailable to the

aphids until the plants recovered turgor when re-watered or when the evaporative power of the air was reduced (e.g. after sunset). The contradictory reports in the literature were in part resolved by postulating that the effect of water strain in the host plant on aphids may be governed by 1 during continuous severe water strain and by 2 during less severe or intermittent water strain, such as often occurs in the field. Wearing and van Emden (1966) further suggested that different aphid species may vary in their dependence on turgor pressure for feeding since the reproduction of B. brassicae on Brussels sprouts suffered from increased water strain in the plants much before that of M. persicae.

Miscellaneous factors

The growth, survival and reproduction of aphids on plants may be affected by i) temperature (Smith, 1937; Rivnay 1938; Markkula, 1953; Kenten, 1955; Lamb, 1961; McMurtry, 1962; Isaak, Sorensoh and Ortman, 1963; Kawada, 1964b; Singh and Painter, 1964, 1965a; Cartier, Isaak, Painter and Sorenson, 1965; Isaak, Sorenson and Painter, 1965; Markkula and Pulliainen, 1965; Murdie, 1965 (aphid size)). This may result from temperature-induced changes in the host plants (McMurtry, 1962); 2) relative humidity (Davidson, 1921a; Isaak, Sorenson and Ortman, 1963), perhaps through influencing the turgidity of the host plant (see Bachmann, 1922). Pilose cultivars of cotton generally sustain larger populations of A. gossypii than glabrous cultivars (Dunnam and Clark, 1938; Pollard and Saunders, 1938;

Khalifa and Sharaf El-Din, 1964). Higher humidity on the pilose leaf surface may contribute to this phenomenon; 3) photoperiod (Dixon, 1963; Banks, 1965; Murdie, 1965), perhaps through influencing the sugar and soluble nitrogen content of the host plant (see Steward, Crane, Millar, Zacharius, Rabson and Margolis, 1959); 4) the hormone content of the plants (Wartenburg, 1953; Maxwell and Painter, 1962a,b,c,d; see also Nuorteva, 1956). Plant hormones are particularly important to aphid species which form galls on the host plant e.g. Eriosoma lanigerum Hausmann on apple (Wartenburg, 1953).

The development of an established population of aphids is therefore strongly influenced by the host plant, particularly the chemical and physical properties of its phloem sap. The effects on the aphids of other features of the host plant, such as its colour and structure, are probably normally of secondary importance. The diversity of the host plant factors affecting the aphids is, however, most important in the development of plant resistance to aphid attack since it may ultimately be possible to incorporate many resistance mechanisms into a single plant. Few of the effects of host plants on the growth, survival and reproduction of aphids have yet been methodically exploited in the development of plant resistance.

c) Departure from the Host Plant

Apterae may depart from the host plant in response to a variety of environmental changes, some seemingly unconnected with the host plant. The greater restlessness of apterae on some plants or plant parts has already been mentioned (p. 36 )

and this may lead to the departure of apterae from the plant. Apterae are particularly restless during the last (4th) larval instar and the pre-reproductive period after the final moult (Taylor, 1959; Ôtake, 1966). The dispersal of apterae from the host plant was shown by Itô (1960a) to increase with the density of the aphids on the plant. Greater contact between individuals under "crowded" conditions was thought to increase restlessness and hence dispersal (see also Itô, 1952a,b; Ôtake, 1954,1966). Dixon (1963,1966), in contrast to Hughes (1963), found that a similar mechanism decreased the reproductive activity of "crowded" D. platanoides by reducing the length of time that the aphids spent feeding (see also Murdie, 1965; Branson and Simpson, 1966). The size of the host plant and its effect on aphid restlessness may, therefore, be important in determining how soon an aphid population becomes "crowded" (see Ôtake, 1966).

However, the departure of aphids from the host plant is primarily achieved by alatae in the progeny of apterae (see eg. Gregory, 1917; Davidson, 1921b; Noda, 1959; Hille Ris Lambers, 1966). Alatae are responsible for the early-summer and autumn migrations of host alternation by heteroecious aphid species and, in addition, play a major role in carrying aphid populations from one host plant to another during the summer. Polymorphism in aphids was recently reviewed by Lees (1961) and Hille Ris Lambers (1966) (see also Smith, 1937; Schaefer, 1938). In the present review, the term 'alatae' refers to alate virginoparae unless otherwise stated.

The role of the host plant in determining the morph of virginoparae, which, according to Johnson and Birks (1960) and others, probably begin development as "presumptive alatae", is difficult to assess. There is abundant evidence that the proportion of alatae in a population, like the dispersalof apterae, increases with aphid density (Wadley, 1923; Ackerman, 1926; Reinhard, 1927; Davidson, 1929; Wilson, 1938; Bonnemaison 1950, 1951, 1958 (sexuales); Rattan Lal, 1952; Kenten, 1955; Kulkarny, 1956; Noda, 1958a, 1960; Paschke, 1959; Dickson and Laird, 1962; Hughes, 1963; Kawada, 1964a; Ôtake, 1966), and particularly when the early instars of aphids are "crowded" (Wadley, 1923; Noda, 1958a; Paschke, 1959). Recently, the direct interaction (contact) between other aphids and 1) the reproducing female or 2) the first instar larvae or 3) between mother and larvae has been shown to stimulate the production of alatae (or suppress the production of apterae) among the progeny of the female or among the larvae respectively (Bonnemaison, 1951; Lees, 1959, 1961; Johnson, 1965). Johnson (1965) pointed out that any increase in the restlessness of aphids (p. 36 ), such as may occur on a deteriorating host plant, may be expected to increase the contact between aphids and, hence, the production of alatae; variation in the handling of and interaction between aphids under different conditions may also have contributed to many of the previously recorded effects of the environment on the formation of alatae (see below).

Thus, the formation of alatae, like the dispersal of apterae, may be influenced by the size and condition of the

host plant in relation to aphid restlessness and the density of the aphid population.

The increased restlessness of "crowded" aphids and aphids on "unsuitable" plants also reduces the time spent feeding by the aphids. The resultant probable lower intake of food may lead to starvation, which has been found to promote the formation of alatae by some species of aphids (Gregory, 1917; Wadley, 1923; Ackerman, 1926; Reinhard, 1927; Shull, 1928; Schaefer, 1938; Rattan Lal, 1952; Noda, 1958a; Kenten, 1955). However, Paschke (1959) and Lees (1961) found no effect of starvation on the formation of alatae by T. maculata and M. viciae respectively. Lees (1961) found that the starvation of aphids in groups, but not in isolation, promoted the formation of alatae, this being a "crowding" effect. This phenomenon may have occurred in the work of earlier authors (above), though some (e.g. Shull, 1928) obtained an effect of starvation in the light but not in the dark. The stimuli for formation of alatae may also vary with the aphid species.

Greater interaction between aphids and starvation resulting from increased restlessness may therefore contribute to the increase in the formation of alatae on certain plants and plant parts (see p. 55 ). The chemical composition of the host plant has also been implicated in the formation of alatae, though, as yet, this has not been confirmed by experiments involving variation in the chemical composition of artificial diets for aphids (see Auclair, 1965; Dadd and Mittler, 1965b).

Nothing is known of the role, if any, of "odd" plant substances in the formation of alatae (cf. selection of the host plant by alatae p. 16 ).

The nitrogen and mineral content of plants

Mittler (1958c) found a greater proportion of alatae among T. salignus reared on willow containing lower quantities of nitrogen in the phloem sap (stylet sap). Similar results were obtained by other workers (Evans, 1938; Lindemann, 1948; Pintera and Ulrychová, 1959; Alikhan, 1960a). Branson and Simpson (1966) measured the percentages of alatae in populations of Rhopalosiphum (Aphis) maidis (Fitch) at equal densities on sorghum receiving high and low levels of nitrogenous fertiliser (in solution). The percentage of alatae was higher on the nitrogen deficient plants. This contrasts with the results of Paschke (1959) who found no effect of nitrogen deficiency in alfalfa on the formation of alatae by T. maculata. The proportion of alatae was increased, however, on potassium- and phosphorus-deficient plants.

Higher levels of soluble nitrogen compounds (and lower S/N ratio) in the plant sap may account in part for the lower percentage of alatae among aphids on young than older leaves (Smith, 1923; Rivnay, 1937; Lindemann, 1948; Davis, 1957 (sexuales); Kennedy et al, 1958; Noda, 1960; Hughes, 1963; Bonnemaison, 1965 (sexuales)) though Paschke (1959) found no effect of leaf age on the formation of alatae by T. maculata.

Some early workers supplied host plants with a variety of compounds in solution, some of which were called

wing-developing substances (e.g. the salts of heavy metals) and others, non-wing-developing substances (e.g. the salts of alkali metals) (e.g. Shinji, 1918). The mechanism of influence of these substances on the aphids via the host plant has not been elucidated.

The carbohydrate content of plants

Sugar solution was one of the wing-developing substances of Shinji (loc.cit.). Alikhan (1960a) found a higher production of alate progeny by apterae of A. fabae on plants with a higher S/N ratio, and later showed (1960b) that by treating host plants with sugar solution, the production of alatae was increased. Evans (1938) found no correlation between the carbohydrate content of cabbages and the proportion of alatae in populations of B. brassicae.

The water content of plants

Periods of drought and the wilting of host plants have long been thought to promote the formation of alate aphids (Kyber, 1815 (in Schaefer, 1938); Woodworth, 1908; Smith, 1923; Davies and Whitehead, 1935; Thomas and Vevai, 1940; Walker, 1954; see also Broadbent and Heathcote, 1961) and experimental work has largely supported this view.

Rivnay (1937) obtained a much higher percentage of alate Toxoptera aurantii Boy. on citrus twigs periodically denied water than on twigs continuously supplied with water. He pointed out that the wing-producing chemicals of Shinji (loc. cit.) may have reduced water uptake by the host plants, while the effect of "crowding" may be to increase the competition

between the aphids for water. Rivnay also obtained greater production of alatae as the relative humidity of the air decreased and saw this as a response of the aphids to their reduced water content resulting from increased evaporation. Rattan Lal (1955), after more extensive experiments, reiterated these views. Other authors have obtained an increased proportion of alatae in the progeny of apterae on host plants with reduced water content (Markkula, 1953; Lamb, 1955; Kennedy *et al*, 1958; Noda, 1960; Weismann, Macko and Fekete, 1960 (= high osmotic pressure)). However, in the experiments of Markkula (1953) aphid density was greater on the water-deficient plants, and Noda (1960) obtained an increased proportion of alate *Aphis maidis* (Fitch) on the wilted plants (*Holcus sorghum* L. var. *japonicus*) only when the aphids were "crowded". Lamb (1955) and Kennedy *et al* (1958) attributed the increased production of alatae on water-deficient plants to their reduced turgor pressure and/or increased sap viscosity lowering the rate of sap uptake by the aphids (see p.34 ).

Shaposhnikov (1959a,b) and Shaposhnikov and Eliseev (1960) related the migration of heteroecious aphids (e.g. *Dysaphis* sp.) in early summer to the deterioration of the primary host plant in at least three ways: (1) a lowering of the soluble nitrogen content of the leaves (also Lindemann, 1948), (2) a decrease of the water content (below 70% fresh weight) and (3) an increase in the osmotic pressure of the cell sap (10 atmospheres +) (also Weismann *et al*, 1960; Weismann, 1960); in response to these stimuli, the aphids migrated to

(secondary) summer host plants providing opposite feeding conditions i.e. high soluble nitrogen content, high water content and low osmotic pressure. In obligately heteroecious species of aphids, these stimuli for migration may have been gradually replaced by a "more perfect mechanism" dependent on a fixed number of spring generations of the aphid to "predetermine the onset of migration" (Shaposhnikov, 1959a). Shaposhnikov, on the other hand, regarded autumn migration from the secondary to the primary host plant as occurring in response to decreasing daylength and temperature independent of aphid nutrition.

Light, temperature and humidity

Among the physical factors of the environment, temperature (Davidson, 1929; Shull, 1942; and others), relative humidity (Rivnay, 1937; Rattan Lal, 1955; and others) and photoperiod (Noda, 1958b; MacGillivray and Anderson, 1964; and others) affect the formation of alatae, including gynoparae and sexuales, (Bonnemaison, 1958). Whether these factors influence the aphids directly and/or through the host plant has not been satisfactorily determined, though Lees (1964) has shown that in M. viciae photoperiod is directly perceived by the aphid. Complex interactions of many environmental factors are frequently involved in morph determination (see Hill Ris Lambers, 1966).

In summary, it would seem that modern research has established the importance of contact between aphids in alary polymorphism and that earlier work requires reappraisal in the light of this (Lees, 1961; Johnson, 1965). Many, if not all, the effects of the host plant, and/or other factors, on the format

ion of alatae may result from effects on aphid activity. For example, the greater restlessness of aphids on resistant than susceptible host plants (p. 37 ) may have contributed to the greater production of alatae on some plants (e.g. Lindemann, 1948; Markkula, 1963; Mosbacher, 1963a); similarly, both restlessness and the formation of alatae increase on wilting plants. Physical factors of the environment as well as starvation are also known to affect the activity of alatae (Broadbent, 1949) and may, therefore, similarly influence apterae.

For the present, doubt has been cast on the role of the host plant in providing direct stimuli for the formation of alatae. However, as an indirect influence at least, its importance is considerable and the host plant may similarly influence the formation of sexuparae, sexuales and other stages in the life history of aphids. Adult alate aphids typically fly away from the host plant on which they developed and do not respond to the plant by feeding and reproducing; a period of flight or other activity is normally required before alatae will settle again on a plant (Johnson, 1958).

While the host plant relations of aphids are imperfectly understood nevertheless, as this review has indicated, plants, including crop plants, offer to the agricultural entomologist an enormous reservoir of diverse resistance mechanisms against aphid attack which merit much greater use. Only during the early part of migration (by alatae) do aphids cease to be strongly influenced by plants. At all other times, mechanisms within the

host plant are available and awaiting manipulation by cultural or plant breeding methods to reduce the population of aphids on plants.

Addendum

The Effects of Water Shortage on Plants

Some of the effects of water shortage on plants are listed below. Those effects which are most likely to or are known to influence aphids, as indicated by the literature on host plant relations, are marked §.

(1) Changes of leaf colour - young leaves become darker (Burman and Painter, 1964); older leaves become more yellow§ (Kennedy et al, 1961); plasmolysis inhibits the production of chlorophyll and may affect production of other pigments (Crafts, Currier and Stocking, 1949).

(2) Greater thickness of cell walls and cuticle § (Curtis and Clark, 1950).

(3) Reduced cell size (Morton and Watson, 1948).

(4) Retarded initiation of leaves (Gates, 1964)

(5) Reduced plant growth § (including reduced cell size and reduced rate of cell growth) (Gates, 1955a,b, 1964; Owen and Watson, 1956; Burman and Painter, 1964; see also Richards and Wadleigh, 1952; Russell, 1961; and others).

(6) Increased abscission of leaves (Russell, 1961), (see 15a).

(7) Acceleration of flowering § (Richards and Wadleigh, 1952; Wearing, 1963).

(8) Reduced turgor pressure of the cell sap § (Bachmann, 1922;

Kennedy and Booth, 1958; Weatherley et al, 1959; Catsky, 1962; and others). Wilting decreases from the base to the apex of the plant § (Herrick, 1933; Tingley, 1944).

(9) Increased osmotic pressure of the cell sap (Kramer and Currier, 1950; Slayter, 1961; and others).

(10) Increased suction pressure of the cell sap (Renner, 1959).

(11) Decreased transpiration (Foster and Tatman, 1940; Russell, 1961; see Richards and Wadleigh, 1952).

(12) Reduced photosynthesis § (Stolzy, Taylor and Mersereau, 1964; see also Richards and Wadleigh, 1952).

(13) Increased sometimes followed by decreased respiration § (Iljin, 1957; Gates, 1964).

(14) Increased release of hydrolytic enzymes resulting from desiccation of the protoplasm (see (15a) and (16a)) (Sisakyan and Kobiakova, 1947 (in Kursanov, 1956); Iljin, 1957; see also Crafts et al, 1949; Richards and Wadleigh, 1952; Gates, 1964).

(15a) Increased hydrolysis (and reduced synthesis?) of proteins leading to increased soluble nitrogen§ in the old (lower) leaves (hastened senescence) (Mothes, 1926; Petrie and Wood, 1938a,b; Fennah, 1960; Subbotina, 1962 (severe wilting)).

(15b) Increased transport of nitrogen § from the old (lower) leaves to the stem but not to the plant apex (Schumacher, 1933; Gates, 1957, 1964, (also phosphorus§); see also Curtis and Clark, 1950).

(16a) Increased hydrolysis of starch and sometimes a rise of sucrose § in the old (lower) leaves (Molisch, 1921; Horn, 1923; Ahrns, 1924; Wadleigh and Ayers, 1945; Iljin, 1957; Gates,

1964; see also Richards and Wadleigh, 1952; Crafts et al, 1949).

(16b) Increased transport of carbohydrates § from the old (lower) leaves to the stem (Curtis and Clark, 1950; Gates, 1964).

(17) Reduced protein synthesis leading to higher levels of soluble nitrogen § in the plant apex and young leaves (Lamb, 1955; Kennedy et al, 1958; Gates, 1964).

(18) Changes in the overall "mineral" content of the plant - Richards and Wadleigh (1952) concluded that a decreasing supply of water to the plant is normally associated with an increase in nitrogen content §, a decrease in potassium content §, and a variable effect on the contents of phosphorus §, calcium and magnesium in the plant tissue (also Mason, 1958); some of these effects may be related to decreased and increased availability of the "minerals" in the moisture films of soil particles; boron deficiency is often associated with dry soils.

(18) Increased alkaloid content § (Sokolov, 1959).

# CHAPTER III

## THE ESTABLISHMENT AND ASSESSMENT OF WATER STRAIN IN THE HOST PLANT

Water strain in a plant describes that condition in which one or more cells of the plant are unable to obtain sufficient water to maintain turgidity. The water strain increases as the number of cells in this condition and their deficit from turgidity increase.

A great number and variety of methods exist for the establishment and assessment of water strain in plants (see Richards and Wadleigh, 1952; Hudson, 1957). No attempt will be made, therefore, to give a comprehensive review of this subject, only an introduction to the methods used in the present work.

Vaadia, Raney and Hagan (1961) have pointed out that plant internal water deficits depend at any given time on the balance between the water lost from the shoot and that absorbed by the roots. The experimental control and measurement of both these factors at one time is extremely complex, so that the water strain in plants is frequently estimated by the measurement or assessment of one only of these factors. This is commonly the water absorbed, or available for absorption, by the roots. There is another quite different and more direct approach to the estimation of the water strain in plants, which takes into account both the water lost and the water absorbed by the plants.

This approach involves measurement of some feature or features of the plant which is/are correlated with its water content, or the direct measurement of the water content itself. Water strain in different parts of the plant may be estimated by many of these methods.

The use of the plant for assessment of water strain

Various aspects of the internal water relations of plants, which can be measured to estimate their water strain include the osmotic pressure of the cell sap (e.g. Sokolov and Sokolova, 1952; Weismann, 1960) and the water content of leaves (e.g. Kennedy, Lamb, and Booth, 1958), and Hudson (1957) also lists guttation rate and the degree of stomatal opening. The last author mentions the following aspects of growth which have been related to water strain in the plant and which could possibly be used to estimate it: Carbohydrate metabolism, the dry weight of leaves, the rate of elongation of stems, the growth of leaves (see p. 96 ) and the development of fruits. The colour of the leaves of plants is also known to change with water strain (Burman and Painter, 1964).

The wilting of leaves and stems is the most obvious symptom of water strain in a plant. In consequence, this symptom has been used extensively by entomologists studying the effects of host plant water shortage on insects (Kennedy, Lamb and Booth, 1958; Maxwell and Painter, 1959; Noda, 1960; McMurtry, 1962). Wilting is a very convenient indicator of extreme water strain in the plant and permits comparison with

plants maintained in a fully turgid condition. It is not possible, however, to distinguish visually by this method any intermediate level between full turgidity and wilting. In addition, variations in the internal structure of different leaves may cause variable wilt symptoms at identical levels of water strain (Koketsu, 1928; Kennedy and Booth, 1958).

Changes in the thickness of leaves reflect changes in the turgor of their constituent cells at all levels of turgidity. Thus, Bachmann(1922) was able to detect changes in the thickness of leaves of Sanchezia sp. and Farfugium sp., even under small fluctuations in the moisture deficit of the atmosphere. For this purpose, he constructed a sensitive "hebelpachymeter" to measure the thickness of the leaves with an accuracy of about 2 microns.

All of these methods for assessment of the water strain in a plant depend on a wholly separate technique to establish this water strain. These techniques are frequently crude (e.g. ceasing to water the plants (Kennedy, Lamb and Booth, 1958)) but quite satisfactory, since the plant or part of the plant which is being measured is acting as an integrating tensiometer for all external water stresses (Hudson, 1957). Where estimation of the water strain in the plant is made from the measurement of the external water which is available to, or lost from, the plant, a more refined and carefully controlled technique is required to establish water strain.

The use of soil moisture for assessment of water strain in the plant

As previously indicated (p. 63 ), indirect estimation

of water strain in a plant is most frequently made by measuring the degree of availability of water for absorption by the roots. Many soil moisture constants have been proposed for convenient representation and standardisation of soil moisture conditions. However, Veihmeyer and Hendrickson (1950) considered that, for a given soil, only two of these constants were worthy of practical consideration. These were field capacity and the permanent wilting percentage. Field capacity is the amount of water held by a soil when excess has been removed under the influence of gravity; the permanent wilting percentage is the amount of water held by a soil when a plant, growing in the soil, permanently wilts, i.e. will not recover when put in an atmosphere saturated with water vapour. The water which is held between the permanent wilting percentage and field capacity is, therefore, termed the available water.

There have been problems in deciding whether permanent wilting should include, for example, the whole plant or only the basal leaves. Russell (1961) has pointed out, however, that these large differences in the plant (over the "wilting range") are brought about by only a very small decrease in the water content of the soil, which causes a large increase in the soil moisture tension by which water is held in the soil. As the water content of a soil decreases from field capacity, the soil moisture tension, which holds the water in the soil, and the osmotic pressure of the soil water increase slowly at first and then rapidly, making the water less and less available to the plant. The soil moisture tension and the osmotic pressure

of the soil water constitute the "total soil moisture stress". At and below the permanent wilting percentage this stress is too great for the plant to obtain water by further increase in the diffusion pressure deficit or suction pressure of the leaves. Plants vary in their ability to obtain water depending on their osmotic characteristics. For general purposes, plants with similar osmotic characteristics may be associated with a particular value of moisture content in a given soil at the permanent wilting percentage.

Other important factors which influence the availability of soil water to the plant are the volume of soil exploited by the roots of the plant, the number of root hairs, the rate of growth of the roots and the rate at which water is able to enter the roots. Where possible, the available water should be measured per unit volume of soil, to take into account variations in the density of different soils and a single soil at different depths.

Constant "total soil moisture stress"

In many experiments it is desirable to subject the plants to constant moisture conditions, either to reduce water strain in the plants as a variable environmental factor, or to study plants suffering a consistent water strain. Such techniques as have been developed for this purpose can, as yet, only be operated at low moisture stress near to field capacity, e.g. aerated water culture solutions and "constant water level" methods (Hudson, 1957), and are, therefore, of limited

application. The ability of plants to adjust to external water stress, as for example by increasing the osmotic pressure of their cells (Kramer and Currier, 1950), is well known, particularly when this stress does not increase rapidly. Visible symptoms of water strain, such as wilting, are most often associated with rapid increase in the external water stress, as near the permanent wilting percentage in a drying soil, or when the evaporative power of the air is suddenly increased. Thus, even water culture solutions of high osmotic pressure can only be used to impose water stress on plants for a short period (Groenewegen and Mills, 1961; Slatyer, 1961). Furthermore, the experiments of Richards and Loomis (1942) have shown that it is not possible to maintain a constant percentage of moisture in a soil at or near the surface of active roots.

Fluctuating "total soil moisture stress"

In order to subject plants to measurable soil moisture stress at levels throughout the whole range of available water, it is necessary to allow controlled variation of the soil moisture stress. Thus, Hudson, Salter and Majmudar (1955) considered that in studying the effect of soil moisture on plants, one should "grow plants under different, defineable and reproducible water regimes in each of which the soil moisture is allowed to fluctuate only between two predetermined conditions, one being field capacity and the other a measurable stage in the drying cycle." Water regimes of this type have been used in many experiments investigating the effects of total

soil moisture stress on different crops, and a variety of methods have been used for measurement of the drying cycle (see Stanhill, 1957).

In general, the methods for assessment of soil moisture for the control of water regimes fall into two types: 1) Those which measure the amount of available water in, or the amount of water lost from the soil, and 2) those which measure the tension at which water is held in the soil. Water regimes which are based on measurement of the amount of available water lost before restoration to field capacity do not permit comparison between different soils, since the same amount of water is held at different tensions in different soils. However, water regimes of this type are particularly suitable for pot experiments which use a single growing medium, since a tensiometer (or similar device) is not required for each pot. In addition, in a pot, the roots of a plant are often well distributed throughout the growing medium so that an average figure for the moisture content of the medium is satisfactory.

Water regimes have been little used by entomologists (Kennedy and Booth, 1959; Specht, 1965). Other water treatments, which are based on the watering of plants at fixed intervals of time or with fixed quantities of water (e.g. Markkula, 1953) give only a poor estimate of the water strain in the plants.

Water strain in the plants of the present experiments

Furr and Taylor (1939) stated that "with decreasing soil moisture, a turgor deficit arises before the first visible signs of wilting appear, or before there is a decrease in transpiration rate, and increases progressively as the soil dries out until the plant is completely wilted." Thus, a primary aim of the present work has been to study the effects on aphids of host plants suffering three levels of water strain: low (turgid), high (wilting) and medium, and to do this throughout the life of the aphids (several weeks). Water regimes clearly provided the most suitable technique for this work and, since the host plants were grown in pots, the regimes were controlled by allowing predetermined amounts of available water to be lost from the growing medium before watering to field capacity (see p. 87 ).

For short-term experiments, to study the effects on aphids of continuously increasing water strain in the host plants, the changing water strain in the plants was estimated from the concurrent changes in the available water of the growing medium (see p. 202).

Apart from the limitations regarding the accuracy of the above techniques (e.g. dependence on a uniform distribution of the roots in the growing medium), the following reservations must be made: The degree of correlation between water strain in the plant and the available water of its growing medium is difficult to assess. This correlation may be quite close where

variation in the loss of water from the plant is at a minimum, as in growth rooms with constant temperature and humidity. But in the field the water strain in the plant may fluctuate wildly with changes in the evaporative power of the air while the water content of the soil remains unchanged. Secondly, the available water gives an estimate of the general water strain in the plant only and does not take into account variations in different plant parts. Water strain in a plant is normally minimal at the apex and increases towards the base (e.g. Herrick, 1933). Thirdly, close to the permanent wilting percentage a small decrease in the water content of the soil causes a large increase in the total soil moisture stress. Greater accuracy of measurement of the available water of a very dry regime is thus needed to keep variability of the total soil moisture stress within the same limits as in wetter regimes. These limitations have been assumed to be of minor importance in the extreme water regimes of the present investigation, which has been conducted in a constant temperature growth room (see p. 76 ).

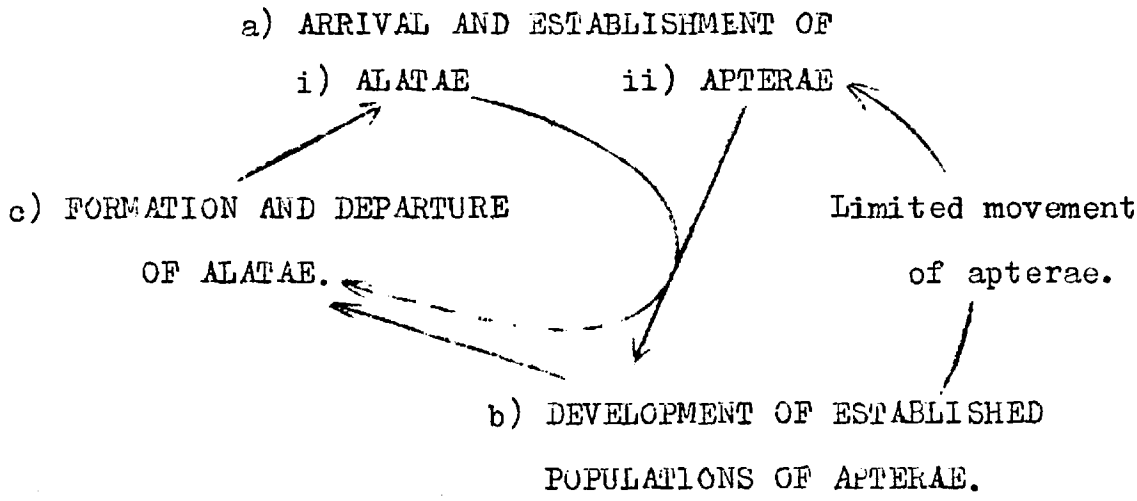
# CHAPTER IV

## EXPERIMENTATION

### PART 1. THE RESPONSES OF THE APHIDS *Myzus persicae* (Sulz.) AND *Brevicoryne brassicae* (L.) TO HOST PLANTS CONDITIONED BY INTERMITTENT WATER STRAIN OVER A LONG PERIOD:

### Section A Introduction

It is convenient and logical to examine the relationship between aphids and their host plants in stages corresponding to the cycle of movement of aphid populations from one host plant to another:




---- Alatae often produce only apterous progeny (e.g. Noda, 1959)

This simple cycle is frequently complicated by the different generations of the aphids and by alternation between a primary and a secondary host plant. Nevertheless, the same basic pattern often emerges, perhaps once or many times in a single year, in which the host plant is essentially a temporary oasis for an aphid population in a desert of largely unsuitable vegetation. The suitability of a plant as a host for an aphid depends on the relationship between the two organisms at all the stages in the cycle and on the co-ordination of the different stages. These arguments apply equally well on a smaller scale to the relationship between aphids and the different parts of a host plant where the movement from one part to another may be readily achieved by apterae.

The experiments of Part 1 were designed to investigate the influence of intermittent water strain in the host plants over a long period on virginoparae of two species of aphids, M.persicae and B.brassicae during each stage of the above cycle. Kennedy, Lamb and Booth (1958) and Kennedy and Booth (1959) made a comprehensive study of the responses of A.fabae to water shortage in host plants in pots and in the field respectively. In this work many aspects of the different stages of the above cycle were examined as well as the importance of the leaf age of the host plants. Markkula (1953) studied some aspects of stages b) and c) of the cycle for B.brassicae on cabbage using short-term experiments in which the plants were submitted to some intermittent though largely continuous water strain. The leaf

age water strain interaction was not considered. Other workers studying aphids and the water status of their host plants have been concerned solely with 1) the distribution of aphids in stage a)ii) (Weismann, 1960), 2) the reproduction of aphids for short periods in stage b) (e.g. McMurtry, 1962), 3) stage c) (e.g. Lal, 1955; Noda, 1960) or 4) honeydew production by the aphids (Maxwell and Painter, 1959). This and related literature is discussed more fully in the Review of the Literature (p. 10 ) and the Discussion (p.292 ).

With the exception of a paper by Wearing and van Emden (1966), no published reports could be found describing experimental work with M.persicae and the water status of its host plants, or long term experiments of the type to be described with any species of aphid. Unlike the work of Wearing and van Emden (1966), on free-living populations of aphids on the host plants, the present study, with the exception of the selection of the host plants by alatae, was conducted with individual aphids and small groups of aphids in cages. In all experiments the effects on the aphids of the age of the leaves of the host plants were measured. This approach to the study of the host plant relations of the aphids was based on the principles first enunciated by Kennedy and Booth (1950).

It was anticipated that the variability in the product of an interaction between two populations of living organisms would be greater than the variability in a population of one only of these organisms. For this reason, great emphasis was

placed on the production of aphids and plants as uniform as possible for use in the experiments.

## Section B General Materials and Methods

### Sub-Section 1 The Constant temperature growth room

For uniformity, all experiments were conducted in a day length of 16 hours in a constant temperature growth room at 20°C ± 1°C. Relative humidity was not precisely controlled. All aphids and experimental host plants were reared in these conditions, thus minimising the change in the physical environment of the aphids when they were moved from the culture to the experimental plants.

Light in the constant temperature room was provided by six "Daylight" fluorescent tubes, which were placed three feet above the bench. Heat and humidity were supplied in circulated air.

### Sub-section 2 The insects

Two species of aphid were studied throughout the experiments: The green peach aphid, Myzus persicae (Sulz.) and the cabbage aphid, Brevicoryne brassicae (L.) (Aphididae). These species were chosen for 1) the ease with which they could be reared and studied on the same species of host plant (see below), and 2) their contrasted sites of feeding on this plant. M.persicae most frequently feeds on the senescing leaves while B.brassicae occupies the young apex of the host plant.

Stock clonal cultures of both species of aphid (begun in October 1963) were reared continuously as apterous and alate virginoparae, i.e. viviparous, parthenogenetic females producing, and produced by, viviparous, parthenogenetic females

(see Hille Ris Lambers, 1966). Clonal cultures were developed from single alatae in an attempt to decrease the variability of the aphid populations. The cultures were housed in standard, cylindrical, insect-rearing cages made with cellulose acetate, between a metal base and lid. Butter muslin, which covered holes in the lid and walls, gave ample ventilation. The cages measured 38 cm. in height by 22 cm. in diameter.

When used in selection experiments, alatae were permitted to leave the cultures unassisted (see pp. 106 and 158). In contrast, apterae, which were feeding on the culture plants, were stroked gently with a camel-hair brush until they withdrew their stylets, before being lifted, on the brush, to the experimental plants. The apterae did not appear to suffer from this treatment.

Sub-section 3 The host plants

One species of plant, Brussels sprout, Brassica oleracea L. var. bullata gemmifera (Cruciferae), was used both to culture the experimental animals and to act as host in all experiments. Both species of aphid fed and reproduced satisfactorily on Brussels sprout, which had the added advantage of being an important crop plant. With the exception of one early experiment (p. 133 ), when the Brussels sprout cultivar "Irish Elegance" was used, the only cultivar employed was "Jade Cross" (Sutton and Sons Ltd., Reading). While the former is commercially important, the latter is genetically more uniform, a greater advantage in the present circumstances. To reduce the variability of the host plants still further, only those

seedlings showing visual uniformity were selected for experimental use.

Seeds of Brussels sprout were sown in silver sand in clay pots. At approximately the first leaf stage, the seedlings were pricked out into John Innes Potting Compost No. 1 in 3½" clay pots, where they were allowed to remain, either permanently for use as culture plants, or for 1 - 2 weeks before use in an experiment. By this time, the plants had acquired 6 - 8 true leaves. They were transferred to the experimental pots and allowed to establish for 1 week before being randomly allocated to the water regimes (p. 87 ). By growing the plants in this manner in a constant temperature and daylength, by using a uniform cultivar, and by roguing unusual plants at the seedling stage, it was possible to obtain extremely uniform Brussels sprouts for experimental work. This growing method also supplied plants of convenient size, which carried leaves of all ages, young, mature and old, (see p. 93 ), at the same time. This greatly assisted the experimental comparison of aphids which fed on different aged leaves.

Host plants, which were used to culture the aphids, were changed every 2 - 3 weeks, and were transferred to a greenhouse for the period from pricking out until their use. In winter, the greenhouse temperature and daylength were 55°F (13°C) and 16 hours respectively, but in summer there was no such control.

Sub-section 4 The experimental cages

a) Clip cages

i) The simple clip cage

In order to examine the responses of individual aphids to different leaf ages of their host plant, cages were required which would confine each insect on small, equal areas of leaves without altering the physiology of the leaves to a serious extent. Several workers have described clip cages for this purpose, (MacGillivray and Anderson, 1957; Kennedy, Lamb and Booth, 1958; Noble, 1958; Murdie, 1965). The cages which were used in the present work (Fig.1) were of the design used by Murdie (1965).

The cages were used in the measurement of reproductive and temporal parameters of the aphids (pp. 129 and 176). Figure 3 shows two cages in position on a mature leaf and an old leaf of the host plant. The figure also shows the wire supports which were required for these cages, which would otherwise have put too much strain on the petioles of the leaves. The supports allowed the leaves to remain in their natural positions.

This clip cage was simpler to construct than others previously described in the literature. It also retained the advantages of cheapness and ease of manipulation, and the lid was particularly useful in allowing easy access to the aphids. If left for several days, the cage caused some yellowing of the leaf where the polystyrene foam prevented light from reaching the leaf surface. To avoid this, the position of the cage was altered slightly each day.

FIG. 1. SIMPLE CLIP CAGE. — VERTICAL SECTION.

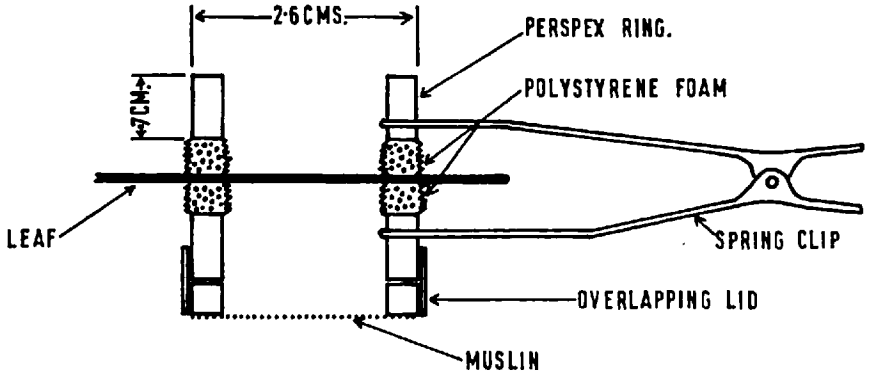



FIG. 2. PREREFERENCE CAGE. — INTERNAL VIEW FROM THE ABAXIAL LEAF SURFACE

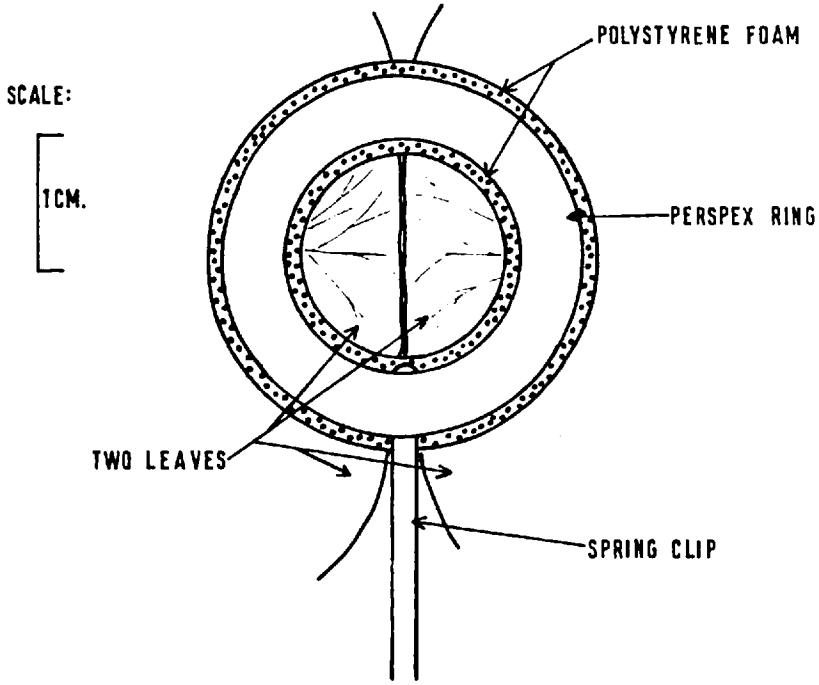


Fig. 3. Simple clip cages and leaf cages in use on a Brussels sprout plant.

Fig. 4. Preference cages in use on Brussels sprout plants.

ii) The preference cage

The preference cage was designed for exactly the same purpose as the one used by Kennedy and Booth (1950) -"to confine aphids to the under-surfaces of two equal areas of different leaves held side by side, the insects being free to move from one to the other and settle down on whichever they preferred". Their cage exposed to the confined aphids two equal areas of different leaves with a short distance between them. Initially a cage of similar design was made and tested, when it was found that the distance between the different leaves was preventing a satisfactory demonstration of preference by the aphids, in the time available. This distance was greater than in the cage of Kennedy and Booth (1950), who pointed out many of the difficulties in the interpretation of results from such experiments. In particular, they indicated that preferences seemed to result from less movement of the aphids on one leaf than on the other (kinesis), rather than from movement towards (or away from) a given leaf (taxis). In addition, the level of activity of the aphids greatly influenced the results.

For these reasons a cage was designed which confined equal areas at the edges of two different leaves which were in contact with each other (see Fig. 2). Great care was required to ensure 1) good contact between the edges of the two leaves to prevent escape of the aphids to the upper surfaces of the leaves, and 2) that equal areas of similar parts of the two leaves were caged. By having the two leaves in contact, the aphids were

able to move very easily from one to the other. Kennedy and Booth (1950) considered that this, and any gregarious tendencies of the aphids, would weaken the expression of preference. However, the proximity of the two leaves probably reduced the confusing influence of the variable activity of the aphids, and also the time required by the aphids to collect preferentially on one leaf. Furthermore, the probe-perceived differences between the leaves may possibly have been intensified by their contiguity.

The preference cage used in preference tests with apterae (pp. 119 and 168) was made by modifying the simple clip cage (see above). The top and bottom (lid) of the cage were each covered with a circle of black filter paper, and, to complete the exclusion of light from the interior of the cage, the outer perspex walls were painted black. This was done, not only to prevent the aphids from moving to the better-lit side of the cage (Kennedy and Booth, 1950), but also to prevent their expression of preferences for different qualities and/or quantities of light transmitted and reflected by different leaves.

Two preference cages are illustrated in use in Fig. 4. Wire supports were provided, where necessary, to avoid straining the petioles of the leaves, and to hold the cage horizontal to avoid any response of the aphids to gravity.

b) <u>The leaf cage</u>

The leaf cage, designed to limit aphids to the lamina of a single leaf, was used in determining the proportion of

alatae in the progeny of apterae (pp. 148 and 191).

The cage (Fig.5) was simply constructed with cellulose acetate and butter muslin. Its double wall was made with two close-fitting rings of cellulose acetate, 3.5 cms. deep, and, in different cages, varying in diameter from about 6.5 cms. to 9.0 cms., in order to accommodate leaves of different sizes. The top of the outer ring and the bottom of the inner ring were covered with muslin, which thus formed the top and bottom of the cage, and gave adequate ventilation.

In use, the inner, bottom half of the cage was moved into position underneath the raised lamina of the leaf. A notch, which was cut down into the cellulose acetate wall, held the petiole of the leaf and allowed the leaf to be lowered into the bottom half of the cage. The outer, top half of the cage could then be placed in position above the leaf. Another notch was cut up into the wall to accommodate the petiole. To prevent aphids escaping from the cage along the petiole, cotton wool was put around the petiole where it passed through the double wall of the cage. The tip of a wire support for the cage was hooked over the lower wall-notch and squeezed so as to grip the wall. The base of the wire was firmly lodged in the growing medium of the plant. In some instances, to avoid dismantling the cage to insert aphids, a small hole was cut in the muslin top. This was plugged with cotton wool when not in use.

Two leaf cages are shown in use in Figure 3.

c) The screen cage

The screen cage (Fig. 6) was essentially a rectangular

FIG. 5. LEAF CAGE — VERTICAL SECTION.

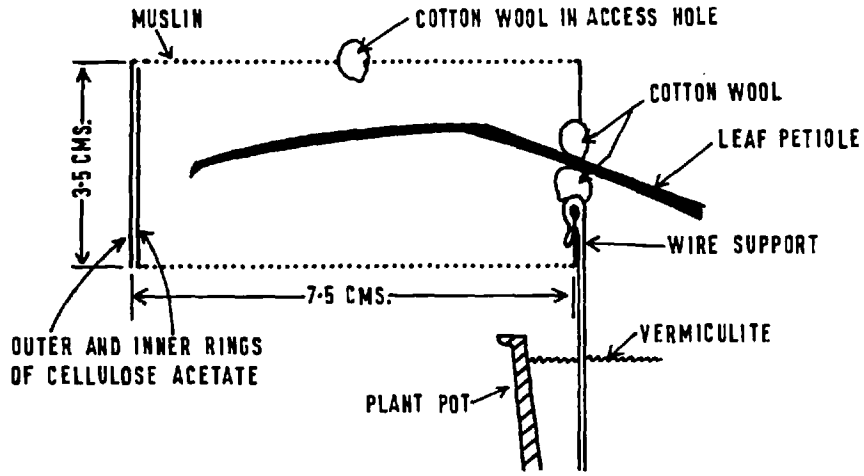
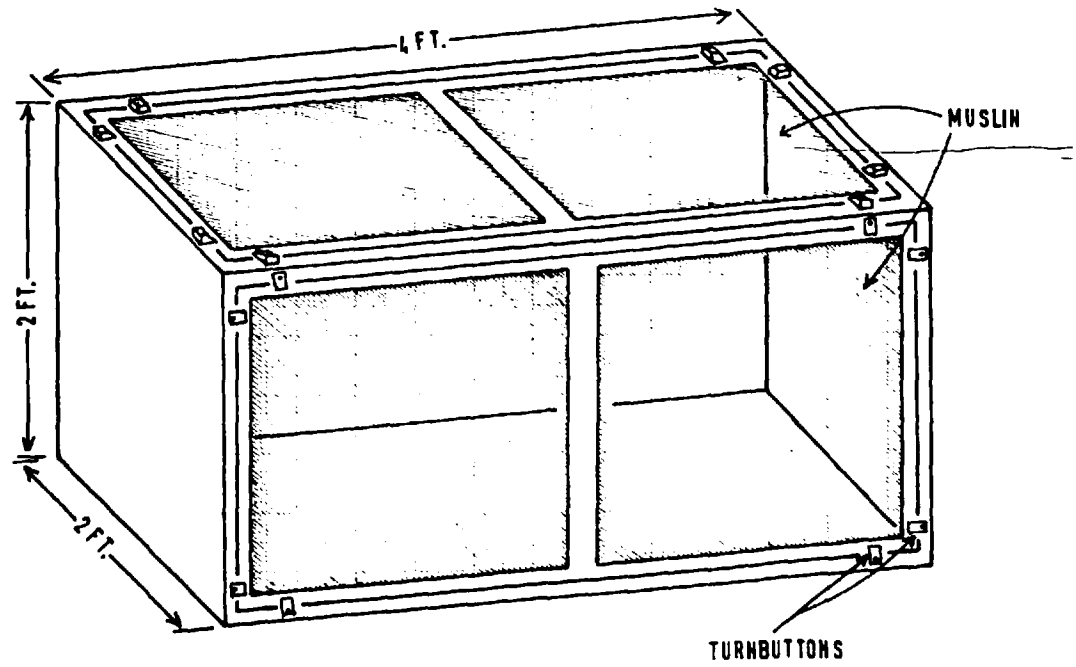



FIG. 6. SCREEN CAGE.



box, which was built on a wooden frame 4'x 2'x 2'. The floor, the back, and the two end walls of the cage were made with hardboard, and were painted white on the inside. The roof and the front of the cage could be removed and each consisted of a wooden rectangular frame carrying butter muslin. The inside of the frame was painted white. Thus the inside surface of the cage was almost uniformly white, reflecting the maximum amount of light. In use, the roof and the front were slotted into the frame of the cage and held in position by turn-buttons.

The cage was used in experiments on host plant selection by alatae (pp. 106 and 158).

Sub-section 5 The water regimes

To establish variable water content of the host plants in the experiments of Part 1, the plants were subjected to a series of water regimes, (see p. 68 ). The growing medium of the plants was allowed to dry out to various predetermined deficits of available water before watering to field capacity.

3½" plastic pots (Chemical Research Horticulture Ltd., Poly-pot No.3A) were chosen, in preference to clay pots, as the containers in which to grow the plants. In earlier work, (Wearing, 1963), it was found that clay pots, because of their porosity, allowed a too rapid and variable rate of drying of the growing medium.

Medium grade vermiculite ("Micafil" granular vermiculite, Dupre Vermiculite Ltd., Hertford) was used as the growing medium. This material was conveniently sterile and uniform, and was capable of holding large quantities of water.

The water regimes were based on the method of Hand (1961). Initially it was necessary to calculate the available water which was held by the vermiculite at field capacity. To do this the following information was required: The amount of water held by the vermiculite i) when air dry, 2) at the permanent wilting percentage, and 3) at field capacity. All calculations of the water content of the vermiculite were made on a wt/wt basis, although using vol./vol. would have given a nearer estimate of the available water in the vermiculite. Such volume measurements are cumbersome and are particularly important only when the compaction of the growing medium varies as in deep soils. The compaction of the vermiculite used here varied little, so that an almost constant factor related the weight of the vermiculite to its volume.

The water content of air-dry vermiculite

Twelve random samples of air-dry vermiculite, each weighing 5.5 grams, were put in an oven at 100°C for 48 hours and re-weighed. Figures for the means of the twelve samples were as follows:

Weight of air-dry vermiculite = 5.50 gms.

Weight of oven-dry vermiculite = 5.20 gms.

∴ Weight of water in 5.20 gms. of vermiculite when air-dry = 0.30 gms.

Therefore, % water content of vermiculite when air-dry (wt/wt basis)

$$= \frac{0.30}{5.20} \times 100$$

$$= \underline{5.77\%}$$

The water content of the vermiculite at the permanent wilting percentage

Hand (1961) gave an average figure for the permanent wilting percentage of fine and coarse grade vermiculite as a water content of 39% (wt/wt basis), for herbaceous mesophytes (e.g. Brussels sprout). At first, the present work was expected to finish after one year and thus the above figure was necessarily adopted for early experiments. For later experiments, the permanent wilting percentage was calculated.

Brussels sprouts were established in plastic pots containing known weights of vermiculite, which were allowed to dry out. A constant check was kept on the weights of the pots until the point was reached when all leaf ages of each plant no longer recovered when put for 48 hours in an atmosphere saturated with water vapour (in desiccators). The weights of the plant pots and their oven-dry vermiculite were known, and the plants were now removed and weighed. With this knowledge, the weight of water, held by the vermiculite in each pot at the permanent wilting percentage, could be calculated. The visual assessment of the permanent wilting of a plant is notoriously difficult, and in this instance a wide range of values for the permanent wilting percentage was obtained. The percentage of water which was held by the vermiculite at the permanent wilting point of Brussels sprout (wt/wt basis) ranged from 25% to 50%, with the mean of fifteen results at 42%. This last figure was adopted for later experiments and is remarkably similar to the earlier figure (39%) of Hand (1961).

The water content of the vermiculite at field capacity

The amount of water held by the vermiculite at field capacity was calculated at the beginning of the work and, because of the ease with which this could be done, also each time that a new bag of vermiculite was used.

On each occasion, six plastic pots were weighed, filled with a known weight of vermiculite, and thoroughly watered. When drainage ceased, each pot was weighed and the water content of its vermiculite was calculated. In a typical example, the percentage of water held by the vermiculite of different pots ranged from 424 to 453%, with a mean value of 438%.

Knowing the water content of the vermiculite at the permanent wilting percentage and field capacity, calculation of the difference between these two figures gave the percentage of available water held by the vermiculite at field capacity.

Calculation of the weights of the experimental pots and their vermiculite, at predetermined percentage deficits of available water (before watering to field capacity), was now possible. The same three water regimes were used in all experiments, namely:

Wet 10% ± 5% deficit of available water before restoration to field capacity.

Medium 50% ± 5% " " " " " " "

Dry 90% ± 5% " " " " " " "

where the available water held by the vermiculite at field capacity is 100%.

The calculation of the weight of the pot and its vermiculite at a given deficit of available water is shown below. This procedure was repeated several hundred times during these experiments, but a single example will suffice. The example is taken from the dry regime of the second series of preference experiments (p. 169 ).

Weight of pot = 30.2 gms.

Weight of pot + air-dry vermiculite = 69.7 gms.

$\therefore$ Weight of air-dry vermiculite = 39.5 gms.

Weight of oven-dry vermiculite $= \frac{39.5 \times 5.2}{5.5}$ gms.

$= \underline{37.3 \text{ gms}}$.

$\therefore$ Percentage of water held by the vermiculite at field capacity = 464%

$\therefore$ Weight of water held by 37.3 gms. of vermiculite at field capacity $= 37.3 \times 4.64$

$= \underline{173.1 \text{ gms}}$.

Permanent wilting percentage of the vermiculite = 42%

$\therefore$ Percentage of available water held by the vermiculite at field capacity = 422%

$\therefore$ Weight of available water held by 37.3 gms. of vermiculite at field capacity $= 37.3 \times 4.22$

$= \underline{157.4 \text{ gms}}$.

This pot was randomly allocated to the -90% $\pm$ 5% water regime.

$\therefore$ Weight of available water lost to the required deficit

$= 157.4 \times \frac{90}{100} \pm 157.4 \times \frac{5}{100} = \underline{141.7 \pm 7.9 \text{ gms}}$.

$\therefore$ Weight of pot and vermiculite at required deficit of available water

$= (30.2 + 37.3 + 173.1 - 141.7) \pm 7.9$ gms.

$= \underline{98.9 \pm 7.9 \text{ gms.}}$ or approximately $\underline{99 \pm 8 \text{ gms.}}$

Long-term experiments were required for measurement of the reproductive and temporal parameters of aphids (pp. 129 and 176) and their alate production (pp. 147 and 190). In these instances, to avoid frequent removal of the cages and their supports which were carried by each pot, their weights were added to the calculated weight of the pot at a given water deficit. Throughout all experiments, the weights of the plants were estimated by comparison with non-experimental plants which had been sown at the same time.

During the experiments, a continual check was made on the weight of each pot, so that, when the required water deficit was reached, the vermiculite was watered to field capacity. Each pot was stood in a 2lb jam-jar (see, for example, Fig.4) which collected and stored the leachate until it was re-circulated at the end of the next drying cycle. The jars were painted black to prevent the growth of algae in the leachate.

U.C. Fertiliser IIa (Baker, 1957) (see Appendix p.354) was mixed with the vermiculite before it was put into the pots. Any nutrients washed out of the vermiculite during watering were collected and stored as described above, and were later passed through the pots again. Apart from symptoms of manganese deficiency, which appeared in a number of plants of one early experiment (p. 177), no plant showed symptoms of nutrient

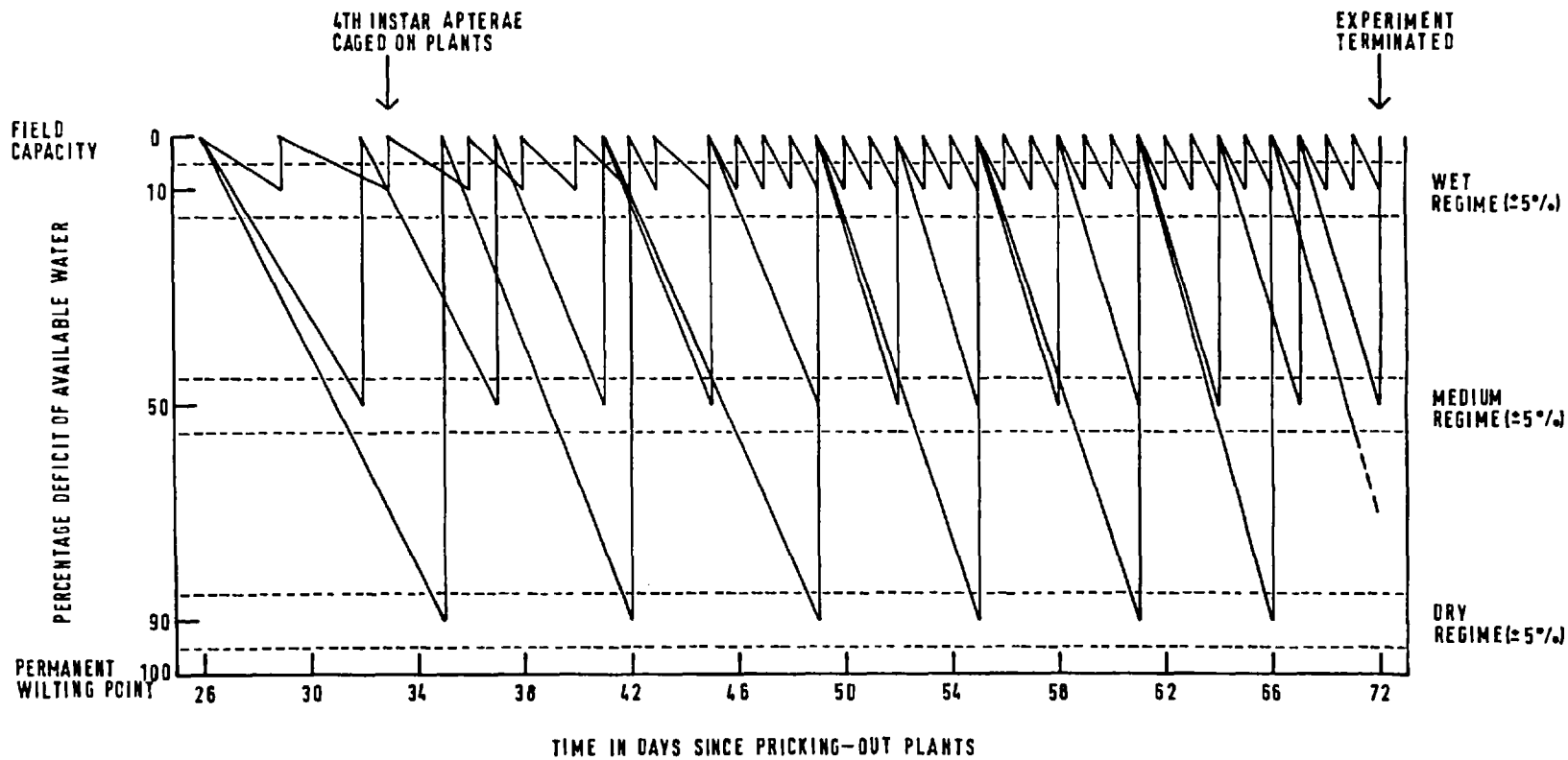
deficiency. The above deficiency symptoms were corrected by watering the plants, at the appropriate time, with a 0.009 molar solution of manganese chloride ($MnCl_2.4H_2O$) as well as the normal leachate.

The water regimes were conveniently recorded in watering frequency diagrams, examples of which are shown in Figure 7.

Sub-section 6 Leaf age

The leaves of the host plants were subjectively classified into three types: Young(Y) - leaves which were still developing and had not yet reached their maximum area; mature (M) - leaves which had grown to their maximum area and showed no yellowing of their green colour; old (O) - leaves which had grown to their maximum area and showed yellowing. This age classification, by leaf growth and colour, was made easier by comparing a given leaf with its neighbours and by taking account of its position on the plant (i.e. near the apex - young, near the base - old), (cf. Kennedy, Ibbotson and Booth, 1950).

FIG. 7. WATERING FREQUENCY DIAGRAMS. — THE PERCENTAGES OF AVAILABLE WATER IN THE GROWING MEDIUM (VERMICULITE) OF BRUSSELS SPROUTS IN WET, MEDIUM AND DRY REGIMES AT 20°C. (EXAMPLE CHOSEN: BLOCK VI, EXPT.3, TABLE 11).



## Section C Experiments

### Sub-section 1 The Host Plants

As shown by the Review of the Literature (p. 60 ), the responses of plants to water shortage have been studied by a great many workers. Nevertheless, it was desirable to have some indication that the plants were responding "normally" to the water regimes of the present work.

Visible symptoms of water strain were evident in the plants of the dry regime. One or more old leaves of these plants always wilted as the available water deficit of the vermiculite approached 90% (see Fig. 9). Occasionally, mature leaves were observed to wilt. Hastened senescence associated with this wilting was visible as a greater rate of abscission of the dying leaves. The old leaves of the plants of one experiment (p. 108), 43 days after pricking-out, were as follows:

| | | Mean No. of Old Leaves per plant (8 replicates) | | Mean No. of Completed Drying Cycles |
|---|---|---|---|---|
| | | Attached | Abscissed | |
| Water Regime | Wet | 3.25 | 3.88 | 9.6 |
| | Medium | 3.00 | 4.50 | 4.2 |
| | Dry | 2.50 | 5.25 | 2.0 |

The apex of each plant in the dry regime developed a stunted appearance as the regime progressed (see Fig. 9), and

the young leaves took on a darker green colouration, (cf. Burman and Painter, 1964). Considerable curling of the edges of the young and mature leaves of the plants was also associated with the dry regime. As a result of these symptoms of water strain the plants of the dry regime looked much smaller than those of the other regimes.

No visible feature distinguished the plants of the wet from those of the medium regime. However, a number of measurements of the plants, and plant parts, revealed differences in their responses to the three water regimes.

Leaf Development

The rate of development of young expanding leaves at the apex of a plant is known to be a sensitive indicator of the moisture conditions surrounding it, (e.g. Higgins, Haun and Koch, 1964). Measurements were made, therefore, of the daily increase in length of the 10th initiated leaf of plants in the three water regimes, (10 replicates). These plants were the same as those used in measuring the feeding preferences of apterae of M. persicae (p.119 ). Measurements were begun as soon as the leaves reached a length of 0.5 cms. and were terminated when no further increase in length occurred. The lamina of the leaf of a Brussels sprout is oval and extends down both sides of the petiole in two narrow strips. Measurement was made from the tip of the lamina to the base of these strips.

Mean daily increase in the length of the leaf decreased linearly with the availability of water to the plant,

in terms of the water regimes, as shown in Fig. 8. A 't'-test of the regression coefficient was very highly significant ($p < 0.001$, 28 df.).

Leaf Area

In determining the selection of host plants by alatae (pp. 106 and 158), it was necessary to measure the areas of the young, mature and old leaves of the plants in the three water regimes.

In view of the large numbers of leaves on the plants, (47 days after pricking-out; 8 replicates), the leaf areas of 12 randomly chosen young, mature and old leaves were measured accurately using a transparent grid of squares, 0.25 sq. cms. in area. The length (see above) and maximum breadth of these leaves were also measured and a second figure for their areas obtained by multiplication. For young, mature and old leaves the true leaf area was found to be 0.87, 0.81 and 0.81 of the estimated leaf area respectively (means of 12). For all future measurements the length and maximum breadth of a given leaf were measured and multiplied to provide an estimated leaf area. This was multiplied by the appropriate conversion factor to obtain an improved estimate of the leaf area. The plant apices were estimated to have a leaf area of 9.5 sq. cms. (mean of 12). This area consisted of that leaf surface which was considered to be available for the settling and feeding of alate aphids and was primarily of significance in the experiments with alatae (p. 107). However, the apices were classified as 'young' for present purposes.

FIG. 8. REGRESSION OF MEAN DAILY INCREASE IN LENGTH OF THE 10TH INITIATED LEAF OF BRUSSELS SPROUTS ON THE PERCENTAGE PERIODIC DEFICIT OF AVAILABLE WATER IN THE GROWING MEDIUM BEFORE RESTORATION TO FIELD CAPACITY.

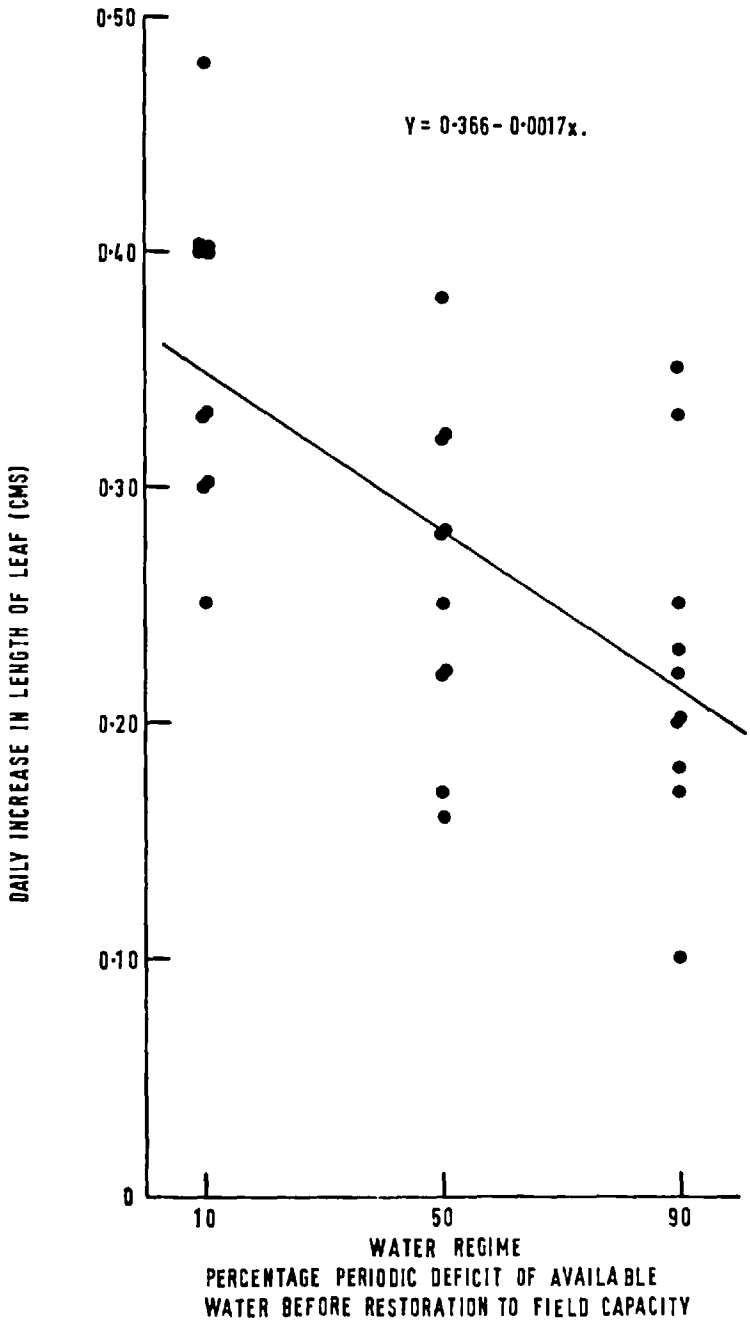


Fig. 9. Brussels sprout plants conditioned by (left - right) wet, medium and dry water regimes (see text).

Fig. 10. Screen cage opened to show an experiment investigating the selection of plants by alatae.


VIII 50
V 90

The areas of the leaves of each plant were summed according to leaf type: "young" (including apex), "mature" and "old". These figures formed sub-plots in an analysis of variance of a split-plot experiment in which the three water regimes were main-plot treatments and were represented once each per block.

Despite a trend towards decreasing leaf area with decreasing availability of water to the plant, no significant differences could be demonstrated between plants in different water regimes:

| | | Mean Leaf Area per Plant (sq. cms.) | | | | Mean No. of completed drying cycles |
|---|---|---|---|---|---|---|
| | | Young | Mature | Old | Total | |
| Water Regime | Wet | 67.30 | 84.95 | 47.86 | 200.11 | 11.0 |
| | Medium | 62.63 | 77.38 | 44.56 | 184.56 | 5.0 |
| | Dry | 58.68 | 75.48 | 36.95 | 171.10 | 2.8 |
| L.S.D. x p.05 | | 23.07 | 23.07 | 23.07 | 46.92 | |

x Least significant difference.

In addition, the linear and quadratic terms of the leaf area responses to the water regimes were not significant in 't'-tests.

Leaf Number

The leaves whose areas were measured above were counted to find out whether the availability of water in the

growing medium influenced the number of leaves per plant. Abscissed leaves were included but the apices of the plants were not dissected.

It was unnecessary to analyse the results of this work. The mean number of leaves per plant in each water regime were:

| | | |
|---|---|---|
| Water Regime | Wet | 17.0 |
| | Medium | 17.0 |
| | Dry | 17.1 |

This consistency of leaf number, regardless of water regime, has been found by other workers (e.g. Morton and Watson, 1948), and Gates (1964), who analysed the apices of lupins, reported impeded initiation of leaves during water stress but good recovery on re-watering.

Fresh Weight of the Plants

At the end of the experiments to determine the selection of host plants by alatae, the plants in each water regime were weighed to facilitate the calculation of the available water in the vermiculite (p. 204). An analysis of variance of these values for the fresh weights of the plants (8 replicates, one per block) revealed a significant effect of the water regimes using the 'F' test ($p < 0.02$). The water treatment means were as follows:

| | | Mean Fresh weight of Plants (gms.) | Mean No. of completed Drying Cycles |
|---|---|---|---|
| Water Regime | Wet | 19.1 | 11.0 |
| | Medium | 16.5 | 5.0 |
| | Dry | 13.5 | 2.8 |
| L.S.D.[x] | p.05 | 4.0 | |

x Least significant difference

By splitting the water regime sum of squares into its linear and quadratic components using orthogonal polynomials, it was possible to demonstrate a highly significant linear component using the 'F' test:

| Var$^{n}$. due to | | df. | ms. | 'F' |
|---|---|---|---|---|
| Water Regime | Linear | 1 | 161.9256 | $11.726^{**}$ |
| | Quadratic | 1 | 2.0419 | $0.148^{n.s.}$ |
| Error | | 14 | 13.8089 | |

'F' for 1 and 14 df. p.01 = 8.86 p.001 = 17.14

n.s. = not significant, ** $p < 0.01$

Conclusions

Both the observations and measurements of the plants suggest that the water regimes used in the present work elicited "normal" responses from the plants. The rate of leaf development and the fresh weight of the plants decreased linearly with the deficit in available water of the vermiculite before restoration to field capacity. Leaf number was not affected.

Leaf area was not significantly reduced by water strain, but it is suggested that further drying cycles would probably have produced a significant reduction (cf. Owen, 1958).

The "normality" of these responses to the water regimes suggests that the known physiological and biochemical effects of water strain in plants, which were not measured, were also proceeding normally in the plants of the dryer regimes. This is essential to any interpretation of the responses of aphids to these plants.

Sub-section 2 Myzus persicae

a) Selection of the Host plant (= Aphid arrival and establishment on the host plant).

i) Alatae

over

i) Alatae

Alate aphids alight, feed and subsequently produce nymphs (= settle) preferentially on leaves of particular ages. In general, young and early senescent leaves are preferred to mature and dying leaves as a result of the responses of the aphids to the colour, and perhaps shape, (Kennedy, Booth and Kershaw, 1961) and nutrient gustatory stimuli of the leaves (Kennedy and Booth, 1951; Kennedy, 1958). 'Token' gustatory stimulants, such as sinigrin, also play an important role in the selection of hosts by aphids (e.g. Wensler, 1962), but probably primarily in the selection of different species or families of host plants (Kennedy and Booth, 1951; Kennedy, 1958).

Kennedy, Lamb and Booth (1958) and Kennedy and Booth (1959) found that settling of alate aphids of A. fabae on spindle and sugar beet was, in general, reduced by water strain in the plants. The same authors reported a tendency for water strain in the host plants to obliterate the normal leaf age preferences shown by alatae in cages. Further consideration of this work is given in the Discussion (p. 29 ) and the Review of the Literature (p. 10 ).

Selection of host plants by alate M. persicae has been studied extensively (Moericke, 1952; Kennedy, Booth and Kershaw, 1959a and 1961; Pospíšil, 1963). This work has established that alate M. persicae typically alight preferentially on yellow surfaces, in particular young and old leaves, rather than surfaces reflecting light with a smaller

long/short wave ratio, such as many mature leaves. Taylor (1955) found that alate M. persicae alighted on the upper leaves of potato before moving to and settling on the old leaves. There are no published reports of the effects of water strain in the host plant on selection by alate M. persicae and an experiment was undertaken to study this (Stage a) i) of the cycle on p. 72 ).

Experimental procedure

Brussels sprouts subjected to the three water regimes (p. 87 ) were placed in a large screen cage (p. 85 )in the constant temperature room (p. 76 ) as shown in Fig. 10 (Feb. 1966). The plants were arranged in 8 randomised blocks, each containing one plant from each water regime. The blocks ran parallel to and either side of the "source" of alate aphids, a row of Brussels sprouts heavily infested with virginoparous M. persicae. To prevent apterae wandering onto the experimental plants, 'Fluon' was painted around the central culture on the floor of the cage and around the top of each jam-jar holding an experimental plant.

With the cage closed alate aphids were permitted to fly from the central culture and settle on the plants in the three regimes. After 24 hours the "source" plants were removed and the cage closed for a further 24 hours to allow flying aphids to settle.

At the end of the second period, the alatae which had settled on the plants were counted, separate records being kept

for each leaf and the apex of each plant. Only aphids which had produced nymphs were regarded as settled (Müller, 1958), though even this is not a complete guarantee that an aphid has settled permanently (Johnson, 1958). Others were recorded separately since many aphids re-take off after alighting on plants (Müller, 1958; Kennedy, Booth and Kershaw, 1959 b). Leaves were numbered from the base of the plant upwards, dead and dying leaves being noted but not used in the analysis of the experiment.

As the aphids on each leaf were counted, the length and maximum breadth of the lamina of the leaf were measured, and an estimated leaf area was calculated as previously described (p. 97 ). Since alatae rarely settled on the upper surfaces of leaves, the area of one surface only was estimated. In the apices of the plants, however, alatae frequently settled on both surfaces of the tightly packed leaves. The estimated leaf areas of the apices, therefore, included the upper and lower surfaces of those leaves which were arbitrarily considered to be available for the settling of the alatae (i.e. not too tightly rolled). Only leaves which were in a vertical position and had a laminal length equal to or less than 1 cm. were included in the counts and measurements of the apices. The mean value for the area of 12 randomly chosen dissected apices was 9.5 sq. cms. and this figure was adopted for all plants. The original counts of alatae per leaf and apex were converted to alatae per sq. cm. to allow a more correct comparison between the selection of different plants and leaves. Leaves were also classified as young, mature and old (see p. 93 ), and numbers and densities of aphids per leaf age were calculated for each plant, with the

apices of the plants forming a fourth leaf age category. These figures were analysed as a split-plot experiment. To remove zeros in the analyses, 1 was added to all numbers and 0.001 to all densities.

With regard to the water regimes, it was clearly necessary in a short experiment of this type that the available water in the vermiculite of the plants in the three regimes should be as different as possible at the time of selection of the plants by the alatae. The experiment was, therefore, timed to fit the drying cycles of the regimes so that the available water deficits in the wet, medium and dry regimes were as near as possible to 10%, 50% and 90% respectively, at the end of the experiment. The mean available water deficits in the vermiculite of the eight plants of each regime were:

| | |
|---|---|
| Wet | 9.0% |
| Medium | 47.4% |
| Dry | 89.2% |

and the mean number of completed drying cycles were 3.0, 6.0 and 12.0 respectively. At the end of the experiment (48 days after the plants were pricked-out), the plants were removed and weighed to facilitate the above calculations of the available water in the vermiculite (see p.204 for the calculation procedure).

## Results

### Alate aphids per plant and per leaf age

#### 1) Numbers

Table 3 gives a summary of the analysis of variance

of the numbers of alatae settled on the plants.

Table 3 Analysis of Variance of Numbers of Alate Aphids

| Varⁿ due to: | df | ss | ms | 'F' |
|---|---|---|---|---|
| Blocks | 7 | 1121.33 | 160.190 | 5.027 x |
| Water | 2 | 60.02 | 30.010 | 0.942 n.s. |
| Error (a) | 14 | 445.98 | 31.856 | |
| Whole Plot | 23 | 1627.33 | | |
| Leaf Age | 3 | 166.58 | 55.527 | 4.968 xx |
| Leaf Agex Water | 6 | 253.73 | 42.288 | 3.783 xx |
| Error (b) | 63 | 704.19 | 11.178 | |
| Total | 95 | 2751.83 | | |

'F' for 2 and 14df p.05 = 3.74 p.01 = 6.51 p.001 = 11.78
7 and 14df = 2.77 = 4.30 = 7.11
3 and 63df = 2.76 = 4.12 = 6.15
6 and 63df = 2.25 = 3.11 = 4.35

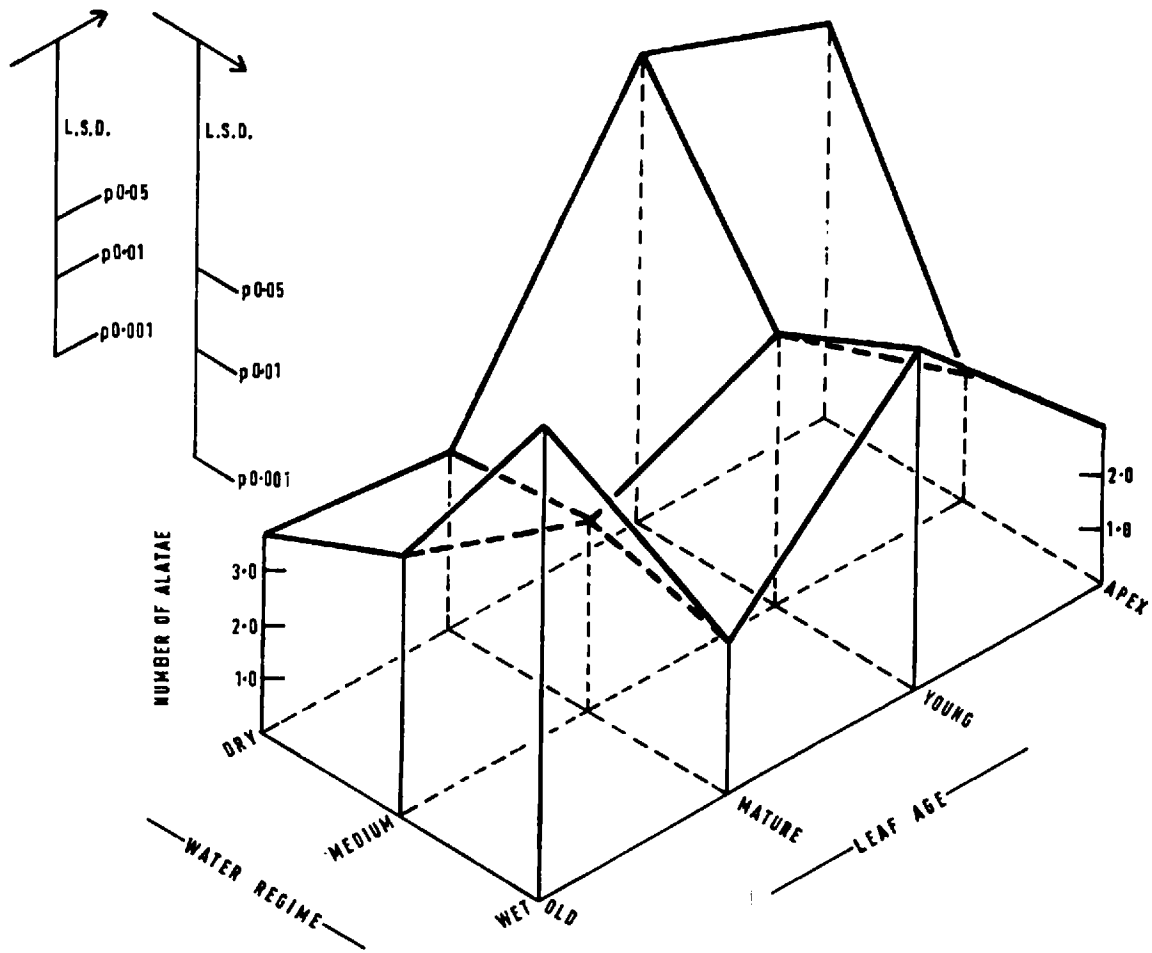
n.s. = not significant
x = $p<0.05$
xx = $p<0.01$

Blocking effectively removed variation due to a gradient in the numbers of settling alatae on either side of the "source" plants.

a) Water regime comparisons

The mean number of alatae settled on the plants of the wet, medium and dry regimes were 20.63, 15.63 and 23.25 respectively, and there were no significant differences between them. However, comparisons of the mean numbers of alatae per leaf age in the different regimes revealed significant differences as shown in Fig. 11 (Left-Right). More alatae settled on the apices of the plants in the dry regime than on

FIG. 11. MEAN NUMBERS OF ALATE VIRGINOPARAE OF M.persicae SETTLED ON DIFFERENT LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.



those of the wet and medium regimes ($p<0.05$). Splitting this effect into its linear and quadratic terms revealed a significant linear term in a 't'-test ($p<0.05$) while the quadratic term was not significant. The reverse situation was found on the old leaves where fewer alatae settled on the plants of the dry regime in comparison with the wet regime ($p<0.05$; linear term $p<0.05$; quadratic term not significant). Within leaf ages, there were no significant differences between the mean number of alatae on young and mature leaves in the three regimes.

b) Leaf age comparisons

The mean numbers of alatae settled on each leaf age, irrespective of water regime, were:

| | | | |
|---|---|---|---|
| | Apex | 4.33 | L.S.D. p0.05 = 1.93 |
| Leaf | Young | 6.63 | 0.01 = 2.57 |
| Age | Mature | 3.17 | 0.001 = 3.34 |
| | Old | 5.71 | |

't'-tests showed that there were more alate aphids on young and old leaves than on mature leaves ($p<0.001$ and $p<0.05$ respectively), and more on young leaves than on the apices of the plants ($p<0.05$).

Within the water regimes, this pattern of distribution varied (see Fig. 11, Front-Back), particularly in relation to the apex and the old leaves. Thus, in the wet regime the mean number of alatae on the old leaves was greater than on the apex ($p<0.001$) while the reverse situation occurred in the dry regime ($p<0.05$) where as many as 18 alatae settled on the apex of a

plant. In the dry regime the old leaves were not significantly preferred to the mature leaves. In the medium regime there were no significant differences between leaf ages.

2) Alate aphids per square cm.

Table 4 summarises the analysis of variance of alate aphids per sq. cm. where blocking is again shown effective in removing variation.

Table 4 Analysis of Variance of Alate aphids per sq. cm.

| Varn due to: | df | ss | ms | 'F' |
|---|---|---|---|---|
| Blocks | 7 | 1.691131 | 0.241590 | 3.275 xx |
| Water | 2 | 0.477620 | 0.238810 | 3.237 n.s. (x) |
| Error (a) | 14 | 1.032773 | 0.073770 | |
| Whole Plot | 23 | 3.201524 | | |
| Leaf age | 3 | 2.366992 | 0.788997 | 14.011 xxx |
| Leaf age x Water | 6 | 1.163086 | 0.193848 | 3.442 xx |
| Error (b) | 63 | 3.547660 | 0.056312 | |
| Total | 95 | 10.279262 | | |

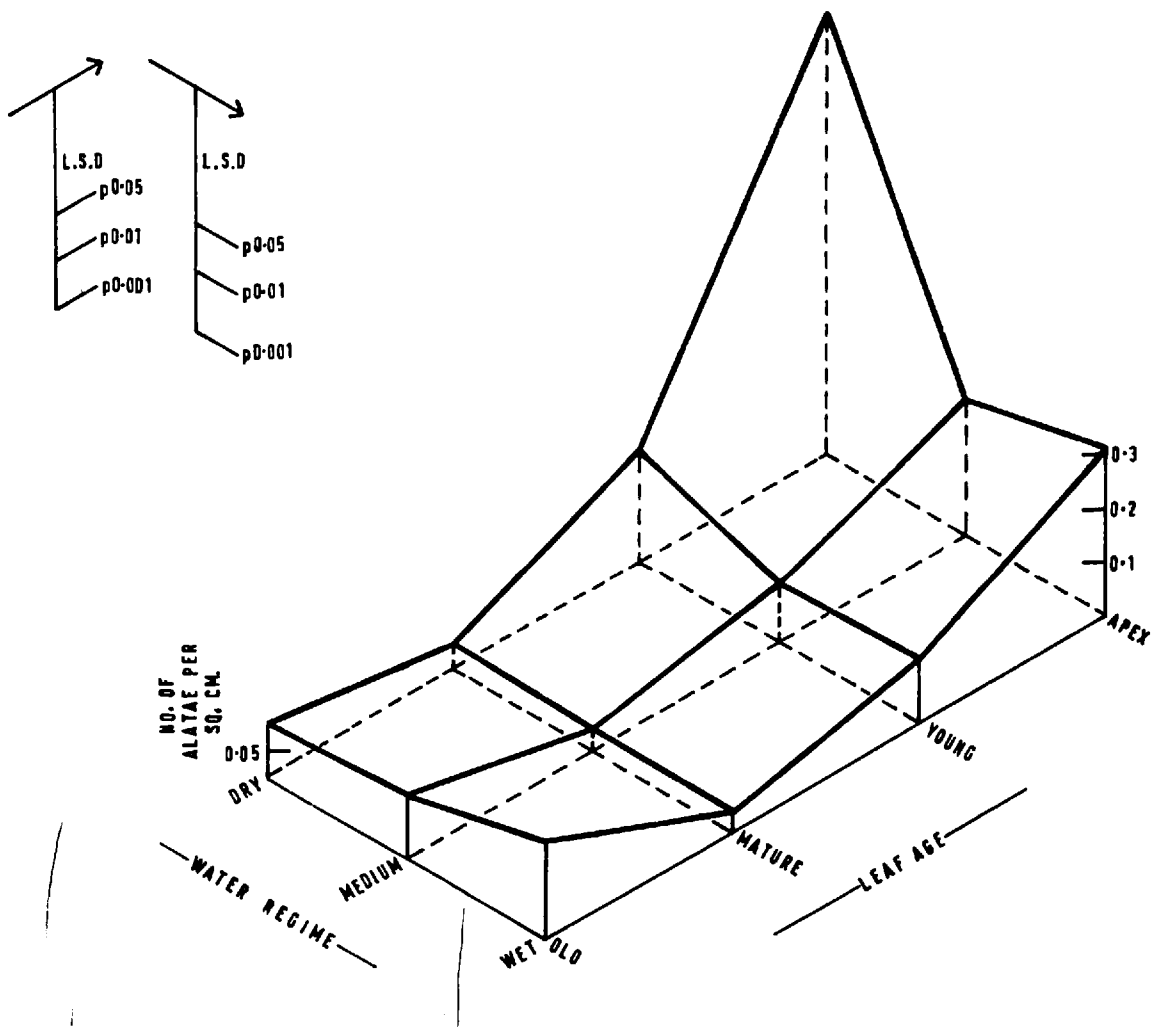
F for 2 and 14 df etc. (see Table 3 )

n.s. not significant x $p<0.05$ xx $p<0.01$ xxx $p<0.001$.

a) Water regime comparisons

Conversion of the above results to alate aphids per sq. cm. altered the significance of differences but did not change the basic trends in the selection of the plants in the three regimes. The overall density of settled alatae was greater on the plants of the dry than on those of the wet (p n.s.) and medium regimes ($p<0.05$):

FIG. 12. MEAN DENSITIES OF ALATE VIRGINOPARAE OF M.persicae SETTLED ON DIFFERENT LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.



| | | Mean alatae/sq.cm./plant | |
|---|---|---|---|
| Water Regime | Wet | 0.157 | L.S.D. p0.05 = 0.146 |
| | Medium | 0.129 | 0.01 = 0.202 |
| | Dry | 0.291 | |

This overall increase in the density of alatae on the plants of the dry regime was due almost exclusively to increased density of alatae on the apices of these plants ($p<0.001$) (see Fig. 12, Left-Right). Within leaf ages there were no other significant differences between water regimes.

b) Leaf Age Comparisons

The mean density of alatae on the apices was much greater than on any other parts of the plants ($p<0.001$) when all plants were considered:

| | | Alatae/sq. cm. | |
|---|---|---|---|
| | Apex | 0.456 | |
| Leaf Age | Young | 0.143 | L.S.D. p 0.05 = 0.137 |
| | Mature | 0.042 | 0.001= 0.237 |
| | Old | 0.130 | |

When the densities of alate aphids on young leaves and the apices were combined and the whole experiment re-analysed, standard errors were reduced and revealed significant differences in the density of alatae settled on old and mature leaves ($p<0.01$):

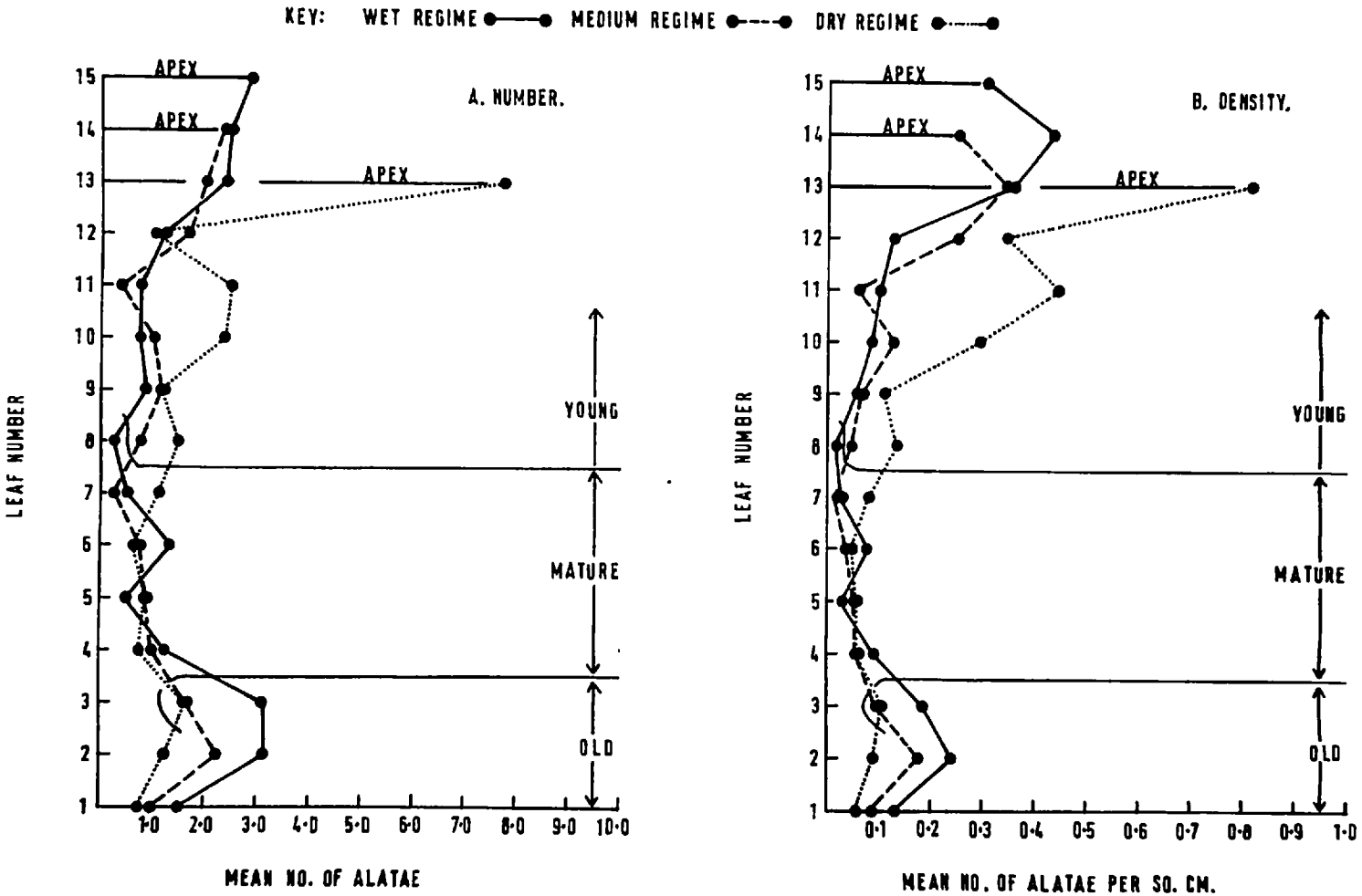
| | | Alatae/sq. cm. | |
|---|---|---|---|
| | "Young" | 0.196 | L.S.D. p 0.05 = 0.058 |
| Leaf Age | Mature | 0.042 | 0.01 = 0.078 |
| | Old | 0.130 | 0.001= 0.102 |

In leaf age comparisons, therefore, the conversion of numbers of alatae to alatae per square cm. disclosed the concentration of aphids on the apices of the plants and their dilution on the mature leaves which had a greater area than the leaves of any other age (see p. 101). This is reiterated when comparing leaf ages within water regimes (Fig. 12, Front-Back) where the high density of alatae on the apices of the plants was most marked in the dry regime. But there were very few significant differences between leaf ages in these comparisons. Lower standard errors were obtained when the densities of alatae on the young leaves and apices combined were calculated and used in the analysis. Under these circumstances the mean density of alatae on the old leaves of the wet regime was higher than on the mature leaves ($p<0.01$) and not significantly different from the young leaves. In the dry regime this pattern was replaced by a mean density of alatae on the old leaves not significantly different from that on the mature leaves and much lower than that on the young leaves ($p<0.001$).

Alate aphids per Leaf

The results described above for the alate aphids settled on each leaf age were confirmed by examination of the vertical distribution of the mean numbers and densities of alatae settled on individual leaves numbered from the base of the plant, (Figs. 13a & b). Separation of sections of the graphs into young, mature and old leaves was based on the mean number of leaves of each age on the plants of each regime. The

FIG. 13. MEAN NUMBERS AND DENSITIES OF ALATE VIRGINOPARAE OF M.persicae SETTLED ON LEAVES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. LEAVES NUMBERED FROM THE BASE OF THE PLANTS. FOR YOUNG, MATURE AND OLD LEAVES — SEE TEXT.



graphs were not statistically analysed.

Figures 13a & b clearly show the "pivot position" of the mature leaves in the distribution of alatae on the plants as water strain changes: Above the mature leaves the numbers and densities of alatae settled on the young leaves were increased by the dry regime, while below the mature leaves the reverse procedure was operating on the old leaves. Relatively low densities of alatae settled on all the mature leaves in all the water regimes. There were no alatae on many of the mature leaves. Other interesting features of the graphs are: 1) Within the old leaves peak numbers and densities of alatae did not occur on the oldest leaves but on the leaves in the early stages of senescence (dead and dying leaves are not included on the graphs). 2) The numbers and densities of alatae on the young leaves increased steadily towards the apices of the plants.

Conclusions

Alate M. persicae settled in greater numbers and densities on young (including apex) and old leaves rather than mature leaves of turgid plants. The density was particularly high on the apex of the plant. Similar effects of leaf age occurred to a lesser extent on plants suffering an intermediate level of water strain, but where water strain was severe the alatae showed an overwhelming preference for the apices and young leaves of the plants while the old leaves were largely

avoided. These effects led to an overall increase in the density, but not number, of alate aphids on plants with severe water strain.

Thus, the overall attractiveness of the plants to the alate aphids was not altered by changing the water status of the plants. Statistically equal numbers of alate aphids settled on the plants. It was only their distribution which changed with the water strain of the host. In these experiments this may have occurred during final alightment, in response to colour changes (and perhaps odour or taste changes) in the leaves of the host plants under water strain (Kennedy, Booth and Kershaw, 1961), and/or more probably, after the aphids had finally alighted on the plants (cf. Taylor, 1955; Johnson, 1958; Dickson & Laird, 1962). The possible causal mechanisms involved in this redistribution are discussed on pp. 309 and 319.

a) Selection of the Host Plant (≡ Aphid arrival and establishment on the host plant)

ii) Apterae

Within an established population of aphids on a plant a definite distribution pattern has frequently been recognised. Aphids are commonly concentrated on the young and old rather than the mature and dying leaves of plants, though this varies with the species of aphid and of host plant (see the Review of the Literature p. 25 ). Ibbotson and Kennedy (1950), when studying free infestations of A. fabae on sugar beet, largely

attributed this variation in the population density with leaf age to the preferences exercised by the adult apterae. This conclusion was supported by subsequent preference tests (Kennedy and Booth, 1954).

With reference to the water status of the host plant, the osmotic pressure of the cell sap is known to be correlated with the distribution of feeding Aphis pomi Deg. (Sokolov and Sokolova, 1952) and A. fabae (Weismann, 1960). Both species were found to accumulate on parts of the plant with low osmotic pressure (and high turgor pressure) such as the apices of stems.

Depending on the species and variety of the host plant and the clone of the aphid, populations of M. persicae may be concentrated on the old leaves and/or the young leaves of the plants, (Kennedy, et al, 1950; Bradley, 1952; Tanaka, 1957; Thurston, 1961; Wyatt, 1965). Dickson and Laird (1962) observed M. persicae feeding primarily on the young leaves of sugar beet and ascribed this to the dry (desert) conditions. However, the preferences of apterous M. persicae for leaves of different ages had not hitherto been measured in direct preference tests, nor had their preferences for plants of different water status. Experiments were undertaken to measure both sets of preferences and their interaction (Stage (a) (ii) of the Cycle, p. 72 ).

Experimental Procedure

Adult apterous virginoparae of M. persicae in darkened

preference cages (p. 83 ) were allowed to chose between leaves of different ages (p. 93 ) on Brussels Sprouts (cv. "Jade Cross") of different water status (p. 87 ) in the constant temperature room (p. 76 ). The paired leaves which were offered for selection were:

Within each water regime (on the same plant) ÷ Y-M, M-O, Y-O.

Within each leaf age (on different plants):

wet - medium regime (10-50)

medium - dry regime (50-90)

wet - dry regime (10-90)

The young leaves used were as young as possible yet sufficiently unfurled to carry half of the cage without being damaged; among the old leaves, dying leaves were not used, and, when testing preferences for different water status, only leaves of similar appearance were caged together (i.e.in a similar stage of senescence). At the time of measuring the preferences, the percentage available water deficits of the vermiculite of the plants in the wet, medium and dry regimes were as near as possible to 10%, 50% and 90% respectively and the basal leaves on the plants of the dry regime were wilting. Further preference tests were made with plants of the dry regime when the vermiculite was at or near field capacity: Y-M, M-O, Y-O, and within each leaf age, 50-90 and 10-90. Preference tests with these plants are marked NW (non-wilting) to distinguish them from the other tests with the dry regime, W (wilting). The same pairs of leaves were used for both sets of tests. This gave a total of 27 leaf-preference experiments.

In each test 12 aphids were put in a cage and left for 24 hours to settle on the leaves. At the end of this period the aphids which were feeding on each leaf were counted and removed. In preliminary work this 24-hour period yielded much more consistent results than the half-hourly readings for two hours used by Kennedy and Booth (1950). In addition, the problems associated with the variable activity of the aphids reported by these workers were minimised by conducting the experiments in a constant temperature growth room (20°C) with controlled daylength (16 hrs.). Each preference test was replicated ten times.

Assuming the null hypothesis that equal numbers of aphids would settle on each of a given pair of leaves, the results were analysed using $X^2$. This was done with replicates summed for each pair of leaves ($X^2_1$) as well as separately in 2 x 10 tables ($X^2_{10}$). A significant $X^2$ was required in both tests before the aphids were considered to have exercised a preference. This occurred in the results described, except where otherwise stated. This method of analysis was simpler than that of Kennedy and Booth (1950) (which was used initially) and attached similar low levels of significance to individual results. When less than 10 aphids were feeding at the time of counting, the test was discarded and repeated.

The experiments were carried out between 34 days and 58 days after pricking out the host plants (Oct.-Nov. 1965). This age range of the plants did not appear to influence the results. The mean number of completed drying cycles in each

regime after 58 days were: Wet - 32.0, medium - 15.6 and dry - 9.1.

Results

The preference experiments fall into groups of three, i.e. between the three leaf ages and three water regimes. In consequence, the results are presented graphically in equilateral triangles each side being composed of the mean percentages of apterae feeding on a given pair of leaves (see Figs. 14 and 15). Each side of the triangles is equivalent to 100%. Those tests involving NW plants of the dry regime are superimposed on the graphs with W plants.

Water regime preferences

Apterous aphids presented with young leaves on plants of different water status expressed preferences in the direction 10 $\longrightarrow$ 50 $\dashrightarrow$ W90, (see Fig. 14a). The preference test, 50-W90, only failed to provide a significant $\chi^2_{10}$ when separate replicates were considered. When the replicates of this test were summed, $\chi^2_1$ was significant ($p<0.01$) suggesting a possible preference of the apterae for the plants of the dry regime (dotted line). The effect of watering the plants of the dry regime was apparently to reduce the attractiveness of their young leaves to the aphids. Thus, almost equal numbers of apterae settled on the two leaves in the 50-NW90 test while the preference for the plants of the dry regime was retained at a lower level of significance ($\chi^2_{10}$, $p<0.05$ in the 10-NW90 test.

An identical pattern of directional preferences as just described was recorded on mature leaves, i.e. 10 ⟶ 50 ⟶ W90, (Fig. 14b) with a similar, though very limited, influence of NW90 plants, which in this instance did not alter the directions of the preferences.

The preferences of the aphids for old leaves of different water status can be summarised 10 ⟷ 50 ⟵ W90, (see Fig. 14c). The old leaves of the plants of the medium regime were preferred to both turgid (10) and wilting (90) old leaves. When the plants of the dry regime were watered, the preferences of the apterae changed to 10 ⟷ 50 ⟵ NW90, which represented a shift in favour of the old leaves of the dry regime, particularly in nullifying the earlier preference for the medium regime. The wet and dry regimes apparently provided plants with old leaves which were equally attractive to the aphids in all tests.

Leaf Age Preferences

Within plants of the wet regime, apterae offered leaves of different ages showed the preferences Y ⟵ M ⟶ O, (Fig 15a). Unexpectedly, the young leaves were not preferred to the mature leaves according to the $X^2_{10}$-test for separate replicates. However, $X^2_1$ was significant ($p<0.01$) when using the summed results, suggesting a weak preference for the young leaves. The marked preference of the apterae for the old leaves, when offered in conjunction with the young leaves ($p<0.001$), was also unexpected, (cf. Wyatt, 1965). Both these responses may

be attributed in part to inability to attach the cages to the youngest (probably most attractive) of the young leaves of the plant, i.e. next to the apex, (Kennedy, et al, 1950).

In the medium regime the different aged leaves of the plants elicited from the aphids similar preference responses to those just described, Y ⟵M⟶ O, (Fig. 15b). Once again old leaves were preferred to the mature and young leaves and, in addition, the young leaves were clearly preferred to the mature leaves.

These preferences of the aphids for the young and old leaves were disrupted in the dry regime (W) to take the form Y ⟷ M⟶ O, (Fig. 15c). In these tests young leaves were preferred to old which were preferred to mature; but in the Y-M test $\chi^2_{10}$ was significant while $\chi^2_1$ was not. This indicates that the direction of preference varied considerably between replicates, a situation which was rarely met in these tests. When the plants were watered and the leaf age preferences re-tested (NW), they were in the directions, Y ⟷M⟷ O, (Fig.15c). Watering the plants, therefore, decreased the relative attractiveness of the mature leaves, and increased that of the old leaves.

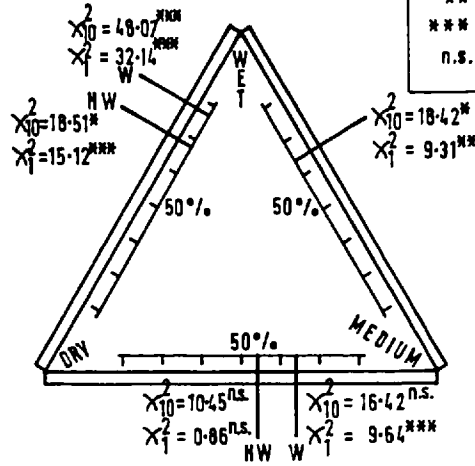
Without attaching significance to the apparent strength of the preferences here described (see Kennedy and Booth, 1950), the directions of preferences between each leaf age and water regime were entered in a three-dimensional diagram (Fig. 16). (A separate scheme was superimposed

FIG. 14. FEEDING PREFERENCES OF ADULT APTEROUS VIRGINOPARAE OF M.persicae BETWEEN BRUSSELS SPROUT LEAVES OF THE SAME AGE ON PLANTS OF DIFFERENT WATER STATUS AT 20°C. EACH SIDE OF THE TRIANGLES INDICATES THE MEAN PERCENTAGE DISTRIBUTION OF FEEDING APHIDS BETWEEN 2 LEAVES IN THE WATER REGIMES STATED.

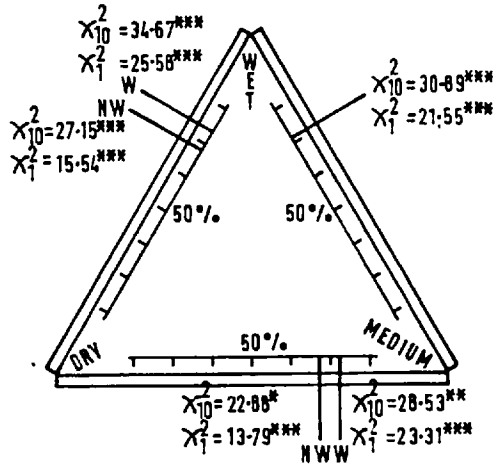
W=WILTING NW=NON-WILTING

| | | | $\chi^2_{10}$ | $\chi^2_1$ |
|---|---|---|---|---|
| * | (>) | p·05 | 18·31 | 3·84 |
| ** | (>) | p·01 | 23·21 | 6·64 |
| *** | (>) | p·001 | 29·59 | 10·83 |
| n.s. | not significant. | | | |

A. YOUNG LEAVES.




B. MATURE LEAVES.




C. OLD LEAVES.

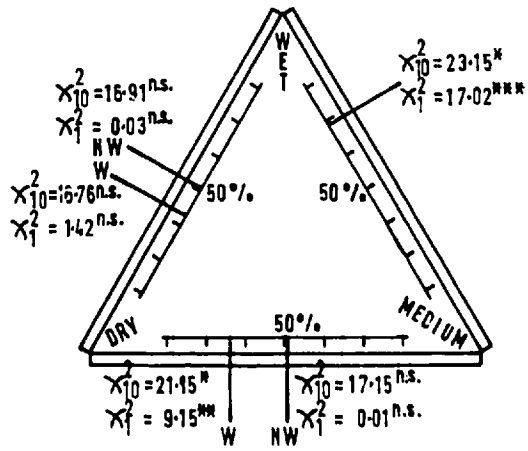
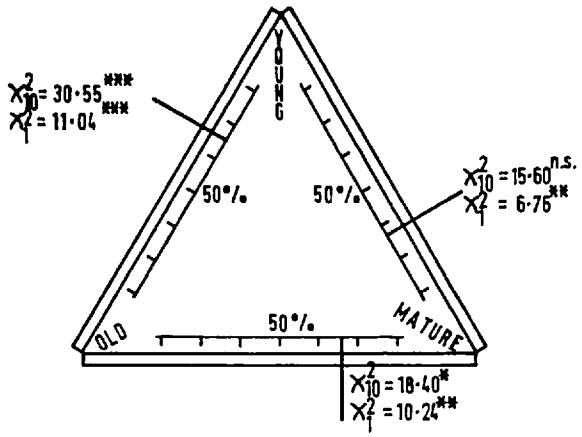


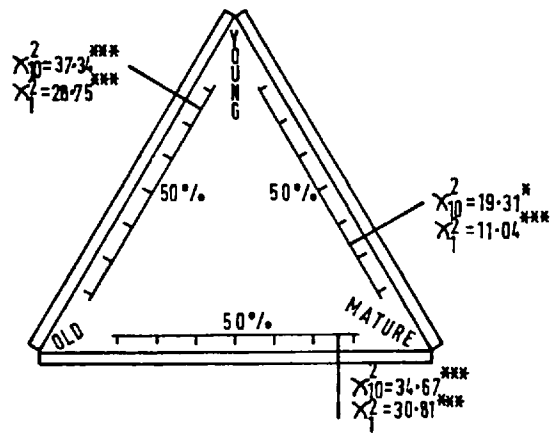
FIG. 15. FEEDING PREFERENCES OF ADULT APTEROUS VIRGINOPARAE OF M.persicae BETWEEN BRUSSELS SPROUT LEAVES OF DIFFERENT AGES AT 20°C. EACH SIDE OF THE TRIANGLES INDICATES THE MEAN PERCENTAGE DISTRIBUTION OF FEEDING APHIDS BETWEEN 2 LEAVES OF THE AGES STATED. EACH TRIANGLE REFERS TO PLANTS OF A DIFFERENT WATER REGIME.

SYMBOLS AS IN FIG.14.

A. WET REGIME.




B. MEDIUM REGIME.




C. DRY REGIME.

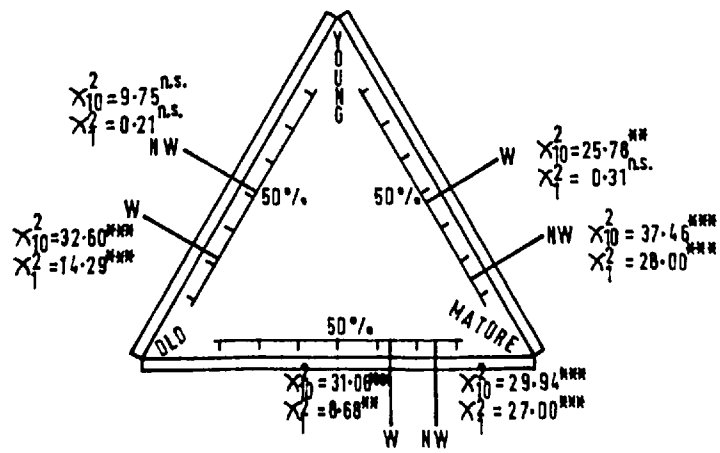
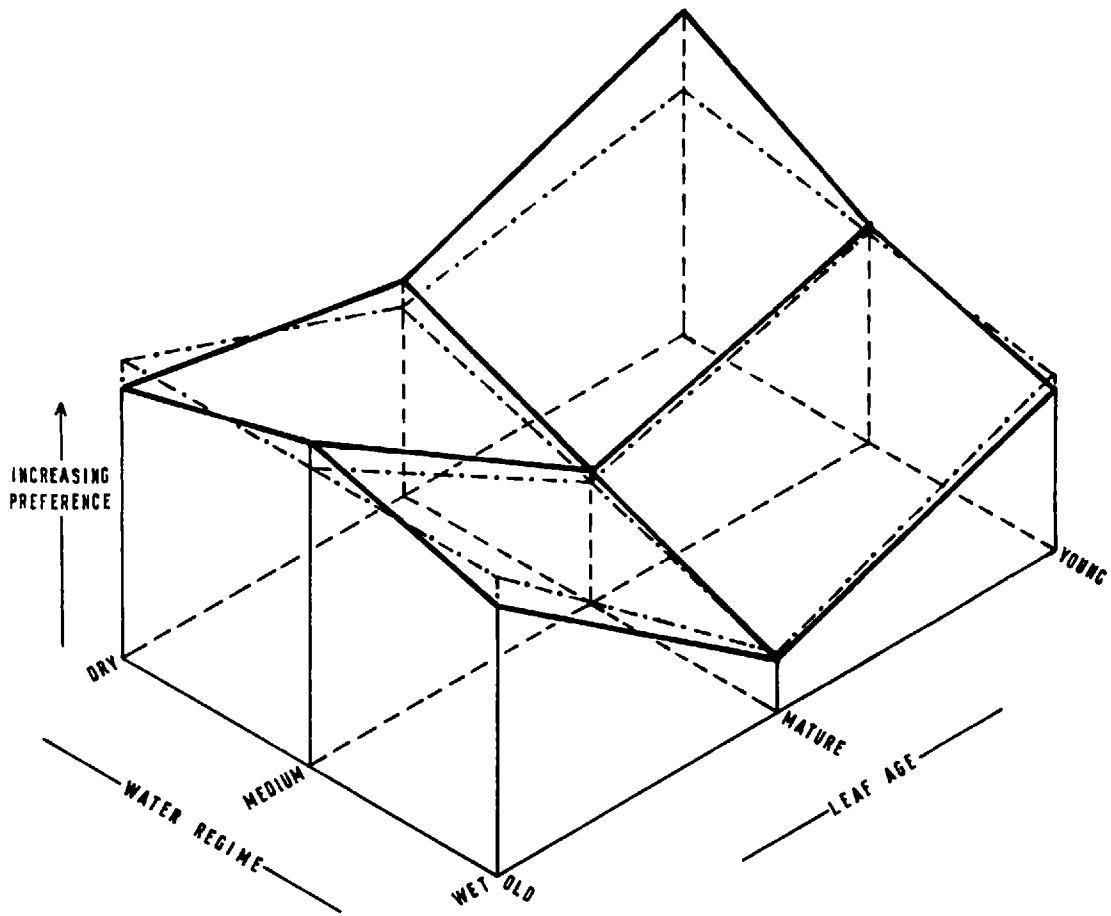


FIG. 16. SCHEMATIC DIAGRAM OF THE DIRECTIONS OF FEEDING PREFERENCES OF ADULT APTEROUS VIRGINOPARAE OF *M. persicae* BETWEEN BRUSSELS SPROUT LEAVES OF DIFFERENT AGES AND WATER STATUS. COMPILED FROM FIGS. 14 AND 15.

KEY: ━━ USING WILTING PLANTS OF THE DRY REGIME (SEE TEXT)
-·- USING NON-WILTING PLANTS OF THE DRY REGIME



involving the NW plants of the dry regime). In this diagram the trends of the preferences may be seen in a form conveniently comparable to the results of other experiments, (see the Summary of Results p. 153).

Conclusions

When offered paired, caged leaves of different ages or water status on which to settle, adult apterous virginoparae of M. persicae showed clear preferences which may be regarded as primarily feeding preferences (Kennedy and Booth, 1950). One preference was consistently in the direction of increasing water strain in the host plant, except for a reversal where water strain was severe in the old leaves (wilting). When water strain was removed from these leaves by watering the plants, this non-acceptance of the leaves by the aphids was nullified, but not reversed. The watering of plants previously under severe water strain also annulled the preference of the aphids for the young leaves of these plants rather than the young leaves of plants of an intermediate water status.

The preferences of the apterae for different aged leaves were in the order old, young, mature, except on plants subjected to extreme water shortage. During the wilting of plants, young leaves were preferred to old (cf Dickson & Laird, 1962) and old to mature while young and mature were equally accepted. The reason for this last-mentioned lack of preferenc is not known but the Y-M test was unusual in providing wide variation in the direction of preference (p. 124). When wiltin g

plants were watered, the aphids exercised more 'typical' preferences for the different leaves except for the absence of a preference between young and old leaves. The results are given further consideration in the Discussion (p.292) and the Summary of Results (p. 163).

b) Reproductive and Temporal Parameters of Apterae

(≡ Development of established populations of apterae on the host plant).

The development of an established population of apterous aphids on a plant is closely related to the physio logical condition of the plant and its different parts (Kennedy, 1958; Review of the Literature, p. 30 ). This relationship results from the influence of the host plant on the fecundity and rate of reproduction of the aphids (reproductive parameters) and on the lengths of the larval, adult, reproductive and post-reproductive periods of their lives (temporal parameters).

In general, aphids reproduce faster on actively growing and senescing parts of the host plant than on more stable parts, such as the mature leaves. Water strain in the host plant has been associated with reduced reproduction (e.g. Weismann and Vallo, 1963), increased reproduction (Markkula, 1953) and unchanged reproduction (McMurtry, 1962) of aphids. This paradox is probably a result of variations in the age and water strain, and accompanying biochemical differences in the tissues of the host plants in different experiments (Kennedy,

et al, 1958; Wearing, 1966) and upon differences between species of aphids (Wearing & van Emden, 1966; Wearing, 1966).

Apterous M. persicae occur in large numbers on the old and young leaves of plants (Kennedy, et al, 1950; Bradley, 1952; Taylor, 1955; Heathcote, 1962), and particularly on the old leaves of Crucifers (Miyashita, 1953; van Emden and Wearing, 1965). With reference to the water status of the host plant, Taylor (1955) related rapid multiplication of M. persicae on one plot of his experiments to drought-induced senescence in the host plants (potatoes). Wearing and van Emden (1966) recorded increased reproduction of M. persicae on plants at an intermediate level of water strain and a fall in reproduction at higher levels. The same water regimes were used as in the present experiments.

All these reports, however, are based on free infestations of M. persicae. The present experiments were undertaken to measure many aspects of individual apterae of M. persicae caged throughout their lives on different aged leaves of Brussels sprouts in different water regimes. (Stage (b) of the Cycle, p. 72 ).

Experimental Procedure

Brussels sprouts in the constant temperature room (p. 76 ) were subjected to three water regimes (p.87 ). When the regimes were established (after one completed drying cycle of the dry regime), simple clip cages (p. 79 ) to contain the aphids were attached to leaves on the plants. In the first

experiment the responses of the aphids to one leaf age only were studied on the plants of each regime. But this was soon superceded by experiments in which two leaf ages were compared using two clip cages per plant. In addition, each plant required two additional matching leaf cages (p. 84 ) for determining the production of alatae (see p. 148). Ideally, leaves of all three ages should be compared in a single experiment, but the plants would not tolerate the numbers of aphids and experimental cages involved (see Fig. 3).

Into each clip cage was placed a 4th instar apterous virginoparous nymph of M. persicae which moulted to adult and larviposited. The first-born larvae, which are thought to be atypical (Murdie, personal communication), were discarded. The adult, too, was discarded after the birth, (on the 2nd or 3rd day of adult life,) of two more larvae which were reared to adult in each cage. One of these adults, always apterous, was used in the experiment, two being reared as insurance against the development of an alate aphid. By rearing the experimental aphid in this way, rather than using a freshly moulted adult direct from the culture, it was hoped to remove, in part, the effects on the experimental results of the previous feeding history of the aphid. At the same time the effects of the treatments on the length of larval life could be determined.

The chosen adult (the first aptera to develop) remained in the clip cage throughout its life. By transferring the cage from leaf to leaf as the leaves aged, the adult aphid always fed

on leaves of one age category. Each day the progeny of the adult were counted and carefully transferred, using a soft brush, to a leaf cage containing a leaf of similar age (Fig. 3). For uniformity, adults, if feeding, were always disturbed during this operation which was done at the same time each day within each experiment. Normally the leaf cage was on the same plant. In the experiments with young leaves, however, to prevent damage to the apices of the plants it was necessary to grow a second set of plants in the three water regimes to hold the leaf cages. For reasons of shelf space, in these experiments the normal 12 replicates were therefore reduced to 10.

The responses of the aphids to the three leaf ages and three water regimes were measured in three experiments. In the first, aphids feeding on old leaves only were studied and the Brussels sprout cultivar "Irish Elegance" was used before the more uniform cultivar "Jade Cross" became available. In the second and third experiments, aphids caged on old and mature, and mature and young leaves, respectively, of plants in the three regimes were compared. The cultivar "Jade Cross" was used for these experiments.

Table 5 shows the ages of the plants at the beginning and end of the experiments and the mean numbers of completed drying cycles in each water regime at the end of the experiments.

Table 5 Host Plants and Water Regimes

| Age of Plants (days from pricking out) | | | Water Regimes Mean No. of Completed Drying Cycles at end of expts. | | |
|---|---|---|---|---|---|
| | +Start (of expts.) | End | Wet | Medium | Dry |
| Expt.1 (cv."Irish Elegance" Oct-Dec 1963. | 27 | 79 | 38.2 | 17.6 | 9.0 |
| Expt. 2 (cv."Jade Cross") Sept-Dec 1964. | 25 | 83 | 52.0 | 24.8 | 10.4 |
| Expt. 3 (cv."Jade Cross") Dec. 1964 - March 1965. | 36 | 92 | 54.2 | 21.0 | 11.1 |

+ Start = when 4th instar nymphs were caged on the leaves.

Records were kept of the length, in days, of the larval, adult, reproductive and post-reproductive periods of the life of each aphid, (The temporal parameters). The daily records of the progeny were summed to measure the fecundity of each aphid. The reproductive rate was calculated as the mean number of progeny produced each day for the first 90% of the progeny of each aphid. The last 10% were not included in the calculations since these nymphs were born erratically, often several days apart, at the end of the reproductive life of the aphid. Their inclusion would have confused the important earlier differences in the rate of reproduction of the aphids in different treatments. The fecundity and reproductive rate constituted the reproductive parameters of the aphids.

The experiment involving one leaf age was designed and analysed as a randomised block experiment consisting of 12 blocks, each containing one plant in each water regime. The linear and quadratic components of the sum of squares for the

water regimes were calculated using orthogonal polynomials and tested in an analysis of variance using an 'F' test. Where two ages of leaf were compared in a single experiment, they formed the sub-plot treatments in a split-plot experiment in which the water regimes (one of each per block) formed the main-plot treatments (12 or 10 replicates). The linear and quadratic terms of the differences between the water regime means in each leaf age were tested using 't'. To reduce the random variation in the plants, they were re-randomised each week within the blocks.

Results

Experiment 2 was marred by the presence of a number of diseased aphids (fungal attack) which occurred almost entirely on the mature leaves of the plants in the dry regime. The results for this treatment are, therefore, excluded from the discussion of the results.

Temporal Parameters

The mean values in the three experiments for the various temporal parameters listed above are shown in Table 6 , where significant differences between means are also indicated. The mean values of these parameters are also presented together in Fig. 17.

Length of Larval Life(LL)

For aphids feeding on old and mature leaves of the plants, the water regimes did not significantly influence their LL, though the linear decrease of LL with increasing water strain in the mature leaves of Experiment 3 was close to

significance ($p>0.05$). Such a linear decrease could be demonstrated on the young leaves of this experiment ($p<0.01$; quadratic term not significant), where the mean LL of the aphids on the plants of the dry regime was significantly less than that of the medium ($p<0.05$) and wet regimes ($p<0.01$).

In general, the aphids developed to adult faster with the increasing age of the leaves, except where water strain in the plants was severe. Thus, the aphids feeding on the mature leaves of the plants in the medium and wet regimes developed to adult faster than the aphids on the young leaves of these plants; the aphids feeding on the old leaves of the plants in the medium regime developed to adult faster than the aphids on the mature leaves of the plants in the wet regime.

Length of Adult Life (AL)

Adult aphids feeding on young leaves lived longer on the plants of the dry regime than on those of the other regimes. On mature leaves the adult aphids lived longer on the plants of the medium and dry regimes when compared with the wet regime (Expt. 3). Within the two leaf ages the linear components of these effects of the water regimes were highly significant ($p<0.01$) while the quadratic components did not approach significance. On the old leaves the AL of the aphids increased from the wet to the medium regime and then decreased to the dry regime, (Expt. 1). The quadratic component of this effect was highly significant ($p<0.01$).

On the plants of the wet regime the age of the leaves did not affect the AL of the aphids. In the medium regime the

AL was shorter on the young than the mature leaves of the plants. In the dry regime it seems probable that had Experiment 2 been wholly successful the AL of the aphids would have been shorter on the old leaves than on the other leaf ages.

Reproductive Life (RL)

The aphids reproduced for most of their adult life. The effects of the water regimes and different aged leaves on the AL, just described, reflected very similar effects on the RL of the aphids, as shown clearly by Fig. 17. These effects will not, therefore, be described again.

Post-reproductive Life (PRL)

The water regimes significantly influenced the PRL of the aphids only when they were caged on the old leaves (Expt. 1) Here, the mean PRL of the aphids on the plants of the dry regime was reduced, when compared with the wet regime, ($p<0.05$); the water regime sum of squares was composed primarily of a linear component which was significant ($p<0.05$) while the quadratic component was not significant, suggesting a linear fall of PRL with increasing water strain in the old leaves.

With the exception of the old leaves in the dry regime, a trend of increasing PRL with the increasing age of the leaves was detectable (cf. larval life), but the comparisons between different leaf ages within each water regime revealed no significant differences. Nevertheless, the mean PRL of the aphids on the young and mature leaves of all plants were compared (in Expt.3) and exposed a longer PRL of the aphids on

Table 6 Mean temporal parameters of apterous virginoparae of M. persicae on 3 leaf ages of Brussels sprouts in 3 water regimes at 20°C

| | | | Larval Life (LL)(days) | | | | Adult Life (AL)(days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Water Regime | | | | Water Regime | | | |
| | | | WET | MED. | DRY | L.S.D(W) | WET | MED. | DRY | L.S.D.(W) |
| Expt. 1. | Leaf age | O | 6.42 | 6.17 | 6.33 | p.05=0.75 | 28.08 | 30.66 | 18.79 | p.05=5.68<br>.01=7.72<br>.001=10.39 |
| Expt. 2. | Leaf age | O | 6.67 | 6.17 | 6.42 | p.05=0.80<br>.01=1.08 | 27.92 | 29.58 | 24.00 | p.05=5.97<br>.01=8.06 |
| | | M | 7.00 | 6.67 | (6.92)* | | 27.58 | 32.75 | (25.33) | |
| | L.S.D. (L) | | p.05 = 0.88 | | | | p.05 = 6.44 | | | |
| Expt. 3. | Leaf age | M | 9.80 | 8.60 | 8.30 | p.05=1.85<br>.01=2.52 | 26.90 | 33.50 | 36.30 | p.05=5.43<br>.01=7.38 |
| | | Y | 11.10 | 10.50 | 8.00 | .001=3.41 | 26.90 | 27.70 | 36.10 | .001=9.94 |
| | L.S.D. (L) | | p.05 = 1.32<br>.01 = 1.78<br>.001 = 2.37 | | | | p.05 = 5.50<br>.01 = 7.43<br>.001 = 9.89 | | | |

* In parenthesis - including diseased aphids (see text).

L.S.D.(W) = least significant difference between two water regime means in a single leaf age or a different leaf age.

L.S.D.(L) = least significant difference between two leaf age means in a single water regime.

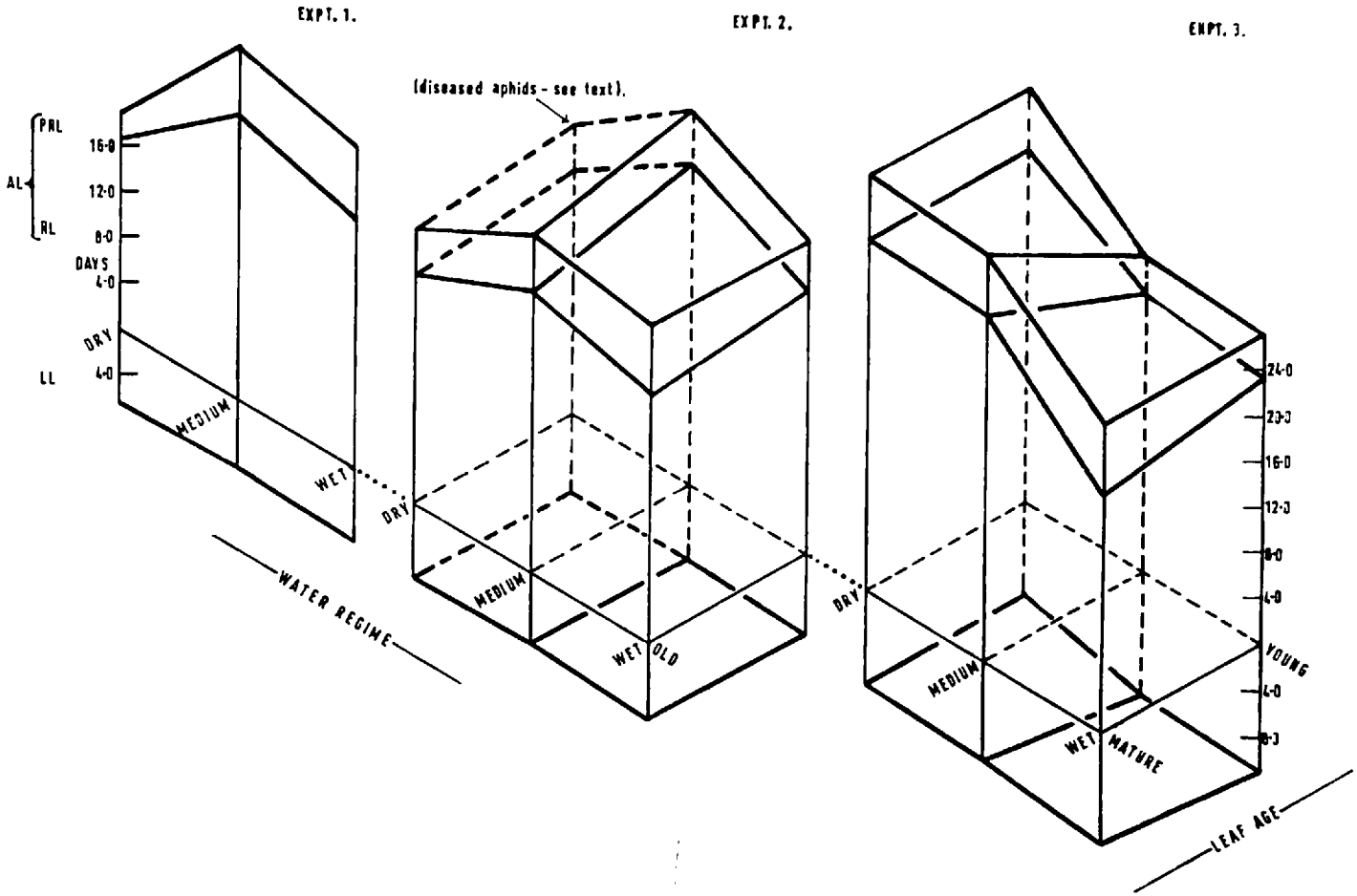
(cont. overleaf)

Table 6 (cont.).

| | | Reproductive Life (RL)(days) Water Regime | | | | Post-reproductive Life (PRL)(days) Water Regime | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | WET | MED. | DRY | L.S.D.(W) | WET | MED. | DRY | L.S.D.(W) |
| Expt. 1. Leaf age | O | 21.83 | 24.75 | 16.50 | p.05=3.69<br>.01=5.02<br>.001=6.75 | 6.25 | 5.92 | 2.25 | p.05=3.76 |
| Expt. 2. Leaf age | O | 21.83 | 24.58 | 19.92 | p.05=4.79<br>.01=6.47<br>.001=8.61 | 6.08 | 5.00 | 4.08 | p.05=2.66 |
| | M | 23.08 | 28.08 | (21.42)* | | 4.50 | 4.67 | (3.92) | |
| L.S.D. (L) | | p.05 = 5.50 | | | | p.05 = 2.21 | | | |
| Expt. 3. Leaf age | M | 20.70 | 30.10 | 30.60 | p.05=4.14<br>.01=5.63<br>.001=7.58 | 6.20 | 5.40 | 5.70 | p.05=2.54 |
| | Y | 23.20 | 24.20 | 30.80 | | 3.70 | 3.50 | 5.30 | |
| L.S.D. (L) | | p.05 = 4.22<br>.01 = 5.70<br>.001 = 7.59 | | | | p.05 = 2.56 | | | |

* In parenthesis - including diseased aphids (see text).
Lettering as in the first half of the Table.

FIG. 17. MEAN LENGTH OF TEMPORAL PARAMETERS OF APTEROUS VIRGINOPARAE OF M.persicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. LL = LARVAL LIFE; RL = REPRODUCTIVE LIFE; PRL = POST-REPRODUCTIVE LIFE; AL = ADULT LIFE. SEE TABLE 6 FOR SIGNIFICANT DIFFERENCES.



the mature leaves ($p<0.05$).

Reproductive Parameters

The mean values for the reproductive parameters of the aphids from each leaf age and water regime are presented in Table 7 and Fig. 18.

Fecundity

The fecundity of the aphids was greatly influenced by feeding the aphids on leaves of different ages and water status (Fig. 18a). Fecundity on young and mature leaves increased with the water strain in the host plant (medium and dry regimes). On both leaf ages the linear components of these increases were significant ($p<0.001$) while the quadratic components were far from significant. On old leaves the fecundity similarly increased on the plants of the medium regime compared with the wet regime (Expt. 2, $p<0.01$), but on the old leaves of the plants of the dry regime, fecundity was considerably reduced compared with the other regimes. Thus, on old leaves, the quadratic components of the effects of the water regimes on fecundity were significant in both Expt. 1 ($p<0.01$) and Expt. 2 ($p<0.001$).

Although the aphids feeding on the young leaves of the plants produced consistently more progeny than those feeding on the mature leaves, (all plants, $p<0.05$), the differences between the two leaf ages within the water regimes were never significant. Except on the plants of the dry regime, the fecundity of the aphids feeding on the old leaves was much higher than on the mature leaves ($p<0.001$) and, presumably, the young leaves. The

results suggest that in the dry regime the fecundity of the aphids on the old leaves would have been lower than the other leaf ages if disease of the aphids had not interfered with Experiment 2.

Reproductive Rate (R)

Variations in the reproductive rate of the aphids (see Fig. 18b) on leaves of different ages and water status closely resembled the variations in fecundity and are not, therefore, described fully. The aphids reproduced much faster when feeding on the old leaves of the plants than on the mature leaves (and probably young leaves), unless water strain in the plants was severe. In general, the aphids reproduced faster on the young leaves than on the mature leaves of the plants, but this was significant only in the medium regime of Expt. 3. This effect of leaf age would seem to be marginal (cf. fecundity).

Figures 19 and 20 show the mean numbers of progeny produced on each day of the lives of the aphids in the different treatments. In Fig. 19 the progeny of each day are presented separately and in Fig. 20 they are accumulated. The Figures show that the differences in the fecundity of the aphids already reported are generally due to differences in the production of progeny throughout the lives of the adult aphids and not, for example, due to differences solely at the beginning of the adult life as a result of effects on the aphids during their larval life.

Table 7 . Mean and Maximum reproductive parameters of apterous virginoparae of M. persicae on 3 leaf ages of Brussels sprouts in 3 water regimes at 20°C

| | | | Fecundity | | | | Reproductive Rate (larvae/day) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Water Regime | | | | Water Regime | | | |
| | | | WET | MED. | DRY | L.S.D.(W) | WET | MED. | DRY | L.S.D.(W) |
| Expt. 1. | Leaf age | 0 | 65.67 | 75.67 | 49.92 | p.05=13.87 | 3.62 | 3.87 | 3.59 | p.05=0.64 |
| | | MAX. | 82 | 102 | 68 | .01=18.85 | 5.0 | 5.2 | 4.9 | |
| | | | | | | .001=25.36 | | | | |
| Expt. 2. | Leaf age | 0 | 56.00 | 70.83 | 45.50 | p.05=10.57 | 3.02 | 3.47 | 2.56 | p.05=0.39 |
| | | MAX. | 79 | 95 | 61 | .01=14.29 | 3.9 | 4.4 | 3.1 | .01=0.52 |
| | | M | 33.08 | 47.50 | (35.58)* | .001=19.08 | 1.67 | 2.00 | (1.98) | .001=0.70 |
| | | MAX. | 61 | 71 | 55 | | 2.6 | 2.5 | 2.7 | |
| | L.S.D. (L) | | p.05 = 9.86 | | | | p.05 = 0.32 | | | |
| | | | .01 = 13.25 | | | | .01 = 0.44 | | | |
| | | | .001 = 17.52 | | | | .001 = 0.58 | | | |
| Expt. 3. | Leaf age | M | 25.60 | 37.30 | 49.50 | p.05=6.96 | 1.47 | 1.37 | 1.81 | p.05=0.29 |
| | | MAX. | 41 | 49 | 68 | .01=9.48 | 2.4 | 1.6 | 3.0 | .01=0.39 |
| | | Y | 31.20 | 38.30 | 54.70 | .001=12.80 | 1.39 | 1.69 | 2.02 | .001=0.52 |
| | | MAX. | 46 | 50 | 70 | | 1.6 | 2.3 | 3.0 | |
| | L.S.D. (L) | | p.05 = 6.27 | | | | p.05 = 0.32 | | | |

* In parenthesis - including diseased aphids (see text).
Lettering as in Table 6 .

FIG. 18. MEAN REPRODUCTIVE PARAMETERS OF APTEROUS VIRGINOPARAE OF M.persicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.

SEE TABLE 7 FOR SIGNIFICANT DIFFERENCES.

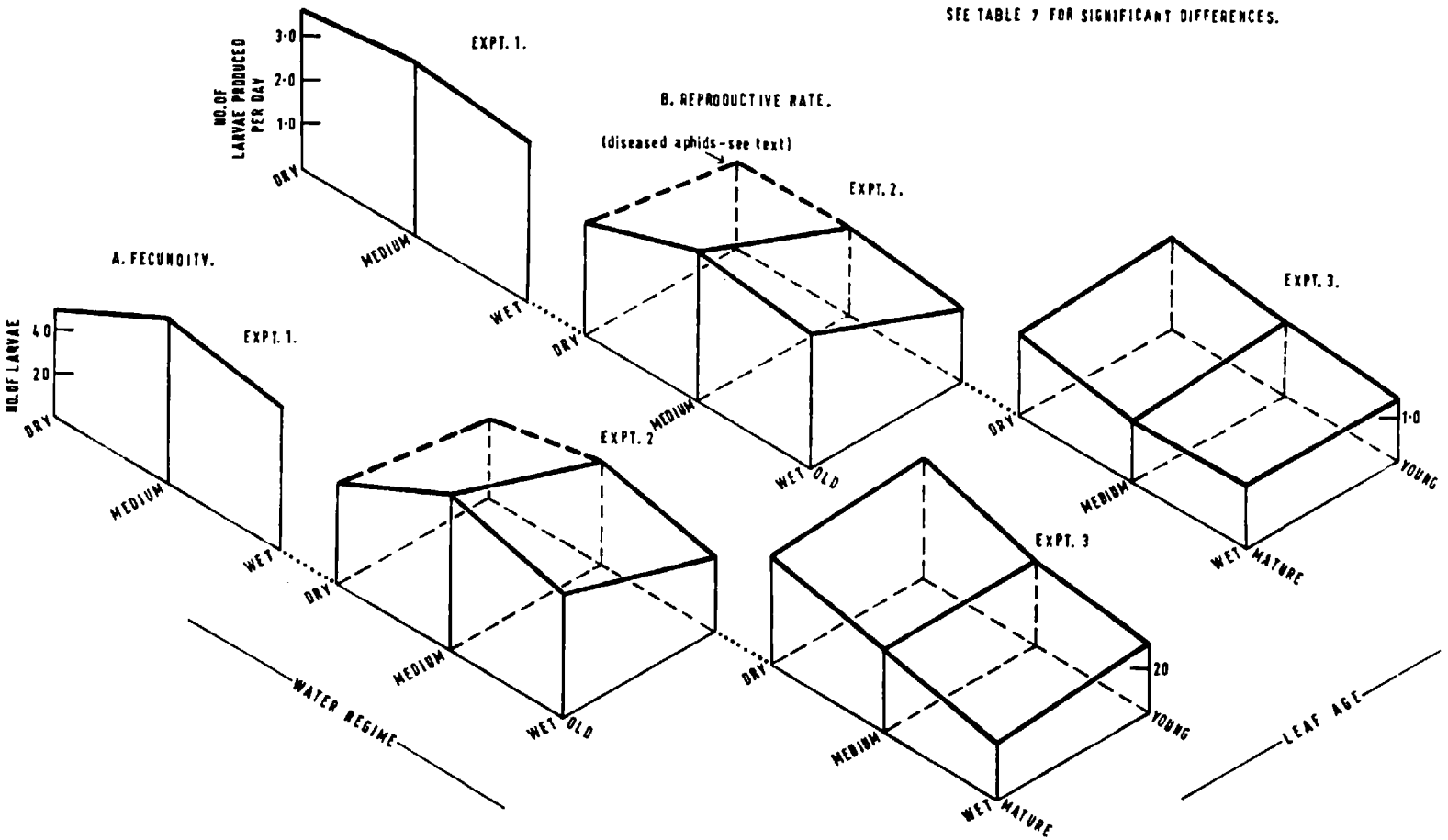


FIG.19. MEAN NUMBER OF LARVAE PRODUCED PER DAY BY APTEROUS VIRGINOPARAE OF M.persicae (DURING THEIR MEAN ADULT LIFE) ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.

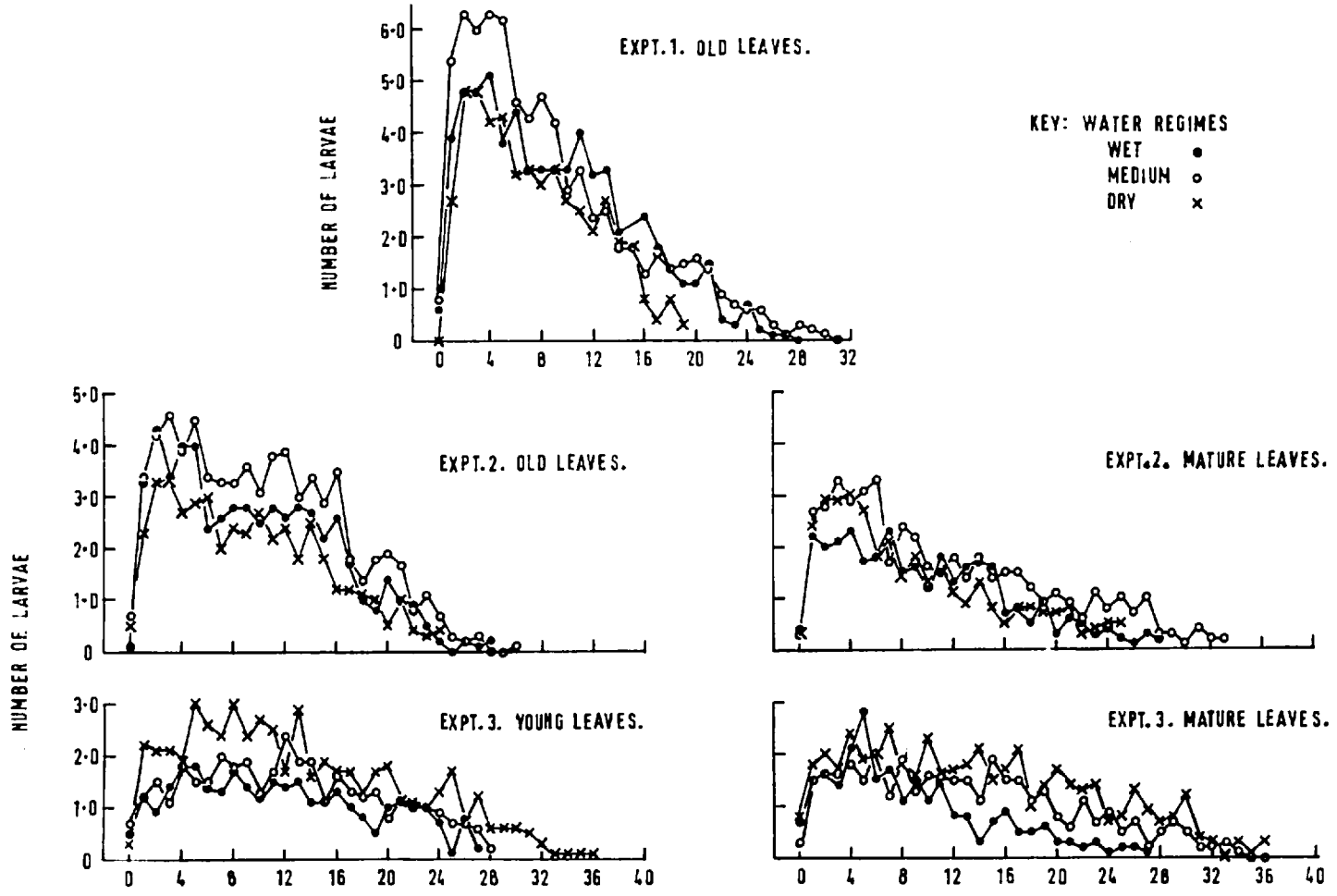
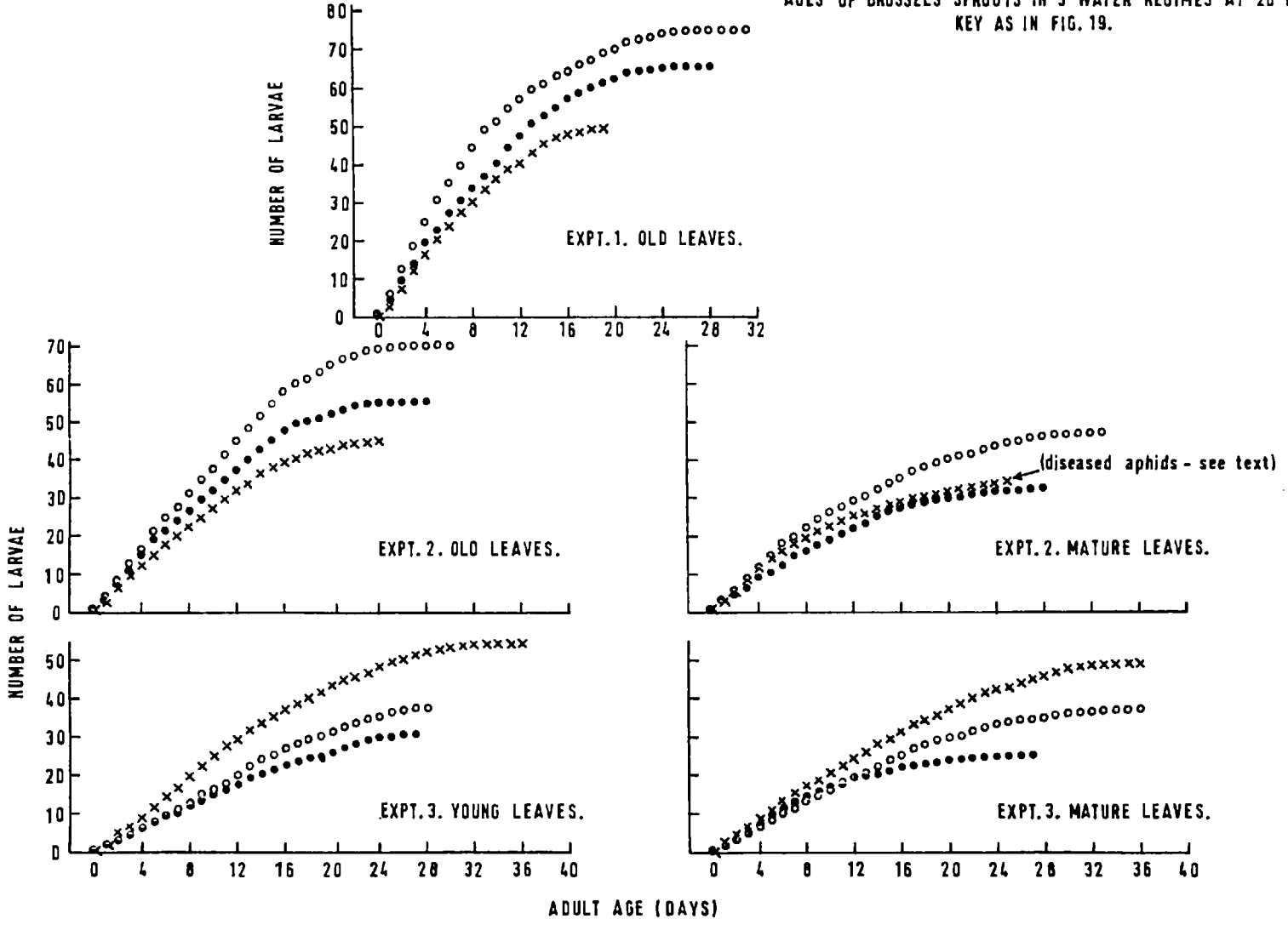


FIG. 20. ACCUMULATED DAILY MEAN NUMBER OF LARVAE PRODUCED BY APTEROUS VIRGINOPARAE OF M.persicae (DURING THEIR MEAN ADULT LIFE) ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. KEY AS IN FIG. 19.



Conclusions

To summarise the results of these experiments:

1). When the aphids were caged on young or mature leaves of the host plants, increasing water strain in the plants was associated with increasing fecundity, reproductive rate, reproductive life and adult life, and decreasing larval life of the aphids. The post-reproductive life of the aphids was not significantly affected.

2). When aphids were caged on the old leaves of the host plants, increasing water strain in the plants was associated with an initial rise (medium regime) in the fecundity, reproductive rate, reproductive life and adult life of the aphids followed by a fall (dry regime) of these parameters. The post-reproductive life of the aphids fell consistently as the water strain in the plant increased but the larval life was not significantly affected.

3). On the plants of the wet and medium water regimes the larval life of the aphids generally decreased and the post-reproductive life generally increased with the age of the leaves upon which the aphids were caged. The fecundity and reproductive rate of the aphids were slightly higher on young leaves and much higher on old leaves than on the mature leaves of the plants. The lengths of the adult life and reproductive life of the aphids were little affected by the age of the leaves.

4). The aphids on the plants of the dry regime responded to the age of the leaves like the aphids on the plants of other regimes (see 3), with the exception of the aphids on the old leaves.

On the old leaves all the parameters of the aphids, save the larval life, were probably smaller than the parameters of the aphids on the other leaf ages, but the failure of one part of one experiment made this comparison difficult and inconclusive.

c) Production of Alatae (≡ Aphid departure from the host plant).

A large number of environmental factors are known to influence the proportion of alatae in a population of aphids, (see Bonnemaison, 1950; Hille Ris Lambers, 1966). Much recent evidence has pointed to the direct interaction between aphids ("crowding") as a major factor determining their morph (Bonnemaison, 1950; Lees, 1961; Johnson, 1965) and Lees (1961) has suggested that much of the earlier work in this field should be re-examined in the light of these findings. Nevertheless, a large body of research has accumulated showing that the proportion of alatae in a population of aphids is influenced by the physiological condition of the host plant, (see Review of the Literature, p. 51 ).

Of particular interest to the present work are the findings that different aged leaves and host plants of different water status affect form determination of aphids, particularly virginoparae. In general, water strain in the host plant (often wilting) has been associated with an increased proportion of alatae in the aphid population(e.g. Markkula, 1953; Kennedy, et al, 1958). Other workers have related an increase in the formation of alate aphids to an increase in the osmotic

pressure (possibly a decrease in the turgor pressure) of the cells of the host plant (Shaposhnikov, 1959a; Weismann, Macko and Fekete, 1960). Noda (1960) found that the proportion of alatae in the progeny of apterous A.maidis increased with the age of the leaves upon which the progeny were fed (see also Smith, 1923; Rivnay, 1937; Kennedy et al, 1958).

The percentage alatae in populations of M. persicae was found to increase with falling levels of sugars and nitrogenous compounds in the host plants (Pintera and Ulrychová, 1959). Davies and Whitehead (1935) observed abundant alatae and alatiform nymphs of M. persicae on wilting cruciferous weeds and attributed the high numbers of alatae to the wilting of the plants. This observation received support from the work of Wearing (1963) who recorded an increased production of alatae in free-living populations of M. persicae on Brussels sprouts suffering intermittent wilting compared with well watered plants. As a further step in this work experiments were undertaken to measure the percentages of alatae in the progeny of individual apterae of M. persicae caged on different aged leaves of Brussels sprouts in three water regimes, (Stage (c) of the Cycle, p. 72 ).

Experimental Procedure

Three experiments were conducted to measure the reproductive and temporal parameters of apterae of M. persicae on three leaf ages of Brussels sprouts in three water regimes, (p. 87 ). As already indicated (p. 132) the progeny of the

apterae were retained in leaf cages, (p. 84 ). Here the nymphs remained until their morph could be readily seen by the presence or absence of wing-pads (3rd - 4th instar). Each leaf cage contained a leaf of an age similar to that on which the mother aptera was caged. The leaf cage and the aphids which it contained were transferred from leaf to leaf as the leaves aged so that the aphids always fed on leaves of one age category. Further details of these experiments are given on p. 130 .

The percentages of alatae in the progeny of each aptera were calculated and transformed to angles using the arc/sin transformation to facilitate analysis (see p. 133 ). In preparation for the analyses, zeros were removed by adding 1 to all the results before the percentages were calculated.

Results

The mean percentages of alatae (transformed to angles) in the progeny of apterous M. persicae in the different treatments of the three experiments are shown in Table 8 , where significant differences between the means are indicated. Fig. 21 shows the mean percentages of alatae calculated from the raw data.

The percentage of alatae in the progeny of apterae caged on the young leaves was not significantly influenced by the water regimes of the host plants. The progeny of the apterae on the mature leaves contained a smaller proportion of alatae as the dryness of the water regime increased. The linear component of this effect was significant ($p<0.01$, Expt.2;

Table 8. Mean percentages of alatae in the progeny of apterous virginoparous M. persicae caged on 3 leaf ages of Brussels sprouts in 3 water regimes at 20°C

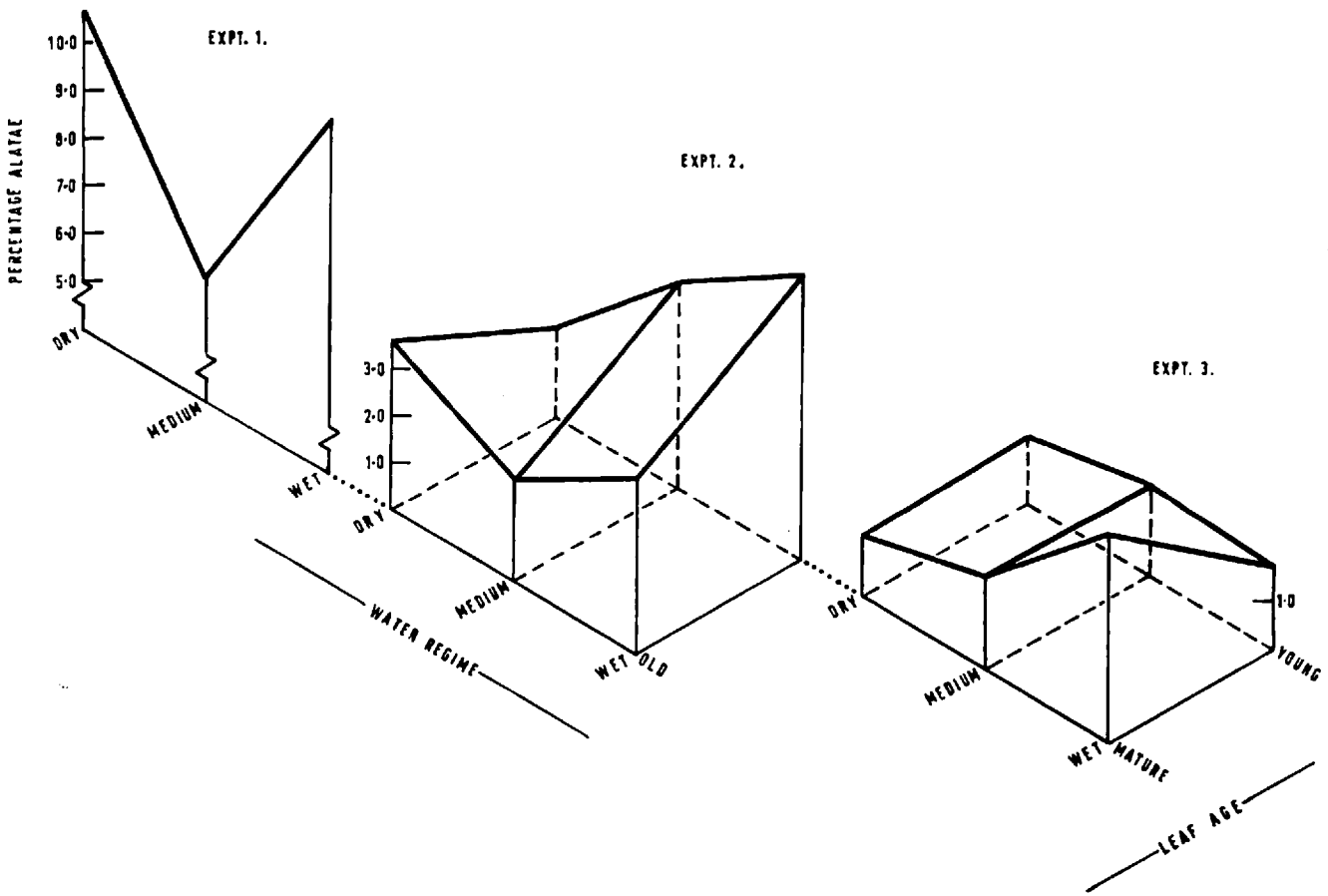
Mean Percentage of Alatae (Transf. to angles in mins.).

| | | Water Regime | | | |
|---|---|---|---|---|---|
| | | WET | MEDIUM | DRY | L.S.D.(W) |
| Expt. 1. Leaf age | O | 19.20(10.82)* | 14.19(6.01) | 17.35(8.89) | p.05=3.41<br>.01=4.63<br>.001=6.23 |
| Expt. 2. Leaf age | O | 11.96(4.30) | 9.87(2.95) | 12.20(4.48) | p.05=3.63<br>.01=4.92<br>.001=6.58 |
| | M | 15.33(6.99) | 13.13(5.17) | 10.25(3.18) | |
| L.S.D. (L) | | | p.05 = 2.66<br>.01 = 3.57 | | |
| Expt. 3. Leaf age | M | 13.77(5.67) | 9.79(2.90) | 7.79(1.86) | p.05=2.06<br>.01=2.80<br>.001=3.78 |
| | Y | 9.96(2.99) | 9.92(2.97) | 8.86(2.40) | |
| L.S.D. (L) | | | p.05 = 1.91<br>.01 = 2.58<br>.001 = 3.44 | | |

* In parenthesis - detransformed percentages.
Lettering as in Table 6 .

FIG. 21. MEAN PERCENTAGE ALATAE IN THE PROGENY OF APTEROUS VIRGINOPARAE OF M.persicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.

SEE TABLE 8 FOR SIGNIFICANT DIFFERENCES.



$p<0.001$, Expt. 3), and the quadratic component was far from significant. On the old leaves a similar initial fall in the percentage of alatae occurred in the progeny of apterae on the plants of the medium regime compared with the wet regime ($p<0.05$, Expt. 1) but in the dry regime the percentage of alatae rose again thereby creating a significant quadratic component in the effect of the water regimes ($p<0.01$, linear component not significant, Expt. 1).

On the plants of the wet regime a higher percentage of alatae developed in the progeny of the apterae caged on the mature leaves compared with the young and old leaves ($p<0.001$ and $p<0.05$ respectively). A higher percentage of alatae was also recorded on the mature leaves (and possibly on the young leaves by extrapolation) compared with the old leaves of the plants in the medium regime ($p<0.05$). There were no significant differences between the percentages of alatae on the different leaf ages of the plants of the dry regime.

Conclusions

The percentage of alatae in the progeny of apterous virginoparous M. persicae caged on the young leaves of Brussels sprouts was not significantly affected by changes of the water strain in the plants. The progeny of the apterae on the mature and old leaves, however, contained a lower percentage of alatae as the water strain in the plant increased, except on the old leaves of the plants in the dry regime. These leaves suffered intermittent wilting and the percentage of alatae was increased

compared with that obtained on the old leaves of the plants in the medium regime.

Higher percentages of alatae were obtained in the progeny of apterae caged on the mature leaves of well watered plants when compared with the young and old leaves. These leaf age effects were absent on plants suffering intermittent severe water strain, while on plants of an intermediate water status the mature leaves were associated with a higher percentage of alatae only in comparison with the old leaves.

Variations in the density of the progeny of different treatments did not appear to influence the results. High percentages of alatae were normally encountered in the progeny of apterae of low fecundity (cf. Fig 18 and Fig. 21).

d) Summary of Results - The Suitability of the Host Plant

As suggested earlier, assuming that the host plants of aphid populations are always temporary, the suitability of a plant as a host for a population of aphids may be estimated from the relationship between the aphids and the plant throughout the cycle of movement of the aphids from one host plant to another (see p. 72 ).

Within the stages of the cycle, the nutritive value of the host plant is generally considered to increase as the rate of development, fecundity and rate of reproduction of the aphids on the plant increase and their production of alatae decreases.

Such changes of nutritive value may result from changes in the quality and/or quantity of the food ingested by the aphids. Taylor (1959) also regarded a long post-reproductive life and low variability of the fecundity of the aphids as indicating that the aphids had fed on a plant of high nutritive value. For a plant to be a suitable host for aphids, the different stages of the cycle - arrival, establishment, development and departure of the aphid population - must be co-ordinated. For example, where this does not occur, the aphids may reject a plant which is nutritionally adequate for their development and reproduction (Wensler, 1962) or, conversely, may accept a plant which is inadequate for their development and reproduction (Taylor, 1959). In the present work, this reasoning is applicable to host plants of differing physiological condition and to the different parts of a host plant.

The experiments have shown that apterous M. persicae generally settled preferentially on those leaves of an age and water status on which other apterae had (i) higher fecundity, reproductive rate, reproductive life and adult life, and (ii) lower variability of fecundity (cf. Taylor, 1959) and production of alatae (compare Fig. 16 and Figs. 17, 18 & 21). This kind of co-ordination has been reported with fewer aphid parameters for A. fabae, (Kennedy and Booth, 1951, 1954, 1959; Kennedy et al, 1958). To a lesser extent the feeding preferences of the apterae paralleled the differences in the rate of larval development and the post-reproductive life of the aphids on the leaves of different water status, but both these aphid

parameters tended to increase with the age of the leaves on which the aphids fed, a trend not wholly consistent with the other results (see Discussion p. 296 ).

The comparable co-ordination between the parameters of the apterae and the selection of the plants by the alate aphids was less precise but was still apparent (compare Figs. 11 & 12 with Figs. 17, 18 & 21) (cf. Kennedy and Booth, 1954). The preferences of alatae for different plants are presumably more important in the future development of the aphid population than their preferences for the different leaf ages of a single plant: the latter role is soon taken over by their apterous progeny. However, similar numbers of alatae settled on the plants in the different water regimes. The alatae assisted only in distributing the population on those leaves of greatest nutritive value on each plant, as reflected by the fecundity and other parameters of the apterae. Even these leaf age preferences were over-shadowed by relatively high densities of the alatae on the apices of all the plants.

In view of the close co-ordination between the stages of the cycle, the suitability of the leaves of different age and water status as hosts for the aphids was reflected in the parameters of the apterae. Thus, the host suitability (= nutritive value) of the different leaves was considered to increase with decreases in the production of alatae and the larval life of the apterae and with increases in their other parameters.

The results of the whole series of experiments may be

summarised in terms of the host suitability of the different leaves and plants:

1. The host suitability of the young leaves increased with intermittent water strain in the plant. The feeding preferences of the apterae (Fig. 14a) suggested that the suitability of the young leaves was particularly increased during the wilting of the old leaves of the plants.

2. The host suitability of the mature leaves increased with intermittent water strain in the plant.

3. The host suitability of the old leaves increased with intermittent mild water strain in the plant (except in terms of the post-reproductive life of the aphids), but decreased again with intermittent severe water strain in the plant. The preference of the apterae for the former rather than the latter leaves persisted only as long as the latter suffered severe water strain (wilting) and, in view of the co-ordination of the preferences and parameters already cited, it would seem that the decreased suitability of the leaves was associated particularly with the wilting period. The rate of larval development of the apterae was similar on all the old leaves and cannot therefore, always be equated with rate of reproduction (Taylor, 1959). Any detrimental effect of wilting on larval development, inferred from the other results, was apparently compensated during non-wilting periods.

4. On turgid plants and plants subjected to intermittent mild water strain, the host suitability of the leaves increased in the order - mature, young, old. However, the rate of larval

development and the post-reproductive life of the apterae on the different leaves increased in the order - young, mature, old - and this may reflect a separate aspect of the nutritive value of the leaves.

5. On plants subjected to intermittent severe water strain, the host suitability of the young leaves was greater than that of the mature and old leaves. The preferences of the apterae (Fig. 15c) suggested that actual wilting of the old leaves reduced their host suitability (see 3 above) and increased that of the young and mature leaves.

6. The results showed that the overall host suitability of the plants increased with water strain in the plants, even perhaps to the level of intermittent severe water strain, where the decreased suitability of the old leaves was more than compensated by the increased suitability of the young and mature leaves.

## Sub-section 3 Brevicoryne brassicae

### a) Selection of the Host Plant (≡ Aphid arrival and Establishment)

#### i) Alatae

In addition to the general remarks regarding selection of the host plant by alate aphids (pp. 12 and 105), specific

comments may be made concerning the aphid B. brassicae.

Wensler (1962) found that the mustard oil glucoside, sinigrin, is a specific stimulus for selection of the host plant by B. brassicae.

The visual stimuli of host finding by alate B. brassicae in the field were studied by Kennedy, et al (1961). It was found that, like M. persicae, alate B. brassicae were attracted to yellow surfaces, such as young and senescing leaves, in preference to surfaces reflecting light with a smaller long/short wave ratio (cf. Moericke, 1955b). This preference for yellow was, however, slightly weaker than that shown by M. persicae. In earlier work Kennedy, et al (1959b), found that alate B. brassicae, after final alightment, accumulated at the apices of cabbage plants in the field. Similarly, Broadbent (1954) recorded the distribution of settling alatae on cauliflowersin the field. Over 75% of the aphids settled on the heart, upper leaves and upper middle leaves of the plants.

Variation in the water status of the host plant is not known to influence the settling of alate B. brassicae. This question was studied in a single experiment, (Stage (a) (i) of the Cycle, p. 72 ).

Experimental Procedure

The experiment was conducted and the results were analysed in the manner described for M. persicae (p.106 ).

At the time of counting the settled alatae, the mean available water deficits in the vermiculite of the eight plants

in each regime were:

Wet 10.7%

Medium 45.2%

Dry 86.5%

and the mean number of completed drying cycles were 11.0, 5.0 and 2.8 respectively. At this time, 47 days had passed since pricking out the host plants (cv. "Jade Cross") (March, 1966).

## Results

### Alate aphids per plant and leaf age

1) Numbers

The analysis of variance of the numbers of alatae settled on the experimental plants is summarised in Table 9. Blocking was effective in removing variations due to a gradient of settling alatae on either side of the 'source' plants.

Table 9 Analysis of Variance of Numbers of Alate Aphids

| Var$^{n}$ due to: | df | ss | ms | 'F' |
|---|---|---|---|---|
| Blocks | 7 | 2683.17 | 383.310 | 11.742 ** |
| Water | 2 | 248.81 | 124.405 | 3.811 * |
| Error (a) | 14 | 457.02 | 32.644 | |
| Whole Plot | 23 | 3389.00 | | |
| Leaf Age | 3 | 2244.08 | 748.027 | 16.960 ** |
| Leaf Age x Water | 6 | 228.86 | 38.143 | 0.865 n.s. |
| Error (b) | 63 | 2778.56 | 44.104 | |
| Total | 95 | 8640.50 | | |

'F' for 2 and 14 df etc. - see Table 3.
symbols as in Table 4.

a) Water Regime Comparisons

The mean number of settled alatae per plant in the wet, medium and dry regimes were 26.0, 33.1 and 17.4 respectively. In a 't'-test the numbers on the plants of the dry regime were significantly less than on those of the medium regime ($p<0.01$). This pattern was consistently repeated on each leaf age of the host plants in the three regimes (see Fig.22 Left (L) - Right (R)). However, significant differences between regimes were present only on the mature leaves where fewer alatae settled on the plants of the dry regime compared with the wet and medium regimes ($p<0.05$ and $p<0.01$ respectively).

b) Leaf Age Comparisons

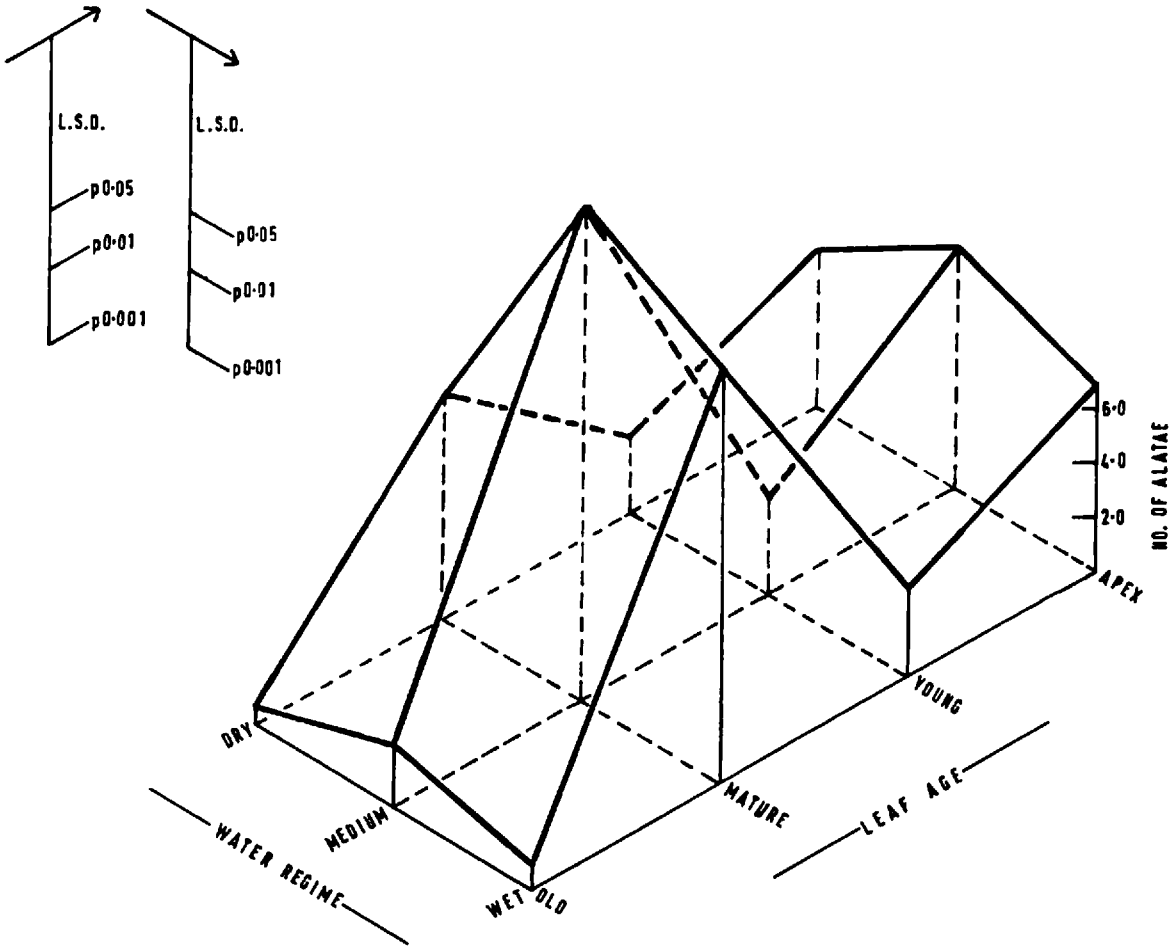
The mean number of alatae settled on each leaf age of the plants in all the water regimes were:

| | | |
|---|---|---|
| Apex | 7.208 | |
| Young | 3.167 | L.S.D. p 0.05 = 3.832 |
| Mature | 13.875 | 0.01 = 5.096 |
| Old | 1.250 | 0.001= 6.626 |

More alate aphids settled on the mature leaves than the leaves of any other age ($p<0.001$). Numbers on the apices of the plants were greater than on the young ($p<0.05$) and old leaves ($p<0.01$).

Figure 22 (Front (F) - Back (B)) shows the comparisons between numbers of alatae per leaf age for each water regime. Apart from slight changes in significant differences, the same pattern of distribution of alatae as just described was repeated within the water regimes, but in the dry regime the only

FIG. 22. MEAN NUMBERS OF ALATE VIRGINOPARAE OF B.brassicae SETTLED ON DIFFERENT LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20° C.



significant difference was between the numbers of alatae on the mature and old leaves ($p<0.05$).

2) Alate aphids per square cm.

Table 10 summarises the analysis of variance of alate aphids per square cm. settled on the plants.

Table10 Analysis of Variance of Alate Aphids/sq. cm.

| Var$^{n}$ due to: | df | ss | ms | 'F' |
|---|---|---|---|---|
| Block | 7 | 3.904942 | 0.557849 | 13.700 *** (*) |
| Water | 2 | 0.250236 | 0.125118 | 3.073 n.s. |
| Error (a) | 14 | 0.570045 | 0.040718 | |
| Whole Plot | 23 | 4.725223 | | |
| Leaf Age | 3 | 7.995388 | 2.665129 | 28.901 *** |
| Leaf Age x Water | 6 | 0.283055 | 0.047176 | 0.512 n.s. |
| Error (b) | 63 | 5.809516 | 0.092215 | |
| Total | 95 | 18.813182 | | |

F for 2 and 14 df etc. - see Table 3 . Symbols as in Table 4 .

Blocking again removed variation in the settling of the alatae due to a gradient on either side of the 'source' plants.

a) Water Regime Comparisons

The mean densities of alate aphids on the plants of the three regimes were:

Alate aphids/sq. cm.

| | | |
|---|---|---|
| Wet | 0.245 | L.S.D. p0.05 = 0.108 |
| Medium | 0.332 | 0.01 = 0.150 |
| Dry | 0.210 | 0.001 = 0.208 |

The density of alatae on the plants of the dry regime was significantly lower than on those of the medium regime ($p<0.05$) (cf. numbers of alatae per plant). The quadratic term of the differences between the three water regime means was significant in a 't'-test ($p<0.05$) in contrast to a very small linear term which was not significant.

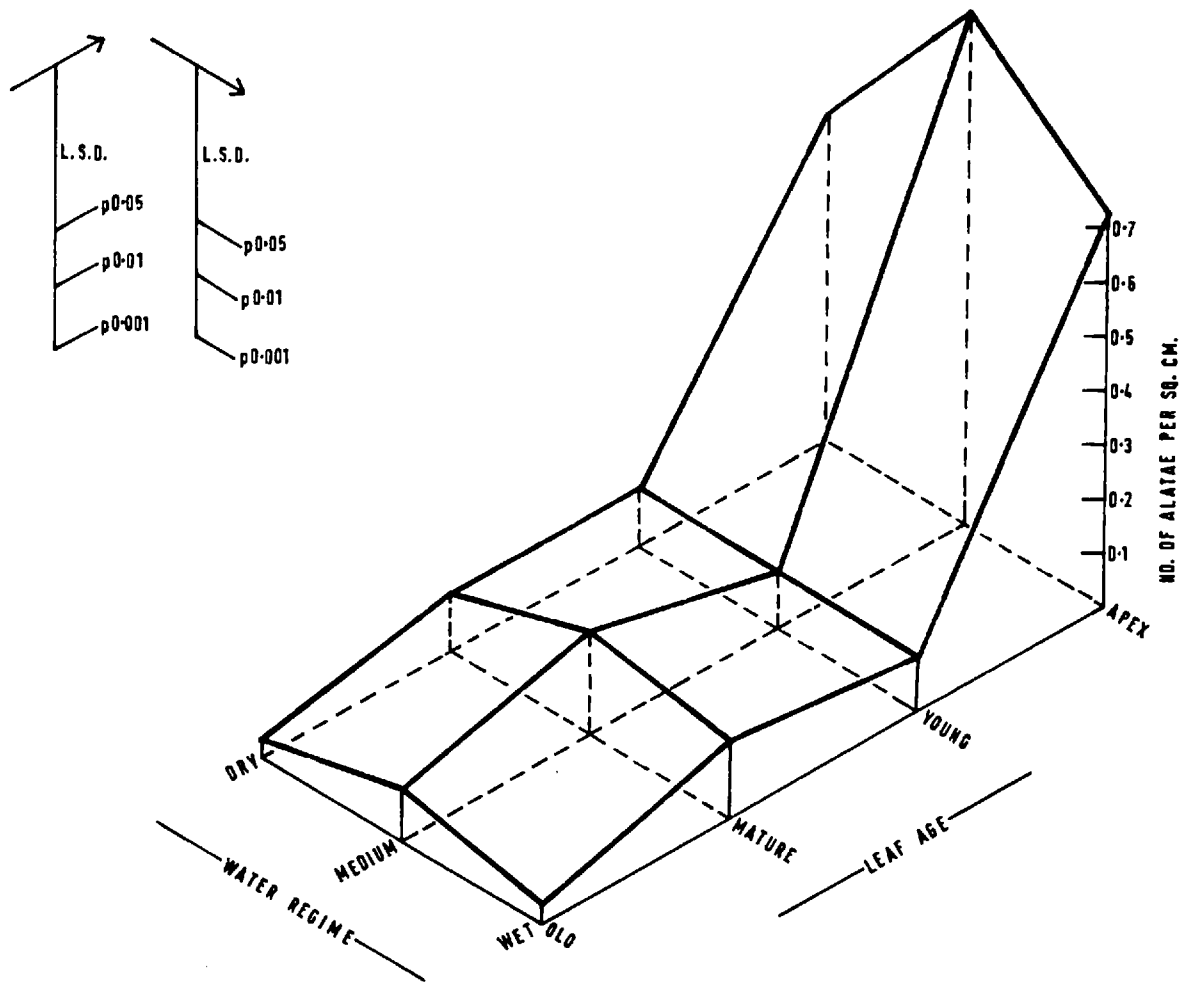
The mean density of alate aphids settled on each leaf age varied with the water regimes as shown in Fig. 23, (L-R). Unlike water regime comparisons with numbers of alatae(Fig. 22, L-R) there were no significant differences in the densities of alatae on the mature leaves. The only significant differences in densities occurred on the apices, between the medium and dry regimes ($p<0.05$). This formed part of a significant quadratic term in comparing all three regimes in a 't'-test ($p<0.05$), while the linear term was far from significant.

b) Leaf Age Comparisons

Conversion of numbers of alatae per leaf age to alatae per sq. cm. per leaf age essentially revealed that the alate aphids were most dense on the apices of the plants and that the large numbers of aphids on the mature leaves (Fig. 22) were spread thinly over a large leaf area. The mean density of alatae settled on each leaf age in all three water regimes combined were:

| | Alate aphids/sq. cm. | |
|---|---|---|
| Apex | 0.759 | |
| Young | 0.097 | L.S.D. p 0.05 = 0.175 |
| Mature | 0.143 | 0.01 = 0.233 |
| Old | 0.050 | 0.001= 0.303 |

FIG. 23. MEAN DENSITIES OF ALATE VIRGINOPARAE OF B.brassicae SETTLED ON DIFFERENT LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.



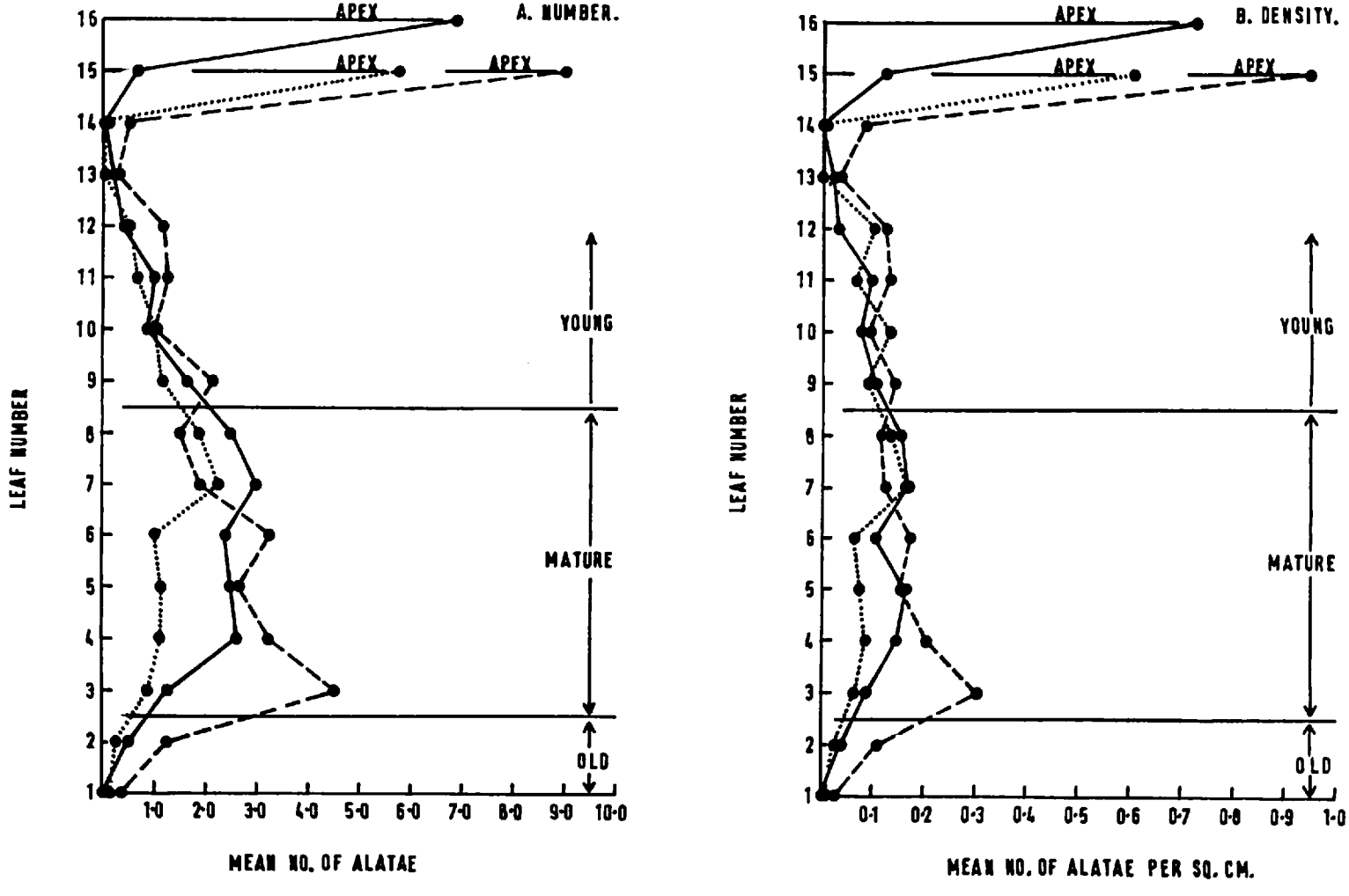
The density of alate aphids on the apices of the plants was greater ($p<0.001$) than on the other leaf ages, which were not significantly different from one another. Similar relationships were obtained within each water regime (Fig.23, F-B), there being little interaction between leaf age and water treatment (see Analysis of Variance).

Alate aphids per leaf

The mean numbers and densities of alate aphids settled on each leaf of the plants (numbered from the base) in the three water regimes are presented in Figs. 24 a and b. Division of the graphs into sections of young, mature and old leaves was based on the mean number of leaves of each age on the plants of each regime. The graphs were not statistically analysed.

In the light of the analyses already described it is possible to recognise the following important features of the graphs: 1) In general, greater numbers and densities of alatae settled on the plants of the medium regime, particularly compared with the dry regime, 2) reduced numbers and densities of alatae on the plants of the dry regime, particularly on the apices and lower mature leaves (N.B. Although the statistical analysis showed no significant reduction in the density of alatae on the mature leaves in the dry regime, it would seem probable that there was a reduction confined to the lower mature leaves), 3) overall very high numbers and densities of alatae on the apices of the plants in all regimes, 4) overall low numbers and densities of alatae on the old leaves of the plants in all regimes, 5) a generally even density of alatae on the

FIG. 24. MEAN NUMBERS AND DENSITIES OF ALATE VIRGINOPARAE OF B.brassicae SETTLED ON LEAVES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. FOR DETAILS SEE FIG. 13.



young and mature leaves though declining gradually with decreasing leaf age, 6) high numbers of alatae on the mature leaves (excl. dry regime) with a steady decline in numbers with decreasing leaf age on to the young leaves of the plants.

Conclusions

When the whole plant is considered, alate B. brassicae settled in greater numbers and densities on host plants suffering an intermediate level of water strain between full turgidity and wilting of the basal leaves. The main components of this quadratic effect were found in the similar (quadratic) responses of the alate aphids to the water regimes when settling on the apices and mature leaves of the plants.

Alate B. brassicae responded to leaf age by settling in greatest numbers on the mature leaves of the plants, (except where water strain in the plant was severe). However, it seems probable that these high numbers of alatae were there mainly because of the large area of mature leaves available for settling. It would appear that the alatae settled preferentially on the apices of the plants, irrespective of water regime, since it is on the apices that by far the greatest densities of alate aphids occurred. Alate B. brassicae settled in very low numbers and densities on the old leaves of the plants whatever their water status.

As with M. persicae (p.118 ), it is worth noting that these measured settling responses of alatae to the leaf ages of the host plants may have occurred during and/or after the final alightment on the plants. The possible causes of the host plant

selection here described are discussed later (see p. 292).

a) Selection of the Host Plant (≡ Aphid arrival and establishment on the host plant)

ii) Apterae

The distribution of populations of aphids on plants has been briefly discussed (p. 25 ). With reference to B. brassicae, colonisation of the host plant generally originates in the apex of the plant and spreads from there to leaves of other ages, (Markkula, 1953; Miyashita, 1953; van Emden, 1965; Ôtake, 1966). This was seen in the cultures of B. brassicae used in the present work. In addition, high densities of aphids have been noted on flower - and seed-heads of the host plant, (Markkula, 1953). Observations such as these suggest that the apex of the host plant is the preferred site of feeding of B. brassicae, including apterae. However, direct evidence in support of this is not available. Experiments were, therefore, carried out to measure the preferences of apterous virginoparae of B. brassicae for leaves of different ages and the interaction of these preferences with the water status of the host plant, (Stage (a) (ii) of the Cycle, p. 72 ).

Experimental Procedure

Apterous aphids of B. brassicae were permitted to select leaves of different ages and water status by the method already described for M. persicae, (p. 119). To recapitulate, the paired leaves in the preference tests were:

Within each water regime (on the same plant): Y-M, M-O, Y-O, with both wilting (W) and non-wilting (NW) plants in the dry regime.

Within each leaf age (on different plants):

10-50, 50-W90, 10-W90, 50-NW90, 10-NW90.

The results were analysed using $\chi^2$, (see p.121 ). The experiments were conducted in two series. The age of the plants (cv. "Jade Cross") during the experiments and the mean numbers of completed drying cycles of each regime in the two series were:

| | Plants | Water Regimes | | |
|---|---|---|---|---|
| | No. of days from pricking-out | Mean no. of completed drying cycles | | |
| | | Wet | Medium | Dry |
| Series 1. (Sept.-Oct. 1965) | 38 - 53 | 26.0 | 13.0 (after 53 days) | 6.5 |
| Series 2. (Nov.-Dec. 1965) | 39 - 48 | 22.0 | 12.3 (after 48 days) | 6.7 |

The age range of the plants did not appear to affect the results.

## Results

The data are again summarised graphically in equilateral triangles (see p. 122 ). In these experiments it was not uncommon to obtain a significant $\chi^2_{10}$ for the separate replicates of a preference test, and a $\chi^2_1$ for the summed replicates which was not significant, (e.g. Mature leaves, 10-50, Fig. 25b).

This indicated that the direction of preference between two specified leaves varied considerably in different replicates. This happened rarely with M. persicae, (p. 124). The difference between the two species of aphid may be associated with the greater tendency of B. brassicae to form aggregates when feeding on plants.

Water regime preferences

Between young leaves of different water status the apterae preferred the leaves of plants in the dry regime, and the wet and medium regime were accepted equally, 10 ⟷ 50 ⟶ W90, (Fig. 25a). Watering the plants of the dry regime did not alter the direction of preference in the 10-90 test. However, the 50-NW90 test yielded no significant difference in the distribution of the apterae on the two leaves.

Very different preference responses were obtained between mature leaves, 10 ⟵ 50 ⟵ W90, (Fig. 25b). The movement of the aphids away from the plants of the dry regime was a complete reversal of the preferences exercised between young leaves. Watering the plants of the dry regime nullified all preferences between the mature leaves, i.e. 10 ⟷ 50 ⟷ NW90 (Fig. 25b).

The directions of the preferences just described for mature leaves were repeated in extenso in tests with old leaves of different water status: 10 ⟷ 50 ⟵ W90 and 10 ⟷ 50 ⟷ NW90 (Fig. 25c). The aphids completely avoided the wilting old leave in many tests. Early experiments were unsuccessful when using old leaves in the late stages of senescence (entirely yellow).

The aphids did not settle on such leaves in sufficient numbers for a $X^2$ test to be applied. Thereafter the tests were carried out with old leaves in the early stages of senescence.

Leaf age preferences

Old leaves in all stages of senescence (excl. dying) were used in tests of the preferences of the aphids for different aged leaves.

On plants in the wet regime the aphids preferred to feed on the young and mature leaves, Y $\overleftarrow{\leftarrow}$ M $\leftarrow$ O, (Fig. 26a), but more aphids fed on the young leaves in the Y-M test only according to the $X_1^2$ analysis ($p<0.05$). This is thought to represent a weak preference for the young leaves.

The aphids showed similar preferences for the different leaf ages of the plants in the medium regime but young and mature leaves were apparently equally acceptable to the aphids, Y $\overleftarrow{\leftrightarrow}$ M $\leftarrow$ O, (Fig. 26b).

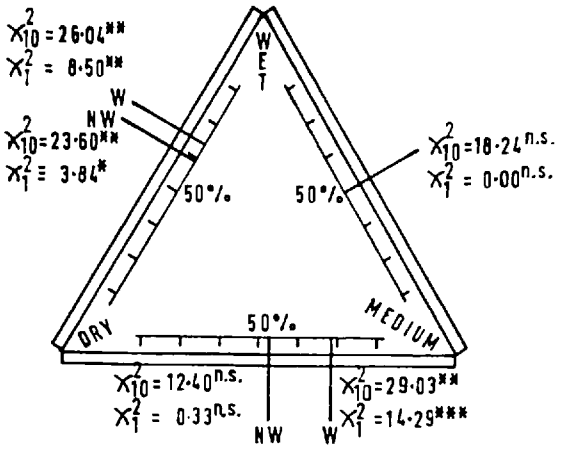
The aphids on the plants in the dry regime (W) exercised an overwhelming preference for the young leaves and a striking avoidance of the old leaves, Y $\overleftarrow{\leftarrow}$ M $\leftarrow$ O, (Fig. 26c). Very few aphids were observed to feed on the wilting old leaves, (less than 5% in the Y-O test) and young leaves were clearly preferred to mature leaves. When the plants were watered (NW), the aphids demonstrated a pattern of preferences very similar to that on the other regimes, with old leaves, as always, largely unacceptable to the aphids: Y $\overleftarrow{\leftrightarrow}$ M $\leftarrow$ O, (Fig. 26c).

The directions of the preferences between each leaf age-water regime category were entered in a 3-dimensional

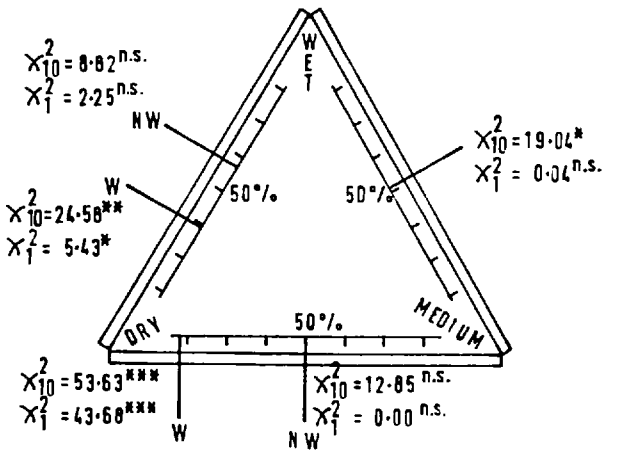
FIG. 25. FEEDING PREFERENCES OF ADULT APTEROUS VIRGINOPARAE OF B.brassicae BETWEEN BRUSSELS SPROUT LEAVES OF THE SAME AGE ON PLANTS OF DIFFERENT WATER STATUS AT 20°C. EACH SIDE OF THE TRIANGLES INDICATES THE MEAN PERCENTAGE DISTRIBUTION OF FEEDING APHIDS BETWEEN 2 LEAVES IN THE WATER REGIMES STATED.

SYMBOLS AS IN FIG. 14.

A. YOUNG LEAVES.




B. MATURE LEAVES.




C. OLD LEAVES.

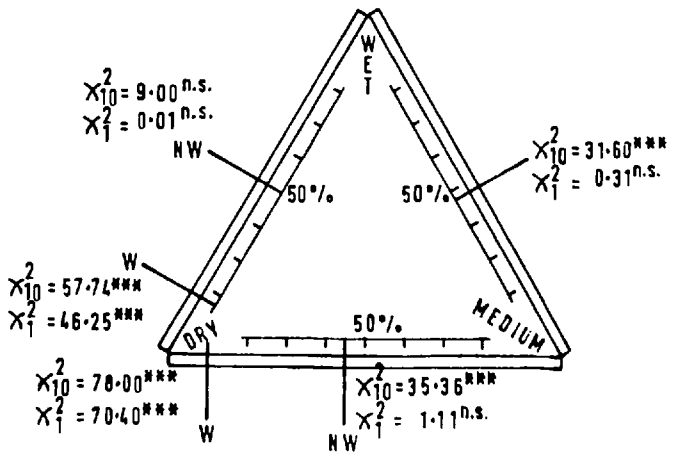
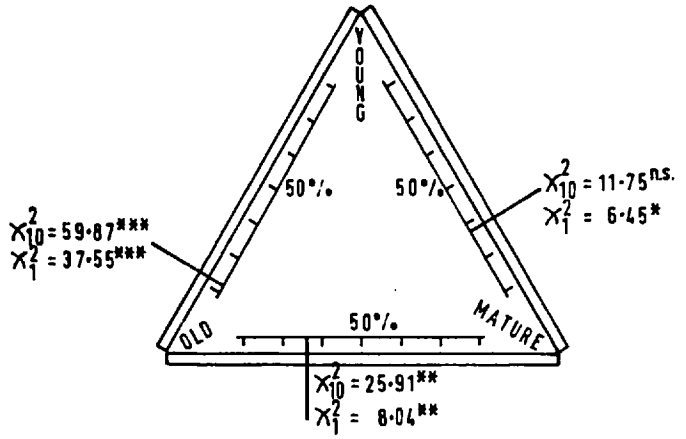
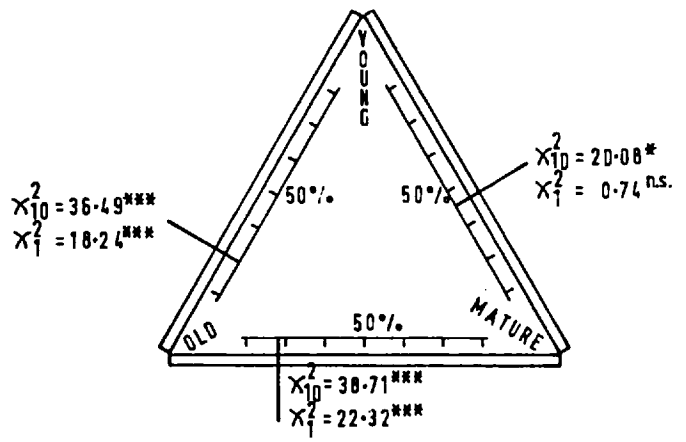


FIG. 26. FEEDING PREFERENCES OF ADULT APTEROUS VIRGINOPARAE OF B.brassicae BETWEEN BRUSSELS SPROUT LEAVES OF DIFFERENT AGE AT 20°C. EACH SIDE OF THE TRIANGLES INDICATES THE MEAN PERCENTAGE DISTRIBUTION OF FEEDING APHIDS BETWEEN 2 LEAVES OF THE AGES STATED. EACH TRIANGLE REFERS TO PLANTS OF A DIFFERENT WATER REGIME.
SYMBOLS AS IN FIG. 14.

A. WET REGIME.




B. MEDIUM REGIME.




C. DRY REGIME.

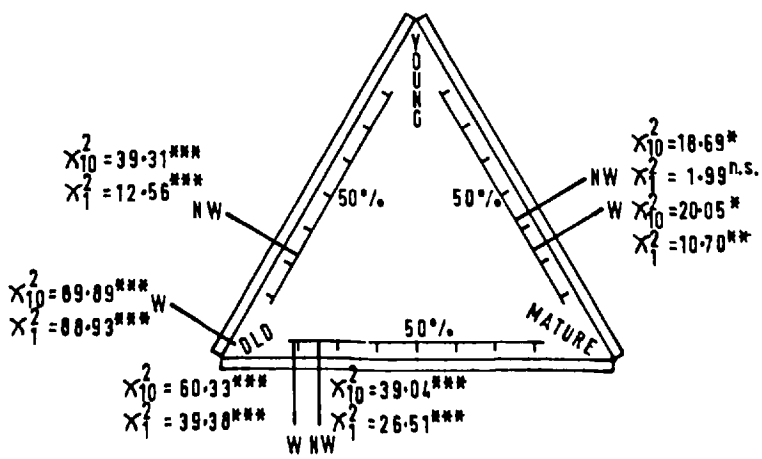
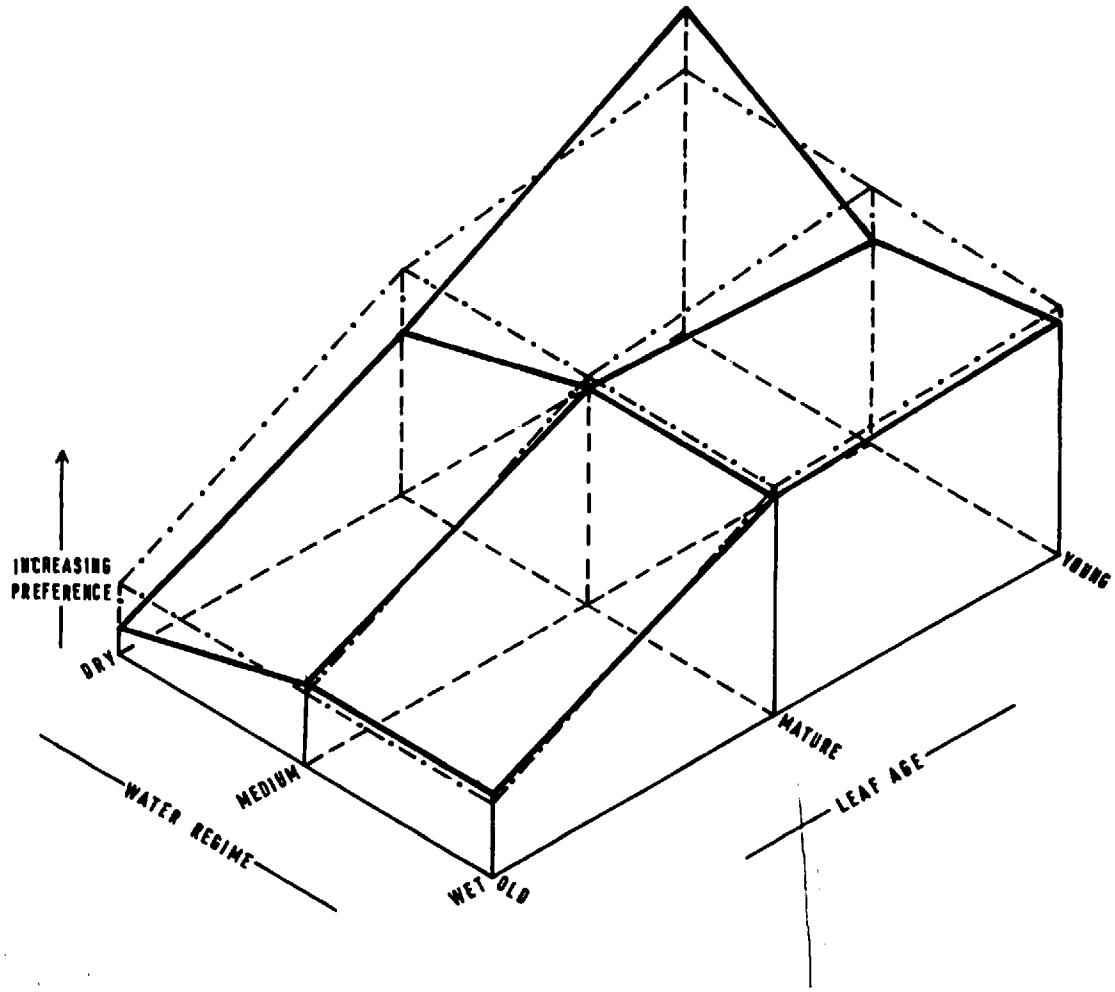


FIG. 27. SCHEMATIC DIAGRAM OF THE DIRECTIONS OF FEEDING PREFERENCES OF ADULT APTEROUS VIRGINOPARAE OF B.brassicae BETWEEN BRUSSELS SPROUT LEAVES OF DIFFERENT AGES AND WATER STATUS. COMPILED FROM FIGS. 25 AND 26. KEY AS IN FIG. 16.



diagram, (Fig. 27). In this form the preferences could be easily, though only approximately, compared with the results of other experiments, (Summary of Results p. 185).

Conclusions

Adult apterous virginoparae of B. brassicae demonstrated preferences for different aged leaves of Brussels sprouts in different water regimes. Young and mature leaves were preferred to old leaves in all tests, particularly when the old leaves were wilting. Young leaves were preferred to mature leaves only on well watered or wilting plants.

The preferences of the aphids for plants of different water status varied with the age of the leaves but on no occasion did the aphids show a preference when offered the leaves of plants in the wet and medium regimes. Similarly, one preference only, (Y, 10 $\longrightarrow$ NW90), was shown between the leaves of watered plants in the dry regime (NW) and the leaves of plants in other regimes. Preferences of the aphids for plants of different water status were, therefore, almost wholly confined to tests in which one of the plants was in a wilted condition: Young leaves of wilting plants in the dry regime were preferred to the young leaves of the plants in other regimes, while the opposite occurred between mature leaves and between old leaves.

These results are analysed further in the Discussion (p.292 ).

b) Reproductive and Temporal Parameters of Apterae (= Development of established populations of apterae on the host plant).

The development of established populations of apterous aphids on plants has been briefly discussed, with particular reference to aphids on the different leaf ages of host plants of different water status (p. 129). In a study of B.brassicae, Hughes (1963) found considerable retardation of the development of larvae reared on discs cut from immature heart leaves compared with discs from other leaf ages of cabbages. Markkula (1953) recorded greater reproduction of groups of apterous B. brassicae on young and old leaves than on very young, mature and dying leaves of cabbages. Reproduction was particularly high on the young leaves. The same author reported that reproduction of the aphids increased with water strain in the host plants, but the watering treatments altered the physiological age as well as the water status of the leaves on which the aphids were caged. In the field, outbreaks of B. brassicae have been associated with drought conditions (Cottier, 1953).

Experiments were undertaken to study individual apterae of B. brassicae caged throughout their lives on different aged leaves of Brussels sprouts in three water regimes (Stage (b) of the Cycle, p. 72 ).

Experimental Procedure

The procedure and analysis of the present experiments

was the same as described previously for M. persicae (p. 130 ). Four experiments were conducted: In the first and second, apterous aphids were studied on old and mature leaves, respectively, of the plants in the three water regimes; in the third and fourth, aphids were studied on two leaf ages in each experiment - old and mature, and mature and young, respectively. Table 11 shows the ages of the plants (cv. "Jade Cross") during the experiments and the mean numbers of completed drying cycles in each water regime at the end of the experiments.

Table 11 Host Plants and Water Regimes

| Age of Plants (days from pricking-out) | | Water Regimes Mean No. of Completed Drying cycles at end of experiments. | | |
|---|---|---|---|---|
| Start* of expts. | End of expts. | Wet | Medium | Dry |
| Expt. 1. 36 Jan-March 1964 | 77 | 27.0 | 12.2 | 6.3 |
| Expt. 2. 27 April-June 1964 | 71 | 28.0 | 13.7 | 7.0 |
| Expt. 3. 33 June-Sept. 1964 | 72 | 33.2 | 15.0 | 8.1 |
| Expt. 4. 33 April-June 1965 | 88 | 41.0 | 17.6 | 8.4 |

* as in Table 5 .

## Results

### Temporal Parameters

The temporal parameters of the aphids in these

experiments are the lengths, in days, of their larval life, adult life, reproductive life, and post-reproductive life. The mean values of these parameters in the four experiments are shown in Fig. 28 and Table 12 , which also indicates the significant differences between the means.

Length of Larval Life (LL)

When caged on young leaves, apterae of B. brassicae developed to adult faster as the dryness of the water regime surrounding the plants increased, (Expt. 4). The linear component of this effect was very highly significant ($p<0.001$) while the quadratic component was not significant. The LL of apterae on the mature leaves was unaffected by the water regime of the host plant, but on the old leaves in Expt. 3, the aphids developed to adult more slowly on the plants of the medium and dry regimes compared with the wet regime ($p<0.05$), a trend which was also visible in Expt. 1.

Comparisons between the LL of aphids on different leaf ages of the plants indicated that larval development was more rapid on mature than on young leaves, except on the plants of the dry regime. No other leaf age comparisons yielded significant differences.

Length of Adult Life (AL), Reproductive Life (RL) and Post-reproductive Life (PRL)

The length of the adult life of the aphids on the young leaves decreased with dryness of the water regime (Expt. 4). The linear term of this effect was significant ($p<0.05$; quadratic term not significant), and was almost entirely due to

a similar decrease in the PRL of these aphids ($p<0.05$). Their RL was not significantly influenced by the water regimes.

Except in Experiment 2 (see below), the adult life of the aphids caged on the mature leaves was unaffected by the water regimes surrounding the plants. In all the experiments, however, within the AL of the aphids, their RL consistently increased and their PRL consistently decreased from the wet to the medium to the dry regime. For example, in Expt. 2 the linear component of the increasing RL of the aphids was very highly significant ($p<0.001$; quadratic component not significant); in Expt. 3 the linear component of the decreasing PRL of the aphids was significant ($p<0.05$; quadratic component not significant). In all experiments the long PRL of the aphids on the mature leaves of the plants of the wet regime was very striking, and was responsible for the significant difference between the AL of these aphids and those of the medium regime in Expt. 2.

The AL of the aphids caged on the old leaves generally decreased with the availability of water to the host plants. Such a decrease was evident in both the medium and dry regimes of Expt. 1, but only the dry regime of Expt. 3. The RL of these aphids was found to be similarly related to the water regimes, particularly in Expt. 1, where the linearity of the relationship was highly significant ($p<0.01$; quadratic term not significant). But the water regimes had no significant effect on the PRL of the aphids on the old leaves.

Table 12 . Mean temporal parameters of apterous virginoparae of B. brassicae on 3 leaf ages of Brussels sprouts in 3 water regimes at 20°C

| | | | Larval Life (LL)(days) | | | | Adult Life (AL)(days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Water Regime | | | | Water Regime | | | |
| | | | WET | MED. | DRY | L.S.D.(W) | WET | MED. | DRY | L.S.D.(W) |
| Expt. 1. | Leaf age | O | 9.50 | 9.58 | 9.77 | p.05=0.79 | 18.17 | 14.50 | 15.00 | p.05=3.67 |
| Expt. 2. | Leaf age | M | 7.92 | 7.83 | 7.92 | p.05=0.83 | 22.67 | 18.17 | 21.25 | p.05=3.92<br>.01=5.32 |
| Expt. 3. | Leaf age | O | 6.75 | 7.50 | 7.50 | p.05=0.71<br>.01=0.96 | 21.92 | 22.08 | 16.58 | p.05=4.65<br>.01=6.30 |
| | | M | 7.33 | 7.33 | 7.00 | | 22.42 | 21.08 | 22.25 | .001=8.41 |
| | L.S.D. (L) | | | p.05 = 0.71 | | | | p.05 = 4.17<br>.01 = 5.61<br>.001 = 7.42 | | |
| Expt. 4. | Leaf age | M | 8.30 | 7.70 | 7.80 | p.05=0.81<br>.01=1.11 | 29.90 | 30.10 | 30.70 | p.05=5.56<br>.01=7.55 |
| | | Y | 9.80 | 8.80 | 7.80 | .001=1.49 | 26.40 | 24.10 | 19.80 | |
| | L.S.D. (L) | | | p.05 = 0.76<br>.01 = 1.03<br>.001 = 1.37 | | | | p.05 = 5.81<br>.01 = 7.85<br>.001 =10.45 | | |

Lettering as in Table 6 .

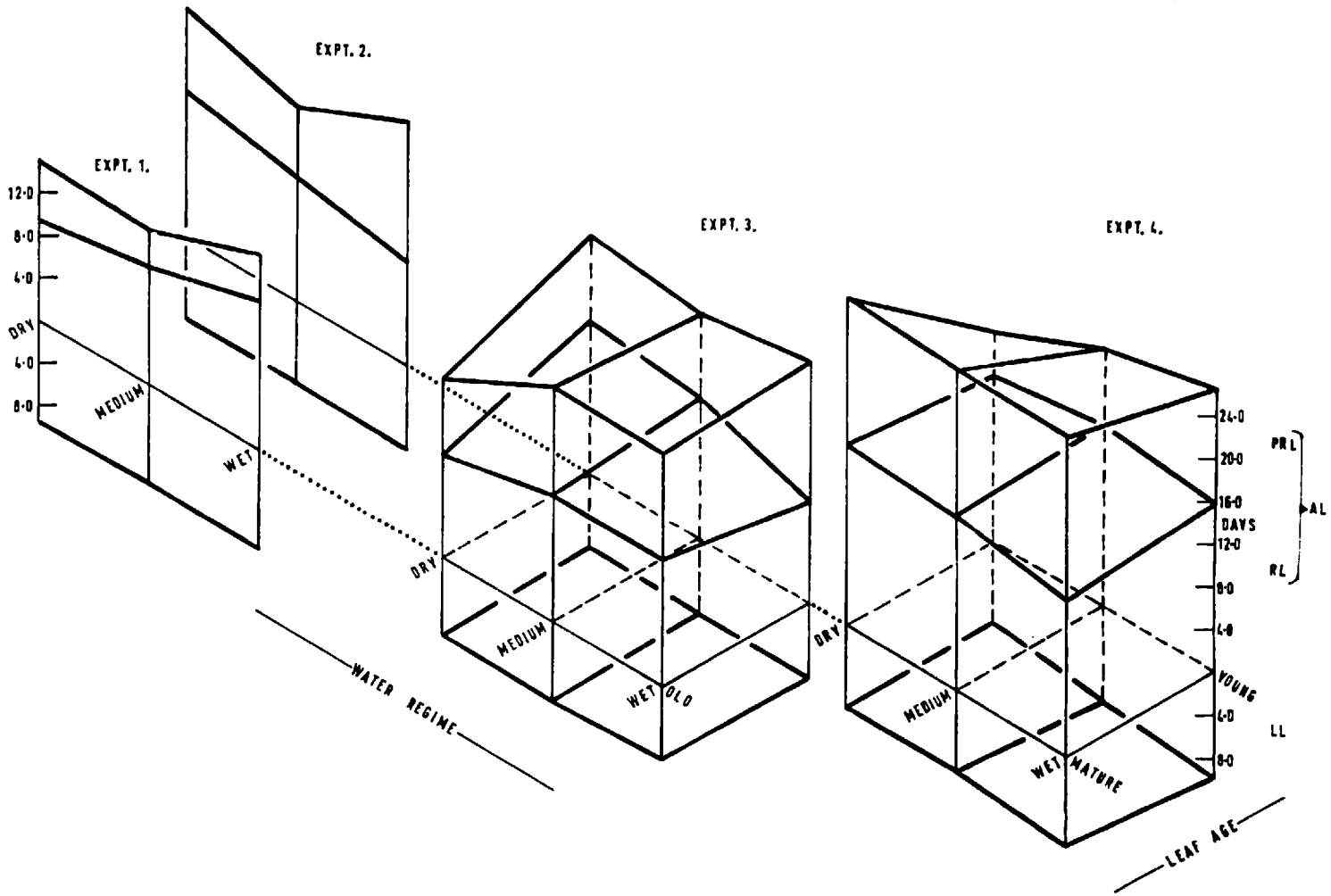
(cont. overleaf)

Table 12 . (cont.)

| | | Reproductive Life (RL)(days) | | | | Post-reproductive Life(PRL)(days) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Water Regime | | | | Water Regime | | | |
| | | WET | MED. | DRY | L.S.D.(W) | WET | MED. | DRY | L.S.D.(W) |
| Expt. 1. | Leaf age 0 | 13.92 | 11.08 | 9.58 | p.05=2.65<br>.01=3.61<br>.001=4.85 | 4.25 | 3.42 | 5.42 | p.05=2.54 |
| Expt. 2. | Leaf age M | 9.75 | 11.50 | 13.58 | p.05=1.91<br>.01=2.60<br>.001=3.50 | 12.92 | 6.67 | 7.67 | p.05=3.62<br>.01=4.92<br>.001=6.62 |
| Expt. 3. | Leaf age 0 | 11.92 | 11.92 | 9.75 | p.05=3.19<br>.01=4.31 | 10.00 | 10.17 | 6.83 | p.05=3.94<br>.01=5.33 |
| | M | 9.75 | 13.33 | 14.33 | .001=5.74 | 12.67 | 7.75 | 7.92 | .001=7.12 |
| | L.S.D. (L) | | p.05 = 3.30<br>.01 = 4.44<br>.001 = 5.87 | | | | p.05 = 3.51 | | |
| Expt. 4. | Leaf age M | 14.50 | 16.30 | 16.90 | p.05=3.86 | 15.40 | 13.80 | 13.80 | p.05=5.23<br>.01=7.12 |
| | Y | 15.90 | 16.90 | 15.40 | | 10.50 | 7.20 | 4.40 | |
| | L.S.D. (L) | | p.05 = 3.37 | | | | p.05 = 4.83<br>.01 = 6.53<br>.001 = 8.69 | | |

Lettering as in Table 6 .

FIG. 28. MEAN LENGTH OF TEMPORAL PARAMETERS OF APTEROUS VIRGINOPARAE OF B.brassicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. LL=LARVAL LIFE; RL=REPRODUCTIVE LIFE; PRL= POST-REPRODUCTIVE LIFE; AL=ADULT LIFE. SEE TABLE 12 FOR SIGNIFICANT DIFFERENCES.



The comparisons between aphids on the different aged leaves revealed that the AL of the aphids was longer on the mature leaves than young leaves, primarily as a result of the longer PRL of the aphids on the mature leaves. The AL of these aphids was also longer than the AL of the aphids on the old leaves of the plants in the dry regime, this time owing to the reduced RL of the latter aphids.

Reproductive Parameters

Two reproductive parameters of the aphids were analysed: Fecundity and reproductive rate (see p. 133). The mean values of these parameters in the 4 experiments are presented in Fig. 29 and Table 13.

Fecundity

Throughout the 4 experiments the effects of the water regimes on the fecundity of the aphids on each leaf age of the plants was very consistent (Fig. 29a).

On the young and mature leaves of the plants in different water regimes the fecundity of the aphids may be summarised, wet < medium < dry regime, and on the old leaves may summarised, dry < medium < wet regime. In all experiments the linear components of these effects were significant ($p<0.001$ in most tests) while the quadratic components were far from significant.

On the plants of all the water regimes the aphids produced more progeny on the young than the mature leaves (wet regime, $p<0.05$; medium and dry regimes, $p<0.001$). In the

wet regime the fecundity of the aphids on the old leaves was also higher than on the mature leaves ($p<0.001$). This relationship was reversed on the plants of the dry regime ($p<0.001$) with the medium regime occupying an intermediate position with no significant difference between the aphids on the two leaf ages.

Reproductive Rate

The reproductive rate of the aphids on leaves of different ages and water status (see Fig. 29b) varied in a form similar to, but less consistent than that described for fecundity. It is, therefore, only necessary to draw attention to the differences between the two parameters. A distinctive feature of the aphids was their high rate of reproduction on the young leaves of the plants in the dry regime. The effects of the water regimes on the reproductive rate of the aphids on the mature leaves was particularly inconsistent; but in two of the three experiments with mature leaves, the reproductive rate was significantly higher on the plants of the dry regime than the other regimes. The aphids reproduced faster on the old than on the mature leaves when the aphids on all the plants were compared in Expt. 2. Within the water regimes of this experiment, a higher reproductive rate of the aphids on the old leaves was found only on the plants of the medium regime.

Figures 30 and 31 show, respectively, the separate and accumulated mean numbers of progeny produced on each day of the lives of the aphids in the different treatments. The

Table 13. Mean and maximum reproductive parameters of apterous virginoparae of B. brassicae on 3 leaf ages of Brussels sprouts in 3 water regimes at 20°C

| | | Fecundity | | | | Reproductive Rate (larvae/day) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Water Regime | | | | Water Regime | | | |
| | | WET | MED. | DRY | L.S.D.(W) | WET | MED. | DRY | L.S.D.(W) |
| Expt. 1. | Leaf age O | 29.58 | 20.17 | 16.83 | p.05=4.46 .01=6.06 | 2.84 | 2.27 | 2.03 | p.05=0.63 .01=0.86 |
| | MAX. | 46 | 28 | 22 | .001=8.15 | 3.7 | 3.7 | 3.7 | |
| Expt. 2. | Leaf age M | 24.58 | 31.25 | 32.08 | p.05=6.33 | 3.10 | 3.32 | 2.80 | p.05=0.61 |
| | MAX. | 40 | 43 | 42 | .01=8.61 | 4.7 | 4.8 | 4.0 | |
| Expt. 3. | Leaf age O | 26.25 | 23.58 | 19.92 | p.05=5.47 | 2.79 | 2.74 | 2.75 | p.05=0.65 |
| | MAX. | 37 | 34 | 34 | .01=7.40 | 4.7 | 4.3 | 4.3 | .01=0.87 |
| | M | 17.08 | 20.92 | 28.83 | .001=9.88 | 2.13 | 1.81 | 2.72 | .001=1.16 |
| | MAX. | 30 | 38 | 43 | | 3.4 | 3.6 | 4.2 | |
| | L.S.D. (L) | | p.05 = 4.91 .01 = 6.60 .001 = 8.72 | | | | p.05 = 0.74 .01 = 0.99 | | |
| Expt. 4. | Leaf age M | 15.10 | 19.50 | 28.40 | p.05=5.63 | 1.38 | 1.65 | 2.40 | p.05=0.66 |
| | MAX. | 20 | 23 | 44 | .01=7.69 | 2.6 | 2.5 | 4.3 | .01=0.90 |
| | Y | 19.00 | 26.60 | 36.60 | .001=10.43 | 1.41 | 1.94 | 3.39 | .001=1.21 |
| | MAX. | 23 | 33 | 64 | | 2.5 | 2.8 | 4.9 | |
| | L.S.D. (L) | | p.05 = 3.74 .01 = 5.05 .001 = 6.73 | | | | p.05 = 0.57 .01 = 0.77 .001 = 1.03 | | |

Lettering as in Table 6 .

FIG. 29. MEAN REPRODUCTIVE PARAMETERS OF APTEROUS VIRGINOPARAE OF B.brassicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.

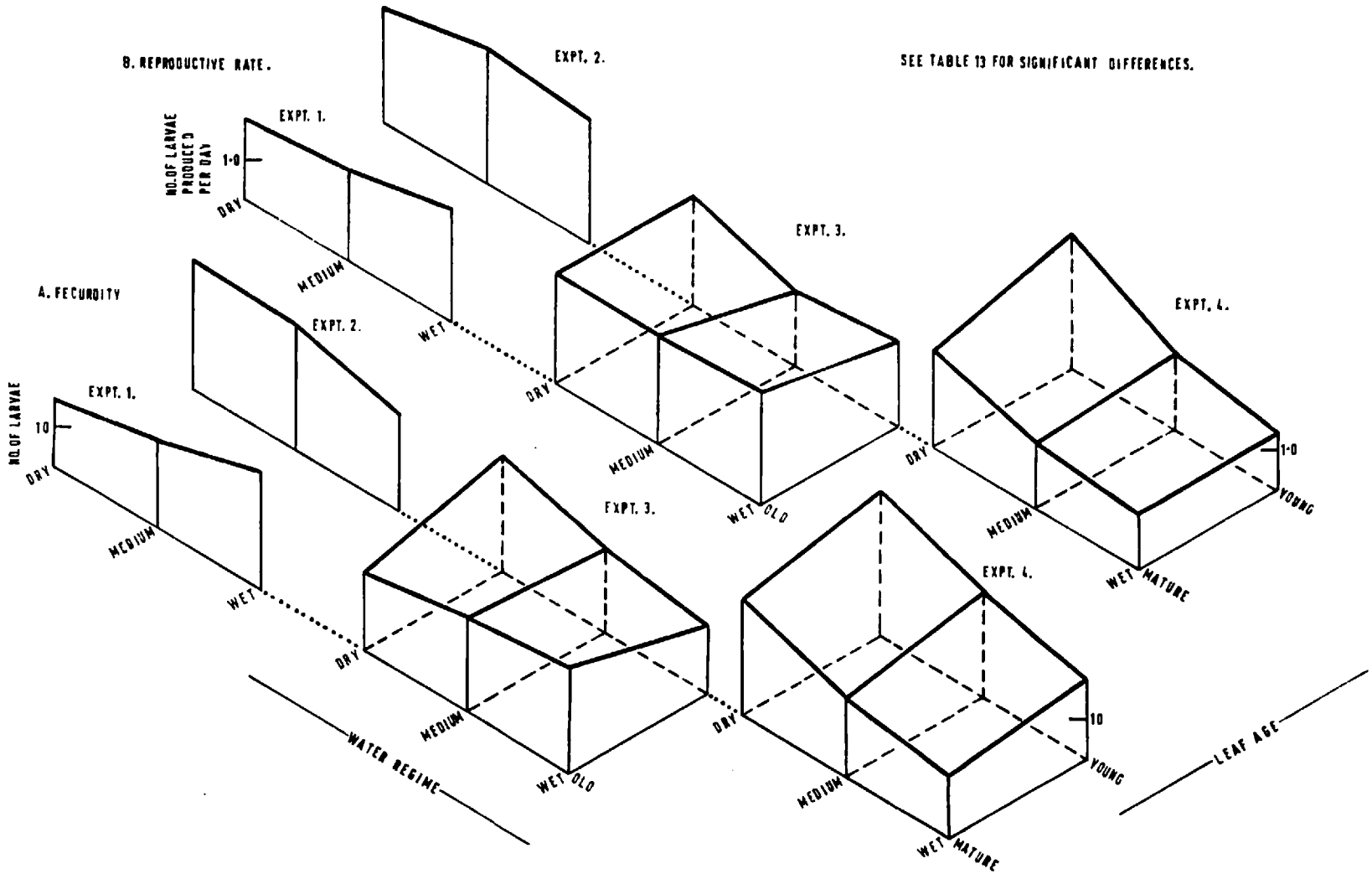


FIG. 30. MEAN NUMBER OF LARVAE PRODUCED PER DAY BY APTEROUS VIRGINOPARAE OF B.brassicae (DURING THEIR MEAN ADULT LIFE) ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. KEY AS IN FIG. 19.

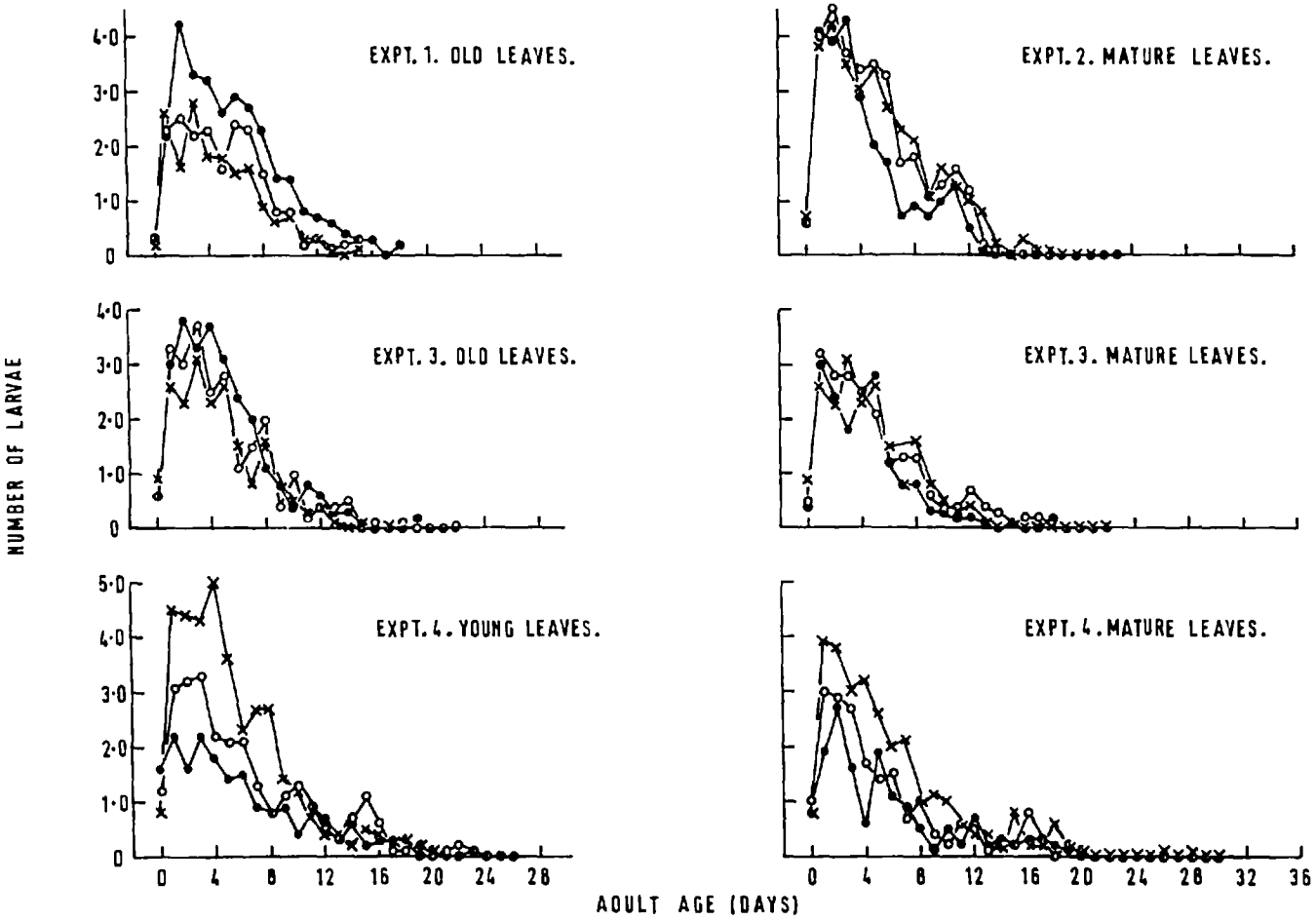
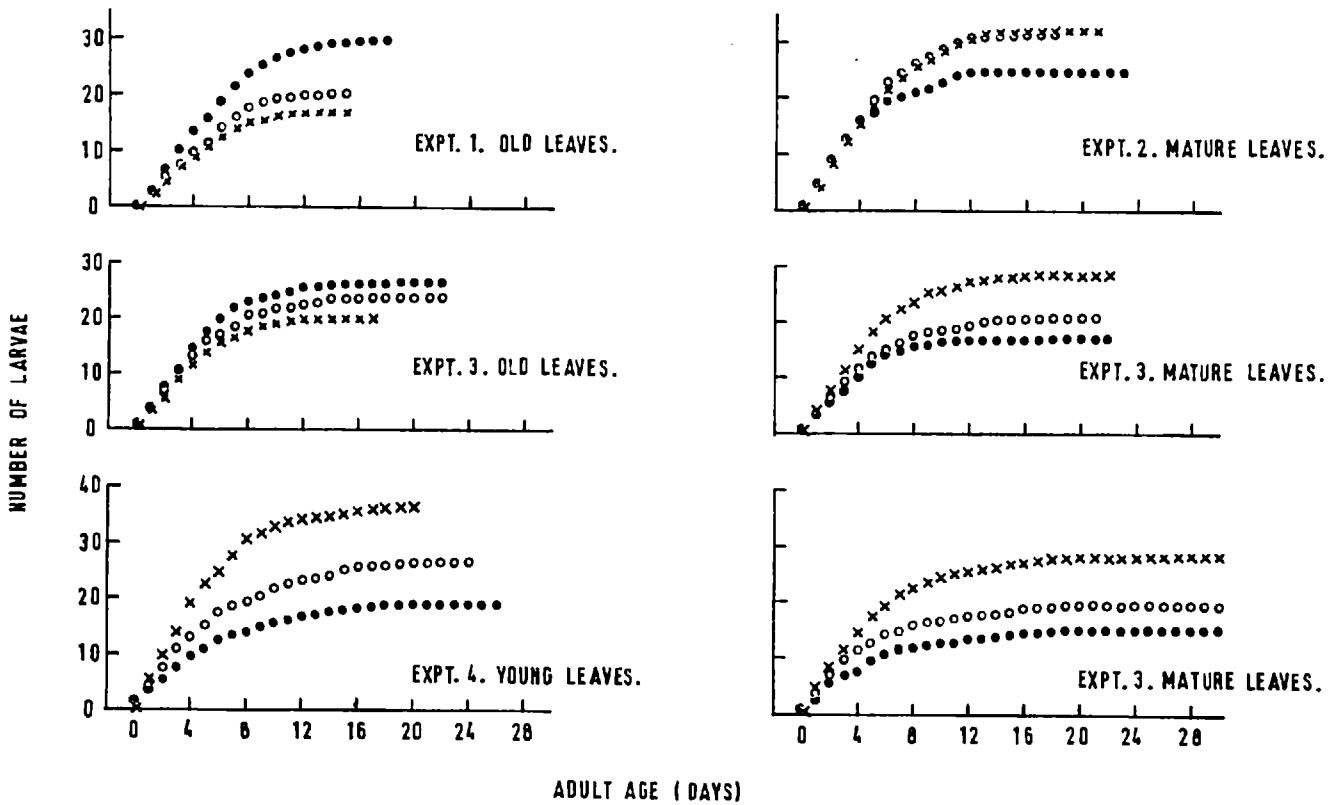


FIG. 31. ACCUMULATED DAILY MEAN NUMBER OF LARVAE PRODUCED BY APTEROUS VIRGINOPARAE OF B.brassicae (DURING THEIR MEAN ADULT LIFE) ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C. KEY AS IN FIG.19.



Figures indicate that the differences in the fecundity and reproductive rate of the aphids arose principally in their early adult life, but frequently persisted beyond the early peak of reproductive activity.

Conclusions

Increasing water strain in the host plants was associated with -

1) Young leaves: increasing fecundity and reproductive rate, decreasing larval life, adult life and post-reproductive life and unchanged reproductive life.

2) Mature leaves: increasing fecundity, reproductive rate and reproductive life, decreasing post-reproductive life and unchanged larval life and adult life.

3) Old leaves: increasing larval life, decreasing fecundity, reproductive rate, reproductive life and adult life, and unchanged post-reproductive life.

Maturation (Y $\longrightarrow$ M) of the leaves of the host plants was associated with:

4) Increasing post-reproductive life and adult life (medium and dry regimes), decreasing fecundity, reproductive rate and larval life (wet and medium regime) and unchanged reproductive life, larval life (dry regime) and adult life (wet regime) of the aphids.

Senescence (M $\longrightarrow$ O) of the leaves of the host plants was associated with:

5) In the wet and medium regimes, increasing fecundity (wet regime) and reproductive rate, and unchanged fecundity (medium regime), larval life, adult life, reproductive life and post-reproductive life of the aphids; in the dry regime, decreasing fecundity, adult life and reproductive life and unchanged reproductive rate, larval life and post-reproductive life of the aphids.

c) Production of Alatae (≡ Aphid departure from the host plant)

Like many species of aphids (see p. 147 and the Review of the Literature), populations of B. brassicae contain variable percentages of alate aphids depending, in part, on the physiological condition of the host plants. Evans (1938) found a rapid increase in the percentage of alatae in populations of B. brassicae as the percentage protein nitrogen in the host plants (cabbages) reached a low level. Changes in the carbohydrate content of the plants did not appear to influence the production of alatae. Woodworth (1908) related observed increases in the production of alatae of Aphis (Brevicoryne) brassicae L. to the wilting of the host plants. This deduction was partly supported by the experiments of Wearing (1963) and Markkula (1953). However, in the experiments of the latter author the density of the aphids on the plants, like the percentage alatae, increased due to increased reproduction of the aphids caged on the wilted plants.

Thus, the increased production of alatae on the wilted plants may have been related as much to the increased "crowding" of the aphids (see Bonnemaison, 1950) as to changes in the plants during wilting. The experiments of Wearing (1963) were conducted with uncaged populations of B. brassicae which complicated the interpretation of the results. Experiments were therefore undertaken to measure the percentage of alate aphids in the progeny of apterae of B. brassicae caged on three leaf ages of Brussels sprouts in three water regimes, (Stage (c) of the Cycle, p.72 ). In field measurements of populations of the cabbage aphid, Hughes (1963) recorded higher proportions of winged progeny among aphids on the old leaves (80%) than on the young and mature leaves (40%) of kale.

Experimental Procedure

The experimental procedure used for the measurement of the production of alatae by B. brassicae was the same as that used for M. persicae (see p.148 ). The temporal and reproductive parameters of the apterae of B. brassicae were measured in 4 experiments. The apterae produced progeny which were sorted into alatiform and apteriform when they had developed to the 3rd or 4th instar. Further details of these experiments may be found on p.176 .

Results

The mean percentages of alatae (transformed to angles) in the progeny of apterous virginoparous B. brassicae on three

leaf ages of Brussels sprouts are presented in Table 14 . Fig. 32 shows the mean percentages of alatae which were calculated from the raw data.

A similar percentage of alatae was contained in the progeny of the apterae caged on the young leaves of the plants of each water regime. On the mature and old leaves, more alatae were produced as the dryness of the water regime of the plants increased, (except Expt. 4 - no effect of the water regimes). The linear component of this effect was significant on the old leaves ($p<0.001$, Expt. 1; $p<0.001$, Expt. 3) and on the mature leaves ($p<0.05$, Expt. 2; $p<0.001$, Expt. 3), while the quadratic component was not significant.

Comparisons between the leaf ages indicated that on the plants of the wet regime the percentage of alatae in the progeny of the apterae increased as the leaves matured ($p<0.01$) and again as they senesced ($p<0.05$). Within the other regimes the percentages of alatae on the young and mature leaves of the plants did not differ significantly; but the apterae on the plants of the medium regime produced a higher proportion of alatae on the old leaves than on the mature leaves ($p<0.01$).

## Conclusions

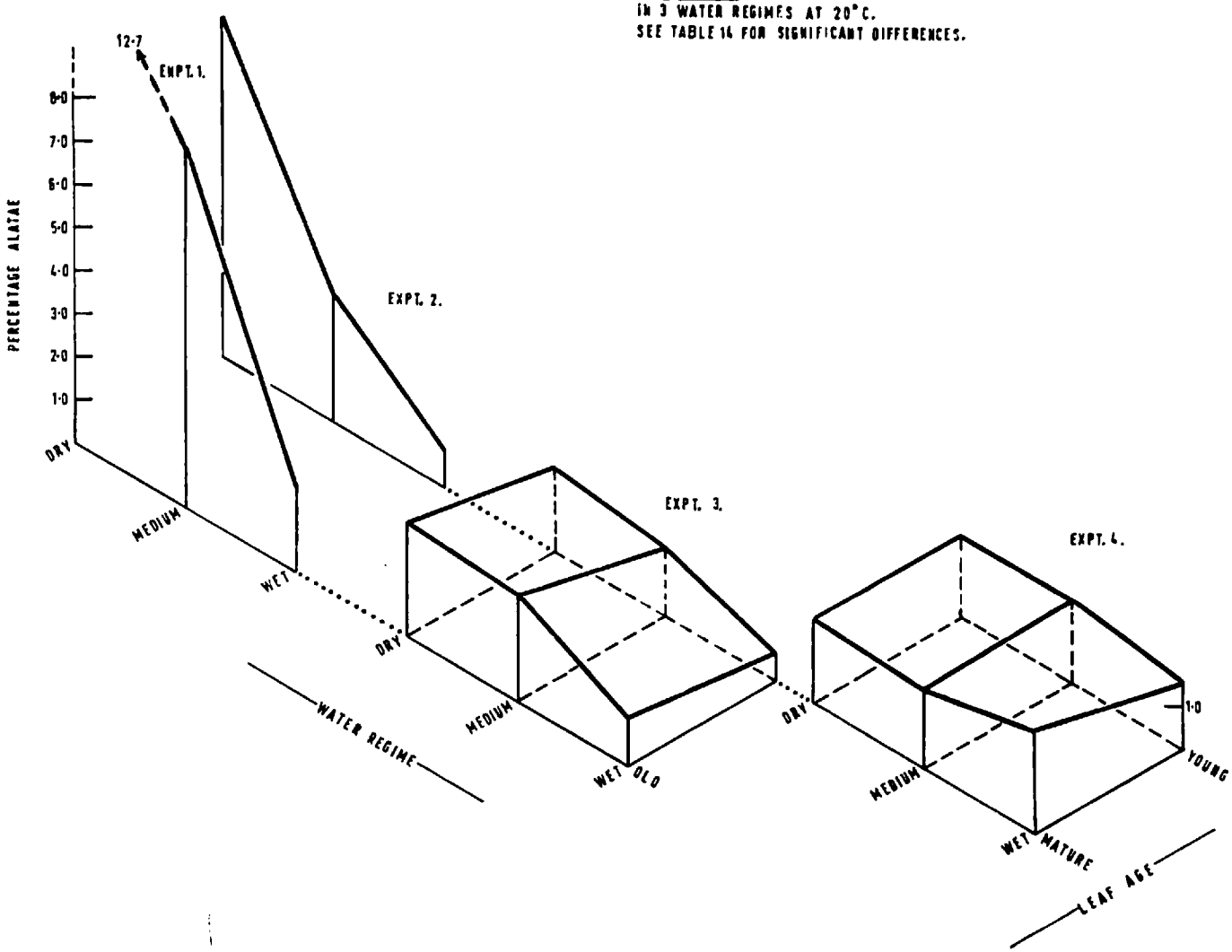
Apterous virginoparous *B. brassicae* caged on the young leaves of Brussels sprouts produced progeny containing a similar percentage of alatae irrespective of the water strain in the plants. However, on the mature and old leaves of the plants the apterae produced an increasing proportion of alatae

Table 14. Mean percentages of alatae in the progeny of apterous virginoparous B. brassicae caged on 3 leaf ages of Brussels sprouts in 3 water regimes at 20°C

| | | | Mean Percentage of Alatae (Transf. to angles in mins.) | | | |
|---|---|---|---|---|---|---|
| | | | Water Regime | | | |
| | | | WET | MEDIUM | DRY | L.S.D.(W) |
| Expt. 1. | Leaf age | U | 14.27(6.08)* | 22.02(14.06) | 26.62(20.07) | p.05=3.18<br>.01=4.33<br>.001=5.02 |
| Expt. 2. | Leaf age | M | 12.94(5.02) | 14.55(6.32) | 18.35(9.92) | p.05=4.78<br>.01=6.50 |
| Expt. 3. | Leaf age | O | 7.68(1.81) | 11.03(3.68) | 11.05(3.69) | p.05=1.85<br>.01=2.50 |
| | | M | 5.59(0.97) | 8.22(2.05) | 9.69(2.85) | .001=3.33 |
| | L.S.D. (L) | | | p.05 = 2.05<br>.01 = 2.75<br>.001 = 3.64 | | |
| Expt. 4. | Leaf age | M | 8.60(2.26) | 7.26(1.64) | 7.32(1.66) | p.05=2.60 |
| | | Y | 6.47(1.31) | 6.93(1.50) | 7.26(1.64) | |
| | L.S.D. (L) | | | p.05 = 1.45<br>.01 = 1.95<br>.001 = 2.60 | | |

* In parenthesis - detransformed percentages.
Lettering as in Table 6

FIG. 32. MEAN PERCENTAGE ALATAE IN THE PROGENY OF APTEROUS VIRGINOPARAE OF B.brassicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER REGIMES AT 20°C.
SEE TABLE 14 FOR SIGNIFICANT DIFFERENCES.



as the water strain in the plants increased.

On turgid plants the percentage of alatae in the progeny of the apterae increased with the age of the leaves on which they were caged. This effect of the age of the leaves was gradually reduced by increasing the water strain in the host plants until the apterae on plants suffering intermittent wilting were producing similar percentages of alatae on all leaf ages.

Any differences in the density of the progeny in different treatments did not seem to influence the results. High percentages of alatae were obtained in the progeny of apterae of low and high fecundity alike (cf. Figs. 29 & 32).

d) Summary of Results - The Suitability of the Host Plant

Estimation of the suitability of the Brussels sprout leaves of different age and water status as hosts for aphids was used in the summary of the results of the experiments with M. persicae (see p. 153). The same technique is here used for B. brassicae, where host suitability is assessed from the various measured parameters (e.g. fecundity) of apterous aphids caged on the leaves and the co-ordination of these parameters with the estimated arrival and establishment on, and departure from the leaves, (i.e. co-ordination of the stages of the Cycle on p. 72 ).

Unlike M. persicae, the co-ordination between the

stages of the Cycle was very erratic (compare Figs. 23, 27, 28, 29, 32), so that assessment of the host suitability of the different leaves was complex and could not be based solely on the parameters of the apterae.

Of the different aged leaves of turgid plants, the young leaves appeared to have the greatest host suitability for B. brassicae. Apterae preferred these leaves to all others and alatae settled on the young leaves and apices of the plants at much greater densities than on any other leaf age (Figs. 23 and 27). In addition, the apterae on the young leaves had higher fecundity and reproductive rate and lower production of alatae than apterae on the mature leaves (Figs. 29 and 32). However, the apterae on the young leaves had a slower rate of larval development and a shorter post-reproductive life than the apterae on the mature leaves (cf. M. persicae). Again, on turgid plants the apterae on the old leaves had greater fecundity and reproductive rate than apterae on the mature leaves (Fig. 29). This immediately suggested a greater host suitability of the old leaves, particularly since there were no significant differences between the other parameters of the apterae on the two leaf ages. However, the alatae settled in much lower numbers (and densities) on the old rather than the mature leaves (Fig. 22), the apterae fed preferentially on the mature leaves (Fig. 27) and the apterae produced a higher proportion of alatae on the old than on the mature leaves (Fig. 32). Thus, the greater reproduction of the apterae

forced to feed on the old leaves suggested that the nutritive value of these leaves was higher than that of the mature leaves. Nevertheless, it is proposed that the latter had the greater host suitability since the selection, preference and alatae production experiments pointed to a massive rejection of the old leaves by the aphids, possibly in response to a non-nutritional factor. In summary, therefore, it would seem that for B. brassicae on a turgid plant the host suitability of the leaves decreased with age.

The effects of water strain in the plants on the host suitability of the leaves varied. The low suitability of the old leaves was even further reduced by intermittent water strain in the plants, particularly when the water strain was severe. Under these conditions, the rejection of the old leaves by the apterae and alatae was enhanced, the larval development of the apterae was slower, and all the other parameters of the apterae were reduced. The preference tests with apterae suggested that this reduced host suitability of the old leaves was particularly associated with actual wilting of the leaves (Fig. 27). In contrast, water strain in the plants appeared to increase the nutritive value of the young and mature leaves, as indicated by the increased fecundity and reproductive rate and, on mature leaves only, of the reproductive life of the apterae. Apterous aphids also preferred the young leaves of the plants suffering severe intermittent water strain (Fig. 27). However, with increasing

water strain in the plants, the post-reproductive life of the apterae decreased and the variability of their fecundity increased on both leaf ages; and on the mature leaves, their production of alatae increased. Furthermore, when the old leaves were wilting, alatae settled on the mature and young leaves of the plants in reduced numbers (and densities) (Figs. 22 and 23) and apterae preferred the mature leaves of more turgid plants (Fig. 25b). This last-mentioned preference was nullified by watering the wilting plants, suggesting that these detrimental effects of water strain on the host suitability of the mature leaves were associated particularly with the wilting process.

Thus in B. brassicae, the effects of water strain in the plants on the host suitability of the different leaves varied with the position of the leaves on the plant. There seemed to be at least two opposing processes in operation during increasing water strain in the plant (cf. M. persicae), one decreasing host suitability to a minimum in the lowermost leaves, and another increasing host suitability throughout the plant. The preference experiments with apterae suggested that both these processes were particularly associated with the wilting of the old leaves of the plants, (see Fig. 25 a,b,c.). This interpretation of the results is given further consideration in the Discussion (p.292 ).

The net effect of water strain on the host suitability of the whole plant seemed to be an increase, where the plant

suffered mild water strain, and a decrease where the water strain was severe. This pattern of host suitability was paralleled by the selection of the host plants by alate aphids which settled in greatest numbers and densities on plants suffering mild water strain (Figs. 22 & 23).

# PART 2. CONTRIBUTORY FACTORS IN THE MEASURED RESPONSES OF THE APHIDS *Myzus persicae* (Sulz.) AND *Brevicoryne brassicae* (L.) TO INTERMITTENT WATER STRAIN IN THE HOST PLANT.

## Section A Introduction

The two species of aphid, *M. persicae* and *B. brassicae* were chosen for this study partly for their contrasted sites of feeding on the host plant, Brussels sprout, - the old leaves and the apex, respectively. The experiments of Part 1 showed that the contrast between the species in their responses to this host plant is much more extensive. Large differences in responses were found in each stage of the investigation.

Some of the mechanisms which may govern these differences (see Discussion, p. 292 ) were examined and form Part 2 of the Experimentation. This work was focused on the probable importance of the turgor pressure of the plant in the feeding of the aphids (see Kennedy and Mittler, 1953) and the ways in which the two species of aphid might differ in their sensitivity to turgor pressure. To this end, the aphids were studied on plants whose water strain increased continuously (cf. the water regimes of Part 1) and the mechanisms of feeding of the aphids were studied, a) by examination of the disposition of their stylets in the tissues of the host plant, and b) by feeding the aphids on artificial diets under controlled pressures.

The different water regimes of Part 1 undoubtedly produced plants with cell sap of differing chemical composition,

and hence nutritive value. It was necessary to postpone to some future time the enormous task of analysing the nutritive value of the cell sap of the different parts of such plants. Some preliminary attempts were made to obtain "stylet sap" via the severed mouthparts of feeding aphids, (see Discussion, p p. 322).

## Section B Materials and Methods

### Sub-section 1 The insects, host plants and experimental cages

The same species of aphid and host plant were used as in the experiments of Part 1, i.e. the aphids, M. persicae and B. brassicae, and the plant, B. oleracea var. bullata gemmifera. The Brussels sprout cultivar, "Jade Cross" was again used almost exclusively. However, M. persicae was sectioned while feeding on leaves of the cultivar "Irish Elegance". There were no changes in the methods of rearing which were given previously (pp.76-78 ).

Simple clip cages (p. 79 ) were used in determining the larviposition of the aphids and their restlessness ( pp. 216, and 261).

### Sub-section 2 The water treatments

The water treatments were devised to compare aphids which fed on continuously turgid plants with aphids which fed on plants subjected to continuously increasing water shortage over a short period. This was done in a single experiment ( p. 216 ).

As in the water regimes (p. 87 ), the plants were grown in vermiculite in 3½" plastic pots, which were stood in blackened jam-jars. The same procedures as before were also followed in calculating the available water which was held by the vermiculite at field capacity, and to provide nutrients for the plants. For greater accuracy, a separate figure was obtained from each experimental pot for the percentage of water which was held by the vermiculite at field capacity (wt/wt basis)

Thus, by subtracting the permanent wilting percentage, a separate figure per pot was also obtained for the percentage of available water which was held by its vermiculite at field capacity.

The ideal objective was to obtain, at the beginning of the experiment, plants growing in vermiculite with percentage deficits of available water of 1) 0%, 2) 20%, and 3) 80%, where the available water held by the vermiculite at field capacity was 100%. Thereafter, in treatment 1) the pots would be weighed and watered every day, while in treatments 2) and 3), the pots would also be weighed daily but no more water would be applied. Treatment 1) (turgid) was readily achieved, but it was not possible to prepare the vermiculite of every plant of treatments 2) (wilt) and 3) (severe wilt) with available water deficits of exactly 20% and 80% respectively, at the beginning of the experiment. The best that could be achieved was to cease watering the plants two days, for the wilt treatment, and nine days, for the severe wilt treatment, before the start of the experiment. Up to these times, all plants were maintained in a turgid condition by watering every day.

During the experiment, the weight of each pot was recorded daily, and hence it was possible to follow the changes in the available water of the vermiculite. The weights of the pots, experimental cages, cage supports, and plants, which were included in the daily weighings, were subtracted to obtain the weight of the vermiculite. The fresh weights of the plants were recorded at the beginning and end of the experiment, and, in the wilt and severe wilt treatments, these weights differed by only

a few grams (see below). In these treatments, therefore, the mean of the two figures for each plant was used as an estimate of the weight of each plant throughout the experiment. In the turgid treatment there were large increases in the weights of the plants during the experiment. For convenience and in view of the shortness of the experiment (12 days), these increases were assumed to be linear in order to calculate the approximate weight of each plant on each day. Inaccuracies arising from this assumption, which disregards the known exponential growth of plants, were of very minor significance in the widely differing water treatments used. The mean fresh weights of the six plants in each treatment at the beginning and end of the experiment were:

| Water Treatment | Beginning | End |
|---|---|---|
| Turgid | 5.2 grams | 27.6 grams |
| Wilt | 5.2 grams | 6.3 grams |
| Severe wilt | 5.2 grams | 3.7 grams |

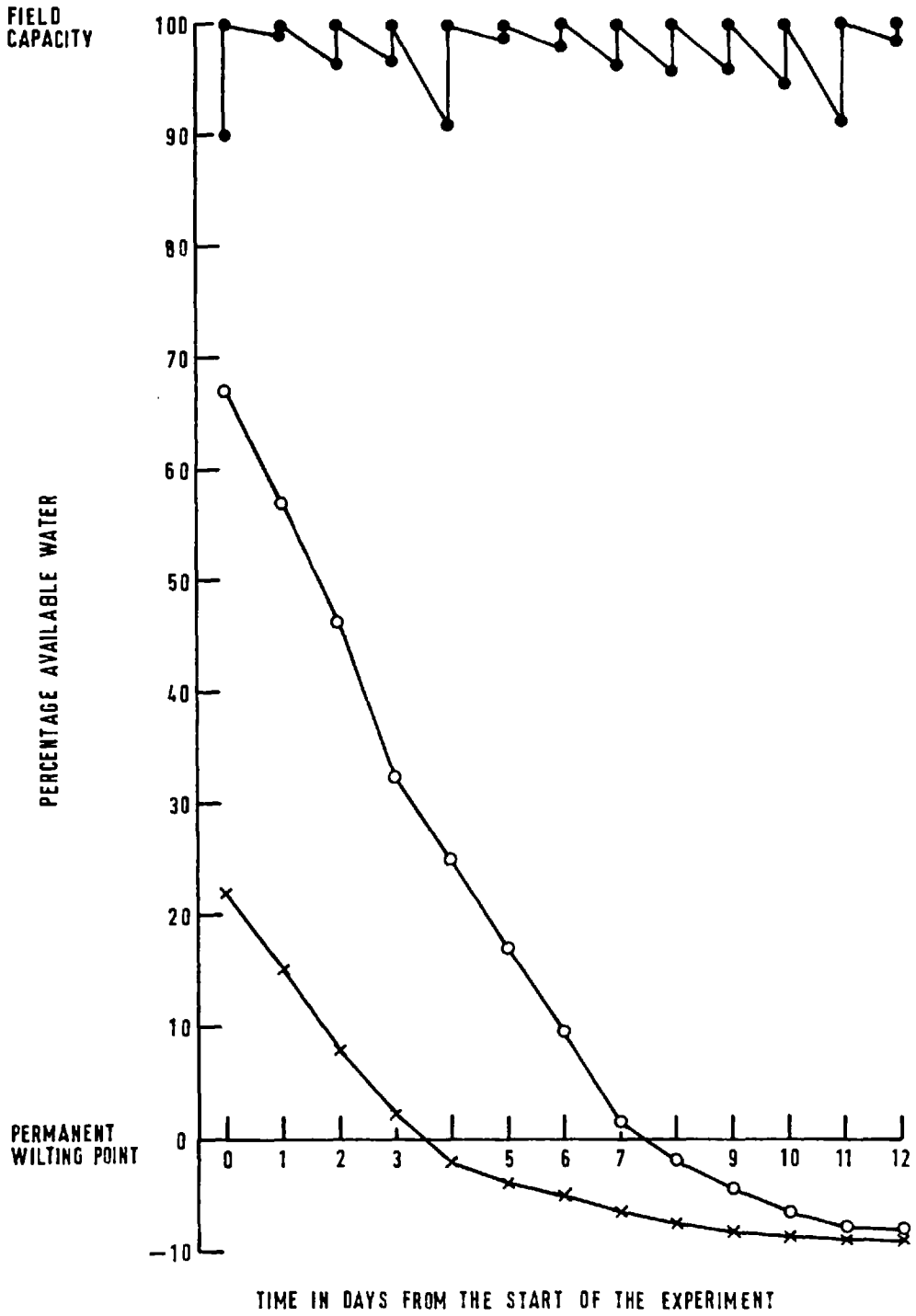
The procedure for calculation of the percentage deficit of available water of the vermiculite of a pot on each day is exemplified below. The example chosen is from the turgid treatment on the 14th day of the experiment.

Initial weighing (Pot + vermiculite + water + plant + cages + cage supports = 328.3 gms.

Weight of cages and supports = 35.1 gms.
Weight of pot = 30.6 gms.
Estimated weight of plant = 26.4 gms.
Weight of oven-dry vermiculite = 39.7 gms.

FIG. 33. WATER TREATMENT DIAGRAMS.— THE PERCENTAGES OF AVAILABLE WATER IN THE GROWING MEDIUM (VERMICULITE) OF BRUSSELS SPROUTS IN 3 WATER TREATMENTS AT 20°C. (EXAMLE CHOSEN: BLOCK VI).

KEY: WATER TREATMENTS — TURGID ●; WILT ○; SEVERE WILT ×.



∴ Weight of water in the vermiculite

= 328.3 - (35.1 + 30.6 + 26.4 + 39.7) gms.

= 196.5 gms.

Percentage of water in the vermiculite

$= \frac{196.5}{39.7} \cdot 100 = 495.0\%$

Permanent wilting percentage = 42.0% (see p. 89 )

Percentage of water available in the vermiculite

= 495.0 - 42.0 = 453.0%

Percentage of water available in the vermiculite at field capacity = 468.6%

If the available water held by the vermiculite at field capacity = 100% (i.e. 468.6 = 100)

Then, percentages of available water in the vermiculite

$= \frac{453.0}{468.6} \cdot 100 = 96.7\%$ or percentage deficit of available water = 3.3%

In many of the pots of the wilt and severe wilt treatments, the water content of the vermiculite fell below the permanent wilting percentage by the end of the experiment. Where this occurred, negative percentages of available water in the vermiculite were calculated in the same manner as the positive percentages. An example of each water treatment is represented in Fig. 33, as the daily changes in the available water of the vermiculite during the experiment.

Sub-section 3 The sectioning of feeding aphids

Both species of aphid, M. persicae and B. brassicae, were killed while feeding on leaves of Brussels sprouts, and were subsequently fixed and serial sectioned. This permitted comparison of the methods of penetration and destinations of the stylets of the aphids in the plant tissues (see pp. 232 and 274).

Fixation

For this work a fixative was required which very rapidly penetrated and killed the tissues of the aphid and the host plant, in order to fix the aphids as nearly as possible in their feeding position. When disturbed, feeding aphids readily retract their stylets from the tissues of the host plant.

Several fixatives were tested - hot alcohol (Rawitscher, 1933), alcoholic Bouin's fixative, Bles's fluid and Carnoy's fixative. Of these, Carnoy's fixative was found most effective in rapidly killing the aphids, and in fixing the plant and aphid tissues. A leaf, carrying feeding aphids, was quickly cut off the host plant and immediately plunged into Carnoy's fixative. About half of the aphids remained attached, by their stylets, to the lower surface of the leaf, (cf. Day and Irzykiewicz, 1954), particularly if this surface was the first to be immersed in the fixative. In fixing, the leaf was cut into 1 cm. squares which remained in the fixative for 3 hours under reduced pressure (Pollard, 1958). To assist the sectioning of B. brassicae, the squares were removed from the fixative after 1½ hours, immersed for 20 minutes in ethyl acetate to remove the wax covering the aphid, and returned to the fixative.

Dehydration and Clearing

Following fixation, the pieces of leaf with their attached aphids were transferred to 90% dioxan under reduced pressure to begin dehydration. After 3 - 4 hours the material was transferred to pure dioxan and was passed through three

further changes of pure dioxan over the next two days (still under reduced pressure). This process cleared as well as dehydrated the material, which was then removed to a 50:50 mixture of dioxan and chloroform at atmospheric pressure. Here the material was allowed to sink before final transfer to pure chloroform, where it was stored (at least 12 hours) until required for embedding.

Embedding and Sectioning

Each piece of leaf was embedded in paraffin wax (M.Pt. 52 - 54°C). About 3,000 serial sections in all, 10μ thick, were cut with a Cambridge rocking microtome. The choice of thickness of the sections was a compromise between over-fragmentation of the stylets in thin sections and lack of clarity at the tip of the stylets in thick sections.

Staining

Sections of plant tissues containing the stylets of aphids ideally require a stain which differentiates the stylets from the cell walls of the plant. To indicate whether the aphids have retracted their stylets when killed, it is also desirable to stain the salivary sheaths secreted by the aphids into the plant tissues. Several stains were tested:- Flemming's triple stain (Pollard, 1958), Chlorazol Black (Entwistle and Longworth, 1963), Chlorazol Paper Brown B, Mallory's triple stain and Johanssen's quadruple stain. Of these, Chlorazol Black and Johanssen's quadruple stain were adopted for further work. In both methods, preparation for staining consisted of removal of the embedding wax from the slides with xylene, and

thence passing the sections through a graded series of ethyl alcohol to 70% alcohol.

To stain with Chlorazol Black, a freshly prepared saturated solution of the stain in 70% ethyl alcohol was used. The sections were stained for 20 - 25 minutes, dehydrated in absolute alcohol, cleared in xylene and mounted in Canada balsam. The stylets and the cell walls of the plant were stained pale green and grey-black respectively. Salivary sheaths were not stained, but, being highly refractive, were often visible.

The method of staining with Johanssen's quadruple stain described by Gray (1964) was modified only by increasing the concentration of the safranin solution from 0.1% to 1.0%. The stylets of both species of aphid, and the cellulose cell walls and cytoplasm of the plant, were coloured orange. The salivary sheaths of both species of aphid were stained bluish-green. Lignin, in the spiral thickening of the xylem vessels, was stained pale red. The aphids showed complex colour reactions to the stain, which was designed primarily for botanical use. Indeed the various plant tissues could be clearly recognised.

Sub-section 4 An apparatus for feeding aphids on an artificial diet under pressure

Despite the findings of Kennedy and Mittler (1953), that turgor pressure of the host plant is probably important in the feeding of aphids, their ability to feed on liquid diets, (under trifling pressures) behind stretched membranes of

parafilm has been well established (e.g. Mittler and Dadd, 1962 et seq). Yet compared with that on host plants, the rate of feeding by aphids on artificial diets would seem to be much lower (Dadd & Mittler, 1965b) and a few attempts have been made to increase this rate by the application of pressure to the diet. These have met with little or no success (see Mittler & Dadd, 1963a). Using parafilm membranes, (Parafilm "M" - American Can Co., N.Y.) an apparatus was designed (Fig. 34) for feeding aphids on a liquid diet under controlled pressure (pp.248 and 284 ). Van Hoof (1958), who was investigating the transmission of viruses by aphids, described a high pressure cell for feeding aphids on virus suspensions and radioactive solutions. In this apparatus, pressures of 6 - 8 atmospheres, from a compressed-air cylinder, were applied to the liquid behind a plastic membrane.

The apparatus which was used in the present experiments, was designed in consultation with Mr. J.W. Siddorn. It was developed from a single-chamber prototype, with which the basic design and a variety of supports for the parafilm membrane were tested. Initially, electron microscope grids were used to support the very thin parafilm (c.10 μ thick), which had been stretched maximally in two directions at right angles. However, the very fine mesh and the ratio of hole to metal of the microscope grids apparently impeded the feeding of the aphids. Preliminary tests with nylon gauze supports revealed no differences between the survival of the aphids, which fed through double-stretched parafilm (as above), and aphids which

fed through single-stretched parafilm (20-30μ thick) (cf. Auclair, 1965). Accordingly, several types of nylon gauze were tested as supports for the single-stretched parafilm, and of these 0.35 mm. mesh gauze was chosen for all future work. Another preliminary test, which was made with the prototype apparatus, involved examination of the effects on the aphids of transmitting a yellow light (Cinemoid No. 1 Yellow filter) through the diet, while all other light was excluded from the feeding chamber. Any increase in the feeding of the aphids, so treated, was small, when compared with aphids which had fed in total darkness, and did not justify further use of the lighting technique. (cf. Mittler & Dadd, 1965a).

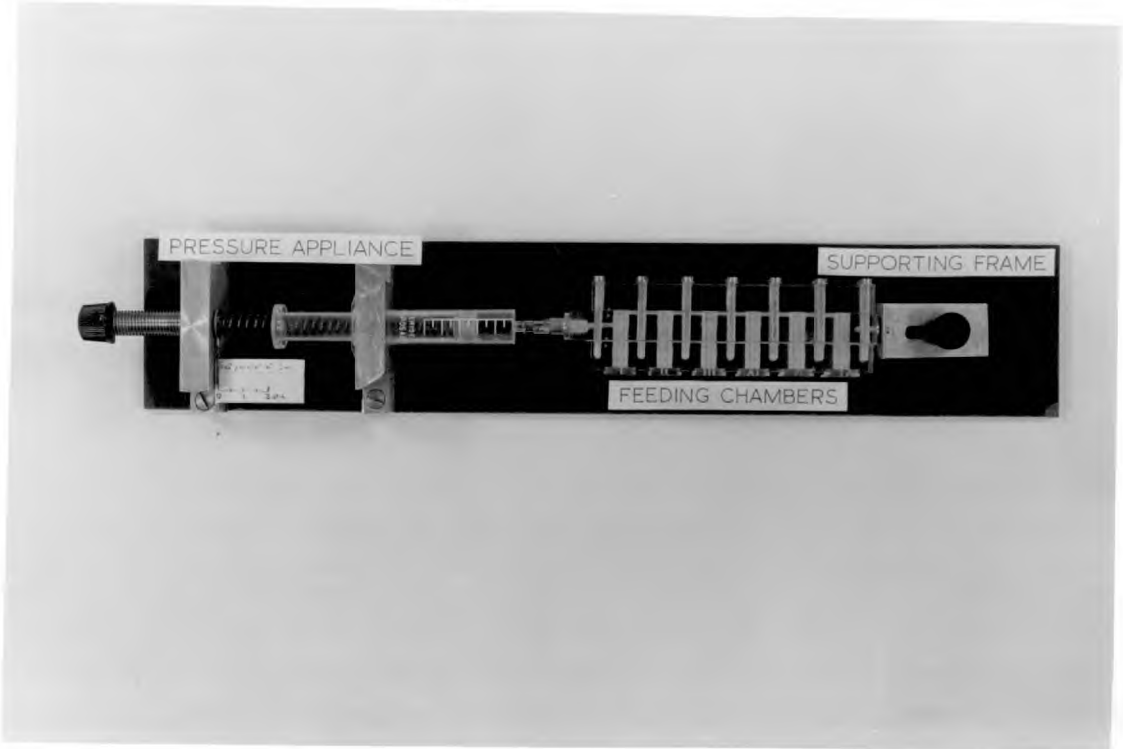
In other respects the basic design of the prototype was found satisfactory and was adopted for the construction of a twelve-chamber apparatus, which allowed adequate replication of treatments. The prototype is not, therefore, described.

The twelve-chamber apparatus consisted essentially of three parts, the supporting frame, the feeding chambers, and the pressure appliance. The apparatus is shown in Figures 34, 35 and 36. In the exploded view of Figure 35 each part of the feeding chambers is seen from its upper surface, except the aphid chambers, which were inverted to show the sliding doors. In assembling the apparatus, the perspex blocks, PVC washers and frame carrying the membranes were screwed together to form the feeding chambers. Using a hypodermic syringe, liquid diet was then admitted through the tube to the first diet chamber. The diet was shaken into the other chambers, beginning with

those distal to the point of insertion of the needle and progressing towards the first diet chamber. This chamber and the entrance tube were filled last, care being taken to remove all air bubbles. The metal connecting nipple filled with diet was screwed into the entrance to the diet chambers. The other end of the nipple was then connected to the large diet-filled syringe which was already attached to the supporting frame. Pressure was applied to the diet by compressing a calibrated metal spring within the piston of the syringe. A scale graduated in atmospheres (up to a maximum of 2) was attached to the supporting frame, and a needle, which was held against the end of the spring by the compression screw, moved along the scale when the spring was compressed.

Fig. 34. Apparatus for feeding aphids on a liquid diet under controlled pressure.

Fig. 35. Apparatus for feeding aphids on a liquid diet under controlled pressure. Exploded view.


PRESSURE APPLIANCE
SUPPORTING FRAME
FEEDING CHAMBERS

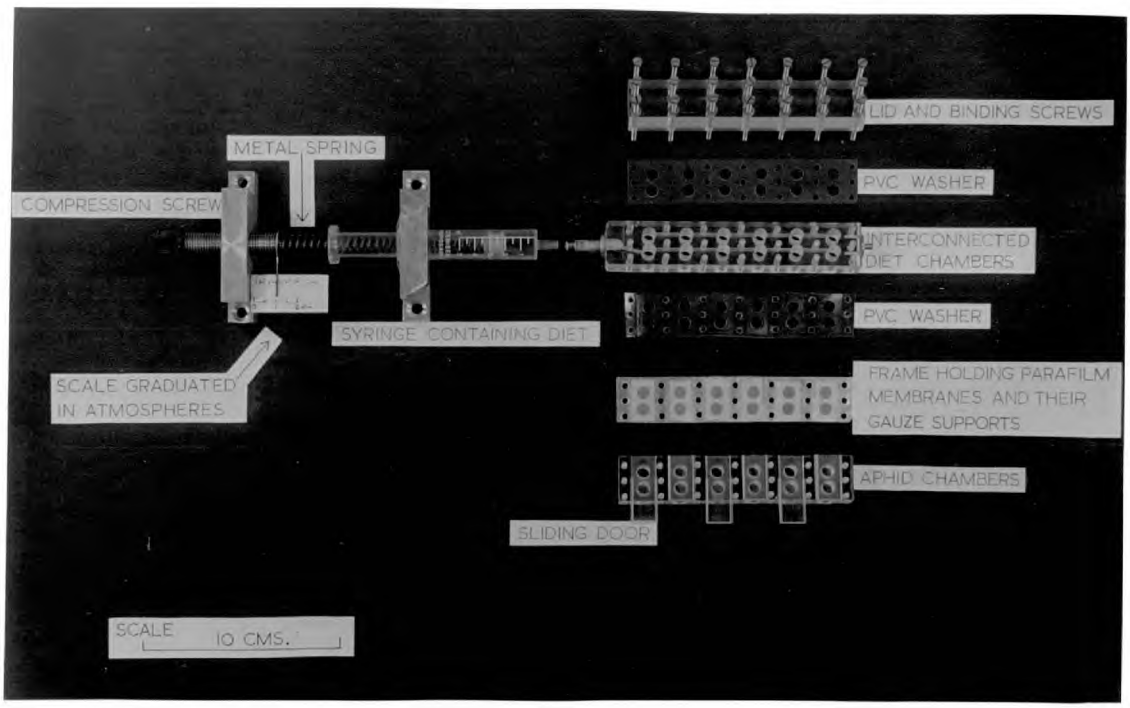
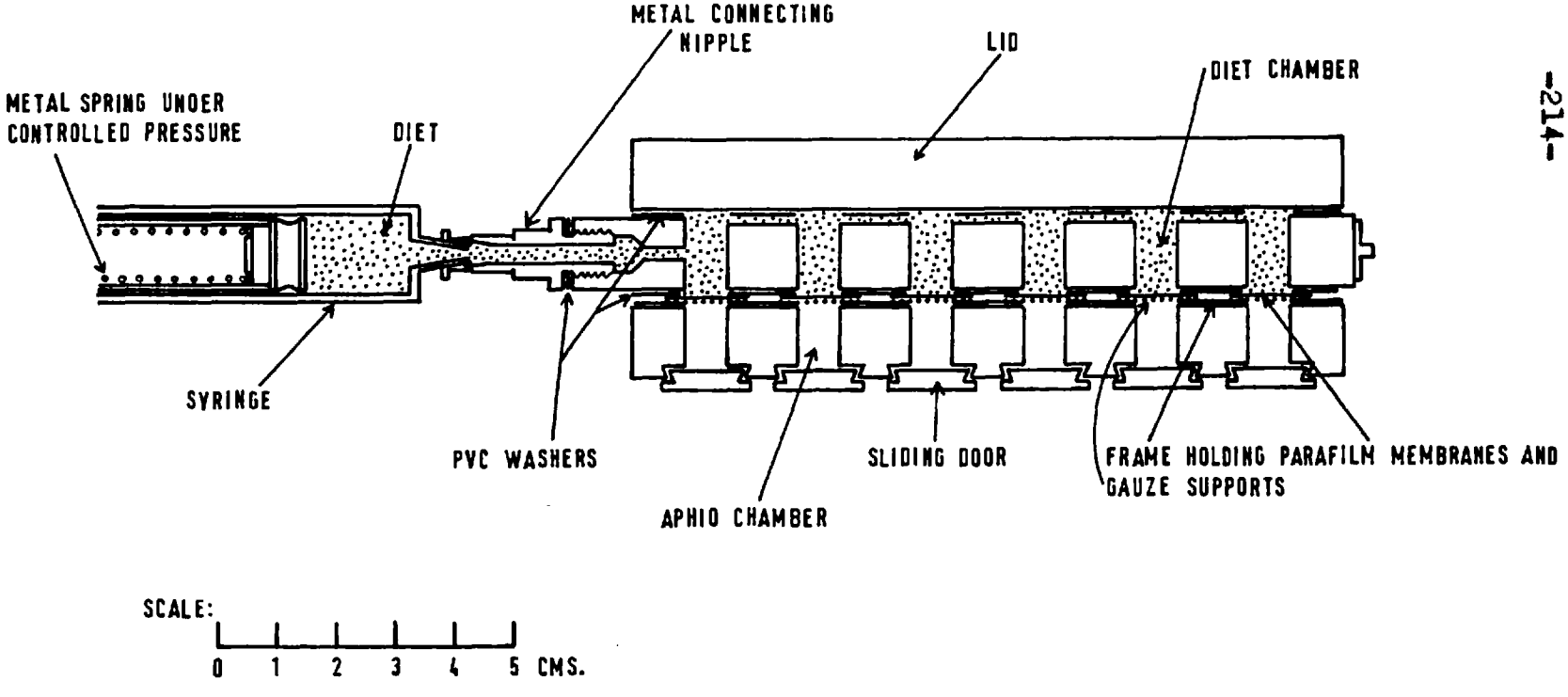

LID AND BINDING SCREWS
METAL SPRING
COMPRESSION SCREW
PVC WASHER
INTERCONNECTED DIET CHAMBERS
PVC WASHER
SYRINGE CONTAINING DIET
SCALE GRADUATED IN ATMOSPHERES
FRAME HOLDING PARAFILM MEMBRANES AND THEIR GAUZE SUPPORTS
APHID CHAMBERS
SLIDING DOOR
SCALE 10 CMS.

FIG. 36. APPARATUS FOR FEEDING APHIDS ON A LIQUID DIET UNDER CONTROLLED PRESSURE.

LONGITUDINAL VERTICAL SECTION TO SHOW THE DIET SUPPLY SYSTEM.



## Section C Experiments

### Sub-section 1 Myzus persicae

#### a) The responses of apterae to host plants subjected to continuously increasing water strain over a short period

The responses of plants to intermittent and continuous water shortage are very different. Water shortage generally reduces the vegetative growth of plants but they have considerable powers of recovery when watered after a period of drought (Gates, 1957; 1964) and at such times may grow even faster than watered control plants (Owen and Watson, 1956). Since the host plants in the experiments of Part 1 were subjected to intermittent water shortage, the responses of the aphids to the short periods of water strain in the plants were difficult to appreciate. The preference experiments with apterae (pp.118,168) revealed differences in the responses of the aphids to the same leaves on a plant in a wilted and a turgid condition (cf. Lamb, 1955; Kennedy, Lamb and Booth, 1958).

An experiment was therefore set up to examine the responses of the aphids to host plants suffering a single period of continuously increasing water strain. Both species of aphid, M. persicae, whose responses are described here, and B.brassicae (see p. 261), were studied in the experiment to obtain a direct comparison between the species. This comparison is drawn in the Discussion (p. 292).

Experimental Procedure

On one day (29.10.65) thirty-six adult apterous virginoparae of M. persicae were permitted to deposit four larvae in simple clip cages (p. 79 ) on three young leaves of twelve Brussels sprouts (40 days since pricking-out). These plants were grown in the constant temperature growth room (p. 76 ) and subjected to the "wet" water regime (p. 87 ). All the deposited larvae thus developed on leaves of similar age on plants of similar water status. Larviposition was timed so that when the larvae moulted to adult they could be immediately transferred on a soft brush to other Brussels sprouts which were just beginning (7.11.65; 45 days since pricking-out) the three water treatments, turgid, wilt and severe wilt, described on p.202 . These water treatments were replicated six times and one adult aptera was put in a simple clip cage on a young, mature and old leaf (see p. 93 ) of each plant. The experiment required, therefore, that 54 of the original 144 larvae should moult to adult apterae on the same day. (54 adult apterae of B. brassicae (see p.261 ), similarly reared on other Brussels sprouts, were distributed individually in the clip cages with the M. persicae). During the experiment the clip cages were moved from leaf to leaf as the leaves aged so that the adult apterae always fed on leaves of one age category.

Larvae deposited during the first 12 days of the adult lives of the apterae were counted and removed daily without disturbing the adults if feeding. At the same time, and again later in each day, records were kept of whether or not

the aphids were feeding. These records were used to assess the restlessness of the aphids (see below). Any mortality of the aphids was noted.

The experiment was designed and initially analysed as a split-split-plot experiment in which each plant constituted a plot consisting of three sub-plots, the three leaf ages. Each leaf age contained two sub-sub-plots, the two species of aphid. Three plants, one from each water treatment, formed a block of which there were six replicates. A split-split-plot analysis was carried out of 1) the total number of progeny produced by each aptera in the first twelve days of adult life, or the whole of its adult life if shorter, 2) the mean number of progeny produced per day (reproductive rate) by each aptera for the first 12 days of adult life, or the whole of its adult life if shorter, and 3) the percentage of occasions when each adult was observed to be not feeding (stylets withdrawn from the leaf during a 5-minute observation period) out of the total of 24 (maximum) observations (see above). In 3), zeros were removed by adding 1 to all figures before calculating the percentages, which were then converted to degrees using the arc-sin transformation.

In almost all the treatments, the daily larviposition of the apterae decreased with their age. But concurrently in the wilt and severe wilt water treatments, the available water in the growing medium of the host plants was decreasing, and this appeared to decrease the larviposition of the aptera still further. By fitting linear regressions of larviposition on the

age of the aphids in each treatment, it was possible to demonstrate significant differences between water treatments, between leaf ages and between aphid species in the rate of decline of larviposition. But such analyses did not permit direct comparison between the effects of the water treatments on the two species of aphid since their rates of decline of larviposition were different even on turgid plants (for each leaf age). Furthermore, the analyses suggested that the rate of decline of larviposition with the age of the aphids depended, in part, on the number of larvae already deposited. This made interpretation of the analyses very difficult.

To obtain a clearer understanding of the relationship between the larviposition of the apterae and the water strain in the host plant, the data from the three water treatments were combined in multiple regressions (Snedecor, 1961 p. 340) of daily larviposition on the age of the aphid and the available water in the growing medium of the host plant. A separate regression was calculated for each species of aphid on each leaf age of the plants. Larviposition was expressed as the mean number of larvae produced each day by the six apterae in each treatment (dead adults included). The age of the adult apterae was expressed in days, day 0 being the day on which the aphids moulted to adult and the experiment was begun. The mean percentage available water (p) in the growing medium of the plants of each water treatment was calculated for the 24 hours preceding a given larviposition figure. (The true lag in the larviposition response to the water strain in the plant was not

known). Since examination of the data indicated that larviposition did not decrease linearly with the percentage available water but decreased particularly in the region of the permanent wilting percentage, the mean percentage available water in the growing medium was transformed to logarithms for the regression analyses (cf. Weismann and Vallo, 1963). Before this was done, 10% was added to all percentages to remove negative values (i.e. below the permanent wilting percentage).

Thus, for each species of aphid on each leaf age of the host plants, a separate regression was fitted based on the equation:

$$y = a + b_1x_1 + b_2x_2.$$

where y = the mean number of larvae produced by the apterae on a given day, $x_1$, in the adult life of the apterae and where $x_2 = \log_{10} p + 10$. From these equations, for aphids of a given age, it was possible to examine the decline in larviposition with the available water in the growing medium of the plants. In addition, the rates of decline (regression coefficients) of larviposition with the age of the aphid ($b_1$'s) and the available water in the growing medium ($b_2$'s) could be compared directly between the species of aphid and leaf ages (using 't'-tests).

In the above analyses of larviposition, apterae which died before the end of the experiment were treated as living apterae failing to produce progeny except in the analyses of the reproductive rate. There is little doubt that any aphid mortality occurred in response to the treatments since on the turgid 'control' plants no adult apterae of M. persicae died

during the experiment. Failure to include the dead aphids of the other treatments in this way (e.g. reproductive rate) reduced the apparent effect of the treatments on the larviposition of the aphids. The adult life of the aphids was not analysed statistically as the lengths of the adult lives of the aphids which survived throughout the experiment were not known. The survival of the aphids was thus presented only graphically (Fig. 37).

## Results

### Survival

The survival of the adult apterae during the experiment is shown in Fig. 37. The results clearly indicate that severe wilting of the plants was associated with increased mortality of the aphids, particularly those caged on the old leaves, where only one aphid survived for 12 days. This contrasts strongly with the survival of all the aphids (for 12 days) on the turgid plants.

### Restlessness

The restlessness of the adult apterae is presented in Fig. 38 and Table 15 . Fig. 38 shows the mean percentages of occasions when the apterae of each treatment were observed to be not feeding (see above) while Table 15 presents the treatment means of the transformed data and the least significant differences between them.

On turgid plants, there were no significant differences between the restlessness of the apterae on different

FIG. 37. NUMBERS OF SURVIVING ADULT APTEROUS VIRGINOPARAE OF M.persicae PLOTTED AGAINST TIME IN DAYS SINCE MOULTING TO ADULT FOR EACH OF NINE TREATMENTS: APTERAE CAGED INDIVIDUALLY ON YOUNG, MATURE AND OLD LEAVES OF BRUSSELS SPROUTS IN TURGID, WILT AND SEVERE WILT WATER TREATMENTS.

KEY: LEAF AGES — YOUNG ●; MATURE ○; OLD ×.

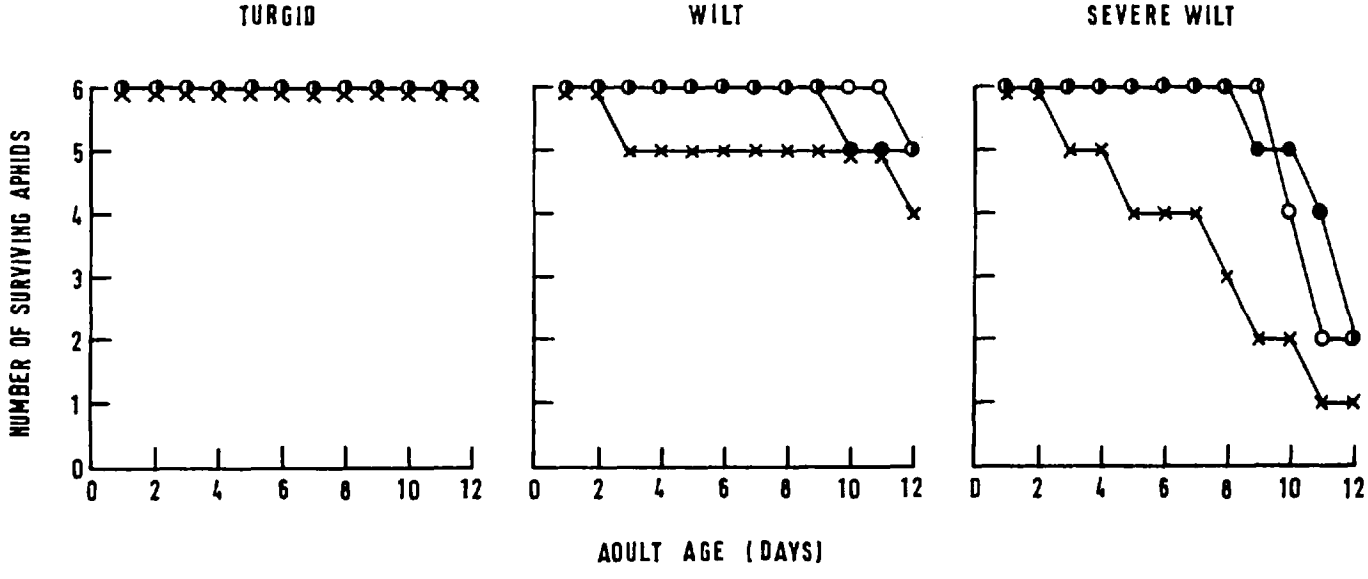


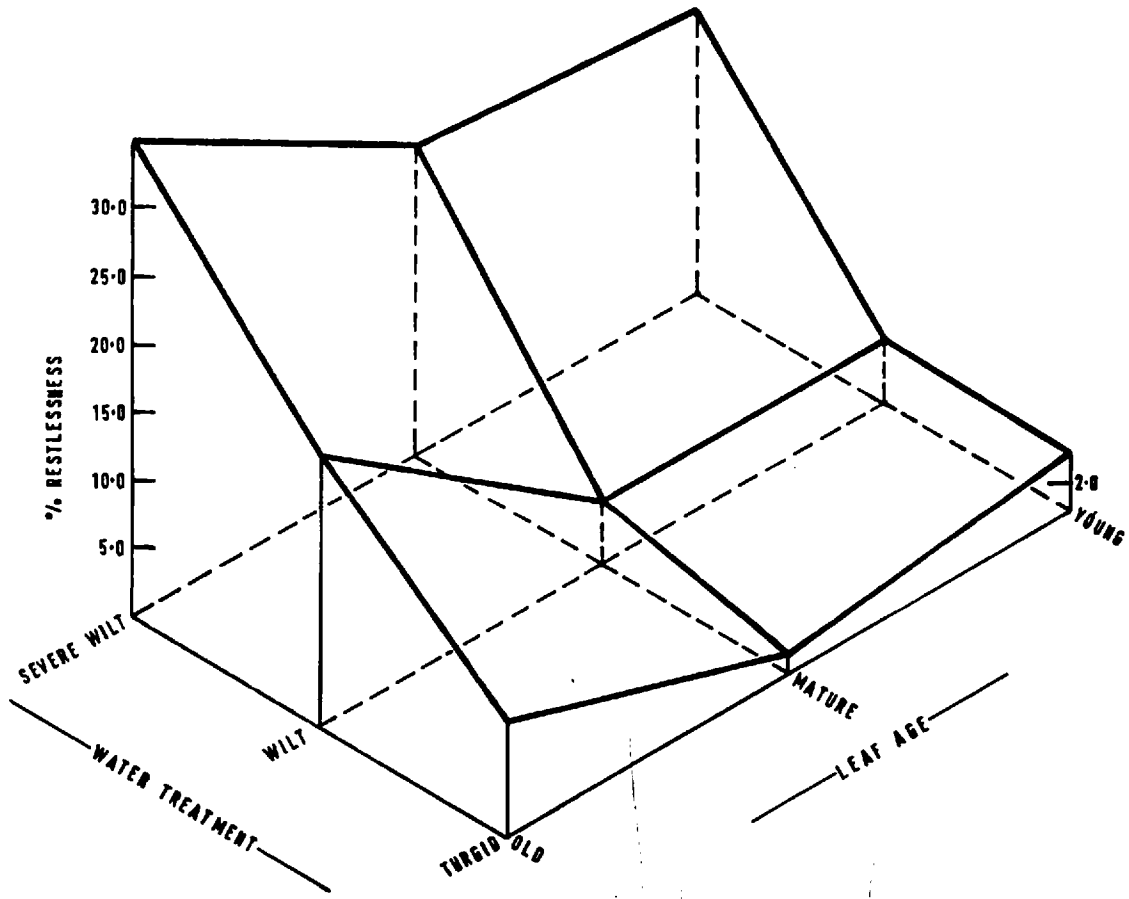
Table 15 . Mean restlessness of adult apterous virginoparae of M.persicae on 3 leaf ages of Brussels sprouts in 3 water treatments at 20°C (For explanation of restlessness, see text).

| | | Mean percentage restlessness (transformed to degrees) | | | |
|---|---|---|---|---|---|
| | | Water Treatment | | | |
| | | TURGID | WILT | SEVERE WILT | L.S.D.(W) |
| Leaf age | O | 21.98(14.0)* | 29.40(24.1) | 39.65(40.8) | p.05=8.46<br>.01=11.59 |
| | M | 16.62(8.2) | 18.78(10.4) | 31.57(27.5) | .001=15.85 |
| | Y | 18.63(10.2) | 19.13(10.7) | 29.65(24.6) | |
| L.S.D. (L) | | | p.05 = 7.48<br>.01 = 10.04<br>.001 = 13.26 | | |

* In parenthesis - detransformed percentages.
Lettering as in Table 6 .

FIG. 30. MEAN RESTLESSNESS OF ADULT APTEROUS VIRGINOPARAE OF M.persicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER TREATMENTS AT 20° C.

FOR AN EXPLANATION OF RESTLESSNESS— SEE TEXT. SEE TABLE 15 FOR SIGNIFICANT DIFFERENCES.



aged leaves. On the old leaves, the restlessness of the apterae increased from the turgid to the wilt (p just$>$0.05) and from the wilt to the severe wilt water treatments ($p<0.05$), (turgid to severe wilt, $p<0.001$). The restlessness of the aphids on the young and mature leaves similarly increased from the wilt to the severe wilt water treatments ($p<0.05$ and $<0.01$ respectively) but not from the turgid to the wilt treatments. As a result of these effects, within the wilt and severe wilt water treatments aphids on the old leaves of the plant were more restless than aphids on the young and mature leaves.

Larviposition

Total Progeny and Reproductive Rate

The mean total progeny and mean reproductive rate of the apterae of each treatment are presented in Table 16, where the least significant differences between the means are also indicated. The effects of the treatments on these two measures of the larviposition of the aphids were very similar, indicating that, as suggested above, the reduced aphid survival in some treatments generally paralleled the reduced larviposition responses of the aphids.

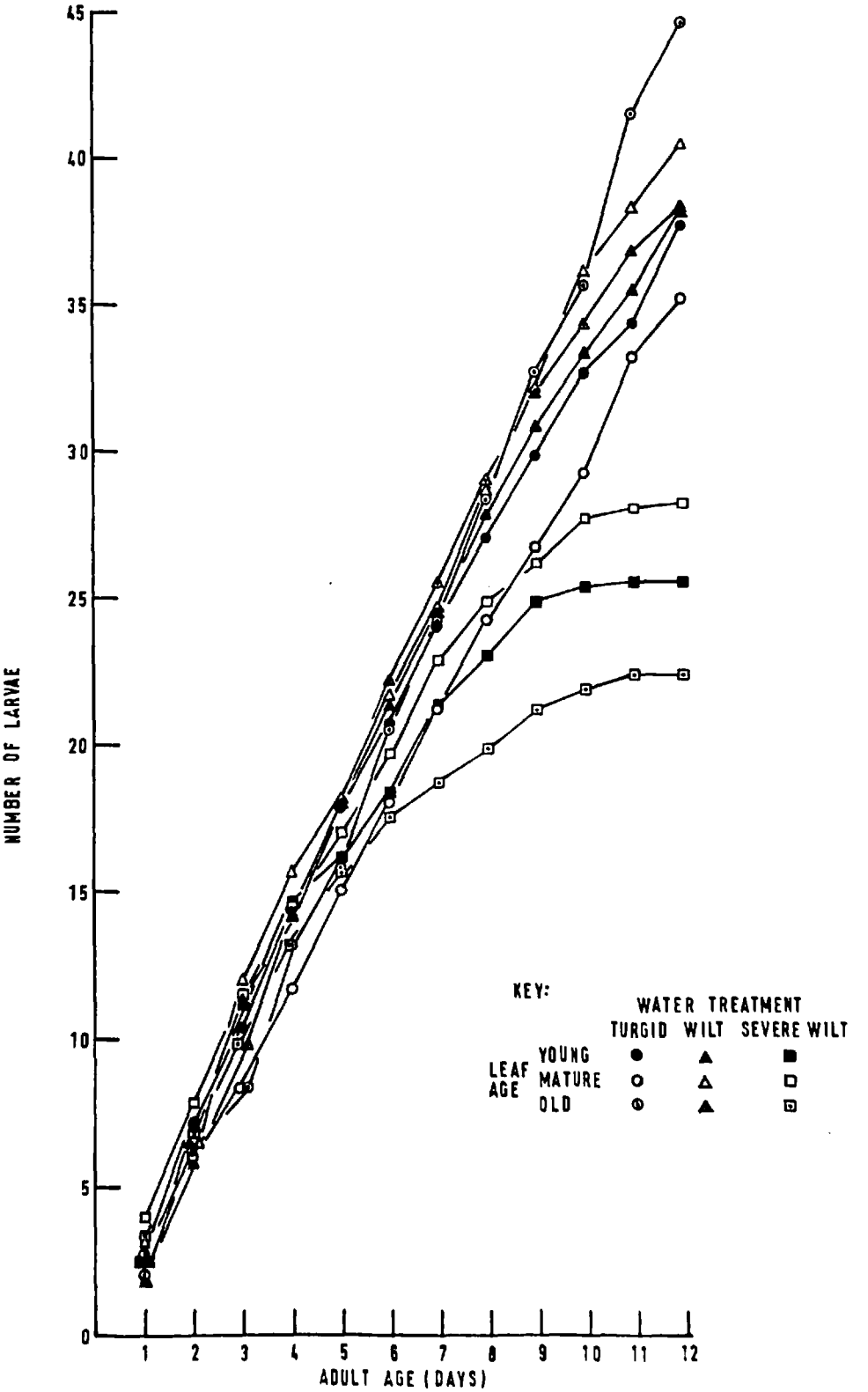
Within each water treatment, the apterae deposited larvae in similar numbers and at similar rates on the three leaf ages of the plants, but on the plants of the severe wilt water treatment, larviposition was consistently reduced compared with the other water treatments, both in terms of total progeny ($p<0.05$) and reproductive rate ($p<0.01$) for the combined leaf ages. Similar effects were found within each leaf age, but

Table 16 . Mean total progeny and mean reproductive rate of adult, apterous virginoparae of M. persicae during the first twelve days of adult life on 3 leaf ages of Brussels sprouts in 3 water treatments at 20°C

| | Total Progeny | | | | Reproductive Rate (larvae/day) | | | |
|---|---|---|---|---|---|---|---|---|
| | Water Treatment | | | | Water Treatment | | | |
| | TURGID | WILT | SEVERE WILT | L.S.D.(W) | TURGID | WILT | SEVERE WILT | L.S.D.(W) |
| Leaf age O | 44.67 | 38.33 | 22.33 | p.05=15.28 .01=20.94 | 3.72 | 3.32 | 2.50 | p.05=1.12 .01=1.53 |
| M | 35.17 | 40.50 | 28.17 | .001=28.61 | 2.93 | 3.38 | 2.55 | |
| Y | 37.67 | 38.17 | 25.50 | | 3.14 | 3.29 | 2.24 | |
| L.S.D. (L) | p.05 = 14.01 | | | | p.05 = 1.15 | | | |

Lettering as in Table 6 .

FIG. 39. ACCUMULATED DAILY MEAN NUMBER OF LARVAE PRODUCED BY APTEROUS VIRGINOPARAE OF M.persicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER TREATMENTS AT 20°C.



only on the old leaves were these effects significant, (see Table 16 ).

The above analyses of larviposition revealed very few significant differences between the apterae of the various treatments, partly because any differences arose almost entirely in the second half of the experiment. This is clearly indicated in Fig. 39 which shows the accumulated mean number of larvae produced on each day by the six apterae of each treatment. Such a delayed response to the treatments might be anticipated from aphids which, prior to the experiment, completed their larval development under very similar conditions. Furthermore, during the experiment the aphids were responding to continuously increasing water strain in the plants of the wilt and severe wilt water treatments.

The multiple regression of daily larviposition on the age of the adult aptera and the percentage available water (p) in the growing medium of the host plant

Fig. 40a shows the linear regression of the mean daily larviposition of apterous M. persicae of a given age on the mean percentage available water (p) in the growing medium of the host plant, expressed as $\log_{10}p+10$ ($x_2$). The figure shows separate regressions for apterae, after 1 and 12 days of adult life ($x_1$), caged on the young, mature and old leaves of the plants. The same regressions are presented in Fig. 40b in which $x_2$ has been de-transformed to p. Table 17 shows the regression coefficients and their standard errors.

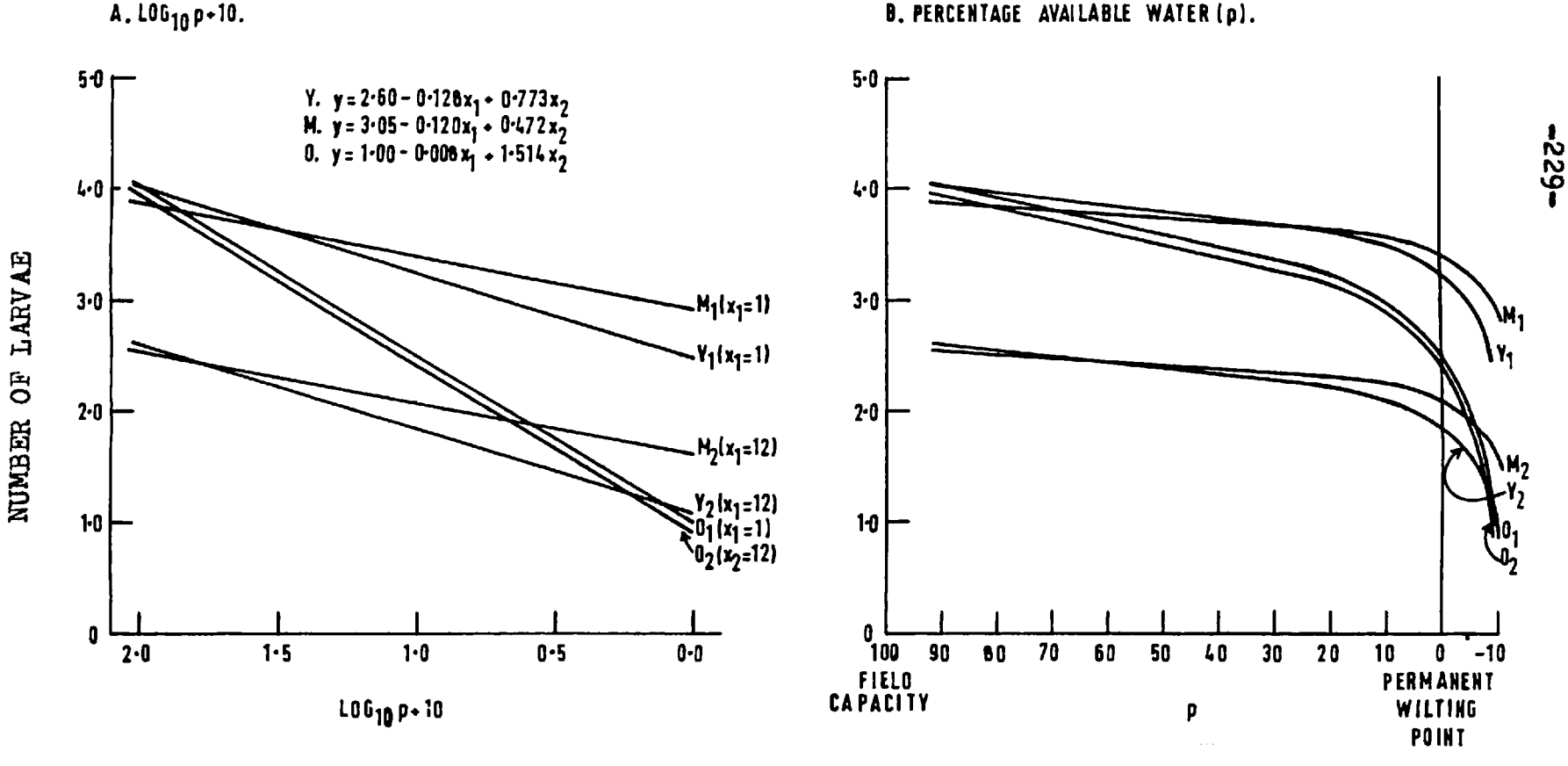
Table 17 Estimated Regression Coefficients and their Standard Errors

| Apterae on: | $b_1$ | $b_2$ |
|---|---|---|
| Young Leaves | $- 0.128 \pm 0.045$ | $+ 0.773 \pm 0.258$ |
| Mature Leaves | $- 0.120 \pm 0.045$ | $+ 0.472 \pm 0.250$ |
| Old Leaves | $- 0.008 \pm 0.049$ | $+ 1.514 \pm 0.277$ |

The mean daily larviposition of the apterae caged on the old leaves did not decline significantly ($b_1$) with the age of the aphids. This contrasted with the mature and young leaves whose apterae, as they aged, produced fewer progeny per day ($p<0.05$ and $p<0.01$, respectively).

Decreasing available water in the growing medium of the plant was consistently associated with decreasing daily larviposition ($b_2$) by the apterae on each leaf age. 'T'-tests of the regression coefficients ($b_2$'s) for each leaf age were significant or nearly so - young, $p<0.01$; mature, p just $>0.05$; old, $p<0.001$. The differences between these $b_2$'s showed that the decline of daily larviposition with the availability of water to the plant was more rapid for apterae on the old leaves than on the mature ($p<0.01$) and young ($p=0.05$). Fig. 40b indicates that larviposition on all leaf ages decreased slowly and steadily as the growing medium of the plant dried out in the upper part of the available water range. Approaching the permanent wilting percentage, however, the decrease in larviposition accelerated sharply, particularly for apterae on the old leaves of the plants, (cf. increasing soil moisture

FIG. 40 MULTIPLE REGRESSIONS OF MEAN DAILY NUMBER OF LARVAE PRODUCED BY APTEROUS VIRGINOPARAE OF M.persicae ON THE ADULT AGE OF THE APHID IN DAYS ($x_1$) AND ON THE PERCENTAGE AVAILABLE WATER (p) IN THE GROWING MEDIUM OF THE HOST PLANT (BRUSSELS SPROUT) EXPRESSED AS $LOG_{10} p+10$ ($x_2$); SEPARATE REGRESSIONS FOR APHIDS CAGED ON THREE LEAF AGES: YOUNG (Y), MATURE (M) AND OLD (O).



tension in a drying soil, e.g. Hudson, 1957).

Conclusions

Continuously increasing water strain in the host plants decreased the survival and increased the restlessness of adult apterae of M. persicae. The more severe was the water strain, the greater were these effects, which reached a maximum among aphids on the old leaves and generally decreased towards the apex of the plant.

In parallel with the increased restlessness and decreased survival of the aphids, the apterae produced fewer progeny more slowly on plants suffering severe water strain compared with turgid plants, particularly when the aphids were caged on the old leaves.

A careful analysis of the relationship between daily larviposition of the aphids, the age of the aphids and the percentage available water in the growing medium of the host plant was carried out: 1) Larviposition decreased linearly with the logarithm of the percentage available water in the growing medium of the plant. The rate of this decrease was greater for aphids on the old compared with the young and mature leaves of the plants. These linear relationships represented essentially a slow, steady decline of larviposition with decreasing percentage available water until approaching the permanent wilting percentage, when there was a sharp decline in larviposition, especially for aphids on the old leaves of the plants. 2) Daily larviposition of the apterae decreased during the first twelve days of adult life when they were caged on young or mature

leaves of the plants, but not when caged on the old leaves.

b) The method of penetration and the destination of the stylets of apterae in the tissues of the host plant

Examinations of the stylets and the salivary sheaths of aphids in the tissues of their host plants have indicated that many species of aphid feed primarily in the vascular bundles, particularly the phloem tissue (e.g. Dykstra and Whitaker, 1938; see Review of the Literature, p. 31 ). However, Esau, Namba and Rasa (1961) (also Banks, 1965) pointed out that there is little direct evidence to support the widespread belief that aphids feed in the sieve tubes of the phloem, (e.g. Auclair, 1963). Most studies of the feeding tracks of aphids in their hosts have been at a tissue rather than a cellular level, while other workers have followed the course of the salivary sheaths of the aphids and not their stylets (e.g. Horsfall, 1923). Even in the very recent study by Hennig (1966), where the tips of the stylets of A. fabae (on Vicia faba) were shown to project beyond the salivary sheaths into the cells of the phloem, the identity of these cells was not clear. In at least one micrograph (Abb. 8) the stylet tips appeared to end in a companion cell. Stylets terminating in individual sieve elements have been found with certainty only for larger species of aphids, such as Longistigma caryae (Harr.) (Zimmermann, 1961) and Tuberolachnus salignus (Gmelin) (Mittler, 1954).

The penetration and destination of the stylets of

M. persicae in the tissues of some host and non-host plants have been examined by a number of workers (Horsfall, 1923; Smith, 1926; Tate, 1937; Dykstra and Whitaker, 1938; Roberts, 1940; Sukhov, 1944; Day and Irzikiewicz, 1954; Esau, Namba and Rasa, 1961). Although most of these workers found that the stylets eventually penetrated to the phloem tissue, none produced clear evidence of the cells in which the stylets terminated, This evidence was required for the present investigation since cells, such as sieve tubes and parenchyma cells, may provide sap at different pressures to assist the feeding of aphids (see Mittler, 1957). A study was therefore undertaken of the penetration and destination of the stylets of feeding M. persicae in the leaves of Brussels sprouts. No published reports could be found describing the disposition of the stylets of M. persicae in the leaves of a brassica.

Experimental Procedure

Apterous virginoparae of M. persicae feeding on early senescent leaves of Brussels sprouts (cv. 'Irish Elegance') were fixed, sectioned and stained by the methods described on p. 205 . All instars of the aphid were used and, prior to being killed, no control was imposed on the length of time that the aphids had been feeding. The locations in the host tissues of the stylets and salivary sheaths of 42 aphids were analysed. Almost all the aphids examined had penetrated deeply into the host and it is assumed that most of those aphids which inserted their stylets only a short distance into the host probably fell

away during fixation (see p.206 ). The tips of the stylets were examined under oil immersion at a magnification of X1000.

Results

Location of the feeding aphids on the leaf

With one exception the aphids fed on the abaxial surface of the leaves, where the veins protruded above the lamina. Every aphid inserted its stylets at a vein, the size of the vein increasing linearly with the length of the stylets of the aphid according to the equation:

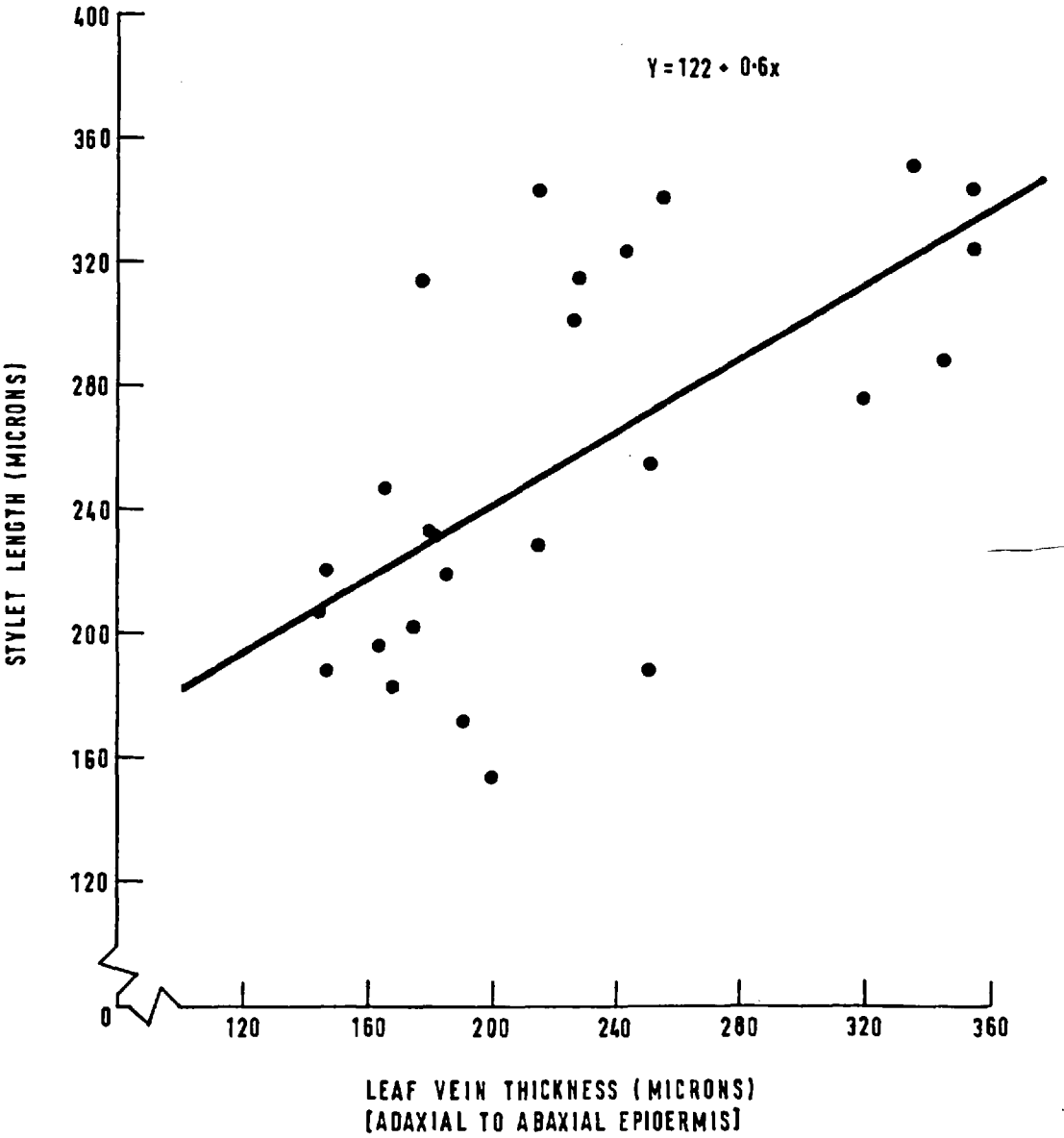
$$y = 122 + 0.6x$$

where y = length of the stylets in microns and x = thickness of the vein in microns (abaxial to adaxial epidermis), for veins up to 360 microns thick (see Fig. 41). Thus, early instars were found to occur at very small veins while adults occurred at larger prominent veins. A 't'-test of the regression coefficient was significant ($p<0.001$). This relationship suggests that a mechanism may exist whereby aphids select veins of a size which permits their stylets to reach the phloem (see below). It is conceivable that the aphids may probe more when astride a vein of a suitable width (see Ibbotson and Kennedy, 1959).

The vascular bundle of the leaf vein of Brussels sprout

A transverse section of a typical leaf vein of Brussels sprout is shown in Fig. 42. The vascular tissues were sheathed on the adaxial (xylem) and abaxial (phloem) surface by unlignified sclerenchyma, the cells of which were markedly elongate in longitudinal section. The amount of the

FIG. 41. REGRESSION OF STYLET LENGTH OF APTEROUS VIRGINOPARAE OF M.persicae ON THE THICKNESS OF THE LEAF VEIN PENETRATED.



sclerenchyma increased with the size of the vascular bundle and was completely absent from very small veinlets. (In the stem the sclerenchyma occurred only outside the phloem and was lignified, and a single layer of collenchymatous cells occurred beneath the epidermis). The cortex surrounding the vascular bundle consisted of closely packed cells, in contrast to the loose cells, with many air spaces, of the mesophyll.

Stylet dimensions and the amount of stylet insertion

The length of the stylets inserted into the leaf varied with the size of the leaf vein and aphid, the instar of the aphid, and the points of insertion and destination of the stylets: from 73 - 216 microns for aphids with their stylets inserted into the phloem. The total length of the stylets of the aphids varied from 153 microns, for a first instar nymph, to 351 microns, for an adult. Accordingly, for aphids reaching the phloem, the proportion of the stylets inserted into the leaf varied from, for example, 31% for a 4th instar nymph on a vein 178 microns thick to 71% for a 3rd-4th instar nymph on a vein 345 microns thick. The mean for all the aphids reaching the phloem was 52%.

The only aphid (4th instar) sectioned while on the adaxial surface of a leaf inserted 79% (206 microns) of its stylets to reach the phloem in a vein 453 microns thick.

In the larger veins, the shortest distance from the abaxial epidermis to the phloem frequently occurred at the side of the vein, where the vein cortex approached the mesophyll. Vertical measurements through the veins showed that the distance

from the adaxial leaf surface to the phloem was usually less than that from the abaxial surface, where the aphids normally occurred (cf. Pollard, 1958). However, many (up to 10) more cells separated the phloem from the adaxial than from the abaxial surface of the leaf.

The diameter of the stylet bundle varied from 3.5 microns (adult) in the epidermis to less than 1 micron at the tip of the stylets.

The destination of the stylets

Of the 42 stylet bundles studied, 38 (91%) terminated in the phloem (Fig. 42,45), 2 (5%) in the epidermis, and 1 each (2%) in the cortex and xylem sclerenchyma. Even allowing for the loss of aphids during fixation, it would appear that the phloem was the primary site of feeding of these aphids (cf. Horsfall, 1923; Smith, 1926; Tate, 1937; Dykstra and Whitaker, 1938; Roberts, 1940; Esau et al, 1961; Guthrie et al, 1962). The tips of the stylets which terminated outside the phloem occupied intercellular positions and many salivary sheaths left by other aphids ended in the phloem.

The tips of the stylets which entered the phloem varied in their destinations as shown in Table 18 .

Table 18 Destination of stylets of M. persicae in the phloem of Brussels sprout leaves

| Type of Phloem Cell | No. of Stylets | Percentage of stylets | Number with tips of stylets intercellular |
|---|---|---|---|
| Sclerenchyma | 3 | 8 | 3 |
| Parenchyma | 4 | 10 | 1 |
| Companion Cell | 1 | 3 | - |
| Sieve Tube | 21 | 55 | - |
| Indefinite | 9 | 24 | 4 |
| Total | 38 | | |

A large proportion (84%) of the stylets, whose destination within a cell could be observed with certainty, ended in the sieve tubes (Figs. 43 & 44), where the stylets extended into the cell beyond the end of the salivary sheath (cf. Miles et al., 1964; Hennig, 1966 for A. fabae). The latter did not enter the sieve tube. Some sections suggested that the inner maxillary stylets only entered the sieve tube while the outer mandibular stylets remained a short distance behind, (Fig. 46) perhaps at the cell wall (cf. Hennig, 1966).

Stylet Penetration

1) Epidermis

The stylets of all the aphids penetrated the epidermis intercellularly (Fig. 42). No stylets entered through stomata. The mean length of the stylets in the epidermis was 7.9 microns and, for aphids later penetrating the cortex, phloem sclerenchyma and phloem, this length was 7.9 of the mean total

inserted length of the stylets.

2) Cortex

The length of the stylets in the cortex and/or mesophyll ranged from 35 microns at small veinlets to 162 microns at a "large" vein, with a mean of 87.2 microns for stylets later penetrating the phloem sclerenchyma and phloem. This mean length constituted 70% of the mean total inserted length of these stylets.

For 80% of the stylets, penetration of the cortex was almost entirely intercellular (Fig. 42), but the stylets sometimes appeared to follow the cell walls both between and within the cells (cf. Entwistle and Longworth, 1963). For the remaining 20% of the stylets, penetration of the cortex was equally inter- and intra-cellular.

3) Phloem sclerenchyma

Stylet penetration of the phloem sclerenchyma (Figs. 42 and 45) was very similar to the penetration of the cortex.

For stylets later reaching the phloem, the mean length of the stylets in the sclerenchyma was 29.6 microns (range, 5 - 67 microns) which was 22% of the mean total inserted length of the stylets.

4) Phloem

Stylet penetration of the phloem (Figs. 43 & 45) was more intra- than inter-cellular, and was commonly (57%) a mixture of both. No stylets penetrated the phloem wholly intercellularly, but for 34% penetration was wholly intracellular.

With one exception (see Table 18 ), the tips of the stylets in the phloem ended within a cell (excluding sclerenchyma).

The mean length of the stylet penetration of the phloem was 6.8 microns (range 2 - 20 microns) for aphids which had already penetrated the epidermis, cortex and phloem sclerenchyma. This length was 5% of the mean total inserted length of the stylets and represented the penetration of between 2 and 3 phloem cells. This shows the usual abrupt termination of penetration when the stylets entered the phloem. Where the stylets penetrated phloem lacking a sclerenchyma sheath (i.e. in small side veins), the mean length of the stylets inserted into the phloem was 16.8 microns (range 3 - 33 microns) or 14% of the mean total inserted length of the stylets. Stylets which had penetrated the phloem deeply often bent back towards the phloem periphery.

5) Adaxial penetration

The stylets of the only aphid sectioned while on the adaxial surface of the leaf penetrated the epidermis, cortex. xylem sclerenchyma and xylem largely intercellularly. The aphid apparently made no attempt to avoid the xylem, whence penetration continued through the cambium to the phloem.

6) Stylet 'excess' during penetration

By measuring the shortest distances from the points of insertion of the stylets to their destinations and comparing these distances with the lengths of the inserted stylets, it was found that the latter exceeded the former by 28% (mean of

38 stylets) (range, 1% - 100%). A large part of this stylet "excess" may be attributed to the intercellular stylet penetration. The cell walls of the host plant frequently appeared to divert the stylets without reference to their eventual destination (cf. van Hoof, 1958).

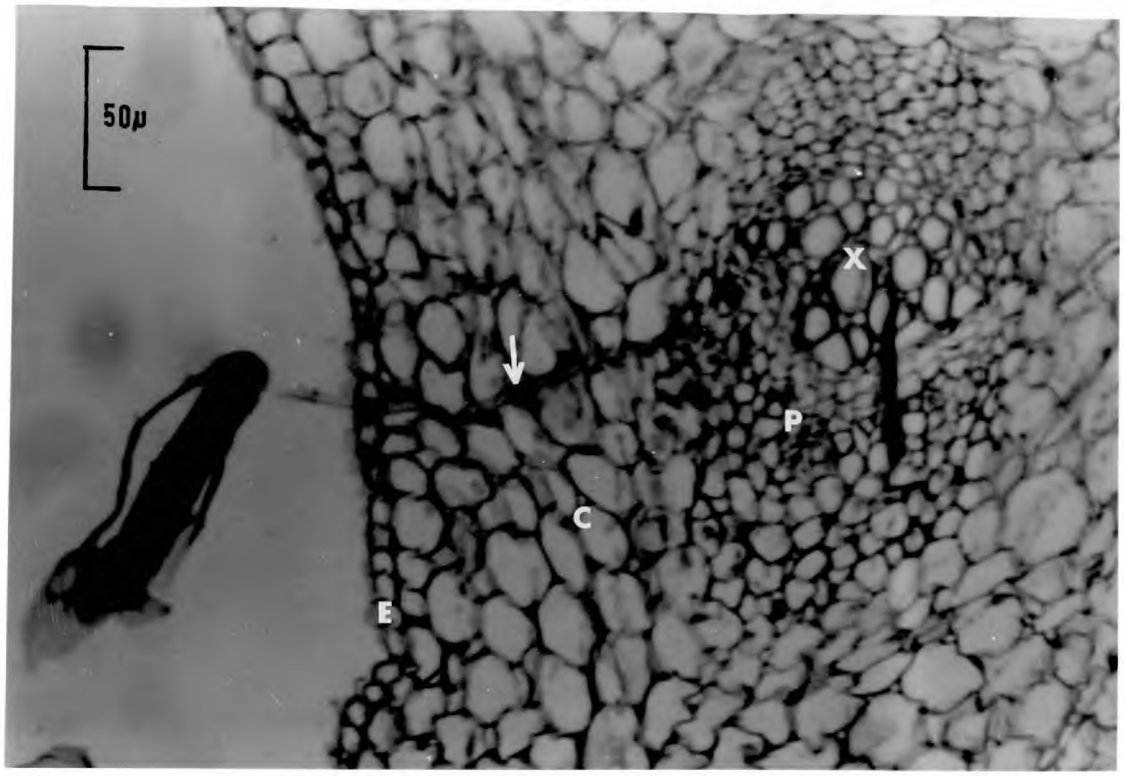
In a similar comparison of 1) the lengths of the inserted stylets with 2) the shortest distances from their points of insertion to sieve tubes in the phloem, the former exceeded the latter by 39% (mean of 34 stylets reaching the phloem, range 4% - 114%). Thus, even allowing for intercellular penetration, the aphids frequently inserted much more of their stylets than was necessary to reach their preferred source of food, the sieve tubes.

The salivary sheath

A salivary sheath normally surrounded the stylet bundle from the point of insertion to the tip, except when the tip entered a cell (see above). The sheath was most prominent within cells (cf. Hennig, 1966) and in the larger intercellular spaces. Branched salivary sheaths occurred near the tips of the stylets. Sometimes these branches terminated at the walls of sieve tubes suggesting that the aphids had "tapped" several sieve tubes in turn (Fig. 47). Very occasionally the salivary sheath, protruding beyond the tips of the stylets, provided evidence of possible slight stylet withdrawal during the killing of the aphid.

Fig. 42. Transverse section of a leaf vein of Brussels sprout (cv. "Irish Elegance") penetrated by the stylets of M. persicae. Arrow indicates the stylets; C = cortex, E = epidermis, P = phloem, X = xylem.

Fig. 43. Destination of the stylets of M. persicae in a leaf vein of Brussels sprout (transverse section). Arrow indicates the tips of the stylets; p = parenchyma, t = sieve tube.


50µ
X
P
C
E

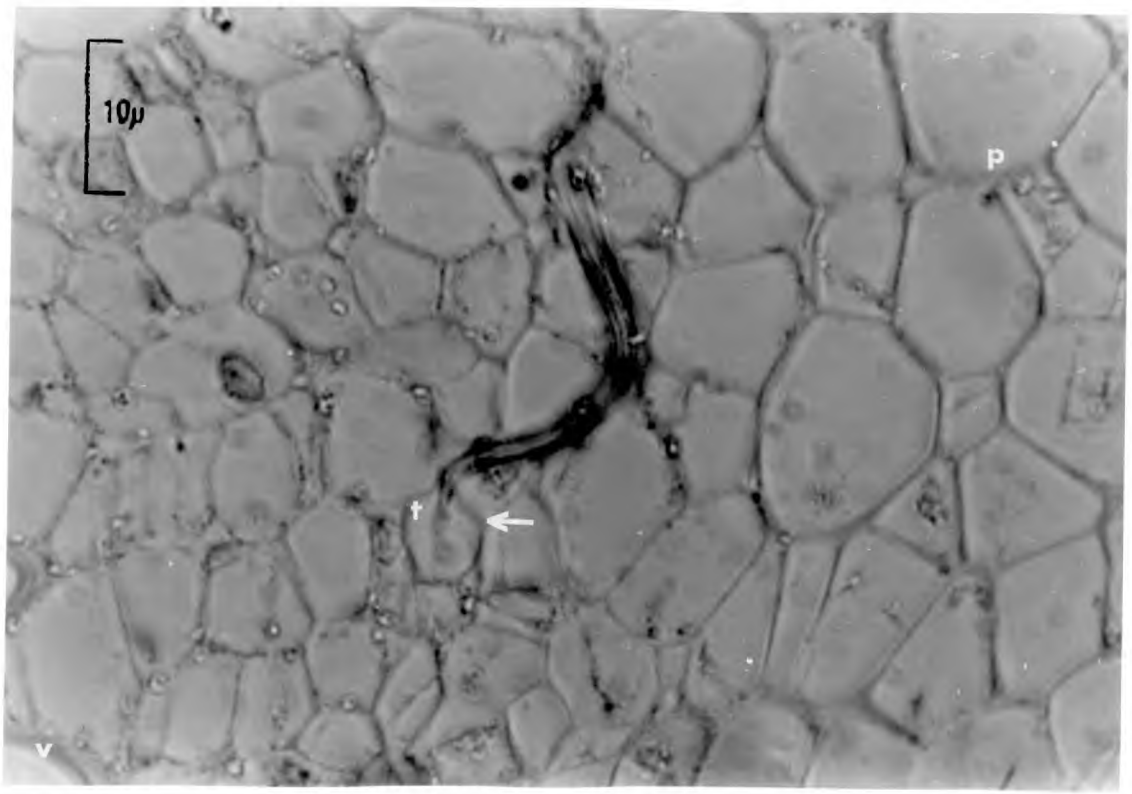
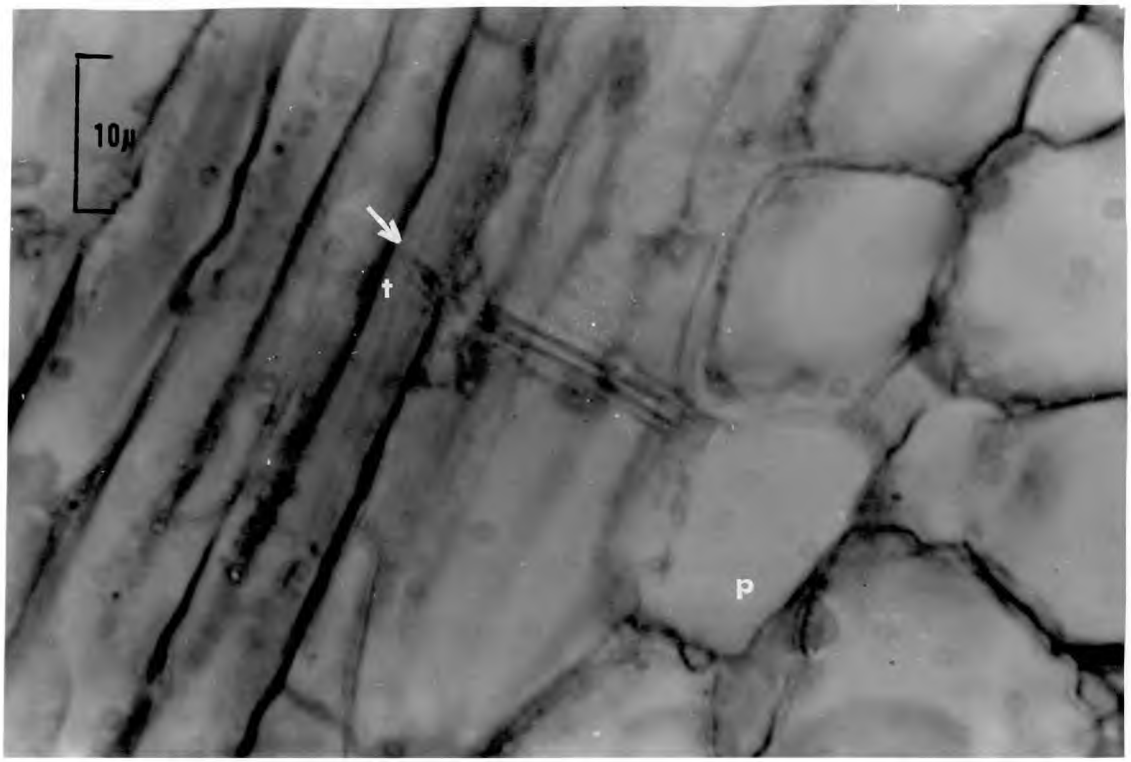

10µ
P
t
v

Fig. 44. Destination of the stylets of M. persicae in a leaf vein of Brussels sprout (longitudinal section). Arrow indicates the tip of the stylets; p = parenchyma, t = sieve tube.

Fig. 45. Method of penetration and the destination of the stylets of M. persicae in a leaf vein of Brussels sprout (transverse section). Arrow indicates the tip of the stylets; e = sclerenchyma, p = parenchyma, P = phloem.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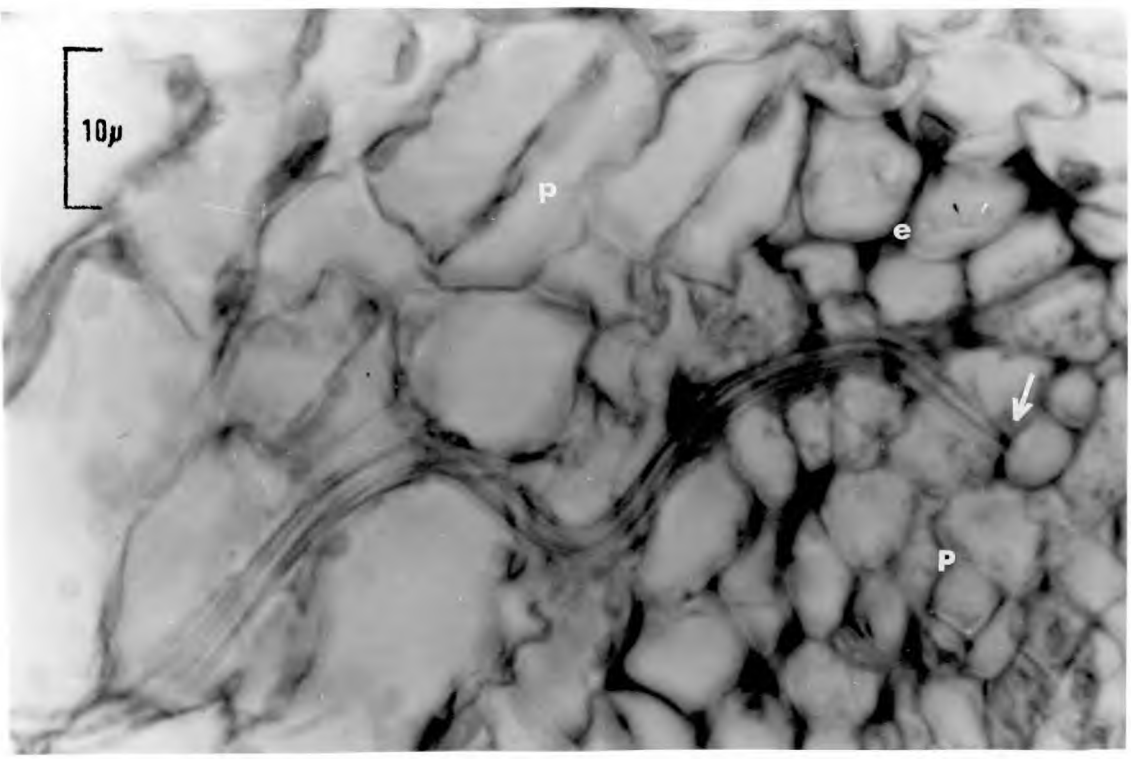
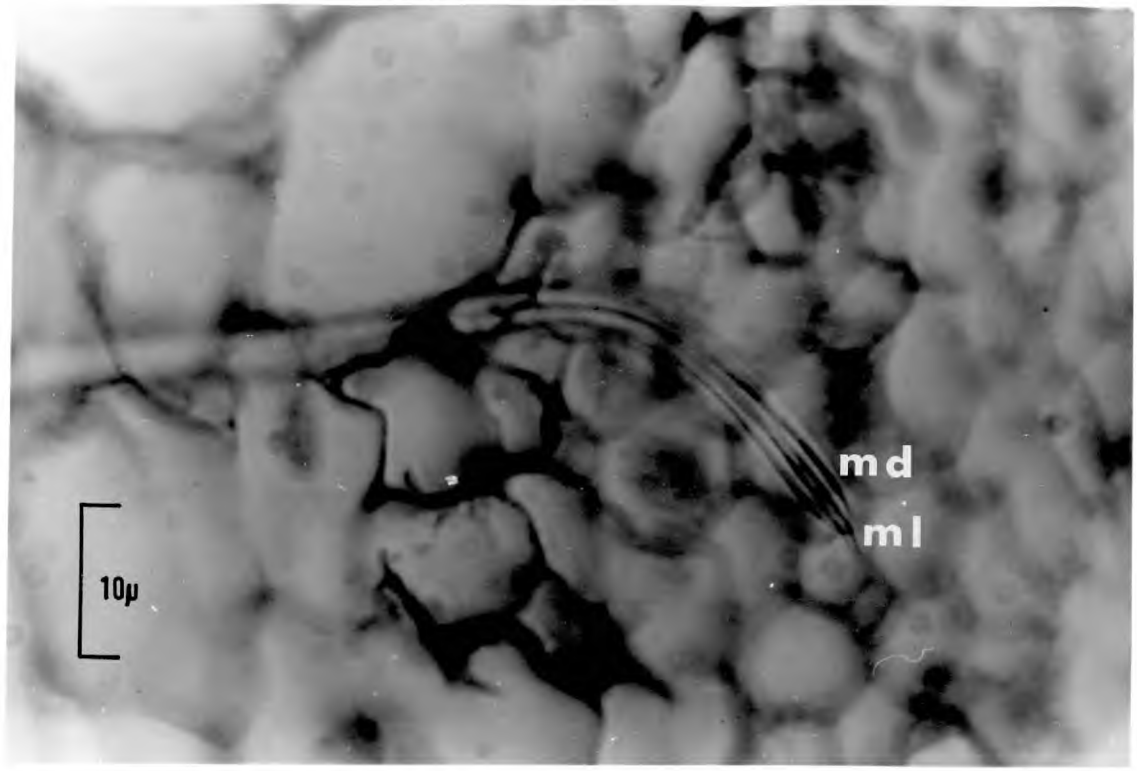
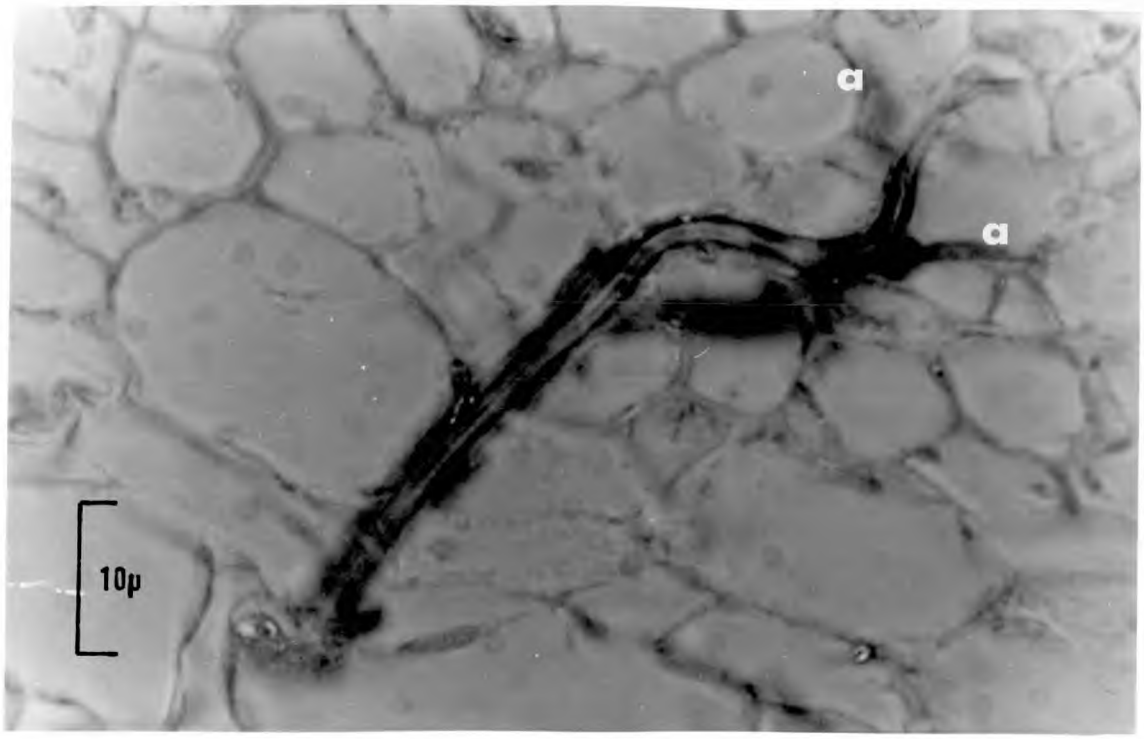

10µ
p
e
P

Fig. 46. Tips of the stylets of M. persicae in the phloem of a leaf vein of Brussels sprout (transverse section). md = mandibular stylets, ml = maxillary stylets.

Fig. 47. Branched salivary sheaths and the end of the stylets of M. persicae in the phloem of a leaf vein of Brussels sprout (transverse section). a = salivary sheath.


md
ml
10µ


a
a
10µ

Conclusions

Apterous virginoparae of M. persicae feeding on Brussels sprouts inserted their stylets into the leaves largely intercellularly, or at least along the lines of the cell walls. Intercellular penetrations of various host plants by M. persicae were observed by Horsfall (1923), Smith (1926), Tate (1937), Dykstra and Whitaker (1938), Esau et al (1961), while Roberts (1940) and Sukhov (1944) found that penetration by this aphid was largely intracellular. Intra-cellular penetration by the stylets was prominent only where they entered the phloem. Stylets, whose tips were within individual cells (i.e. the stylets of feeding aphids), ended only in the phloem, indicating that this tissue may be the sole source of plant sap for the aphids. Using the same argument, the sieve tubes appeared to be the main source of sap, confirming the evidence of the exudation of stylet sap (van Soest and de Meester-Manger Cats, 1956) which has generally been assumed to originate in the sieve tubes.

Where the tips of the stylets ended within a cell, they were extended beyond the salivary sheath which remained outside the cell. In addition, the maxillary stylets sometimes extended beyond the tips of the mandibular stylets. Branched salivary sheaths suggested that the aphids may "tap" a succession of sieve tubes.

The size of the leaf veins penetrated by the aphids increased with the size of the aphids. The length of the stylets of an aphid was always adequate to reach the phloem of

the vein penetrated. The length of the stylets used by an aphid to reach the phloem usually greatly exceeded the calculated minimum assuming direct penetration.

c) The feeding of apterae on sucrose solutions under controlled pressures

The relative importance of fluid pressure within the plant and the "sucking-power" of the aphid in the uptake of plant sap by feeding aphids have long been disputed (e.g. Zweigelt, 1914; Leonhardt, 1940). Recently, the exudation of plant sap from the severed stylets of feeding aphids (e.g. Kennedy and Mittler, 1953; see Review of the Literautre p. 34 ) has been construed as evidence for the importance of the turgor pressure of the host plant in directly forcing sap up the stylet food canal of the aphid (Kennedy and Mittler, 1953; Mittler, 1957). Banks and Nixon (1958) and Ehrhardt (1963) suggested that the so-called "sucking pump" of the aphid may act simply as a valve, normally closed, which could be opened to admit plant sap under pressure. On the other hand, the ability of aphids to feed through membranes on artificial diets in solution under trifling pressures (e.g. Mittler and Dadd, 1962; Auclair and Cartier, 1963) has clearly demonstrated that aphids are able to suck, perhaps by the use of the "sucking pump" (Forbes, 1964). It would seem, therefore, that both the turgor pressure in the cells of the plant and sucking by the aphid take part in aphid feeding.

The probable importance of the turgor pressure of the host plant in the feeding of aphids has occasionally prompted the application of pressure to liquid diets behind membranes in

attempts to increase the uptake of liquid by aphids (Mittler, 1954; van Hoof, 1958). Van Hoof (1958) obtained uptake of radioactive liquids by M. persicae, placed for 15 hours on a plastic membrane, only when the liquid behind the membrane was under a pressure of 6 - 8 atmospheres.

In the present investigation, differences of turgor pressure in the host plants of the different water treatments may have contributed to the responses of the aphids to the plants. As a first step in examining this problem, aphids were fed on sucrose solutions under controlled pressures.

Experimental Procedure

The apparatus described on p. 208 (see Figs. 34, 35 & 36) was used to confine individual aphids in chambers where they had access to sucrose solutions via parafilm membranes. In each experiment, two twelve-chambered devices of this kind were used, one as a "control", where the liquid diet was at atmospheric pressure, and the other to contain the same diet under a constant pressure between 0 and 2 atmospheres (see below). In each experiment a second "control" was conducted in which twelve aphids were starved individually in small glass vials of approximately the same volume (386 cu.mm.) as the other chambers.

For each experiment, 36 adult apterous virginoparae of M. persicae, which were actively reproducing on Brussels sprouts were collected and stored individually in glass tubes. 2 hours after collection (see Mittler and Dadd, 1963a), the aphids were

randomly allocated to the chambers and glass vials of the three treatments. All experiments were conducted in the dark in the laboratory at a temperature of $20^{\circ}C \pm 3^{\circ}C$. The aphids given access to liquids through the membranes were required to feed in an inverted position to prevent fouling of the membranes with honeydew and the cast-skins of larvae, which were produced by the adult aphids.

Each experiment lasted less than a week during which time the daily larviposition of the adults and the mortality of the adults and larvae were recorded. The experiments were repeated 3 or 4 times to give a total of 36 or 48 replicates respectively. 'T'-tests were used 1) to compare the mean number of larvae produced by the aphids of each treatment and 2) to compare the mean lengths of life (in days) of the larvae of each treatment.

To analyse the mortality of the adult aphids the probits of percentage aphid mortality were plotted against i) the logarithms of the access feeding time in days, (i.e. the total time of exposure of the aphids to the membrane), or ii) the access feeding time in days, depending on which produced the more linear relationship in a given series of experiments. Regressions were fitted using a common regression coefficient for the three treatments. The parallelism of the regressions was tested using $x^2$. Log. LT 50's (log m) or LT 50's (m) (median lethal times) were calculated for each regression. The LT 50's estimated the time taken for 50% of the aphids in a

treatment to die. To compare the mortality of the aphids in any two treatments their "relative dosage value" (here called the relative lethal-time value) was calculated (Finney, 1952) and its significance was assessed using 't'-tests. The relative lethal-time value (M or log M) represented the difference in the lengths (or log. lengths) of access feeding time which were associated with the same aphid mortality in two treatments (e.g. $m_1 - m_2$ or $\log.m_1 - \log.m_2$). For regressions involving the log. access feeding time, the relative potency of two treatments (Finney, 1952) was obtained as the antilog. of M, which represented the ratio of the lengths of access feeding time which were associated with the same aphid mortality in the two treatments (e.g. $\frac{m_1}{m_2}$ ).

Feeding solutions

The feeding solutions were made initially with distilled water but preliminary tests suggested that using tap water as a solvent enhanced the survival of the aphids. The tap water, unlike the distilled water, was alkaline (see Auclair, 1965) and was used for all the experiments to be described. The feeding solutions contained 5% or 15% sucrose with or without 0.1% sinigrin (Koch-Light Laboratories, Colnbrook, Bucks.). Sinigrin was at first included in the solutions in attempting to improve the survival of B. brassicae (see p. 284 ), but was later included in feeding solutions for M. persicae to facilitate comparison between the two species of aphid.

Pressures

The pressures applied to the solutions were 1 or 2 atmospheres. Such low pressures minimised the problem of supporting the parafilm membranes. Slight modification of the apparatus, such as the use of a stronger spring, would have permitted the application of higher pressures to the diets if required. No leakage of the sucrose solutions occurred through the punctures in the parafilm left by aphids after feeding, possibly as a result of blocking by collapsed salivary sheaths.

Results

15% Sucrose solution (Preliminary experiments)

Feeding solutions containing 15% sucrose without sinigrin were used only for preliminary experiments in which a pressure of 1 atmosphere was applied to the diet (June 1965). There were no significant differences between the survival and larviposition of the aphids feeding on the pressurised and non-pressurised solutions (Table 19 ).

Table 19 Survival and larviposition of adult apterae and survival of larvae of M. persicae feeding on 15% sucrose soln. with and without pressure (36 replicates)

| Treatment | Mean length of survival of adult aphids (days) | Mean no. larvae born per adult | Mean length of life of larvae (days) |
|---|---|---|---|
| 1. Starvation | 2.22 ± 0.10 | 0.58 ± 0.16 | 0.82 ± 0.11 |
| 2. 15% sucrose soln. without pressure. | 4.00 ± 0.14 | 1.03 ± 0.20 | 0.92 ± 0.14 |
| 3. 15% sucrose soln. under 1 atm. pressure. | 3.94 ± 0.13 | 1.14 ± 0.20 | 1.76 ± 0.23 |

Since in these preliminary experiments the aphids failed to respond significantly to pressure applied to the diet, the pressure was doubled (to 2 atm.) and the concentration of the diet was reduced by a factor of 3 (to 5%). Mittler and Dadd (1963b) found that the survival of M. persicae on sucrose solutions increased with the concentration of the sucrose up to an optimum of about 20%. It seemed possible, therefore, that if the effect of pressure on the diet was to force diet up the stylet food canal of the aphid, maximum benefit to the aphid would accrue when the diet was at sub-optimal concentration for unpressurised feeding.

5% Sucrose solution

Series A

5% sucrose solution, without sinigrin, at a pressure of 2 atmospheres was made available to the aphids in a series of 4 experiments (Nov. 1965). The larviposition of the aphids

in each treatment is presented in Table 20 .

Table 20 Larviposition of adult apterous M. persicae feeding on 5% sucrose soln. with and without pressure. (48 replicates)

| Treatment | Mean no. larvae born per adult | Differences between means 1-2, 2-3 | | 1-3 | |
|---|---|---|---|---|---|
| 1. Starvation | 3.36 ± 0.43 | 2.98±0.72 | *** | 1.67±0.84 | * |
| 2. 5% sucrose soln. without pressure. | 0.38 ± 0.10 | 1.31±0.61 | * | | |
| 3. 5% sucrose soln. under 2 atm. pressure. | 1.69 ± 0.27 | | | | |

't' for 94 d.f. p.05=1.989 p.01=2.635 p.001=3.411.
Symbols as in Table 4 .

The starved aphids produced more progeny than the aphids feeding on 5% sucrose solutions, with or without pressure. However, of more importance to the present study was the finding that the aphids feeding on the sucrose solution under 2 atm. pressure produced more progeny than aphids feeding on the sucrose solution without pressure.

That the higher larviposition of the aphids on the pressurised diet was not akin to the higher larviposition of starved aphids is suggested by the survival of the aphids in the three treatments (Fig. 48). $\chi^2$ revealed no evidence against the parallelism of the regressions of probit percentage mortality on the access feeding time (log.days) for the three treatments. The equations of the regressions were:

$y_1 = 0.676 + 11.812x_1$

$y_2 = -0.538 + 11.812x_2$

$y_3 = -1.340 + 11.812x_3$

The regression coefficient was significant in a 't'-test ($p<0.001$). The log. LT 50's, relative lethal-time values and relative potencies of the three treatments are shown in Table 21.

Table21 Log.LT 50's, relative lethal-time values and relative potencies of 1)starvation, 2)5% sucrose soln. and 3) 5% sucrose soln. under 2 atm. pressure.

| | | | |
|---|---|---|---|
| Log.LT 50 (log.m) | log $m_1$ = 0.3660 | log$m_2$ = 0.4688 | log$m_3$=0.5367 |
| Antilogarithm.(days) | 2.322 | 2.943 | 3.441 |
| Relative lethal time value(log.m) | $M_{1,2}$ = 0.4688-0.3660 = | $0.1028 \pm 0.0179$ | *** |
| | $M_{1,3}$ = 0.5367-0.3660 = | $0.1707 \pm 0.0173$ | *** |
| | $M_{2,3}$ = 0.5367-0.4688 = | $0.0679 \pm 0.0164$ | *** |
| Relative potency (P) (days) | $P_{1,2}$ = 1.27 | | |
| | $P_{1,3}$ = 1.48 | | |
| | $P_{2,3}$ = 1.17 | | |

't' for $\infty$ d.f. p.05=1.96 p.01=2.58 p.001=3.29.
Symbols as in Table 4 .

The analyses showed i) that the aphids survived significantly longer when fed on 5% sucrose solutions than when starved, and ii) that the aphids survived significantly longer (1.17x) when fed on 5% sucrose solution under 2 atm. than when fed on 5% sucrose solution without pressure. The mean lengths of life of the larvae produced by the adults of each treatment

were 1) 0.88, 2)1.00 and 3) 1.23 days and there were no significant differences between them.

Series B

The experiments of series A were repeated but with the addition of sinigrin to the solution (Jan. 1966). The larviposition of the aphids in the three treatments is shown in Table 22 .

Table 22 Larviposition of adult apterous M. persicae feeding on 5% sucrose + 0.1% sinigrin soln. with and without pressure, (36 replicates).

| Treatment | Mean no. larvae born per adult |
|---|---|
| 1) Starvation | 0.25 ± 0.08 |
| 2) 5% sucrose + 0.1% sinigrin soln. without pressure. | 0.19 ± 0.07 |
| 3) 5% sucrose + 0.1% sinigrin soln. under 2 atm. pressure. | 0.47 ± 0.23 |

Differences between the larviposition of the aphids in the three treatments were not significant. Likewise, the regressions of probit percentage mortality against access feeding time (days) revealed no significant difference between the survival of the aphids on the pressurised and non-pressurised diets (Fig. 49 and Table 23 ). According to $\chi^2$ there was no evidence against the parallelism of the three regressions, whose equations were:

$$y_1 = 2.636 + 1.356x_1$$

$$y_2 = 1.905 + 1.356x_2$$

$$y_3 = 1.800 + 1.356x_3$$

The regression coefficient was significant in a 't'-test ($p<0.001$).

Table 23 LT 50's, relative lethal-time values and relative potencies of 1) starvation, 2) 5% sucrose + 0.1% sinigrin soln. and 3) 5% sucrose + 0.1% sinigrin soln. under 2 atm. pressure.

| | | |
|---|---|---|
| LT 50 (m) (days). | $m_1$ =1.7430 $m_2$=2.2818 $m_3$=2.3590 | |
| Relative lethal-time value (M) | $M_{1,2}$=2.2818-1.7430=0.5388±0.1528 | *** |
| | $M_{1,3}$=2.3590-1.7430=0.6160±0.1535 | *** |
| | $M_{2,3}$=2.3590-2.2818=0.0772±0.1507 | n.s. |
| Relative potency (P) (days) | $P_{1,2} = \frac{2.2818}{1.7430} = 1.31$ | |
| | $P_{1,3} = \frac{2.3590}{1.7430} = 1.35$ | |
| | $P_{2,3} = \frac{2.3590}{2.2818} = 1.03$ | |

't' for $\infty$ d.f. - see Table 21.
Symbols as in Table 4 .

The analyses showed that the aphids survived longer when fed on the solution than when starved, but that survival was not significantly altered by the application of 2 atmospheres to the solution. The mean lengths of life of the larvae born in each treatment were not significantly different: 1) 0.89, 2) 0.57 and 3) 0.94 days.

Series C

The experiments of series A were repeated with the reduction of the pressure applied to the diet from 2 to 1 atmosphere (Feb. 1966). The larviposition of the aphids in the three treatments is presented in Table 24.

Table 24 Larviposition of adult apterous M. persicae feeding on 5% sucrose soln. with and without pressure. (48 replicates).

| Treatment | Mean no. larvae born per adult |
|---|---|
| 1) Starvation | $0.10 \pm 0.04$ |
| 2) 5% sucrose soln. without pressure. | $0.19 \pm 0.10$ |
| 3) 5% sucrose soln. under 1 atm. pressure. | $0.23 \pm 0.10$ |

Differences between the larviposition of the aphids in the three treatments were not significant. The regressions of probit percentage mortality against the access feeding time (log.days) are shown in Fig. 50. The regression equations were:

$$y_1 = 3.581 + 6.775x_1$$

$$y_2 = 2.083 + 6.775x_2$$

$$y_3 = 2.150 + 6.775x_3$$

$\chi^2$ revealed no evidence against the parallelism of the three regression. The regression coefficient was significant in a 't'-test ($p<0.001$). Table 25 shows the Log LT 50's, relative lethal-time values and relative potencies of the three treatments.

FIGS. 48-50. REGRESSIONS OF PROBIT MORTALITY OF ADULT APTEROUS VIRGINOPARAE OF M.persicae ON THE TIME OF EXPOSURE TO PARAFILM MEMBRANES BEHIND WHICH WERE VARIOUS LIQUIDS AT CONTROLLED PRESSURES; COMPARED WITH A STARVATION 'CONTROL' TREATMENT(●).

FIG. 48. 5% SUCROSE SOLN. AT 0(○) AND 2(×) ATM. PRESSURE.

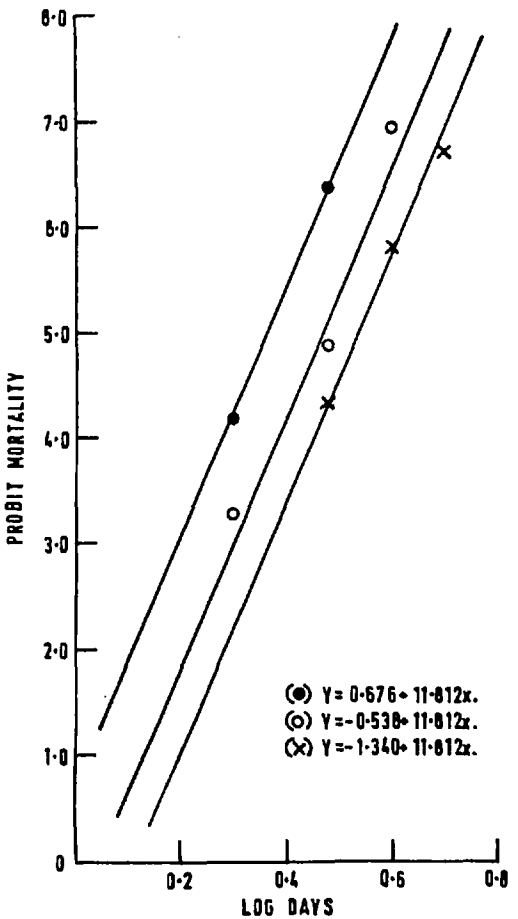



FIG. 49. 5% SUCROSE + 0·1% SINIGRIN SOLN. AT 0(○) AND 2(×) ATM. PRESSURE.

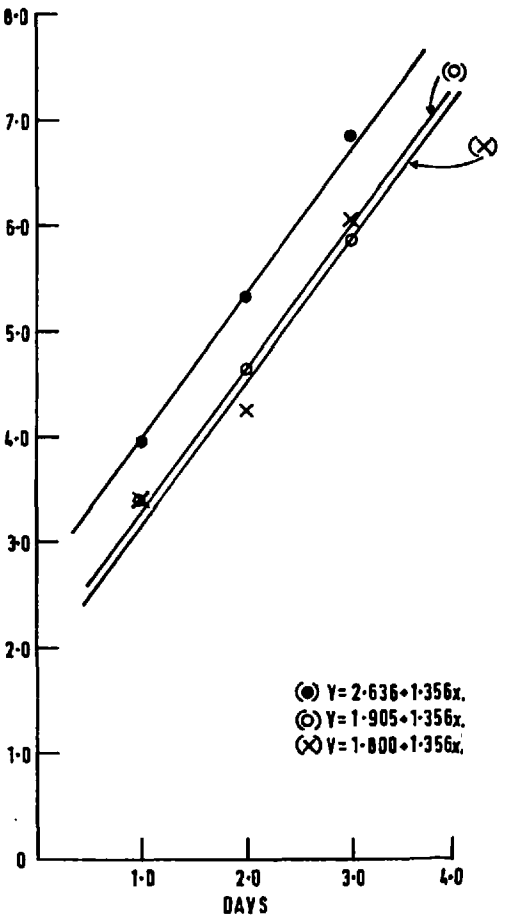



FIG. 50. 5% SUCROSE SOLN. AT 0(○) AND 1(×) ATM. PRESSURE.

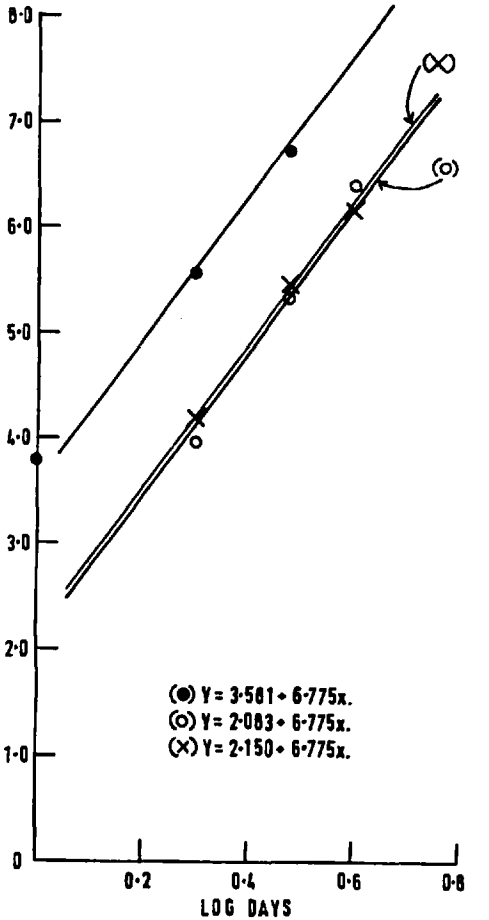


Table25 Log LT 50's, relative lethal-time values and relative potencies of 1) starvation, 2) 5% sucrose soln. and 3) 5% sucrose soln. under 1 atm. pressure

| | | |
|---|---|---|
| Log.LT 50 (logm) | $\log m_1 = 0.2095$ | $\log m_2 = 0.4305$ $\log m_3 = 0.4207$ |
| Antilogarithm (days) | 1.620 | 2.694 2.634 |
| Relative lethal-time value (logM) | $M_{12} = 0.4305 - 0.2095 = 0.2210 \pm 0.0276$ | *** |
| | $M_{13} = 0.4207 - 0.2095 = 0.2112 \pm 0.0275$ | *** |
| | $M_{23} = 0.4305 - 0.4207 = 0.0098 \pm 0.0249$ | n.s. |
| Relative potency (P) (days) | $P_{12} = 1.66$ | |
| | $P_{13} = 1.63$ | |
| | $P_{23} = 1.02$ | |

't' for $\infty$ d.f. - see Table 21 .
Symbols as in Table 4 .

The analyses revealed that the adult aphids survived longer when fed on the 5% sucrose solution than when starved. But the survival of the aphids feeding on the solution was not significantly altered by the application of 1 atmosphere pressure to the solution. There were no significant differences between the mean lengths of life of the larvae born in each treatment: 1) 0.80, 2) 0.89, and 3) 0.82 days.

## Conclusions

Adult apterous virginoparae of M. persicae survived longer and produced more progeny when feeding on 5% sucrose solution under a pressure of 2 atmospheres than when feeding on

the same solution without pressure. These differential responses of the aphids were not present if i) 0.1% sinigrin was added to the sucrose solution, or ii) the pressure was reduced to 1 atmosphere, or iii) the pressure was reduced to 1 atmosphere and the concentration of the sucrose solution was raised to 15%. These results are considered further in the Discussion (p. 307).

When compared with starved aphids, aphids feeding on 5% or 15% sucrose solutions survived longer but did not produce more progeny. Occasionally, starved aphids deposited more larvae than aphids feeding on sucrose solutions.

The lengths of life of the larvae produced by the adult aphids were similar in all treatments but the results of some experiments suggested that larval survival tended to increase when pressure was applied to the sucrose solutions.

## Sub-section 2 Brevicoryne brassicae

### a) The responses of apterae to host plants subjected to continuously increasing water strain over a short period

The responses of adult apterous B. brassicae to Brussels sprouts suffering continuously increasing water strain were studied as part of a joint experiment with M. persicae. The experiment is described on p.216 and the experimental procedure was the same for both species of aphid, an adult

aptera of each species being contained in each cage. From observations of the aphids and from the experimental results, no evidence was found that caging the two species together adversely affected either of them.

## Results

### Survival

The survival of the adult apterae of B. brassicae during the experiment is shown in Fig. 51. Although not statistically analysed (see p.220 ), the survival of the adult apterae was clearly reduced on the old leaves of the plants in the wilt water treatment and on all leaf ages of the plants of the severe wilt water treatment compared with the turgid plants. On the latter, only one aphid failed to survive the twelve days of the experiment.

### Restlessness

The restlessness of the apterae is apparent from Figure 52 and Table 26 . Fig. 52 shows the mean percentage of occasions when the apterae of each treatment were not feeding during daily observations (see p.217 ) and Table 26 shows the treatment means of the transformed data and the least significant differences between them.

On each leaf age of the plants, the restlessness of the aphids increased from the turgid to the wilt to the severe wilt water treatments. The magnitude of this effect was maximal among aphids on the old leaves (e.g. turgid to wilt, $p<0.001$) and decreased towards the apex of the plant, where, on

FIG. 51. NUMBERS OF SURVIVING ADULT APTEROUS VIRGINOPARAE OF B.brassicae PLOTTED AGAINST TIME IN DAYS SINCE MOULTING TO ADULT FOR EACH OF NINE TREATMENTS: SEE FIG.37.



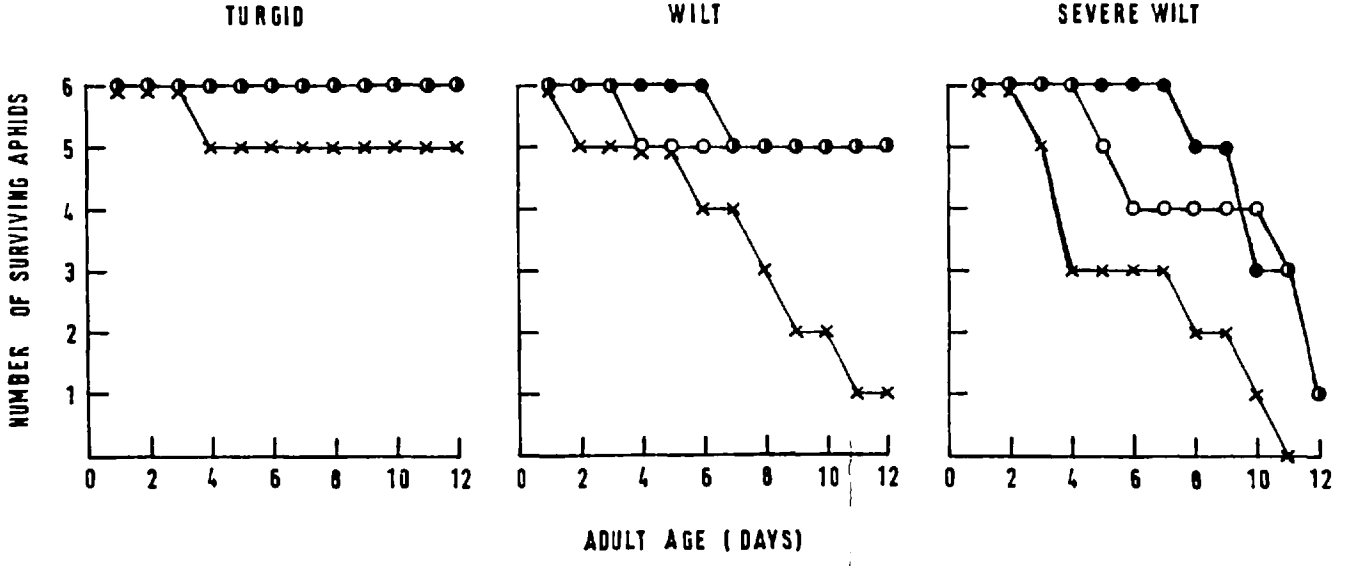


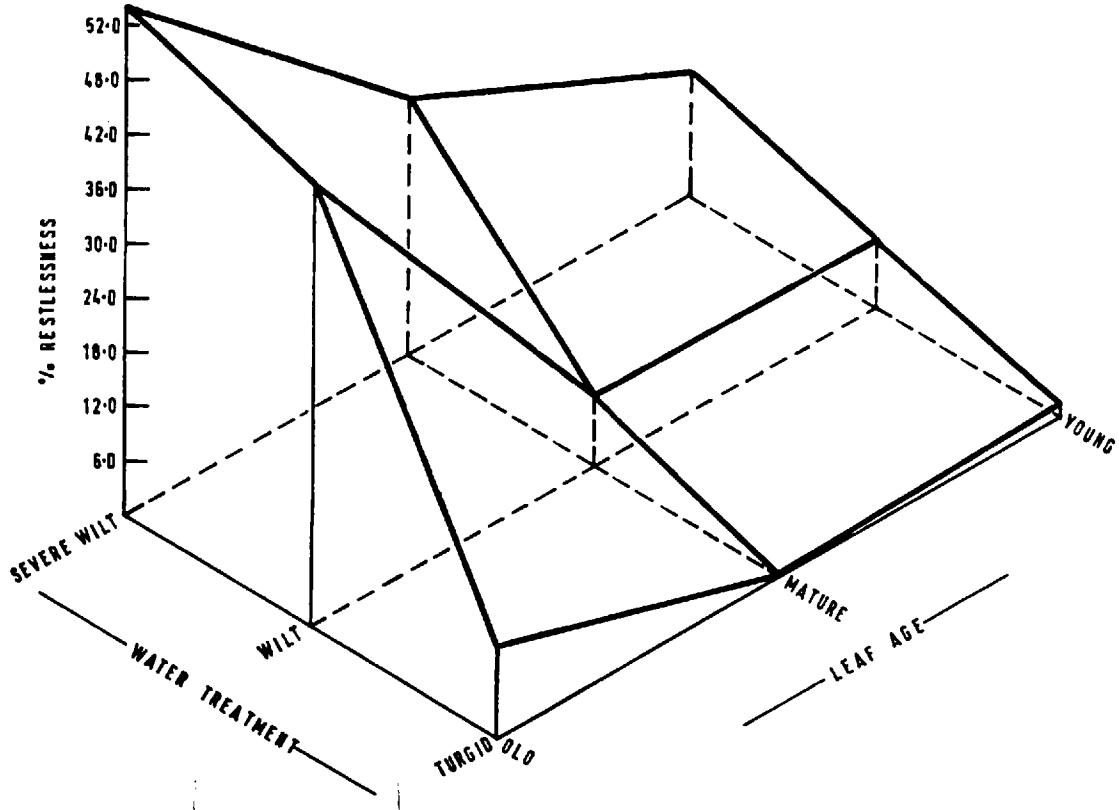
Table 26 . Mean restlessness of adult apterous virginoparae of B. brassicae on 3 leaf ages of Brussels sprouts in 3 water treatments at 20°C. (For explanation of restlessness - see text).

| | | Mean percentage restlessness (transformed to degrees) | | | |
|---|---|---|---|---|---|
| | | Water Treatment | | | |
| | | TURGID | WILT | SEVERE WILT | L.S.D.(W) |
| Leaf age | O | 24.82(17.6)* | 45.10(50.1) | 50.83(60.0) | p.05=8.46 |
| | | | | | .01=11.59 |
| | M | 15.50(7.2) | 24.73(17.4) | 35.18(33.1) | .001=15.85 |
| | Y | 16.62(8.2) | 22.35(14.5) | 25.90(19.1) | |
| L.S.D. (L) | | | p.05 = 7.48 | | |
| | | | .01 =10.04 | | |
| | | | .001 =13.26 | | |

* In parenthesis - detransformed percentages.
Lettering as in Table 6 .

FIG. 52. MEAN RESTLESSNESS OF ADULT APTEROUS VIRGINOPARAE OF B.brassicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER TREATMENTS AT 20°C.

FOR AN EXPLANATION OF RESTLESSNESS—SEE TEXT. SEE TABLE 26 FOR SIGNIFICANT DIFFERENCES.



the young leaves, the restlessness of the aphids was significantly increased only from the turgid to the severe wilt water treatment ($p<0.05$). In addition, even on the turgid plants (cf. M. persicae), the apterae on the old leaves were more restless than apterae on the young and mature leaves, so that, in combination with the above effects of water strain, on the plants of the severe wilt water treatment, the restlessness of the aphids increased from the young to the mature ($p<0.05$) to the old ($p<0.01$) leaves.

Larviposition

Total progeny and reproductive rate

Table 27 shows the mean total progeny and mean reproductive rate of the apterae of each treatment and the least significant differences between them. Since survival of the aphids was generally reduced in those treatments which also lowered the reproductive rate of the aphids, the two measures of larviposition paralleled one another in the different treatments.

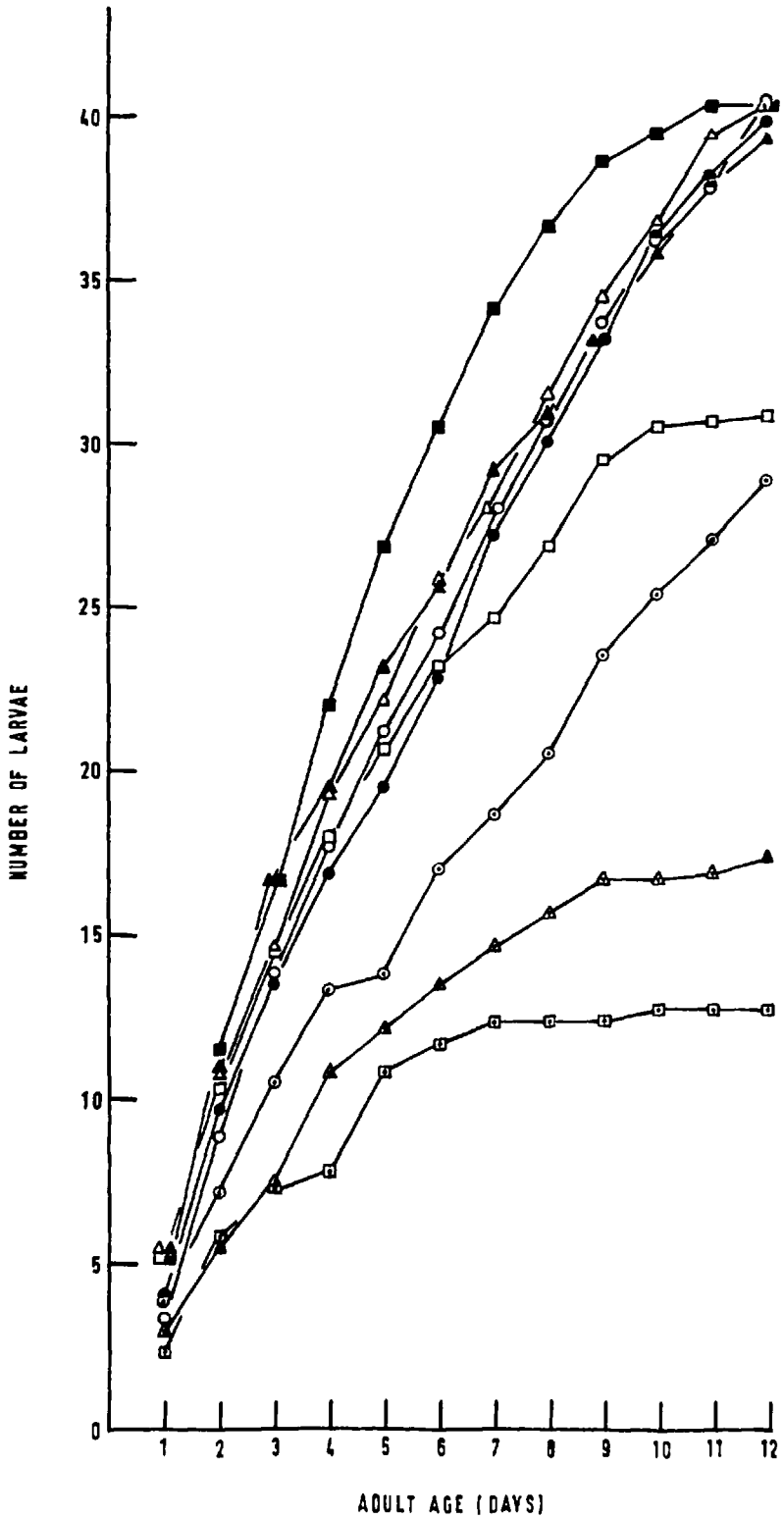
There were no significant differences within and between the larviposition of the aphids on the young and mature leaves of the plants in the different water treatments. Apterae on the old leaves of the plants produced consistently fewer progeny more slowly than the apterae on the other leaf ages. This was most marked on the plants of the severe wilt water treatment, since the larviposition of the apterae on the old leaves was reduced on these plants compared with the turgid plants.

Table 27 . Mean total progeny and mean reproductive rate of adult, apterous virginoparae of B. brassicae during the first twelve days of adult life on 3 leaf ages of Brussels sprouts in 3 water treatments at 20°C

| | | Total Progeny | | | | Reproductive Rate (larvae/day) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Water Treatment | | | | Water Treatment | | | |
| | | | | SEVERE | | | | SEVERE | |
| | | TURGID | WILT | WILT | L.S.D.(W) | TURGID | WILT | WILT | L.S.D.(W) |
| Leaf age | O | 28.83 | 17.33 | 12.67 | p.05=15.28<br>.01=20.94 | 2.57 | 1.75 | 1.54 | p.05=1.12 |
| | M | 40.50 | 40.33 | 30.83 | | 3.38 | 3.78 | 3.19 | |
| | Y | 39.83 | 39.33 | 40.33 | | 3.32 | 3.58 | 3.90 | |
| L.S.D. (L) | | | p.05 = 14.01<br>.01 = 18.83<br>.001 = 24.88 | | | | p.05 = 1.15<br>.01 = 1.55<br>.001 = 2.05 | | |

Lettering as in Table 6 .

FIG. 53. ACCUMULATED DAILY MEAN NUMBER OF LARVAE PRODUCED BY APTEROUS VIRGINOPARAE OF B.brassicae ON 3 LEAF AGES OF BRUSSELS SPROUTS IN 3 WATER TREATMENTS AT 20°C. KEY AS IN FIG. 39.



These effects of the treatments on the larviposition of the aphids are shown in Fig. 53 which depicts the accumulated mean daily larviposition of the six apterae of each treatment. In calculating the mean daily larviposition, dead aphids were treated as live aphids failing to produce larvae (see p. 219). Figure 53 shows other important features of the larviposition of the aphids not apparent from the analyses. Larviposition was reduced particularly towards the end of the experiment in a number of treatments, as 1) the available water in the growing medium of the plants and 2) the influence on larviposition of the pre-experimental feeding of the aphids reached a minimum, and 3) as the aphids aged. Two of these factors (1 and 3) are taken into account in the multiple regression analyses of the results (see below). An interesting feature of Fig. 53 is the early high rate of reproduction of the apterae on the young leaves of the plants in the severe wilt water treatment (see p310)

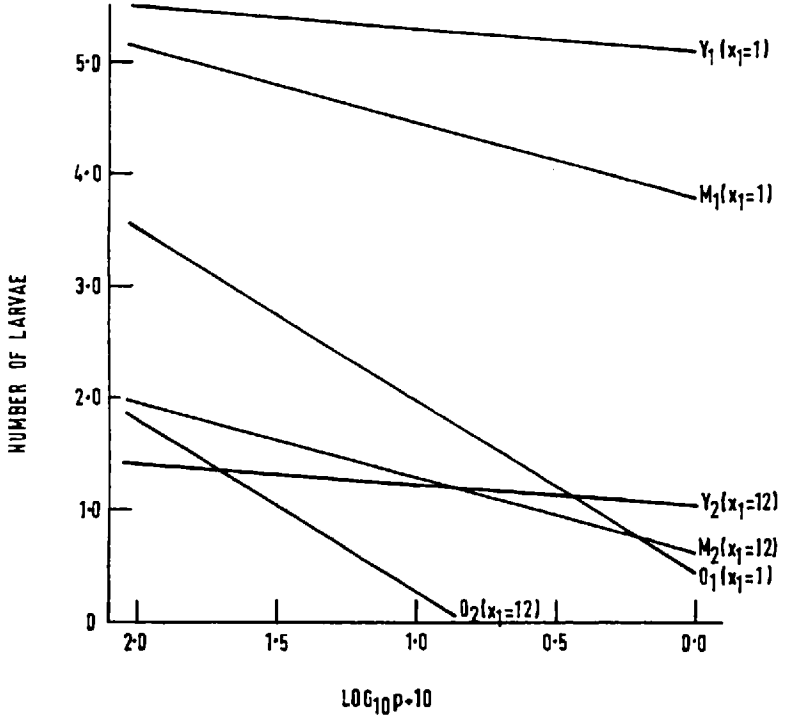
The multiple regression of daily larviposition on the age of the adult aptera and the percentage available water (p) in the growing medium of the host plant

Fig. 54a shows the linear regressions of the mean daily larviposition of apterous B. brassicae of a given age on the mean percentage available water (p) in the growing medium of the host plant, expressed as $\log_{10}p+10$ ($x_2$). The Figure shows separate regressions for the apterae, after one and twelve days of adult life ($x_1$), caged on the young, mature and old leaves of the plants. The same regressions (general equation, $y = a + b_1x_1 + b_2x_2$) are presented in Fig. 54b, in which $x_2$

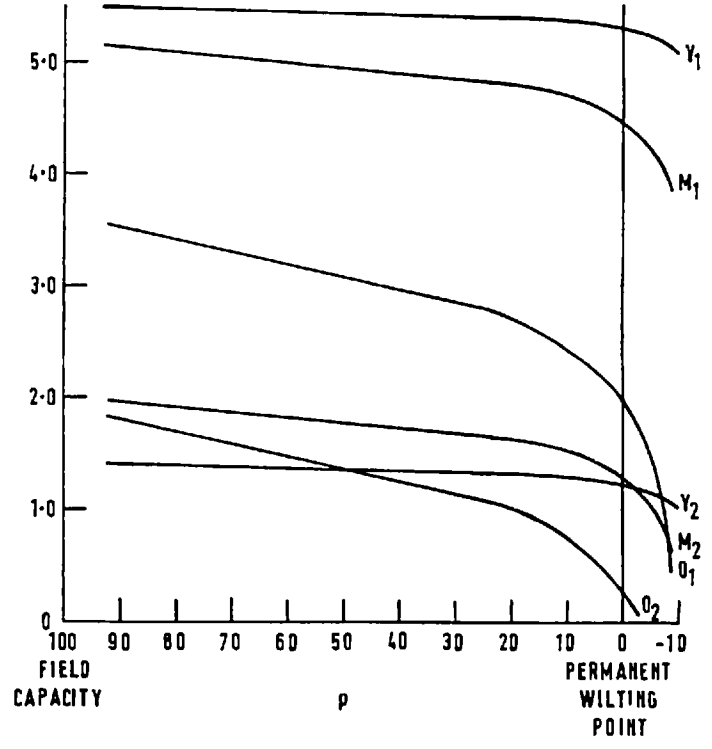
FIG. 54 MULTIPLE REGRESSIONS OF MEAN DAILY NUMBER OF LARVAE PRODUCED BY APTEROUS VIRGINOPARAE OF B.brassicae ON THE ADULT AGE OF THE APHID IN DAYS ($x_1$) AND ON THE PERCENTAGE AVAILABLE WATER (p) IN THE GROWING MEDIUM OF THE HOST PLANT (BRUSSELS SPROUT) EXPRESSED AS $LOG_{10}p+10$; SEPARATE REGRESSIONS FOR APHIDS CAGED ON THREE LEAF AGES: YOUNG (Y), MATURE (M) AND OLD (O).

A. $LOG_{10}p+10$.

Y. $y = 5{\cdot}48 - 0{\cdot}370x_1 + 0{\cdot}188x_2$
M. $y = 4{\cdot}07 - 0{\cdot}288x_1 + 0{\cdot}674x_2$
O. $y = 0{\cdot}60 - 0{\cdot}156x_1 + 1{\cdot}549x_2$




B. PERCENTAGE AVAILABLE WATER (p).



has been de-transformed to p. Table 28 shows the regression coefficients and their standard errors.

Table 28 Estimated regression coefficients and their standard errors

| Apterae on: | $b_1$ | $b_2$ |
|---|---|---|
| Young Leaves | $-0.370 \pm 0.044$ | $+0.188 \pm 0.249$ |
| Mature Leaves | $-0.288 \pm 0.038$ | $+0.674 \pm 0.214$ |
| Old Leaves | $-0.156 \pm 0.025$ | $+1.539 \pm 0.141$ |

The daily larviposition of the apterae decreased with their age. 'T'-tests of the regression coefficients ($b_1$'s) for each leaf age were significant ($p<0.001$) while differences between the $b_1$'s indicated that the decline of larviposition was more rapid on the young and mature leaves than on the old leaves of the plants ($p<0.05$). The larviposition of the aphids recorded in this experiment cannot, therefore, be equated with their fecundity (see p. 183). Moreover, all the apterae were reared to adult on the young leaves of Brussels sprouts and the change to the old leaves as a feeding site for some of the aphids may have adversely affected their larviposition, though this was not found with M. persicae (see Fig. 40 ).

Decreasing available water in the growing medium of the host plant was consistently associated with decreasing daily larviposition by the apterae on each leaf age. 'T'-tests of the regression coefficients ($b_2$'s) were significant for the aphids on

the mature ($p<0.01$) and old ($p<0.001$) leaves but not for aphids on the young leaves of the plants. Differences between the $b_2$'s showed that the rate of decline of larviposition with the availability of water to the host plant increased from the apex to the base of the plant, notably from the young and mature to the old leaves ($p<0.001$ and $p<0.01$, respectively.) Fig. 54b shows the logarithmic fall of daily larviposition with the percentage available water in the growing medium. In the upper part of the available water range, larviposition decreased steadily. But approaching the permanent wilting percentage the decrease of larviposition accelerated, particularly for apterae on the old leaves of the plants, (cf. M. persicae).

Conclusions

A short period of continuously increasing water strain in the host plants decreased the survival and increased the restlessness of adult apterae of B. brassicae. The more severe was the water strain, the greater were these effects, which reached a maximum among the aphids on the old leaves and decreased towards the apex of the plant. In addition, even on turgid plants, the restlessness of the apterae was greater on the old leaves than on the other leaf ages.

The mean reproductive rate (throughout the experiment) and the total progeny of the apterae of each treatment paralleled their restlessness and survival only on the old leaves, where fewer progeny were produced more slowly than on other leaf ages, particularly on the plants of the wilt and

severe wilt treatments. However, a detailed analysis of the changes in daily larviposition with the age of the aphids and the availability of water to the host plant revealed a greater parallelism between the larviposition, restlessness and survival responses of the aphids. For aphids of a given age, larviposition on the mature and old leaves fell linearly with the logarithm of the percentage available water in the growing medium of the host plant. The rate of this fall increased with the age of the leaves on which the apterae were caged. These logarithmic regressions represented essentially a slow, steady decline of larviposition with decreasing available water until approaching permanent wilt when the rate of decline increased rapidly, especially for aphids on the old leaves of the plants. On leaves of all ages daily larviposition of the apterae decreased with their age during the first twelve days of adult life. The rate of this decrease was lower for aphids on the old leaves than for those on the young and mature leaves of the plants.

b) <u>The method of penetration and the destination of the stylets of apterae in the tissues of the host plant</u>

Some general consideration has already been given to the methods of penetration and the destinations of the stylets of aphids in their host plants (p.231 and Review of the Literature, p. 31 ).

With reference to <u>B. brassicae</u>, little is known of the disposition of the stylets of this aphid in its host plants.

Day and Irzikiewicz (1954) reported, but did not describe, differences between the salivary sheaths of M. persicae and B. brassicae on Chinese cabbage. A study was, therefore, undertaken of the penetration and destination of the stylets of feeding B. brassicae in the leaves of Brussels sprouts.

## Experimental Procedure

Apterous virginoparae of B. brassicae feeding on mature leaves of Brussels sprouts (cv. "Jade Cross") were fixed, sectioned and stained by the methods described on p.205 . Adult and late-instar aphids only were used. The locations in the host tissues of the stylets and salivary sheaths of 9 aphids were analysed. Further experimental procedure was similar to that described for M. persicae.

Fewer B. brassicae were sectioned than M. persicae because the former were studied first and individual aphids were sectioned at that time. It was later found with M. persicae that a quicker and more comprehensive study could be made by fixing and sectioning groups of feeding aphids.

## Results

### Location of the feeding aphids on the leaf

All the sectioned aphids were situated on the abaxial surface of the leaf where they had inserted their stylets into leaf veins. The aphids varied in size insufficiently to establish any correlation between the length of the stylets of the aphids and the thickness of the vein at which they were

found (see M. persicae). Nevertheless, the aphids, which were adults and late-instars, occurred almost exclusively at prominent veins.

The vascular bundle of the leaf vein of Brussels sprout

The vascular bundle of a leaf vein of Brussels sprout (cultivar 'Irish Elegance') was briefly described on p.233 . The typical leaf vein of 'Jade Cross' (Fig. 55) differed only in being broader in transverse section and in having a cortex of more straight-walled cells and an epidermis of larger cells than did the leaf vein of 'Irish Elegance'.

Stylet dimensions and the amount of stylet insertion

The total length of the stylets of the aphids varied from 242 to 341 microns (mean of 9 aphids = 286.8 microns). The aphids inserted into the leaf from 110 to 275 microns of their stylets (mean of 9 aphids = 205.0 microns) depending on the size of the leaf vein and aphid, the instar of the aphid, and the points of insertion and destination of the stylets. The proportion of the stylets which was inserted thus varied from 46% for a 4th instar nymph on a vein 220 microns thick (adaxial to abaxial epidermis), to 85% for a 4th instar nymph on a vein 668 microns thick. The mean percentage for all the aphids was 71%, reflecting the greater thickness of the veins here sectioned (mean thickness = 402.9 microns) when compared with those previously sectioned with feeding M. persicae (p. 235).

In the leaf vein of 'Jade Cross' the phloem was usually closer to (but more cells away from) the adaxial surface than the abaxial surface, where the aphids were normally found (cf. 'Irish Elegance').

The diameter of the stylet bundle varied from 3 - 4 microns in the epidermis to less than 1 micron at the stylet tips.

The destination of the stylets

The phloem was the destination and presumably the site of feeding of all the stylets studied (Fig. 55, 57). Many salivary sheaths left by other aphids also terminated in the phloem. Within the phloem the tips of the stylets varied in their destination as shown in Table 29 .

Table 29 Destination of stylets of B. brassicae in the phloem of Brussels sprout leaves

| Type of phloem cell | No. of stylets | % of stylets | No. of stylets with tips intercellular |
|---|---|---|---|
| Sclerenchyma | 1 | 11 | - |
| Parenchyma | - | - | - |
| Companion cell | - | - | - |
| Sieve tube | 5 | 56 | - |
| Indefinite | 3 | 33 | 2 |
| Total | 9 | | |

Five of the six stylet bundles (83%), whose destination within a cell could be clearly seen, ended in the sieve tubes (Fig. 56, 57, 58). The tips of the stylets were just behind, just beyond or level with the ends of the salivary sheaths, which also appeared to enter the sieve tubes (cf. M. persicae).

Stylet penetration

1) Epidermis

Stylet penetration of the epidermis was usually intercellular (67%) (Fig. 55), but intracellular penetration did occur (33%). The mean length of the stylets in the epidermis was 12.5 microns, which was 6% of the mean total inserted length of the stylets.

2) Cortex

The stylets penetrated the cortex both inter- and intra-cellularly, though predominantly by the latter course (Fig. 55). Sometimes penetration was wholly intracellular (22%), while at other times penetration was equally inter- and intracellular (33%). The stylets within the cells often appeared to be guided by the cell walls (Fig. 55) (cf. Entwistle and Longworth, 1963).

The length of the stylets in the cortex varied from 77 to 183 microns with a mean for the 9 aphids of 142.5 microns. This mean length was 70% of the mean total inserted length of the stylets.

3) Phloem sclerenchyma

Stylet penetration of the phloem sclerenchyma was commonly (67%) a mixture of inter- and intra-cellular (Fig. 55) but occasionally was exclusively one or the other. The mean length of the stylets in the sclerenchyma was 42.3 microns (range, 12 - 110 microns), which was 19% of the mean total inserted length of the stylets.

4) Phloem

For 89% of the stylets, penetration of the phloem was

wholly intracellular (Fig. 56). The tips of the stylets normally terminated within a sieve tube (see Table 29 ). The length of the stylets in the phloem ranged from 3 to 16 microns with a mean insertion length of 7.8 microns. This was 4% of the mean total inserted length of the stylets and represented a depth of penetration of the phloem of 2-3 cells. Stylet penetration thus generally terminated abruptly when the stylets reached the phloem.

5) Stylet 'excess' during penetration

Comparison of the inserted lengths of the stylets with the shortest distances from their points of insertion to their destinations in the leaves showed that the former exceeded the latter by 11% (mean of 9 stylets; range, 3-22%). This consistently "small excess" may be attributed, in part, to the limited intercellular penetration by the stylets (cf. M. persicae).

By measuring the shortest distances from the points of insertion of the stylets to sieve tubes in the phloem and comparing these distances with the lengths of the inserted stylets, the latter was found to exceed the former by 19%. (mean of 9 stylets; range, 5-29%). The aphids thus reached their preferred site of feeding, the sieve tubes (Table 29 ), with consistently few diversions (cf. M. persicae).

The salivary sheath

A salivary sheath normally surrounded the stylet bundle from the point of insertion in the epidermis to the tip (see above). The sheath was prominent within cells and in large

intercellular air spaces (Fig. 55). Branching of the sheath occurred at all stages during penetration, notably where the aphid appeared to have inserted the stylets on one course, found this course unsuitable, withdrawn slightly, and re-inserted the stylets in another direction (Figs. 55, 56). Branched sheaths sometimes occurred near the tips of the stylets in the phloem, where the aphids may have fed in turn in a number of sieve tubes.

It was difficult to assess whether the aphids had withdrawn their stylets during killing. The tips of the stylets were always very close to the end of the salivary sheaths. This may have been due to slight consistent stylet withdrawal (cf. M. persicae) or may represent the normal feeding position of the stylets. Further analysis of the problem is required.

Conclusions

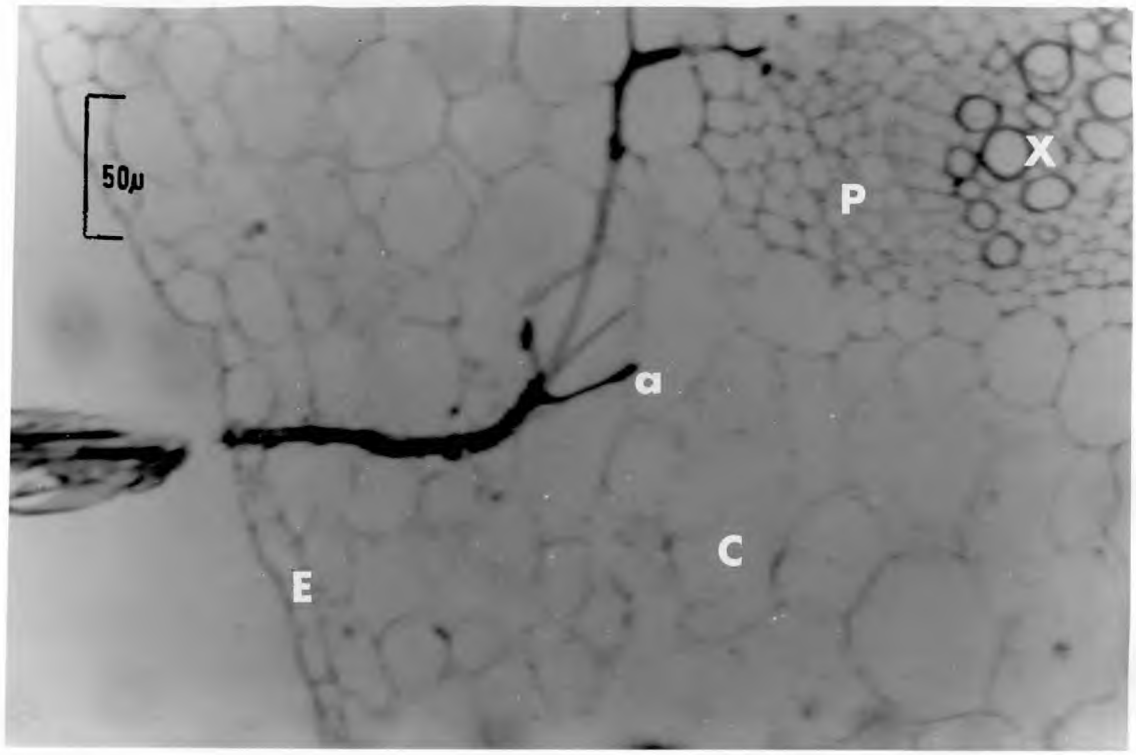
Apterous virginoparae of B. brassicae feeding on Brussels sprout leaves inserted their stylets into the leaves both inter- and intra- cellularly, but predominantly by the latter method. For this reason, the stylets were normally inserted on a straight, rather than a sinuous, course.

All the stylets terminated in the phloem, suggesting that this tissue was the sole source of plant sap for the aphids Within the phloem, the stylets ended almost always in the sieve tubes which would, therefore, seem to be the main source of sap for the aphids.

Both the tips of the stylets and the end of the salivary sheath apparently entered the sieve tube. No evidence was found that the mandibular and maxillary stylets differed in their penetration of the sieve tubes. Branching of the salivary sheaths in the phloem suggested that the aphids may feed in a succession of sieve tubes. Branching of the salivary sheaths in the cortex and other non-vascular tissues appeared to represent unsuccessful penetration followed by slight withdrawal and re-insertion of the stylets in a new direction.

Fig. 55. Transverse section of a leaf vein of Brussels sprout (cv. "Jade Cross") penetrated by the stylets of B. brassicae. a = branch of the salivary sheath, C = cortex, E = epidermis, P = phloem, X = xylem.

Fig. 56. Destination of the stylets of B. brassicae in a leaf vein of Brussels sprout (transverse section). Arrow indicates the tip of the stylets; a = branch of the salivary sheath, c = sclerenchyma, t = sieve tube, v = xylem vessel.


50µ
X
P
a
C
E

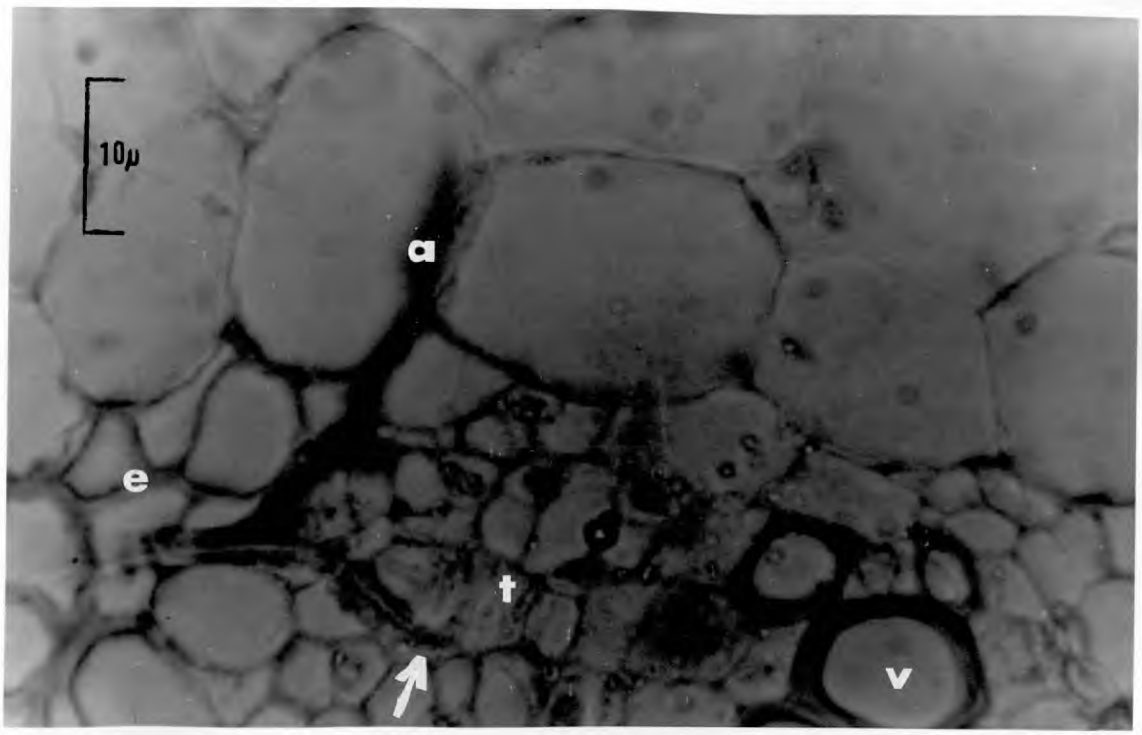

10µ
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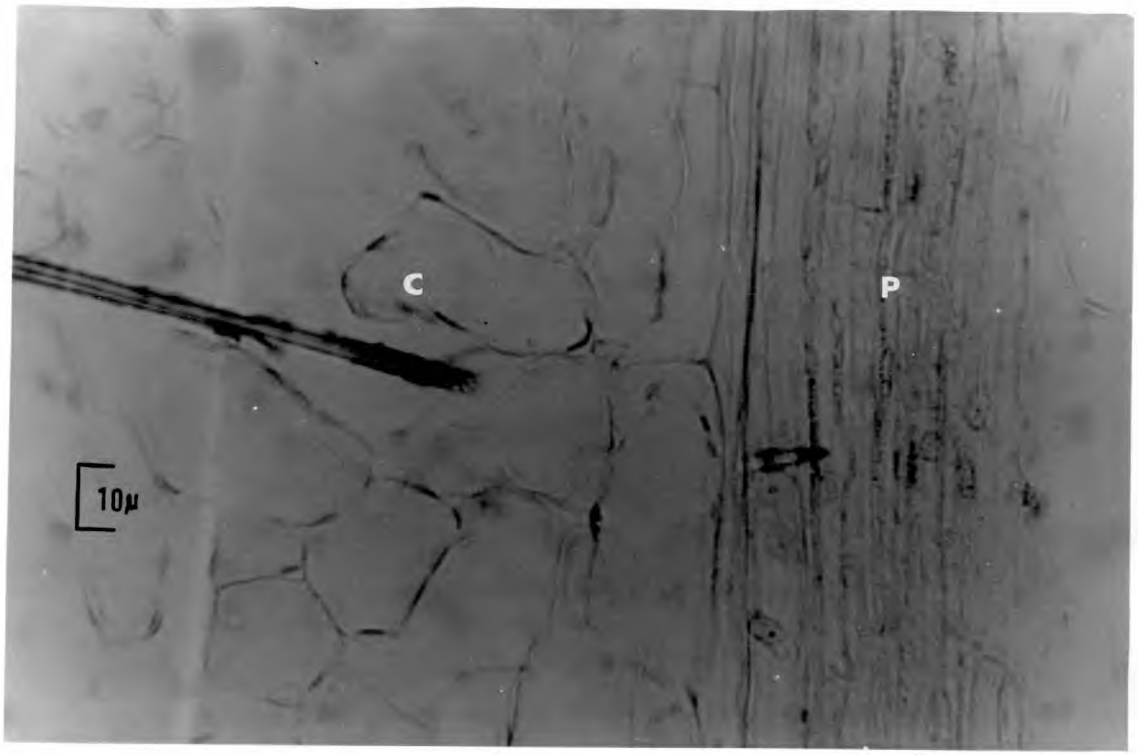
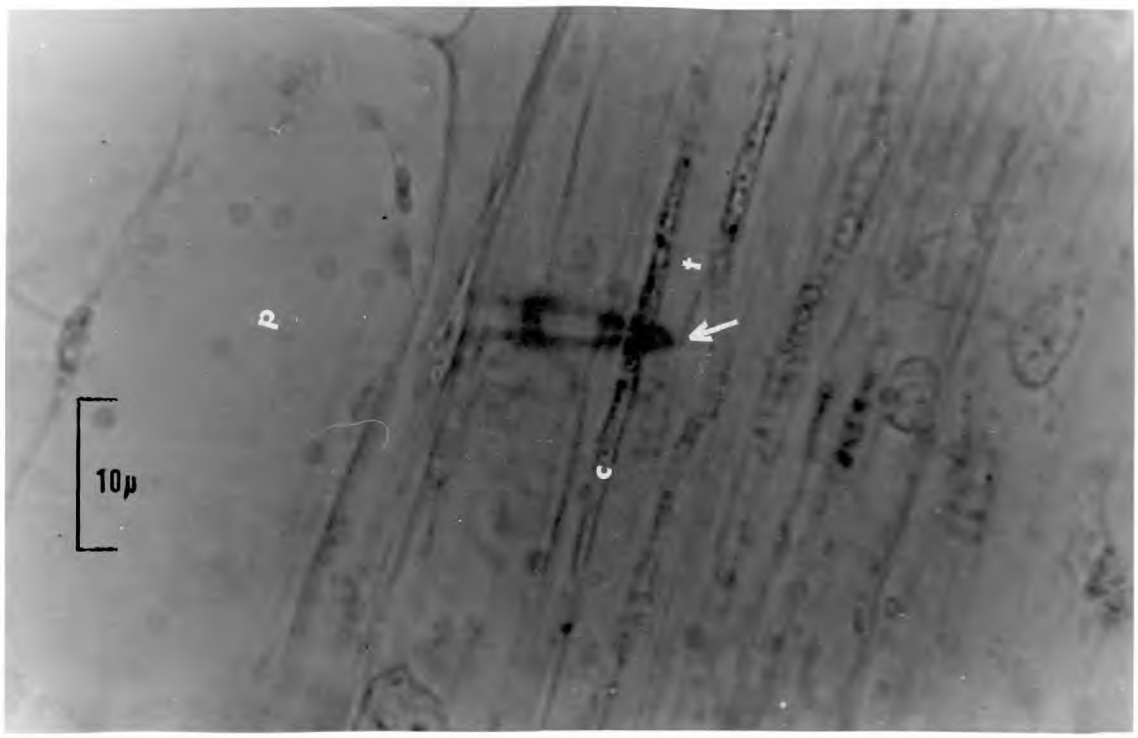
Fig. 57. Penetration and destination of the stylets of B. brassicae in a leaf vein of Brussels sprout (longitudinal section). C = cortex, P = phloem.

Fig. 58. Destination of the stylets of B. brassicae - see Fig. 57. Arrow indicates the tip of the stylets; c = companion cell, t = sieve tube, p = parenchyma.


C
P
10μ


P
C
10μ

c) The feeding of apterae on sucrose solutions under controlled pressures

In view of the probable importance of the turgor pressure of the host plant in the uptake of plant sap by feeding aphids (see p. 248 and Review of the Literature), experiments were undertaken to investigate the responses of B. brassicae to pressure applied to sucrose solutions available to the aphids feeding through parafilm membranes. Changes of turgor pressure accompany changes in the water status of plants, such as those used in the present investigation, (p. 87 and p. 202 ).

Experimental Procedure

The experimental procedure with B. brassicae was similar to that described for M. persicae (p. 249 ) (see also p. 208 ).

Results

15% Sucrose solution (Preliminary experiments)

In a series of preliminary experiments, 15% sucrose solution, without sinigrin, at a pressure of 1 and 0 atmospheres was made available to the aphids (Aug. 1965). The larviposition and survival of the adult aphids and the survival of the larvae are shown in Table 30.

Table 30 Survival and larviposition of adult apterae and survival of larvae of B. brassicae feeding on 15% sucrose soln. with and without pressure (36 replicates).

| Treatment | Mean length of survival of adult aphids (days) | Mean no. of larvae born per adult | Mean length of life of larvae (days) |
|---|---|---|---|
| 1) Starvation. | 2.04 ± 0.09 | 2.71 ± 0.36 | 0.88 ± 0.12 |
| 2) 15% sucrose soln. without pressure. | 4.83 ± 0.19 | 0.41 ± 0.13 | 1.36 ± 0.20 |
| 3) 15% sucrose soln. under 1 atm. pressure. | 4.92 ± 0.21 | 0.41 ± 0.12 | 1.27 ± 0.19 |

The aphids feeding on the sucrose solution produced fewer progeny ($p<0.01$) and survived longer ($p<0.001$) than the starved aphids. There were no significant differences between the lengths of life of the larvae of different treatments. The adult aphids responded similarly to the pressurised and non-pressurised sucrose solution.

The lack of response of the aphids to the 1 atmosphere pressure applied to the diet prompted an increase of the pressure and a decrease of the concentration of the sucrose for subsequent experiments (see M. persicae p. 253 ).

5% Sucrose solution

Series A

Aphids were given access to 5% sucrose solution at pressures of 0 and 2 atmospheres (Oct. 1965). The mean numbers of larvae produced by the aphids of each treatment (48 replicates) were:

| Treatment | |
|---|---|
| 1) Starvation | 0.52 |
| 2) 5% Sucrose soln. without pressure. | 0.00 |
| 3) 5% Sucrose soln. under 2 atm. pressure. | 0.00 |

and there were no significant differences between them.

The regressions of probit percentage mortality on the access feeding time (log. days) for the aphids of the three treatments are presented in Fig. 59. The regression equations were:

$$y_1 = 2.867 + 7.966x_1$$
$$y_2 = 1.925 + 7.966x_2$$
$$y_3 = 1.912 + 7.966x_3$$

A 't'-test of the regression coefficient was significant ($p<0.001$). $x^2$ revealed no evidence against the parallelism of the regressions. The log LT 50's, relative lethal-time values and relative potencies of the three treatments are shown in Table 31.

Table 31 Log LT 50's, relative lethal-time values and relative potencies of 1) starvation, 2) 5% sucrose soln. and 3) 5% sucrose soln. under 2 atm. pressure (48 replicates).

| | | |
|---|---|---|
| Log LT 50 (logm) | $\log m_1 = 0.2678$ | $\log m_2 = 0.3681$ $\log m_3 = 0.3877$ |
| Antilogarithm (days) | 1.853 | 2.433 2.442 |
| Relative lethal-time value (logM) | $M_{1,2} = 0.3861 - 0.2678 = 0.1183 \pm 0.0254$ | *** |
| | $M_{1,3} = 0.3877 - 0.2678 = 0.1199 \pm 0.0254$ | *** |
| | $M_{2,3} = 0.3877 - 0.3861 = 0.0016 \pm 0.0234$ | n.s. |
| Relative potency (P) (days) | $P_{1,2} = 1.31$ | |
| | $P_{1,3} = 1.32$ | |
| | $P_{2,3} = 1.00$ | |

t for $\infty$ d.f. - see Table 21.
Symbols as in Table 4 .

The aphids feeding on the sucrose solution survived longer than the starved aphids. The survival of the aphids feeding on the sucrose solution was not significantly altered by the application of a pressure of 2 atmospheres to the diet (cf. M. persicae).

Series B

The experiments of series A were repeated with the addition of 0.1% sinigrin to the sucrose solution (Dec. 1965). Sinigrin was added to the diet in an attempt to stimulate the feeding of B. brassicae (see Wensler, 1962) and hence improve the survival of the aphids. The larviposition of the aphids in the three treatments is presented in Table 32.

Table 32 larviposition of adult apterous B. brassicae feeding on 5% sucrose + 0.1% sinigrin soln. with and without pressure (72 replicates).

| Treatment | Mean no. larvae per adult |
|---|---|
| 1) Starvation. | $0.93 \pm 0.19$ |
| 2) 5% sucrose + 0.1% sinigrin soln. without pressure. | $0.03 \pm 0.02$ |
| 3) 5% sucrose + 0.1% sinigrin soln. under 2 atm. pressure. | $0.08 \pm 0.04$ |

The difference between the larviposition of the aphids on the sucrose + sinigrin solution with and without pressure was not significant. However, the regressions of probit percentage mortality on access feeding time (log.days) revealed differences between the survival of the aphids feeding on the pressurised and non-pressurised solutions, (see Fig. 60). The regression equations were:

$$y_1 = 2.039 + 9.230x_1$$
$$y_2 = 1.047 + 9.230x_2$$
$$y_3 = 0.628 + 9.230x_3$$

and $\chi^2$ revealed no evidence against the parallelism of the three regressions. The regression coefficient was significant in a 't'-test ($p<0.001$). Table 33 shows the log. LT 50's, relative lethal-time values and relative potencies of the three treatments.

FIGS. 59-60. REGRESSIONS OF PROBIT MORTALITY OF ADULT APTEROUS VIRGINOPARAE OF B.brassicae ON THE TIME OF EXPOSURE TO PARAFILM MEMBRANES BEHIND WHICH WERE VARIOUS LIQUIDS AT CONTROLLED PRESSURES; COMPARED WITH A STARVATION 'CONTROL' TREATMENT (●).

FIG. 59. 5% SUCROSE SOLN. AT 0 (○) AND 2 (×) ATM. PRESSURE.

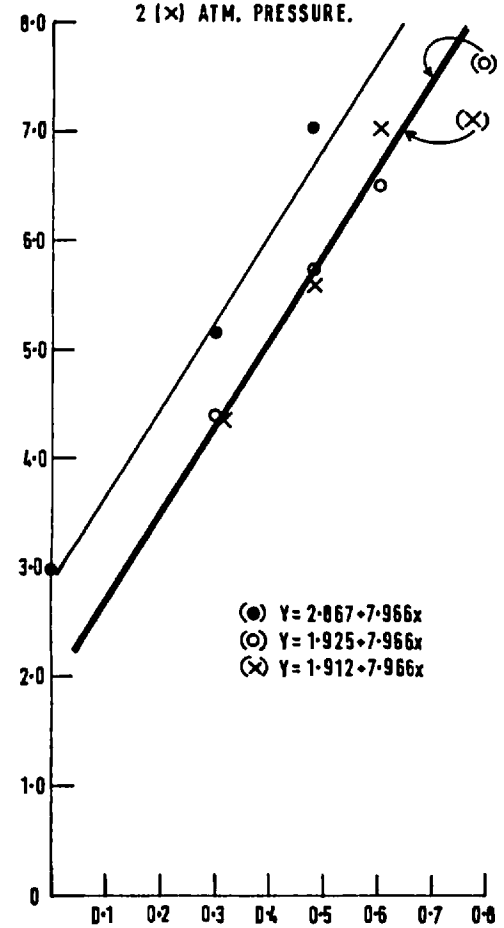
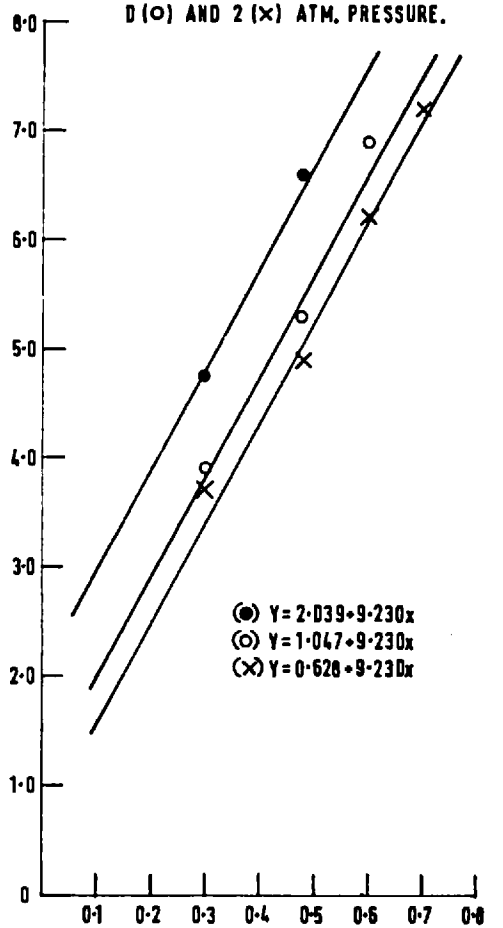



FIG. 60. 5% SUCROSE + 0·1% SINIGRIN SOLN. AT 0 (○) AND 2 (×) ATM. PRESSURE.




PROBIT MORTALITY

ACCESS FEEDING TIME (LOG DAYS)

Table 33 Log LT 50's, relative lethal-time values and relative potencies of 1) starvation, 2) 5% sucrose + 0.1% sinigrin soln., and 3) 5% sucrose + 0.1% sinigrin soln. under 2 atm. pressure

| | | |
|---|---|---|
| Log LT 50 (logm) | $\log m_1 = 0.3208$ | $\log m_2 = 0.4283$ $\log m_3 = 0.4737$ |
| Antilogarithm (days) | 2.093 | 2.681 2.976 |
| Relative lethal-time value (logM) | $M_{1,2} = 0.4283 - 0.3208 = 0.1075 \pm 0.0182$ | *** |
| | $M_{1,3} = 0.4737 - 0.3208 = 0.1529 \pm 0.0176$ | *** |
| | $M_{2,3} = 0.4737 - 0.4283 = 0.0454 \pm 0.0165$ | ** |
| Relative potency (P) (days) | $P_{1,2} = 1.28$ | |
| | $P_{1,3} = 1.42$ | |
| | $P_{2,3} = 1.11$ | |

t for $\infty$ d.f. - see Table 21.
Symbols as in Table 4 .

The aphids feeding on the sucrose + sinigrin solution survived longer than the starved aphids. In addition, the application of a pressure of 2 atmospheres to the solution significantly increased the survival of the aphids (1.11X). The mean lengths of life of the larvae born in each treatment were 1) 0.58, 2) 0.00 and 3) 1.17 days. Insufficient numbers of larvae were produced by the aphids of 2) and 3) (see Table 32) to make comparison of the lengths of life of the larvae worth while.

## Conclusions

Adult apterous virginoparae of B. brassicae survived longer when given access to 5% sucrose + 0.1% sinigrin solution

under a pressure of 2 atmospheres than when given access to the same solution without pressure. Such a difference in the survival of the aphids did not occur if the sinigrin was not present in the solution. No difference was found between the survival of the aphids feeding on 15% sucrose solution under a pressure of 1 atmosphere and the survival of the aphids feeding on the same solution without pressure.

There were no significant differences i) between the larviposition of the aphids and ii) between the lengths of life of the larvae which fed on sucrose ($\pm$ sinigrin) solution with and without pressure.

Starved aphids survived a shorter time but produced more progeny than aphids given access to sucrose solutions.

# CHAPTER V

# DISCUSSION

## The responses of M. persicae and B. brassicae to turgid host plants

Comparison of the responses of M. persicae and B. brassicae to turgid Brussels sprouts (see Experimentation, Part 1) reveals an integrated colonisation of the plants by the two aphid species. The segregation of the two species on Brassica sp. was observed by Miyashita (1953) (also Ôtake, 1966), B. brassicae being concentrated on the apex and young leaves and M. persicae on the old leaves of the plants. In the present work a similar segregation was begun by the settling alatae, continued and improved by the leaf age preferences of the apterae and intensified yet further by differences between the species in their reproduction and alate production (i.e. the proportion of alatae in the progeny of apterae) on different leaf ages.

As was noted earlier (p.154 ), in M. persicae the selection of the plants by alatae, the leaf age preferences of the apterae and the reproduction and alate production by the apterae on different leaf ages are closely co-ordinated, emphasising the "unsuitability" of the mature leaves and the "suitability" of the young and old leaves for the aphids (cf. Table 2). It would seem that in every experiment the

aphids responded to stimuli indicative of the nutritive value of the leaves as measured by aphid reproduction. However, the fecundity and reproductive rate of the apterae are particularly high on the old leaves (Fig. 18), and the apterae, in darkened preference cages, exercise an overwhelming preference for the old > young > mature leaves (Fig. 15a).

Higher reproduction by aphids may result from the uptake of a greater quantity and/or a better quality of plant sap by the aphids. From the present work it is not possible to estimate whether apterous M. persicae feed at a greater rate on the young and old leaves than on the mature leaves of the plants. However, if Kennedy and Booth (1950) (also Mittler and Dadd, 1964) were correct in thinking that feeding preferences, similar to those demonstrated by the apterae here, resulted from less movement of the apterae on one leaf than another (see p. 83 ), then the parallelism of the feeding preferences and reproductive parameters of the apterae on different leaves (cf. Figs. 16 and 18) points to a behavioural component in the differing parameters: apterae, being less restless on the preferred old (and young) leaves would perhaps ingest greater quantities of the sap of these leaves. This reasoning was used by Kennedy and Booth (1951) who found a similar parallelism between the feeding preferences and reproduction of A. fabae on different leaf ages of sugar beet. Furthermore, in the present study, the production of alatae by M. persicae is consistently lower on preferred than non-preferred leaves and this, too, may be related to the reduced restlessness of and

interaction (contact) between the aphids on the preferred leaves (see Johnson, 1965). It would seem, therefore, that on non-preferred leaves (e.g. mature) a reduced frequency and/or duration of feeding by the apterous M. persicae probably contributes to their reduced reproduction, and possibly contributes to their production of a greater proportion of alate progeny. This reasoning is used throughout the Discussion. It is unlikely that the different colours of the leaves exert an important influence on the feeding of the apterae (see Cartier, 1965) since in the dark the apterae still express preferences for those leaves on which they produce more progeny.

That aphids reproduce more on young and old than on mature leaves has often been attributed to the better quality of the sap of growing and senescing plant tissues and particularly to their higher levels of soluble nitrogen compounds (e.g. Kennedy, 1958; see Review of the Literature). However, as was pointed out in the Review of the Literature (p. 39 ), the recent artificial feeding of aphids has indicated that sugars, minerals and perhaps vitamins as well as amino-acids may vary in concentration in the sap of plants sufficiently to occur at sub- and supra-optimal levels and influence the reproduction, development and survival of aphids. Furthermore, different aphid species may require diets with different proportions of amino-acids or other nutrients (see Auclair and Cartier, 1963). Until more is known of (1) the specific dietary requirements (including "token" gustatory stimulants and inhibitors) of different species of aphids and (2) the changes

in these requirements in leaves as they age, it is unwise to ascribe the quality component of the nutritive value of the sap of leaves to a particular chemical compound or group of compounds. Nevertheless, while bearing this in mind, in the present discussion plant-sap"nutrient" quality will be considered principally in terms of the soluble nitrogen and carbohydrate content of the sap, both of which (i) are known to be very important in the diet of aphids and (ii) have been studied in detail by aphidologists and plant physiologists. The role of "token" gustatory stimulants and inhibitors in the responses of the aphids to the plants will also be discussed in detail.

To return to the experimental results, more soluble nitrogen compounds (amino-acids and amides) in the young and old than in the mature leaves may contribute to the selection of, preference for, higher reproduction and lower alate production of M. persicae (cf. Pintera and Ulrychová, 1959) on theyoung and old leaves of the Brussels sprouts. However, in apterae, the much higher fecundity and reproductive rate on, and overwhelming preference for, the old leaves would seem to indicate that the quality of the sap of old leaves of Brussels sprouts (cf. Table 2) may be particularly suitable for reproduction by M. persicae. Michel and Chouteau (1963a,b), who obtained a similar result, related this to the higher levels of potassium in the old leaves of tobacco. In artificial feeding experiments, Dadd and Mittler (1965b) found that M. persicae is sensitive to the concentration of amino-acids in the diet but

not to the proportions of the different amino-acids yet studied. Nevertheless, growing and ageing plant tissues, sites of protein synthesis and hydrolysis respectively, are characterized by different amino-acid and amide constituents (Steward et al, 1954; Plaisted, 1956, 1958) which may be of different nutritive value to the aphids. In this context, the trends of increasing reproductive rate (Fig. 18), increasing rate of larval development and increasing post-reproductive life (Fig. 17) of the aphids on leaves of increasing age are noteworthy. However, in addition, the sugar content of leaves may decrease as they age (see Tingley, 1944; Kibbs, Dahlmann and Rice, 1964) and, although sugar in the diet of M. persicae may not be important in determining the reproductive rate and rate of larval development (Dadd and Mittler, 1965b), sugars may possibly influence the length of the post-reproductive life of some aphids. Banks (1965) suggested that sugar-derived solids may gradually block the gut of those aphids which continue to feed slightly but do not excrete during post-reproductive life. It is conceivable, therefore, that blockage may occur more slowly in M. persicae on the older leaves of Brussels sprouts.

The settling of alatae on the plants requires special comment. M. persicae has previously been observed to settle primarily on the old leaves of host plants in the field (Table 1), perhaps to escape disturbance by wind and rain (Taylor, 1955). In the present study in a constant temperature room, alatae settled in similar high numbers on the young and

old leaves of turgid plants but at highest density on the plant apices and adjacent young leaves, (Figs. 12 & 13). Alate virginoparae of M. persicae are "yellow-sensitive" (see p. 13 ) and may therefore be attracted by the more yellow (as well as more nutritious) young and old leaves rather than the mature leaves. The accumulation of settling alatae at the apices of plants has been observed for many other species of aphid (see Table 1) suggesting that the apex of a plant may offer feeding sites particularly suited to the needs (nutritional, protective, microclimatic ?) of alatae. In M. persicae the alatae settle on the young leaves in decreasing numbers and densities away from the plant apex (Fig. 13) which may be in response to a decreasing gradient of sugars (see Hibbs et al, 1964) and/or soluble nitrogen compounds in the plant sap (see Mittler, 1958c). A similar falling gradient of sugars occurring during the late stages of senescence (see Plaisted, 1958; Weatherley et al, 1959) may contribute to the low numbers of alatae settling on very old leaves (Fig. 13). Sucrose is an important phagostimulant for M. persicae (Mittler and Dadd, 1965a).

At least two further host plant factors may contribute to the higher "suitability" of the young and old leaves rather than the mature leaves of Brussels sprouts for M. persicae. First, Way and Murdie (1965) found that a non-waxy variety of Brussels sprout was more susceptible to settling by M. persicae than a waxy variety. The cuticle of M. persicae does not have a prominent wax covering (cf. B. brassicae). Microscopic examination of the leaves of the plants in the present work

revealed that much wax occurred on the surface of the mature leaves, less on the young leaves and least on the old leaves. Secondly, Kennedy and Booth (1951) suggested that adaptation of an aphid to a host plant increased as the aphid acquired preference for the specific "flavour" of the plant, which was thought to be strongest in the mature leaves (see p. 12 ). On this basis, M. persicae would be poorly adapted to Brussels sprout and it is possible that the "flavour" of the mature leaves is repellent to the aphids (see also p. 29 ); the falling gradient of settling alatae from the young to the mature leaves may occur in response to an increasing gradient of "flavour" as much as to a decreasing gradient of "nutrient" in the leaves.

Kennedy et al (1959b) suggested that M. persicae, being polyphagous, may be more sensitive to plant condition than to plant kind. The close co-ordination of high aphid reproduction on young and old leaves with the other responses of the aphids (e.g. selection) and the overall rejection of the mature leaves supports the view that, in the "dual discrimination theory" of host plant selection (Kennedy and Booth, 1951; see p. 11 ), M. persicae responds particularly to the "nutrient" stimuli of the host plant.

The responses of B. brassicae to turgid Brussels sprouts contrast strongly with those of M. persicae. In B. brassicae, the co-ordination between reproduction and selection, feeding preference and alatae production for the different leaf ages is incomplete. Like M. persicae, apterous B. brassicae are more fecund on the young and old leaves and reproduce faster on old

leaves than on mature leaves, (see Fig. 29) (cf. Markkula, 1953). However, it was concluded (p. 197) that the "suitability" of the leaves for the aphids decreases with age, since in all other experiments (with one exception to be noted) the aphids rejected older leaves in favour of younger ones. Thus, apterae settle preferentially and produce fewer alatae on young leaves than on mature leaves (Fig. 26a,32) suggesting that, as with M. persicae (see above), increased restlessness of B. brassicae and poorer sap quality may contribute to the lower fecundity and higher alate production of the aphids on the mature leaves. However, apterae (in the dark and in the light - preliminary experiments) and alatae settle preferentially, and apterae produce a lower percentage of alatae on mature leaves compared with the old leaves despite the greater nutritive value of the latter inferred from higher aphid reproduction and the possibly greater visual attraction (more yellow) of the old leaves to the aphids (see Kennedy et al, 1961). A similar situation was foreseen by Kennedy and Booth (1951) in their "dual discrimination theory" of host plant selection (see p. 11 and above): An aphid species, having become highly adapted to a host plant and its specific "flavour" stimulus particularly in the mature leaves, "would then find the youngest and oldest leaves too flavourless, and would colonize leaves nearer to maturity for preference, even if they were less nutritious". This appears to describe in part the responses of B. brassicae to the leaves of Brussels sprouts particularly since the mustard oil glucoside, sinigrin, occurs in Brassica sp. and is known to be a specific ("flavour")

stimulus for host plant selection by this aphid (Wensler, 1962). One may speculate that sinigrin occurs in old and young leaves at lower levels than in the mature leaves of the plants, while the young leaves possess the optimum combination of "nutrient" and "flavour" (sinigrin) gustatory stimulants, at least for the apterae. It should be noted that alatae settle in falling number and densities below and above the mature leaves (Fig. 24) (except on the plant apex - see below) which may indicate a response to falling levels of sinigrin ("flavour") on each side of the mature leaves. This would agree with the conclusion of Kennedy and Booth (1954) that alate virginoparae (of A.fabae) were more sensitive to plant kind ("flavour") and less to leaf age ("nutrient") than apterous virginoparae. The high attraction of the plant apex to alatae has already been mentioned( also Kennedy et al 1959b, Broadbent 1954) with reference to M. persicae (p. 297), but in B. brassicae the attraction is even more striking when contrasted with the adjacent relatively unattractive young leaves (Figs. 23 & 24). This suggests that the alatae respond to a unique property of the apex rather than a gradient of, for example, "nutrients" from the young leaves to the apex (cf. M. persicae, above): The tightly curled leaves of the apex may provide tactile stimuli to arrest the movements of the alatae so that they remained in a protected feeding site. High tactile sensitivity of this kind might be expected in B. brassicae which, in contrast to M. persicae, normally forms compact aggregates on the host plant.

The greater fecundity and reproductive rate of

B. brassicae on old leaves than on mature leaves occurs despite greater aphid restlessness on the old leaves inferred from non-preference (and higher alate production). This suggests that the quality of the sap of the old leaves, like that of the young leaves, is very suitable for the reproduction of B. brassicae (cf. M. persicae) as long as the aphids also complete their larval development on the old leaves (cf. "Turgid" plants, Experimentation, Part 2). In B. brassicae, as in M. persicae, larvae develop faster with increasing leaf age (Fig. 28) (for discussion see p. 296 ). Hughes (1963) similarly obtained retarded development of B. brassicae on discs cut from young leaves of cabbages compared with other leaf ages. The post-reproductive life of B. brassicae may be as long as or longer than the reproductive life (Fig. 28)(cf. M. persicae, Fig. 17) The shorter post-reproductive life of the aphids on the young leaves than on the mature (and old?) leaves (Table12 ) may conceivably be related to earlier blockage of the aphid gut by the higher concentration of sugars in the sap of young leaves (see M. persicae above; Banks, 1965; Tingley, 1944).

On the above evidence, therefore, the rejection of the old leaves by the alatae and apterae does not appear to be guided by the quality of the sap of the leaves for aphid reproduction and development; it is suggested that the old leaves may lack (i) sufficient sinigrin (see above), and/or (ii) sufficient surface wax (see p. 297 ) for the aphids to develop adequately their typical wax covering (cf. M. persicae). Non-waxy varieties of Brassica spp. have been found to be more

resistant to colonization by B. brassicae (though equally attractive to settling alatae) than waxy varieties (Thompson, 1963; Way and Murdie, 1965). Furthermore, larvae deposited by apterous B. brassicae feeding on sucrose solutions (p.284 ) did not develop the wax covering which was observed to appear on larvae during the 24 hours after birth on plants.

On the other hand, the preference of the apterae for the young leaves compared with the mature leaves (on which reproduction was lower) suggests that the aphids on this occasion responded to the better quality of the sap of the growing tissues. In selecting host plants, B. brassicae may thus require, in addition to sinigrin and perhaps wax, amino-acids and amides (or other nutrients) particularly associated with protein synthesis and found in growing tissues (see Steward et al, 1954).

B. brassicae is oligophagous, occurring only on Cruciferae and Resedaceae (Markkula, 1953), and thus might be expected to be more sensitive to plant species and perhaps less sensitive to plant condition than a polyphagous aphid such as M. persicae (see Kennedy et al; 1959b). This view would seem to be borne out by the above interpretation of the responses of B. brassicae to turgid Brussels sprouts. On this host plant both aphid species were shown to feed in the phloem and particularly the sieve tubes. Differences between the species in their sites of feeding within the host plant cannot therefore account for the different responses found.

To summarise, both aphid species, when caged on leaves

of turgid Brussels sprouts (i) are more fecund on the young and old leaves than the mature leaves, (ii) have, in general, a decreasing post-reproductive life, rate of reproduction and rate of development on leaves of decreasing age, (iii) have a similar adult life and a similar reproductive life on all leaf ages, and (iv) prefer, and produce fewer alatae, on young leaves than on mature leaves. However, in terms of the "dual discrimination theory" of host plant selection, competition between the aphid species on the plant would seem to be reduced by the positive response of M. persicae to increasing levels of "nutrient" stimuli and their negative response to increasing "flavour" stimuli (and wax) of the plant parts and the positive response of B. brassicae both to increasing "flavour" stimuli (and wax) and, to a lesser extent, to increasing "nutrient" stimuli of the plant parts. The old leaves appear to be particularly suited to colonization by M. persicae and the young leaves to colonization by B. brassicae. The two sites (i) may contain optimal combinations of "flavour", "nutrients" and wax for the two aphid species, and (ii) may reflect differential amino-acid requirements of the two species. The outcome of these responses of the aphids to the plants would be to concentrate M. persicae on the senescing leaves and B. brassicae on the young leaves and apex of the plant (cf. Miyashita, 1953).

Higher alate production occurs consistently on those leaves, usually but not always of lower nutritive value, which stimulate greater restlessness of the apterae as inferred from

preference experiments. This is also found in the responses of the aphids to plant water strain (see below) and strongly supports the suggestion of Johnson (1965) that increased contact between aphids on less suitable host plants may be a primary mechanism stimulating greater alate production.

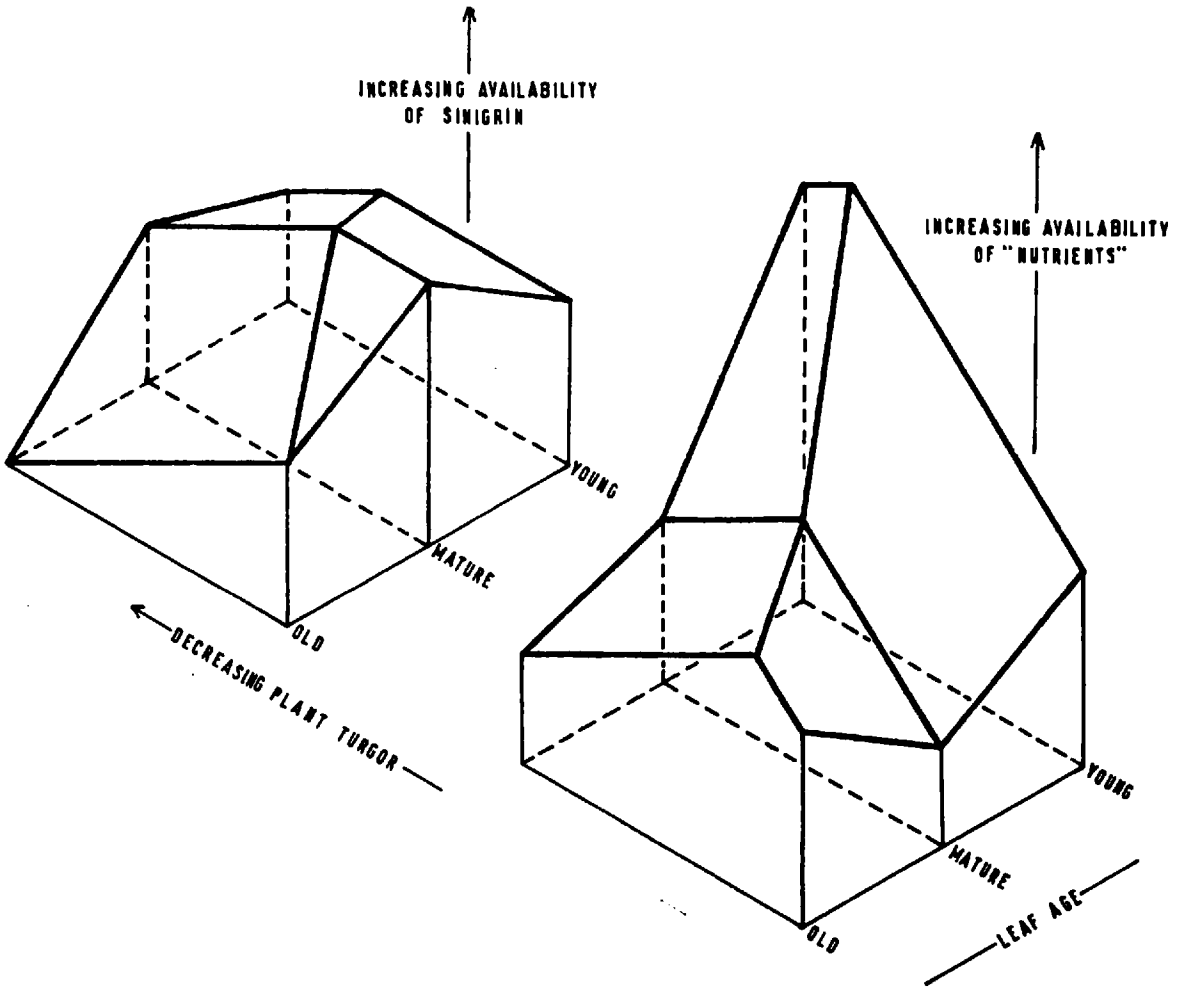
Aphids and the Water Status of the Host Plant

As noted in the Review of the Literature (p. 49 ), Lamb (1955), Kennedy et al (1958) and Kennedy and Booth (1959) reconciled many of the contradictory reports of the responses of aphids to host plants suffering water strain. As a result of comprehensive studies of the responses of A. fabae to beans, sugar beet and spindle subjected to water shortage, these authors concluded that aphids respond to two processes occurring in plants during water strain: (i) reduced cell turgor pressure and/or increased viscosity of the sap of the plants, and (ii) increased concentrations of soluble nitrogen compounds (from retarded protein synthesis and increased protein hydrolysis) in the plant sap. While (i) reduces the quantity of sap ingested by aphids, which are thought to depend to some extent on turgor pressure for feeding (see Kennedy and Mittler, 1953), (ii) increases the quality of the sap. It was argued that the net effect on aphids of these two processes varies with the severity of the water strain, (i) overriding (ii) during prolonged and severe water strain when (ii) would not be available to the aphids (e.g. Kennedy et al, 1958), and (ii) overriding (i) during less severe and intermittent water strain (e.g. Markkula, 1953).

Although a number of other changes, which may be important to aphids, occur in plants during water strain (see Addendum, p. 60 ), the above hypothesis would seem to be applicable to a large part of the results of the present work when allowances are made for a) variation in the water strain in different parts of the plant and b) variation between aphid species in their dietary requirements. Wearing and van Emden (1966) (also Wearing, 1966) suggested that B. brassicae may be more dependent on turgor pressure for feeding than M. persicae since the former suffered adversely from increasing water strain in Brussels sprouts before the latter. This phenomenon was found in the present work in certain circumstances (to be described) and it will be shown that this apparent difference between the species may be related to their different dietary requirements. It was concluded that on turgid plants M.persicae generally responded positively to increasing "nutrients" (particularly those in the old leaves) and negatively to increasing "flavour", while B. brassicae responded positively both to increasing "flavour" and, to a lesser extent, "nutrients" (particularly those in the young leaves). The probable effects of increasing water strain in different parts of the plant on the availability to the aphids of "nutrients" and "flavour" in the plant sap are summarised graphically in Figure 61.

Water strain in a plant normally decreases from the base to the apex (see Addendum, p. 60 ). Aphids feeding on old leaves would therefore be expected to suffer adversely from falling turgor pressure before aphids on the young leaves of the same plant. Thus, in Fig. 61, as plant turgor decreases,

FIG. 61. PROPOSED AVAILABILITY TO APHIDS OF SINIGRIN AND "NUTRIENTS" IN THE SAP OF BRUSSELS SPROUT LEAVES OF DIFFERENT AGE AND WATER STATUS. FOR EXPLANATION - SEE TEXT.



the availability of "flavour" and "nutrients" decreases progressively earlier in the young, mature and old leaves. There is no evidence that the production of the "flavour" of a plant (which it is thought may be sinigrin in Brussels sprout) is affected by water strain. Falling plant turgor may thus be expected to reduce immediately the availability of "flavour" to the aphids. In contrast, during water strain in the plant, "nutrients" are released in greater concentrations, which may be expected to delay the effect of falling turgor of reducing the availability of "nutrients" to the aphids.

On the basis of the above arguments, one would expect B. brassicae to suffer adversely from increasing water strain in the plant before M. persicae, except on the young leaves whose sap is thought to provide "nutrients" particularly required by B. brassicae.

Evidence supporting this sinigrin/"nutrient" hypothesis was provided by the experiments with aphids fed on liquid diets under controlled pressures. Apterous B. brassicae fed on 5% sucrose + 0.1% sinigrin solution under 2 atmospheres pressure survive longer than aphids fed on the same solution without pressure. Without sinigrin in the diet the survival of the aphids is unaffected by pressure (Figs, 59, 60). In contrast, apterous M. persicae fed on 5% sucrose solution under 2 atmospheres pressure survive longer than aphids fed on the same solution without pressure. With the addition of 0.1% sinigrin to the diet, the benefit of pressure is obliterated

(Figs. 48, 49), supporting the view, already expressed, that sinigrin may be repellent to M. persicae. Thus in these preliminary experiments the two aphid species benefitted equally from pressure which apparently increases the uptake (availability) of their appropriate primary phagostimulants (see Wensler, 1962; Mittler and Dadd, 1965a). No evidence was found of a true difference between the aphids in their dependence on turgor pressure for feeding.

In the discussion which follows, the responses of M. persicae and B. brassicae to water strain in Brussels sprouts are shown to agree closely with the above hypothesis. Both aphid species were shown to feed primarily in the sieve tubes of the phloem and therefore would experience similar changes in turgor pressure with changing water strain in the plant. Throughout the experiments, non-preference of the aphids for a given leaf (increased restlessness) usually paralleled increased alate production and, less often, decreased reproduction by the apterae (see p.293 ).

Water strain in young leaves

The young leaves of plants suffering intermittent water strain (Experimentation, Part 1) showed no wilt symptoms. This indicates that cell turgor deficits were probably small in the young parts of the plants even during the periods of water strain inflicted by the dry regime. It is not surprising therefore that on the young leaves many of the responses of both aphid species to water strain are positive - apterae settle preferentially on the young leaves of water-deficient

plants rather than more turgid plants (Figs. 14a, 16, 25a, 27); apterae of both species develop and reproduce more rapidly and produce more progeny, and apterous M. persicae reproduce for a longer period as the intermittent water strain in the plants increases (Figs. 17, 18, 28, 29); alate M. persicae accumulate particularly on the apex and young leaves of water-deficient plants (Figs. 11,12,13) (cf. Dickson and Laird, 1962); alate B. brassicae settle in greater numbers and densities on the apices of plants suffering mild intermittent water strain (medium regime) than on turgid plants (Figs. 22,23,24). As already noted (pp. 156 and 198), the preference tests with apterae suggest that the increased "suitability" of the young leaves of plants of the dry regime is particularly marked during the periods of water strain, when the old leaves are wilting (see Figs. 14a and 25a).

All these positive responses of the aphids to water strain may be related to the higher concentrations of soluble nitrogen compounds occurring in the young leaves of water-deficient plants as a result of retarded protein synthesis in the growing tissues during water strain (Lamb, 1955; Kennedy et al, 1958; Gates, 1964) and as a result of the recirculation of the products of more rapid proteolysis in the ageing leaves. During water strain in plants, nitrogen compounds are transported from the old leaves to the stem whence the nitrogen may be transported to the apex when the plants are again watered (Gates, 1957, 1964).

It was suggested (p. 303) that M. persicae may require

in particular the amino-acids resulting from proteolysis and B. brassicae the amino-acids associated with protein synthesis. From the above evidence, both sets of nitrogen compounds may be expected to accumulate (to the benefit of both aphid species) in the young leaves of plants suffering intermittent water strain. In contrast, in plants suffering continuous water strain, the amino-acids associated with protein synthesis only would be expected to accumulate (to the benefit of B. brassicae) in the apex and young leaves of the plants. This would seem to have occurred in the experiments with plants suffering continuous water strain (Experimentation, Part 2): On the young leaves of the plants of the "severe wilt" water treatment, B. brassicae reproduce more rapidly than M. persicae ($p<0.001$) (cf. Tables 16 and 27, Figs. 39 and 53) despite the similar restlessness of the two species ($p>0.05$) (cf. Figs. 38 and 52). Similar differences between the aphids do not occur on the leaves of any other age or water treatment. In addition, on the young leaves the rate of fall of larviposition with increasing water strain in the host plants tends to be greater ($p$ just $> 0.05$) for M. persicae ($b_2 = 0.773$, see Fig. 40) than for B. brassicae ($b_2 = 0.188$, see Fig. 54).

On the young leaves not all the responses of the aphids to intermittent water strain are positive. 1) Apterous B. brassicae have a similar length of reproductive life and, 2) within each aphid species, apterae produce a similar proportion of alate progeny on the young leaves of the plants in the three water regimes (cf. Bonnemaison, 1951). Such lack of response

of the aphids to water strain is difficult to understand. 1 may perhaps be related to the already high "suitability" of the young leaves when turgid; 2 would seem to indicate that the restlessness of the aphids varied between the treatments insufficiently to influence alatae production.

Earlier in this Discussion, the length of the post-reproductive life of the aphids was tentatively related to the sugar content of the plant sap (p. 296). Accordingly, the decreasing post-reproductive life of B. brassicae on young leaves suffering increasing intermittent water strain (Fig. 28) may conceivably result from the increasing concentrations of sugars in the plant sap. In the lower leaves of the plants, water strain increases the hydrolysis of starch to sugars which may be transported to the plant apex when turgor is restored (cf. nitrogen) (see Addendum, p. 60 ). Unlike B. brassicae, the post-reproductive life of M. persicae is not altered by intermittent water strain in the young leaves (Fig. 17). On the young leaves of turgid plants the post-reproductive life of M. persicae is already very short (3.7 days), perhaps close to the minimum.

On young leaves, B. brassicae shows a further negative response to (intermittent) water strain: alatae settle in lower numbers and densities on the apices of the plants in the dry regime (when the old leaves were wilting) compared with the medium regime (Figs. 22,23,24). It was suggested earlier (p.300) that alate B. brassicae may particularly respond to sinigrin ("flavour") rather than plant "nutrients" in settling. If this

is so, the lower turgor pressure in the apices of the plants of the dry regime may reduce the uptake of sinigrin by the alatae sufficiently to reduce settling. The optimum ratio of "nutrients" to sinigrin for the alatae is apparently obtained in the apices of the plants in the medium regime.

In accordance with the findings of Kennedy et al (1958) for A. fabae, apterous M. persicae and B. brassicae on the young leaves of plants subjected to continuously increasing water strain (Experimentation, Part 2) survive a shorter time and, particularly in M. persicae (see below), produce fewer progeny as the water strain in the plants increases. Decreased larviposition is most marked as the young leaves wilt, which occurs only as the falling water content of the growing medium comes close to the permanent wilting percentage (Figs. 40,54). The tension at which water is held in a drying soil (or vermiculite) increases rapidly near the permanent wilting percentage (see p. 66 ). This may be expected to cause a similar rapid decrease in the turgidity of a plant growing in such a soil. The decreasing larviposition may thus be related to the falling cell turgor pressure and/or increasing sap viscosity reducing the uptake of sap by the aphids and increasing their restlessness (Figs. 38,52).

Compared with B. brassicae the greater rate of fall of larviposition by M. persicae with increasing water strain in the young leaves is thought to result from different amino-acid requirements of the two aphid species (see p. 310 ). Under these circumstances M. persicae appears to be more dependent

on turgor pressure for feeding than B. brassicae (cf. mature and old leaves).

Water strain in mature leaves

On the plants of the dry regime, mature leaves occasionally wilted (Experimentation, Part 1), indicating that compared with the young leaves, the mature leaves of plants subjected to intermittent water strain suffer greater turgor deficits. In accordance with the sinigrin/"nutrient" hypothesis, the responses of B. brassicae to increasing intermittent water strain in the mature leaves are more often negative than positive and M. persicae is apparently less adversely affected than B. brassicae by falling turgor.

As on young leaves, many of the responses of M. persicae to increasing intermittent water strain in the mature leaves are positive --- apterae settle preferentially (and thus are probably less restless) on the mature leaves of water-deficient plants rather than more turgid plants (Figs.14b, 16); apterae reproduce (and tend to develop) more rapidly, reproduce for a longer period and produce more progeny containing a lower proportion of alatae as intermittent water strain in the plants increases (Figs. 17,18,21). These positive responses of the aphids to water strain in the mature leaves may be related to the increasing concentrations of soluble nitrogen compounds (from reduced protein synthesis and/or increased proteolysis) and sugars (from increased starch hydrolysis) in the plant sap. Furthermore, falling turgor in the leaves may reduce the uptake of sinigrin by the aphids; it

was suggested earlier that sinigrin in the mature leaves may be repellent to M. persicae (see p. 298). It would appear, therefore, that intermittent water strain in the mature leaves, to the benefit of apterous M. persicae, decreases the sinigrin/"nutrient" ratio of the ingested sap. Alate M. persicae settle in similar low numbers and very low densities on the mature leaves of the plants in the three regimes (Figs. 11,12,13). However, the alatae, being free to move between leaf ages, accumulate particularly on the young leaves and apices of water-deficient plants. This may prevent any visible response of the alatae to water strain in the mature leaves. In addition, the wax of the mature leaves may reduce settling by the alatae whatever the water status of the leaves (see the Discussion, p. 397; Way and Murdie, 1965).

As mentioned above, compared with M. persicae, the responses of B. brassicae on mature leaves to intermittent water strain in the plants are more often negative, suggesting that B. brassicae is more dependent on plant turgor pressure for feeding (cf. Wearing and van Emden, 1966). Nevertheless, probably in response to increasing concentrations of soluble nitrogen compounds in the plant sap, apterous B. brassicae caged on the mature leaves generally reproduce and tend to develop more rapidly, reproduce for a longer period and produce more progeny as the intermittent water strain in the plants increases (Figs. 28,29). In contrast, apterae settle preferentially (and thus are probably less restless) and alatae settle in greater numbers and densities on the mature leaves

of plants in the wet and medium regimes compared with the dry regime, when the old leaves are wilting (Figs. 22,23,24,25b,27). This reduced settlement of alatae on water-deficient plants is particularly marked on the lower mature leaves (Fig. 24) which suffer the greatest water strain (some wilted). The rejection by apterae of the mature leaves of the plants in the dry regime persists only as long as the old leaves are wilting (Fig.25b). Apterae on mature leaves also produce a greater proportion of alate progeny as intermittent water strain in the plants increases. This overall rejection of the mature leaves suffering water strain may be related to the lower availability of sinigrin resulting from lower cell turgor pressure and/or higher sap viscosity (reducing sap uptake) of the plants suffering water strain. On turgid plants, B. brassicae, highly adapted to Brussels sprout, rejects the more nutritious old leaves in favour of the supposedly stronger "flavour" (sinigrin) of the mature leaves. Similarly, B. brassicae here rejects the more nutritious water-deficient mature leaves in favour of the more turgid leaves, which perhaps by their greater turgor pressure, assist in a greater uptake of sinigrin by the aphids.

Kennedy et al (1958) suggested that there may be an intermediate stage in the development of plant water strain when impeded sap uptake was turning an increasing proportion of aphids into alatae while sap enrichment "was still ensuring the rapid production of large vigorous aphids." In the present work, this would appear to describe the responses of B. brassicae to intermittent water strain in the mature leaves (cf. Markkula,

1953) except that increased alate production is considered to result from increased restlessness of and contact between the aphids. These differential responses of B. brassicae to a supposedly falling sinigrin/"nutrient" ratio during water strain may be a subtle mechanism whereby, despite the increasing nutritive value of the leaves, a greater proportion of aphids are turned into alatae at a time when migration has the greatest chance of success, i.e. in dry, warm weather. M. persicae, less adapted than B. brassicae to Brussels sprout, (see p. 298) would seem to lack this ability.

As on young leaves (see p. 311), the shorter post-reproductive life of apterous B. brassicae on mature leaves with increasing intermittent water strain in the plant (Fig.28) may be related to the increasing concentrations of sugars (from starch hydrolysis) in the plant sap.

Continuously increasing water strain in the mature leaves (Experimentation, Part 2) elicits consistently negative responses from M. persicae and B. brassicae (cf. Kennedy et al, 1958; Weismann and Vallo, 1963). The two aphid species show similar increases in restlessness from the turgid to the wilt to the severe wilt water treatments (Figs. 38,52). The rate of fall of larviposition with increasing water strain in the plants tends to be greater for B. brassicae ($b_2$= 0.674, Fig. 54) than for M. persicae ($b_2$= 0.472, Fig. 40)(cf. young leaves) but this difference is not significant. The water strain in the mature leaves of the plants of the wilt and severe wilt treatments was intense, particularly towards the end of the experiment.

Wilting of the mature leaves usually occurred several days before that of the young leaves. In both aphid species, reduced sap uptake resulting from reduced turgor pressure and/or increased sap viscosity was probably so great as to override any differential responses of the aphids to changes in the chemical composition of the sap during water strain.

As on young leaves, but at an earlier stage in the development of plant water strain, larviposition by the aphids on the mature leaves declines sharply as the water content of the drying vermiculite approaches the permanent wilting percentage. This could be related to a rapid decline in plant turgor, and hence sap uptake by the aphids and a consequent increase in aphid restlessness. Once again no evidence was found to indicate a difference between the aphid species in their dependence on turgor pressure for feeding.

Water strain in old leaves

The old leaves of the plants in the dry regime (Experimentation, Part 1) wilted towards the end of each drying cycle so that turgor deficits were probably greater in the old leaves than in the mature and young leaves of the plants suffering intermittent water strain.

B. brassicae shows only negative responses to increasing intermittent water strain in the old leaves (see Figs. 27,28,29,32). In accordance with the sinigrin/"nutrient" hypothesis, this may be related to the reduced uptake of sinigrin by the aphids (see Fig. 61). In preference tests apterous B. brassicae reject the old leaves of the plants in the dry

regime in favour of other regimes only as long as the former are wilting (Fig. 25c). Sinigrin was thought to occur at sub-optimal concentrations in the old leaves even of turgid plants (see p. 300) so that any reduction of turgor in such leaves might be expected to be immediately discouraging to B.brassicae. Alatae settling on plants and free to move between leaf ages strikingly avoid the old leaves whatever their water status (Figs. 22,23,24).

On old leaves, the preferred feeding site of M. persicae on turgid plants, apterae of this species show a consistent positive response to mild intermittent water strain in the plants (medium regime) (Figs. 14c,16,17,18,21). In accordance with the sinigrin/"nutrient" hypothesis, this may be interpreted as a response to increasing concentrations of soluble nitrogen compounds and sugars in the sap, (from hastened proteolysis and hydrolysis of starch respectively), and decreasing availability of sinigrin during water strain. Sugar and nitrogen compounds are transported out of the leaves via the sieve tubes of the phloem, where the aphids feed (see p.247 ) (Gates, 1957; Schumacher, 1933). Previous evidence has suggested that M. persicae, in contrast to B. brassicae (see above), may particularly require the amino-acids and amides resulting from proteolysis.

However, on the old leaves M. persicae, like B. brassicae, shows consistently negative responses to the intermittent water strain in the plants of the dry regime compared with the medium regime (Figs. 14c,16,17,18,21); non-

preference of the apterae for the former rather than the latter persists only as long as the old leaves of the dry regime are wilting (Fig. 14c). In addition, settling alatae appear to move from the old leaves to the apex of the plants as water strain in the plants increases. These, the only, negative responses of M. persicae to intermittent water strain in the plant may be related to the much greater temporary turgor deficits in the old leaves of the plants in the dry regime compared with other leaf ages and regimes. It would seem that sap uptake is impeded by falling turgor sufficiently even to overcome the benefits for M. persicae of the enrichment of the sap with "nutrients". The preference of apterous M. persicae in the dark for wilting old leaves rather than mature leaves on the same plant is of interest: (Fig. 15c). The aphids appear to show preference for the lower "flavour" of the old leaves despite the greater nutritive value of the mature leaves (in contradistinction to the behaviour of B. brassicae).

Thus, as on mature leaves, B. brassicae on the old leaves suffers adversely from increasing plant water strain before M. persicae, which appears less dependent on turgor pressure for feeding. This again may be interpreted as a differential response of the aphids to a falling sinigrin/"nutrient" ratio in the old leaves suffering increasing water strain. The falling post-reproductive life of both aphid species as intermittent water strain in the old leaves increases may be related to the increasing concentrations of sugars in the sap from hastened starch hydrolysis (see previous discussion).

Continuously increasing water strain in the old leaves (Experimentation, Part 2) elicits consistently negative responses from both species of aphid: The water strain reduces aphid survival (Figs. 37,51). The rates of fall of larviposition with decreasing water content of the growing medium of the plants is similar for the two species, and takes the same logarithmic form, but is much greater than for other leaf ages (see Figs. 40, 54). These negative responses of the aphids to water strain may be related to the falling turgor pressure and/or increasing sap viscosity in the old leaves as the water content of the growing medium decreases. Loss of turgor (wilting) was evident in the old leaves before it was visible in the mature and young leaves.

However, apart from these similarities between the aphids, increasing water strain in the old leaves reduces the survival of B. brassicae more than M. persicae (Figs. 37,51); and while the two aphid species are equally restless on the old leaves of turgid plants, B. brassicae is more restless than M. persicae on the old leaves of the plants of the wilt ($p<0.001$) and severe wilt ($p<0.001$) treatments. This shows that, as in intermittent water strain, continuously increasing water strain in the old leaves adversely affects B. brassicae more than M. persicae, which probably benefits from the increased proteolysis and starch hydrolysis. The mean reproductive rate of B. brassicae on the old leaves of the turgid, wilt and severe wilt plants is lower than that of M. persicae ($p<0.01$, $p<0.001$, $p<0.05$ respectively) which prefers old leaves to leaves of any other age. This is further evidence suggesting

that the "nutrient" content of the old leaves is particularly suited to M. persicae.

In summary, the responses of B. brassicae and M. persicae to water strain in Brussels sprouts have been shown to agree, in general, with the proposed sinigrin/"nutrient" hypothesis. It would seem that the interpretation of the responses of aphids to water strain in plants must take into account not only the nitrogen content and viscosity of the plant sap and the turgor pressure of the cells but also the "flavour" or "token" chemicals and sugar content of the sap. True differences between aphids in their dependence on turgor pressure for feeding may occur but have not been found conclusively in the present work. The largely intracellular undeviating penetration of the leaves by the stylets of B. brassicae suggests that this aphid may follow a turgor or chemical gradient in locating the sieve tubes for feeding; on the other hand, the largely intercellular, sinuous penetration of the leaves by the stylets of M. persicae would seem to indicate a "trial and error" method of locating the sieve tubes whose sap may act as an 'arrestant' (see Dadd and Mittler, 1965b; Mittler and Dadd, 1965a).

A systematic study of the sap collected from the severed stylets of aphids feeding on leaves of different age and water status is essential to substantiate the "flavour" (sinigrin)/"nutrient" hypothesis. Measurement of the rate of exudation and the viscosity of the sap would provide details of the availability of the sap to the aphids, while chemical

analyses of the sap (and similar analyses of the honeydew) would provide details of aphid nutrition. Preliminary attempts to obtain such sap through the stylets of B. brassicae were unsuccessful, perhaps owing to parting of the maxillary stylets preventing the exudation of sap (cf. M. viciae, von Dehn, 1961b). Sap has been collected from the cut stylets of M. persicae (van Soest and de Meester-Manger Cats, 1956). Other pertinent information would be obtained from artificial feeding experiments using more complex diets and higher pressures than were used in the present work. Detailed study of the effects of plants of different water status on aphid restlessness and interaction is required to determine their role in the production of alatae; similar studies using artificial diets of different constitution would assist in separating a possible direct nutritional stimulation of alate formation from the proposed behavioural stimulation.

The economic significance of aphid response to water strain in plants

Kennedy et al (1958) concluded that "Water strain usually develops more or less gradually and intermittently in the field so that the effect on aphids on herbs may usually be favourable, with the eventual unfavourable effect when water strain becomes acute coming too late to have any economic interest". Although no field trials were conducted in the present work, this conclusion would seem to be largely substantiated by the laboratory experiments. In general,

M. persicae and B. brassicae benefitted from intermittent water strain in the plants unless it was severe, and even under these circumstances the feeding preferences of the aphids would seem to enable them to move to those parts of the plant offering the most nutritious feeding conditions; this would delay still further the detrimental effect on the aphids of increasing plant water strain. However, compared with M. persicae B. brassicae would seem to depart earlier from certain parts (e.g. mature leaves) of a plant suffering increasing water strain. This probably contributed to the results of Wearing and van Emden (1966) who obtained (in greenhouse experiments) decreasing numbers of aphids in free infestations of B.brassicae on plants subjected to incresing intermittent water strain. Even this detrimental effect of water strain on the aphids however, is unlikely to be of economic interest for aphid control in view of the concurrent detrimental effects of water strain on the plants.

A more likely source of aphid control, not considered by Kennedy et al (1958), is the deleterious effect on aphids of turgid plants, as compared with water-deficient plants. In other words, irrigation may be a useful cultural method of aphid control by preventing the development of plant water strain and 1) its favourable effects on aphids and 2) its unfavourable effects on crop plants (see e.g. Stanhill, 1957). It is dangerous to extrapolate from laboratory experiments to field conditions but there is evidence that, as expected from the present work and that of Kennedy and his co-workers, the

type of water strain normally experienced by field crops during drought is associated with aphid outbreaks (Davies and Whitehead, 1935; Leonhardt, 1940; Thomas and Vevai, 1940; Wilson, 1946; Cottier, 1953; Walker, 1954; Downes, 1956; Cumber and Todd, 1959; Broadbent and Heathcote, 1961; Nielson and Barnes, 1961). Furthermore, observations have suggested that irrigation during dry weather may prevent, or considerably delay, the build up of aphid numbers to levels which might cause economic damage to crops (Hobbs, Holmes, Swailes and Church, 1961; Unpublished reports of Advisory Entomologists at the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden (Nov.-Dec. 1964)). The present work suggests, in general, that, compared with plants suffering mild water strain, irrigated plants may delay the build up of aphids in a crop 1) by reducing the proportion of alighting alatae which remain on the plants (B. brassicae), 2) by reducing the growth, reproduction and survival of the aphids, and 3) by turning an increasing proportion of the aphids into alatae (M. persicae). Further injurious effects of irrigation on aphids may include 4) washing aphids off the plants, 5) assisting the development of fungal parasites of the aphids (see Nielson and Barnes, 1961), and 6) assisting the uptake of systematic aphicides by the crop.

Hobbs et al (1961) concluded that the pea aphid on alfalfa in Southern Alberta did not appear to require chemical control if adequate water was supplied to the plants. Irrigation may not often be as valuable as this in aphid control. However, as part of an integrated programme for the

control of aphids, irrigation of crops during dry weather may assist predators, parasites and resistant varieties of crops etc. in greatly delaying the need for the application of aphicides (see van Emden and Wearing, 1965). In many parts of the world, including Britain (see Penman, 1963), the weather is sufficiently dry to warrant the regular use of irrigation to improve the yield of many crops, but irrigation is frequently expensive. By assisting aphid control as well as improving crop growth, irrigation may become an economic proposition where previously it was thought to be uneconomic.

# CHAPTER VI

## SUMMARY

The responses of Brussels sprouts to intermittent water strain

1. The rate of leaf growth and the fresh weight of Brussels sprouts decreases linearly with increasing periodic deficit of available water in the growing medium, i.e. with increasing intermittent water strain in the plants.

2. The initiation of leaves (leaf number) is not significantly affected by intermittent water strain.

The responses of M. persicae to Brussels sprouts subjected to intermittent water strain.

1. Settling by alatae. On turgid plants or plants suffering only mild intermittent water strain, alate virginoparae of M. persicae settle in greater numbers and densities on young and old leaves than on mature leaves. Alatae settle at particularly high density on the apices of the plants. On plants subjected to severe intermittent water strain settling alatae accumulate even more markedly on the plant apices and avoid the wilting old leaves.

2. Preferences of apterae (a) Adult apterous virginoparae of M. persicae on turgid plants and plants suffering only mild intermittent water strain prefer leaves of different age in the order old > young > mature. On plants whose old leaves are wilting the order is young > old > mature; when such plants are watered, young and old leaves become equally acceptable.

(b) Apterae prefer leaves of plants subjected to intermittent water strain to those of more turgid plants, except when the leaves are markedly wilting. The aphids reject these leaves until they recover turgor.

3. Larval development. On turgid plants and plants suffering only mild intermittent water strain the rate of development of larval apterae increases with increasing age of the leaf on which they feed. This response is obliterated by severe intermittent water strain in the plants: On mature and young leaves but not on old leaves, the rate of larval development is increased by intermittent water strain in the plants.

4. Adult and reproductive life. On turgid plants, the adult life and reproductive life of apterae are unaffected by the age of leaf on which they feed. On young and mature leaves, apterae live and reproduce longer with increasing intermittent water strain in the plants. However, on old leaves which wilt periodically, apterae live and reproduce for a shorter time than apterae on more turgid old leaves.

5. Post-reproductive life. On turgid plants, the post-reproductive life of apterae increases in relation to increasing age of the leaf on which they live. Intermittent water strain does not affect the post-reproductive life of apterae except on the old leaves, where it is shortened by periodic wilting.

6. Reproduction. On turgid plants, apterae reproduce more on the old and young leaves than on mature leaves and on the old leaves they reproduce faster. On young and mature leaves, apterae produce more progeny more rapidly as intermittent

water strain in the plants increases. On old leaves, apterae respond similarly to mild intermittent water strain, but fecundity and reproductive rate decrease if there is periodic wilting of the old leaves.

7. Alate production. On turgid plants, apterae on young and old leaves produce a smaller proportion of alatae than on mature leaves. On young leaves alate production by the apterae is not significantly affected by intermittent water strain in the plants. On mature and old leaves a smaller proportion of alatae are formed as intermittent water strain increases, except on old leaves which wilt periodically. On these leaves, alate production is increased compared with that on old leaves suffering less severe water strain.

8. Summary-(M. persicae). The suitability of Brussels sprout as a host for M. persicae is increased by intermittent water strain in the plants; even when intermittent water strain is severe, the decreased suitability of the old leaves is more than compensated for by the increased suitability of the young and mature leaves.

The responses of B. brassicae to Brussels sprouts subjected to intermittent water strain.

1. Settling by alatae. More alate virginoparae of B. brassicae settle on the apices and mature leaves than on young and old leaves of Brussels sprouts whatever their water status. Most settle on the apices. More alatae settle on plants conditioned by mild intermittent water strain than on turgid plants or plants conditioned by severe intermittent water strain.

2. Preferences of apterae. (a) irrespective of the water status of the plants, adult apterous virginoparae of B. brassicae prefer young and mature rather than old leaves and show a weak preference for young over mature leaves. When the old leaves are wilting, these preferences are enhanced.

(b) Apterae prefer young leaves of plants subjected to severe intermittent water strain to young leaves of more turgid plants. Apterae prefer mature and old leaves of turgid plants to corresponding leaves of plants on which the old leaves are wilting.

3. Larval development. On turgid plants and plants subjected to only mild intermittent water strain, apterae mature more slowly on young than on older leaves. On young leaves, apterae develop more rapidly as intermittent water strain in the plants increases. Intermittent water strain does not significantly influence the rate of development of apterae on mature and old leaves.

4. Adult, reproductive and post-reproductive life. (a) On turgid plants, the adult life and reproductive life of the apterae do not vary significantly with the age of the leaf on which aphids feed. The post-reproductive life is longer on the mature and perhaps old leaves than on the young leaves of turgid plants.

(b) The life of adult apterae on young leaves shortens as intermittent water strain increases because post-reproductive life is shortened; on mature leaves, reproductive life is lengthened and post-reproductive life shortened, and, on old

leaves, both reproductive and post-reproductive life are shortened by increasing intermittent water strain.

5. Reproduction. On turgid plants, apterae reproduce more on old and young than on mature leaves, and, on old leaves, they reproduce quicker. On the young, and, to a lesser extent, on the mature leaves, apterae produce more progeny more rapidly as intermittent water strain in the plants increases: On the young leaves of the plants suffering severe intermittent water strain, the apterae reproduce more than twice as fast and produce almost twice as many progeny as apterae on the young leaves of turgid plants. On the old leaves, apterae produce fewer progeny more slowly as intermittent water strain in the plants increases.

6. Alate production. On turgid plants, alate production by apterae increases with the age of the leaf on which the aphids feed. On the young leaves, alate production is not significantly affected by intermittent water strain in the plants. On the mature and old leaves, alate production increases with increasing intermittent water strain in the plants.

7. Summary-(B. brassicae). The suitability of Brussels sprout as a host for B. brassicae would appear to be increased by mild intermittent water strain in the plants but decreased by severe intermittent water strain.

The responses of M. persicae and B. brassicae to continuously increasing water strain in Brussels sprouts.

1. The restlessness of apterae of both aphid species increases and their survival decreases with increasing water strain in the host plant. These responses are most marked for apterae on the

old leaves and decreases towards the apex of the plant.

2. Larviposition by apterae decreases logarithmically with decrease in the percentage of available water in the growing medium of the host plant. The rate of fall of larviposition is usually greater for apterae on the old leaves (which suffer greatest water strain) than for those on the mature leaves, which in turn is greater than for those on the young leaves (which suffer the least water strain). Larviposition is particularly reduced on all leaf ages as the available water in the growing medium approaches the permanent wilting point.

The methods of penetration and the destinations of the stylets of M. persicae and B. brassicae in Brussels sprouts.

1. The stylets of apterous M. persicae penetrate Brussels sprout leaves largely intercellularly whereas those of B. brassicae penetrate intracellularly.

2. In both species, the tips of the stylets pierce phloem cells, particularly the sieve tubes, which are thus thought to be the main source of food. The sieve tubes appear to be penetrated by all four stylets and the salivary sheath of B. brassicae and, in contrast, only by the maxillary stylets of M. persicae.

3. Apterous M. persicae of increasing size penetrate leaf veins of increasing size. It is suggested that aphids select veins whose phloem is within the reach of the stylets.

The responses of M. persicae and B. brassicae to liquid diets under controlled pressures.

1. Adult apterous M. persicae feeding on 5% sucrose solution

under 2 atmospheres pressure survive significantly longer than apterae feeding on the same solution without pressure. This response to pressure is not obtained if (a) 0.1% sinigrin is added to the diet, (b) the applied pressure is halved, or (c) if the applied pressure is halved and the sucrose concentration is trebled.

2. Adult apterous B. brassicae feeding on 5% sucrose solution + 0.1% sinigrin solution under 2 atmospheres pressure survive significantly longer than apterae feeding on the same solution without pressure. This response to pressure is not obtained (a) if sinigrin is removed from the diet or (b) if sinigrin is removed from the diet, the applied pressure halved and the sucrose concentration trebled.

Possible causes of the different responses of M. persicae and B. brassicae to Brussels sprouts.

1. It is suggested that M. persicae and B. brassicae respond differently to Brussels sprouts because they have different dietary requirements, i.e. the two aphid species respond differently to changes in the availability of sinigrin and "nutrients" in different parts of the plants and in plants of different water status.

2. Restlessness and hence contact between aphids is suggested as the cause of alate production.

3. It is postulated that the length of the post-reproductive life of the aphids is related to the presumed concentration of sugar in the ingested sap.

The economic significance of aphid response to plant water strain

Although severe water strain in the host plant may be detrimental to aphids, it is suggested that a more likely factor for aphid control is the detrimental effect on aphids of turgid plants compared with plants suffering mild intermittent water strain. In the field, during dry weather, which normally favours aphid outbreaks, irrigation of crops may prevent or delay economic damage by the aphids.

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# APPENDIX

## University of California Fertiliser IIa. (Baker. 1957) converted to metric units

Per bushes of vermiculite:-

53.8 gms. Hoof and horn.
10.8 gms. Potassium nitrate.
5.4 gms. Potassium sulphate.
53.8 gms. Single superphosphate.
32.3 gms. Dolomite lime.
53.8 gms. Gypsum.