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**The Experimental Study of the Chemotactic Basis of Host-Specificity  
in a Phytophagous Insect, *Aphis fabae* Scop. (*Aphididae: Homoptera*)**

**Studia eksperymentalne nad chemotaktycznymi podstawami fagizmu  
u *Aphis fabae* Scop. (*Aphididae: Homoptera*)**

**Экспериментальные исследования над хемотактическими основами  
фагизма у *Aphis fabae* Scop. (*Aphididae: Homoptera*)**

The high degree of host-specificity of many phytophagous insects is a commonplace yet a remarkable phenomenon. Nevertheless, it has been relatively neglected as a field for experimental study.

According to Thorsteinson (43), the phenomenon is manifest in two ways: the host-preference (i.e. the actual selection of the host for oviposition, etc.), and the host-acceptance (i.e. the ability of the nymph or larvae to feed, reproduce, etc. on these selected plant species). Both these phenomena are believed to consist of a chain of responses to optical, chemotactic and tactile stimuli (53). The object of this study was to investigate the responses to chemical stimuli, in a polyphagous phloem-feeder, in relation to its choice of host-plants. Since the host-acceptance behaviour of a number of phloem-feeding insects has already been followed in detail by Alikhan (1), Kennedy and Booth (28, 29), Maltais and Auclair (34), Mittler (37), and Nourteva (38), the emphasis here is on the preference responses of the apterae and alatae adults of the black bean aphid to specific chemical constituents of their food plants.

The first step in this type of studies was made about 50 years ago when Grevillius (21) found that the larvae of the browntail moth, *Euproctis chryorrhoea* (L.), which feed on chickweed, *Stellaria* sp., could be induced to feed on other plants by smearing the leaves with

a paste containing tannin, which is a constituent of chickweed. It is unlikely that this work was highly critical with respect to the chemical purity of the test preparations, yet basic principle inherent in all experimentation in this field was employed. In other words, a relatively isolated chemical constituent of the host-plant with some ingestible substrate other than the host-plant tissue was offered to the insects. Obviously, the induction of feeding indicated that the test material contained some substance that stimulated the appetite of the insect.

Five years later, Verschaffelt (51) made the observations that the distribution of the mustard oil glucosides among plants coincides with the range of the acceptable host-plants for the larvae of the butterflies, *Pieris rapae* (L.) and *Pieris brassicae* (L.). He naturally inferred that the mustard oil glucosides, or their fission products, stimulate feeding in these insects; and he performed some simple but significant experiment that confirmed this hypothesis. His experimental techniques, however, were the same as those of Grevillius and were thus inadequate to permit a complete analysis of the various factors involved.

This work of Verschaffelt (51) was justly considered to be a classical contribution to our knowledge, and yet it failed to stimulate further significant work for many years. Ultimately, a renewal of interest was stimulated in France by the ravages of the Colorado potato beetle. This insect, as is well known, is oligophagous and hence is even more discriminating than the cabbage butterfly in that it will eat only some species in the plant genus *Solanum* and a few other plants. The French workers, Raucourt and Trouvelot (42), believed that a knowledge of the physiological basis of host-plant selection would be of value in arriving at a system of control of this pest, and consequently set out to isolate the host constituents that induce feeding. Although they introduced an ingenious technique for testing the palatability of their extracts, they were unable to isolate the substance they sought in a highly purified form. This was finally accomplished by Chauvin (3, 5), who described some of the chemical properties of this stimulant and showed that it gives reactions characteristic of flavone glucosides. Its precise identity, however, remains unknown. Chauvin also refined his predecessors' methods for testing the palatability of plant extracts.

In America a significant contribution in this behalf was made by Dethier (8). He studied the influence of certain essential oils on the feeding behaviour of *Papilio* sp. and related these to the probable evolution of species in this genus of insects. Since then he has published

illuminating reviews and discussions on the biological significance of host-specificity (11, 12).

In England and Canada, Thorsteinson (43) investigated the chemotactic basis of host-specificity in the diamondback moth, *Plutella maculipennis* (Curt.). This insect has the same host range as the *Pieris* sp. studied by Verschaffelt (51), and therefore was found to be stimulated to feed by the mustard oil glucosides. However, Thorsteinson was unable to contribute, as Verschaffelt had done, any stimulating properties to the mustard oils, the fission product of mustard oil glucosides.

On the other hand, working independently of each other, Alikhan (1), Kennedy and Booth (28, 29), Maltais and Auclair (34), Mittler (37) and Nourteva (38) reported that insects are also stimulated to feed by „primary plant substances”, like amino acids saccharides and vitamins. Their reports were further confirmed by the work of Chauvin (5) on *Chorthippus longicornis* Latr., Dadd's (7) on *Schistocerca gregaria* Fork., and of Thorsteinson (46) on *Plutella maculipennis* (Curt.) and *Leptinotarsa decemlineata* Say.

Thus there is some difference of opinion as to whether token stimuli or a hypothetical perception of nutritionally adequate food is more important in the perception of the host. In several papers (17, 18, 32) the thesis is pronounced that food-plant selection is determined solely by the „odd” or secondary chemicals (e.g. essential oils, glucosides, alkaloids, etc.), which plants synthesize for no apparent metabolic purpose. A similar view is advanced, less categorically, by Dethier (12). There is, in fact, little doubt that many such substances do repel or inhibit oviposition and feeding, and this fact alone would establish them as very significant determinants of food-plant selection. However, a very few of these substances have been shown to function in reverse, i.e. feeding or oviposition stimulators. Furthermore, since a number of attempts to extend the list of such substances have failed, it is very much doubted if the solution of our problem lies in the presence of „odd” substances in the plant.

The only other hypothesis which seems creditable is that forwarded by Kennedy and Booth (28, 29). The acceptance of this hypothesis implies that in phytophagous, whether monophagous, oligophagous or polyphagous insects there is a capacity to smell and taste the major plant nutrients, i.e. vitamins, amino acids, saccharides, etc., and the quantity of feeding in a given diet is regulated, in part, by the kind and quantity of the nutrients it contains. Of course, it is improbable that all the varied nutritional components could be appraised by insects in this way. However, it is not entirely implausible that some of the

nutrients perceived by the gustatory sense of the insect may serve as indicators of nutritional quality.

To correlate the host-selection and acceptance behaviour of the black bean aphid, *Aphis fabae* Scop., with the qualities and the quantities of the nutrients supplied by the plants, studies were undertaken at the Institute of Zoology, Maria Curie-Skłodowska University, Lublin. A report on the results of these studies forms the subject-matter of my thesis.

#### I. EXPERIMENTAL STUDY OF THE OLFACTORY STIMULI

Thorsteinson (46) points out that food-plant selection, or botanical specificity, is brought about through two phases of insect behaviour, viz. (I) food finding, and (II) food acceptance. In food-finding the first and the foremost part is played by the visual and olfactory stimuli provided by the host. The former group of stimuli constitutes mainly the spectral composition of light reflected from the surface of the plant, while the later includes the odour given out by the plant. None of these stimuli, however, have an effective range beyond a few metres (47).

The observations made by Moericke (c.f. 47), that yellow as a colour has a special attraction for aphid, have long since been utilized by workers for the control of these pests. However, no information is available as to the type and the quality of the olfactory stimulants of botanical origin peculiar to this group of insects.

Dadd (7) and Thorsteinson (46), in their studies on the host-selection behaviour of *Schistocerca gregaria* Fork, and *Leptinotarsa decemlineata* Say, have suggested that some of the soluble nutrients, viz. vitamin C and sucrose, serve as chemotactic (usually gustatory) stimuli to release feeding activity in these insects. On the presumption that these compounds may also act as olfactory stimuli, experiments were undertaken to find out their value as olfactory stimulants for the aphid.

In these studies, besides the pure ascorbic acid (vitamin C pulvis, F. P. III. — a product of the Polish State Department of Pharmaceutical Compounds, Cracow), pure sucrose and glucose (Products of Messrs. Boots Medical Laboratories Limited, England), freshly prepared sugar beet and leaf-extracts were used.

The apparatus used was McIndoo's (36) olfactometer (Figure 1). The principle underlying the construction of this apparatus is that of Flüggé (16) (Figure 2), who observed that *Drosophila melanogaster* has different reactions towards various streams of air bearing different odours. The olfactometer consisted of a cardboard box (G) with two

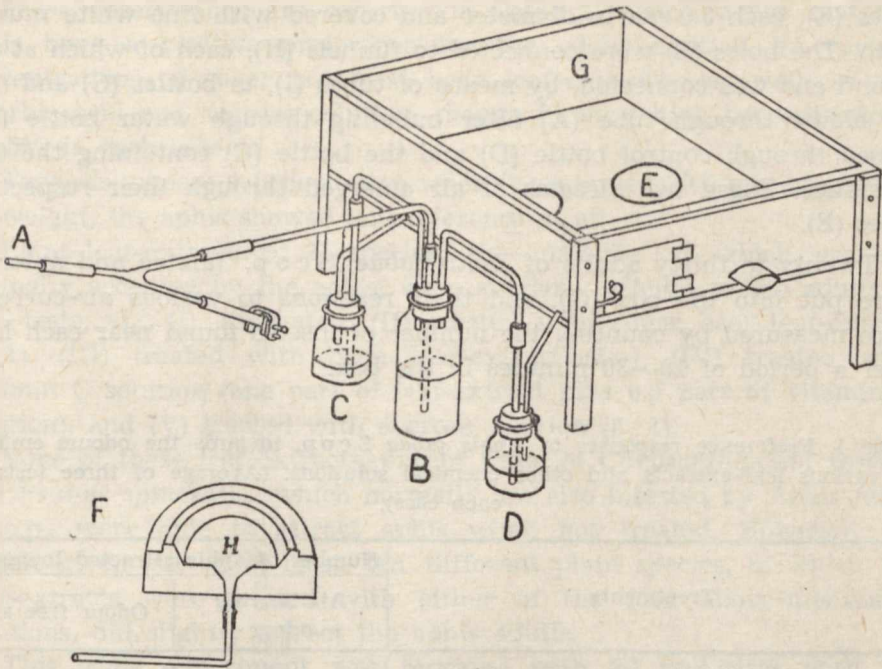


Fig. 1. Insect olfactometer, adapted after McIndoo (36); A — Air tube; B — Water bottle; C — Bottle containing the test substance solution; D — Control bottle; E — Air holes covered with white muslin cloth; F — The air-exit mechanism shown in cross section; G — The cardboard box; and H — Funnel

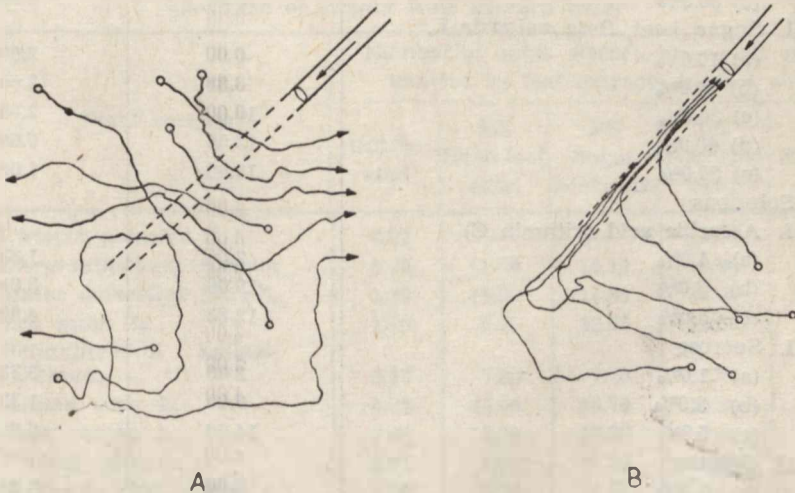


Fig. 2. Diagrams illustrating the response of *Drosophila melanogaster* to a stream of odour-free air A — and to a stream of air bearing an attractive odour B. (Redrawn after Flugge, Z. vergleich. Physiol. (16).

holes (E), each 3.0 cm in diameter and covered with fine white muslin cloth. The holes (E) were connected to funnels (H), each of which at the second end was connected, by means of tubes (I), to bottles (C) and (D), air blown through tube (A) after bubbling through water bottle (B), passed through control bottle (D) and the bottle (C) containing the test substance. These two streams of air emerged through their respective holes (E).

Twenty to thirty adults of *Aphis fabae* Scop. (alatae and apterae) were put into the box (G), and their reactions to various air-currents were measured by counting the number of insects found near each hole after a period of 15—30 minutes in the box.

Table 1. Preference responses to *Aphis fabae* Scop. towards the odours emitted by various leaf-extracts and other chemical solutions. (Average of three tests in each case).

Treatments	Number of aphid attracted towards	
	Air bearing odour	Odour free air
1. Leaf-extracts		
I. Bean, <i>Vicia faba</i> L.		
(a) 10.0%	6.33	2.00
(b) 20.0%	8.33	3.00
(c) 30.0%	11.00	2.66
(d) 40.0%	16.00	2.33
(e) 50.0%	18.00	0.33
II. Sugar beet, <i>Beta vulgaris</i> L.		
(a) 10.0%	6.66	2.00
(b) 20.0%	6.33	3.66
(c) 30.0%	10.00	2.66
(d) 40.0%	13.66	0.66
(e) 50.0%	18.00	1.00
2. Solutions		
I. Ascorbic acid (vitamin C)		
(a) 1.0%	2.00	1.33
(b) 3.0%	10.00	3.00
(c) 5.0%	12.33	4.00
II. Sucrose		
(a) 1.0%	2.00	3.33
(b) 3.0%	5.00	1.33
(c) 5.0%	14.00	6.66
III. Glucose		
(a) 1.0%	2.00	2.33
(c) 5.0%	2.00	1.66
(b) 3.0%	3.00	2.33
(d) 10.0%	3.00	2.33

These observations are reported in Table 1, which shows that the aphid have a positive preference for the odour emitted by various concentrations of sugar beet and bean leaf-extracts, as well as for ascorbic acid and sucrose solution, though in very high concentrations, i.e. 5% in each case.

Towards glucose solution, even when it contained 20.0 gm. of glucose by weight, the aphid showed no preference at all.

Plant leaf-extracts of 21 species, the majority of which are not normally accepted by the aphid, were similarly offered to the adults in five tests, viz. (I), untreated, (II) treated with sugar beet leaf-extract (1:1), (III) treated with bean leaf-extract (1:1), (IV) treated with vitamin C solution (one part of leaf-extract plus 0.5 part of vitamin C solution), and (V) treated with sucrose solution (5:1).

None of these leaf-extracts, except those of *Chrysanthemum roseum* and *Prunus spinosa* L., which normally are also infested by *Aphis fabae* Scop., were able to attract aphid when not treated. However, the odours given out by at least ten different plant species, of which the leaf-extracts were treated with either of the four above-mentioned solutions, did slightly attract the aphid adults.

This same experiment was repeated with 20 replicates with the leaf-extracts of *Cirsium palustre* (L.) Scop. Only in one case did the aphid show a slight preference towards the untreated plant-extracts

Table 2. Preference responses of *Aphis fabae* Scop. — towards the odour emitted by leaf-extracts of various plant species.  
(Average of twenty tests in each case)

	Plant species	Number of aphid attracted towards odour emitted by leaf-extracts treated with				
		Untreated	50% Bean leaf extr.	50% Sugar beet extr.	10% Ascorbic acid	10% Sucrose
1	<i>Cirsium palustre</i> (L.)	3.00	—	—	16.00	15.70
2	<i>Chrysanthemum roseum</i>	5.20	11.60	14.10	10.30	12.00
3	<i>Malus domestica</i> Borb.	2.30	15.30	14.00	16.00	11.50
4	<i>Zea mays</i> L.	1.10	9.60	12.05	14.60	9.80
5	<i>Helianthemum helianthemum</i>	3.10	7.80	7.50	18.70	7.50
6	<i>Allium cepa</i> L.	4.02	10.00	16.70	16.50	8.70
7	<i>Rosa canina</i> L.	3.00	12.20	18.00	7.60	9.10
8	<i>Prunus spinosa</i> L.	6.01	9.60	17.20	9.80	9.00
9	<i>Centaurea rhenana</i> Bor.	4.00	8.50	11.60	8.70	11.70
10	<i>Populus</i> sp.	1.10	13.20	12.40	10.00	13.40
11	<i>Lathyrus vernus</i> (L.) Bernh.	2.30	11.00	10.90	9.30	12.70

of this plant, while 18 of those treated with vitamin C, and 7 of those treated with sucrose, were able to attract the insects.

The data regarding these tests are summarised in Table 2.

Attempts to induce aphid adults (summer forms) to show preference for the leaf-extract of the summer leaves of *Evonymus europaeus* L. were unsuccessful, even when these extracts were treated with vitamin C or sucrose solution. However, when these leaf-extracts were offered in a mixture with the leaf-extracts of either bean or sugar beet, the aphid showed a marked preference for their odour (Table 3).

Table 3. Preference responses of *Aphis fabae* Scop. towards the odour emitted by summer leaf-extracts of spindle tree, *Evonymus europaeus* L. (Average of three tests in each case).

Treatments	Number of aphid attracted towards	
	Air bearing odour	Odour free air
1. control	3.00	3.33
2. Treated with		
(a) sucrose solution (5:1)	3.00	3.00
(b) vitamin C (5:1)	3.33	3.33
(c) bean leaf-extract (1:1)	14.33	4.00
(d) sugar beet leaf-extract (1:1)	12.00	3.00

These findings are in total agreement with the findings of Kennedy and Booth (28) and the natural phenomenon of aphid migration.

On the basis of these observations, it can be said that though vitamin C and sucrose do have some value as olfactory stimulants, this value is not of such a high order as to be of some practical use.

## II. GUSTATORY STIMULI — AN EXPERIMENTAL STUDY

Although nobody has found any evidence of the olfactory role played by primary plant constituents, the gustatory role of quite a number of these substances, viz. vitamin C, sucrose and amino acids, has been reported as gustatory stimulants for *Leptinotarsa decemlineata* Say, *Schistocerca gregaria* Fork., *Plutella maculipennis* (Curt.), leafhoppers and other grasshoppers by Chauvin (4), Dadd (7), Nourteva (38), Thorsteinson (46) and others. However, since the gustatory role of these essential plant substances has not so far been studied in the case of aphid, studies were undertaken to fill this gap.

For this purpose, various freshly prepared plant leaf-extracts and other chemical solutions, viz. sucrose and vitamin C, were offered



to the insects in agar solution (glucose = 6.0%; triolein = 0.5%; soluble casein = 2.0%; yeast = 2.0%; and water (distilled) = 89.5%). Although the use of agar solution assisted a quantitative determination of feeding responses, it did not provide for the experimental control of the physical characteristics of the media. Each experimental diet consisted of test substance incorporated in 50.0 ml. of 3.0% agar solution. The mixture thus obtained was heated to 85.0°C., allowed to cool to 55.0°C., and was offered to the insects in Maltais' aphid feeding apparatus (33).

The responses of the insects to various diets were calculated on the basis of food ingested by the aphid after a 3 to 24 hours' feeding-period on these diets. This was done by weighing the insects before and after the feeding-periods on a Roller-Smith microtorsion balance with a range from 0.002 to 5.0 mg. and a sensitivity of 0.002 mg. Before each weighing the aphid were anaesthetized with carbon dioxide. All these experiments were carried out in the laboratory at a temperature of 20.0°C. to 21.0°C.

Table 4. Loss or gain in weight of 10 aphid after 3 and 24 hours' feeding on various test diets. Aphid were deprived of food overnight prior to feeding.

	Average percentage weight lost or gained after	
	3-hours	24-hours
1. control *	-2.55	-16.44
2. Agar alone (3% solution)	+0.72	+ 4.75
3. Agar + 5% sucrose	-1.68	+ 2.06
4. Agar + 5% ascorbic acid	+2.61	+11.03
6. Sugar beet leaf-extract (50%)	+4.76	+13.70
5. Bean leaf-extract (50%)	+5.08	+17.50

\* Aphid kept in glass vials without food or water for the whole duration of the experiment.

— denotes loss in weight.

+ denotes gain in weight.

A. 5% sucrose solution, 5% ascorbic acid solution, 50% aqueous solution of bean leaf-extract and 50% aqueous solution of sugar beet leaf-extract were offered to insects in a mixture with 3.0% agar solution. Before allowing them to feed on these diets, the insects were starved (without food or water) for about 15 hours.

Table 4 show that the aphid when kept without food or water lost significantly more body weight than when caged on any of the test diets. This implies that some feeding did occur even with unpalatable diets.

However, considered as a whole, the data suggest that the maximum feeding responses occurred with bean or sugar beet leaf-extract, the minimum with agar plus sucrose solution. The same type of effect with sugars has been reported by Fraenkel (17) with regard to leaf-feeding insects and by Nourteva (38) with regard to leafhoppers. Thorsteinson (46) has remarked that a stimulant cannot be also a repellent, since this is contradiction in terms. This may be so. However, there is every possibility that a stimulant may be a toxin, or, in other words, may have some inhibiting effects upon the feeding activity. This appears to be the case with sugars in case of aphids, and thus bears out what has been postulated by Alikhan (1) in his „sugar-nitrogen ratio index” hypothesis.

It is, however, curious that although 3.0% agar solution plus 5.0% ascorbic acid contained practically all the feeding stimulants, and no repellent or feeding inhibitor at all, it was not as palatable as the bean or sugar beet leaf-extracts. An explanation of the insect's behaviour in this case has been given by Thorsteinson (43). According to him, the action of a feeding stimulant is, to a lesser or greater extent, always dependent upon the presence of other, metabolically important or unimportant, substances. To obtain some concrete evidence of this, a study of the feeding responses of aphids adults towards various concentrations of bean leaf (*Vicia faba* L., English Tick beans variety „Niki 7”) extracts were made. The results of these studies are reported below.

B. In this series of tests various concentrations of bean leaf-extracts were offered to insects in 3.0% agar solution. The aphid individuals were not previously starved as in previous experiments, but were transferred directly from the culture plants to the weighing-room, where they were weighed after anaesthetizing with carbon dioxide and allowed to feed on various test diets. At the end of the test period, besides calculating the differential amounts of material taken up by the aphids from various test diets, the number of live insects on each diet was also counted, as occasionally one or more adults died through disease, injury or the poisonous effects of some constituent in the diet. However, since insect losses were relatively infrequent and their effects on the counts could not be estimated, these data are not presented in the tables. As all these experiments were repeated at least three times there was little possibility that this source of error might lead to misinterpretation.

The results obtained in these tests, represented in Table 5, clearly illustrate the typical response of aphids to concentrations of total nutrients. The term „total nutrients” here not only includes the actual

Table 5. Feeding responses of *Aphis fabae* Scop. adults to various concentrations of young bean leaf-extracts offered to insects in 3.0% agar solutions. Aphids were not deprived of food or water prior to feeding

Diet No.	Percentage of leaf-extract	Weight gained (in mg.) by the aphid after feeding for	
		3-hours	24-hours
1	2.0	0.029 (25)	0.077 (10)
2	4.0	0.055 (26)	0.084 (15)
3	6.0	0.070 (38)	0.092 (23)
4	8.0	0.082 (20)	0.130 (16)
5	10.0	0.09 (32)	0.152 (21)
6	50.0	0.096 (21)	0.183 (19)
7	100.0	0.098 (21)	0.207 (11)

Note: Figures in parenthesis indicate the number of aphid weighed to arrive at the figures of weight gain per aphid.

nutrients, viz. amino acids, vitamins, etc., but also the token and feeding stimuli as well as various repellents or toxins.

A clear-cut correlation between feeding responses and concentrations of total nutrients in the range of 2 to 100 per cent is obtained. Since all the nutrients changed in concentration with every dilution of the extract, it cannot be assumed that the feeding-response in this particular case is governed only by the concentrations of specific feeding or other stimuli. This distinction was overlooked by Nourteva (38).

That the effectiveness of a feeding stimulant may be markedly affected by the presence of other (nutrients) constituents was shown by the preliminary experiments (Table 4) in which ascorbic acid did not make agar very attractive in the absence of nutrients. The same Table shows that, however, leaf-extract media containing nutrients, which are themselves only moderately attractive, became highly palatable upon the addition of ascorbic acid.

C. Various authors, like HilleRisLambers (23), have repeatedly attached much significance to the pH value of the cell-sap of a host-plant. According to them, other conditions remaining the same, the leaf-feeders are directed to feed on a plant by its pH value. This, in other words, implies that the pH value of the cell-sap of a host is also a feeding stimulant.

To judge the correct value of the cell-sap pH on aphid feeding behaviour, experiments of the type described below were carried out.

In this series of tests, extracts of the young leaves of various hydrogen ion concentration were tested. The pH values given refer to the

reaction of the distilled water used to make up the diet. Sorensen's buffer mixture (c.f. 43) was used to adjust the pH.

The feeding responses to various buffered diets are reported in Table 6. Although the means do not differ significantly, the feeding responses fall off with an increase in alkalinity and the highest response is obtained at pH 5.1, the value of which is not greatly different from

Table 6. Feeding responses of *Aphis fabae* Scop. adults to bean leaf-extracts (in 3% agar solution) buffered at various hydrogen ion concentrations.

Diet No.	pH	Percentage weight lost or gained after 24-hours feeding	Standard error
1	4.5	+15.73 (25)	0.4
2	5.1	+16.96 (10)	1.2
3	5.9	+14.06 (20)	1.8
4	6.6	+14.04 (30)	2.1
5	7.6	+13.04 (45)	6.6
6	8.5	+12.90 (55)	5.8
7	9.5	+13.11 (60)	6.7
8	10.0	+14.03 (80-90)	8.2

Note: Number of replicates = 3.

\* denotes the percentage of mortality among the adults due to poisonous effects of the diets.

that of the cell-saps of the leaves of beans and sugar beet plants which were found in duplicate determinations to be 5.5 and 6.2 respectively. It is, however, interesting that not even pH 10.0, which is highly poisonous, as can be very well judged from the percentage mortality of the adults feeding upon the diet with its pH at 10.0, released any avoiding activity. Thus, whereas the aphid mortality on this diet was somewhere in the regions of 80 to 90%, the weight gained by the aphid on this diet was equal to that gained by the aphid feeding on diets with pH 5.9 and 6.6. This implies that other conditions remaining the same, the pH value of the cell-sap has neither any stimulating nor any inhibiting or repellent effect upon insect feeding activity.

### III AN EXPERIMENTAL STUDY OF THE ROLE PLAYED BY OLFACTORY AND GUSTATORY STIMULI IN ATTRACTING THE INSECTS TOWARDS LIVING PLANTS.

To study the influence of the various olfactory and gustatory stimuli upon the host-selection and acceptance behaviours of the aphid under natural conditions two series of green-house experiments were con-

ducted, one in which previously starved aphid individuals were used, and another using fully-fed aphids. These experiments were of two types, behaviour and feeding-response studies, often carried out simultaneously on the same set of plants. In each experiment three or more differently treated plants, each represented by several separate plants, were compared.

Bean seeds (*Vicia faba* L., English Tick beans, variety „Niki 7”) were sprouted on wet-trays, and were then transferred to earthen-pots containing sterile sand. For purposes of treatment these loaded earthen-pots were divided into 5 groups, each containing 10 to 13 loaded pots. The methods of treatment were:

- (a) watering with 5.0% vitamin C solution, (Plant Group 1)
- (b) watering with 5.0% sucrose solution, (Plant Group 3)
- (c) watering with 10.0% vitamin C solution (Plant Group 2)
- (d) watering with 10.0% sucrose solution (Plant Group 4), and
- (e) watering with Knop's and Hass and Reed's complete nutrient solutions (Plant Group 5).

In addition, 20 plants growing in normal soil, with a top dressing of farmyard manure and John Innes' potting compost (28) were denoted as plant group No. 6 and used as a control.

For the first series of tests 10 alatae and 10 apterous adults were selected from the culture-plants. These adults were kept without food and water for about 15 hours. Then they were anaesthetized with carbon dioxide, weighed on a Roller-Smith balance, and were let loose on the plants. After 24 hours of feeding activity upon these various groups of plants they were again weighed. The data recorded here are reproduced in Table 7.

Table 7. Behaviour experiments: Percentage majority and the loss and gain in weight of aphids settled on various plant groups

Plant Group No.	Percentage of aphid found settled		Average percentage weight lost or gained	
	Alatae	Apterae	Alatae	Apterae
1	40.0	50.0	+ 9.98	+ 8.22
2	50.0	40.0	+ 9.93	+ 8.43
3	30.0	50.0	+ 3.03	+ 2.96
4	40.0	30.0	+ 1.04	+ 1.93
5	50.0	40.0	+ 9.85	+ 9.04
6	60.0	30.0	+11.53	+10.06

Note: Aphids were deprived of food prior to feeding on the treated plants.

Number of replicates = 10.

For purposes of comparison leaves of these six groups of plants were offered to insects in multi-leaf cages.

In order to obtain data for Table 8 these plants were caged under individual plant-cages (Fig. 3), and each plant was infested individually with ten apterae and alatae females. These females, however, were also starved previously. This experiment was continued for a total period of fifteen days. On the 16th day the strength of aphid-colonies on each plant group was noted. The observations then made are reported in Table 8.

Table 8. Feeding response studies: development of the aphid colonies on various plant-groups.

Plant group No.	Total number of aphid per plant	Percentage of adults per plant	Percentage of alatae female per plant
1	87.9	23.3	2.7
2	148.7	15.8	1.8
3	55.2	9.0	28.3
4	39.6	3.4	53.9
5	152.5	43.8	1.7
6	121.6	41.0	0.20

Number of replicates = 13.

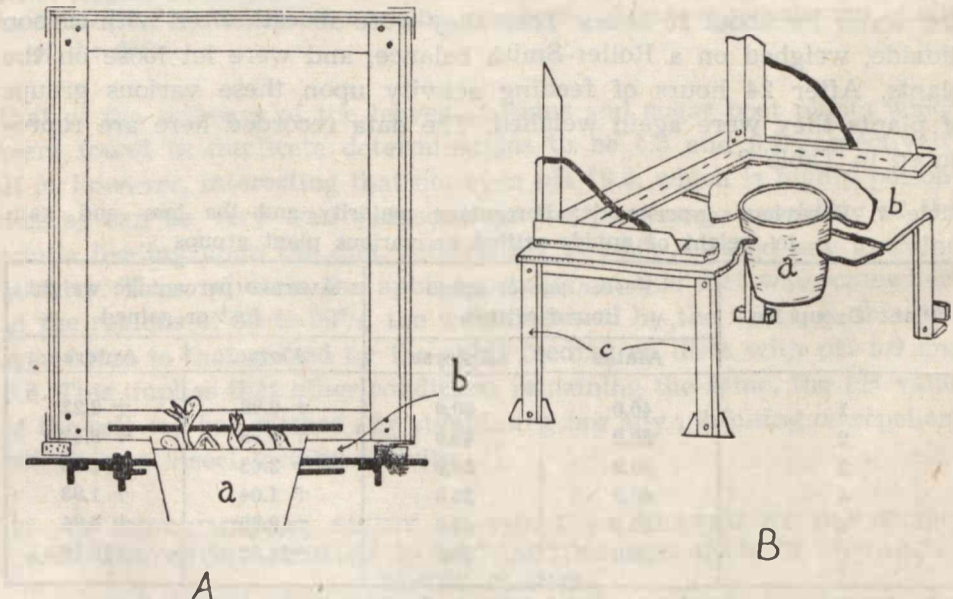


Fig. 3. Single-plant cage (adapted after Kennedy and Booth (28); A — Front view; and B — Cross section, a — Earthen pot containing bean plants; b — Removable base; c — Supports, steel.

From the combined results in Tables 7 and 8 it can be seen that whereas plant-groups 1, 2, 5 and 6 did not differ significantly from each other, they did differ from the plant-groups Nos. 3 and 4. This implies that the plant-groups 3 and 4 were not suitable for the aphid, and since these groups were receiving sucrose treatment the obvious conclusion is, therefore, that the harmful effects to these plant groups were imparted by sucrose.

To confirm this a study of the effects of large quantities of sugars and other treatments of the act of honeydew excretion in the aphid was made. The following subsection specifically deals with this side of the project.

(a). Honeydew excretion versus quality and quantity of nutrients.

The plant material was prepared and denoted by group numbers as in the experiments mentioned above. Hinged 1.0 x 1—1/4 x 3.0 inches bags made of transparent Visking dialyzing tubing (obtained by courtesy of Professor R. H. Painter of the Kansas State College of Agriculture Manhattan, U.S.A.) were adapted to make aphid cages and to provide an efficient means of collecting the droplets of honeydew. Five holes drilled at various places in these bags were covered with fine white muslin cloth to provide ventilation. These bags were notched with a V-shaped groove at one end to facilitate insertion of the leaf, with a damp cotton-wool plug for protection, into the bag. These bags were held in place with the help of wooden stakes.

A representative sample of ten adults each were let loose on various plant groups under these bags, and the number and size of the honeydew droplets excreted by them were recorded at the end of 24 hours. The data collected here are reported in Table 9.

Significant differences occurred between aphid feeding upon plant-groups Nos. 1 and 2, the most acceptable groups, and plant-groups Nos. 3 and 4, the least accepted groups. Highly acceptable plant-groups, viz. Nos. 5 and 6, showed highly significant differences from the plant groups 3 and 4. However, there was no significant difference between plant-groups 1 and 2, and 5 and 6.

It should be understood here that the excretion of the honeydew in aphids is dependent upon two main factors:

- (a) the ability of the aphid to intake a given quantity of the cell-sap and
- (b) the amount of the sugar presented in the ingested cell-sap.

It was observed, during the period of our experimentation on honeydew excretion, that the aphid feeding upon plant-groups 3 and 4 not only excreted a large number of honeydew droplets but also excreted large-size droplets. The reason for this could only be that these plant-groups contained high amounts of sugars. Support for this

Table 9. The influence of the host-plant, subjected to various treatments upon the rate of honeydew deposition by *Aphis fabae* Scopoli after a 24-hours' feeding period

Plant-groups number	No of replicates	Average of droplets per aphid	Treatments on left significantly different from <sup>1)</sup>					
			PLANT - GROUPS NUMBER					
			1	2	3	4	5	6
1	33	13.2		n.s	x	x	n.s	n.s
2	38	12.2			x	x	n.s	n.s
3	24	28.5				n. s	x	x
4	15	30.7					x	x
5	27	12.1						n.s
6	7 <sup>2)</sup>	10.4						

Note: <sup>1)</sup> n. s. = non significant; (x) = significant at 0.1% level.

<sup>2)</sup> The low number of replications used to obtain an average number of honeydew droplets excreted per aphid was due to the high mortality of the aphid individuals on these plants. Many aphids were caged on these plants but only a few lived, and they themselves spent much time in wandering rather than feeding and thus excreted very few honeydew droplets. If zeroes had been included, averages would have been much lower.

conclusion comes from the work of Alikhan (1), who states that the quantity and the quality of honeydew excreted by aphid is directly proportional to the quantity and the quality of the sugars present in the cell-sap of the host on which the aphid is foraging. The statement made by Alikhan (1) is further supported by the work of Kennedy (27) on *Aphis fabae* Scop., and of Mittler (37) on *Tuberolachnus salignus* Gmelin.

The results obtained in the second series of tests, where not starved but fully-fed aphid adults were used, are reported in Tables 10, 11, and 12.

When comparing the observations reported in Tables 10, 11 and 12 with those reported in Tables 7, 8 and 9, one realizes that the aphid preferences appear more markedly in the former than in the latter case. This seems to be because the aphid individuals in the second series of tests, were not starved and therefore had ample time to select the plants which suited them best. The results tabulated in Tables 7 and 10 are very striking in this respect.

Another striking feature is the figures tabulated against plant-groups Nos. 3 and 4 (Tables 7 and 10). It will be noted that whereas the starved individuals gained some weight on these plant-groups (Table 7), they actually lost weight when fully-fed (Table 10). This suggests that the



Table 10. Behaviour experiments: percentage mortality and the loss of gain in weight of aphid settled on various plant-groups. (series 2)

Plant-group No.	Percentage of aphids found settled on		Average per cent wt. lost or gained	
	Alatae	Apterae	Alatae	Apterae
1	16.0	18.0	+ 5.36	+ 3.68
2	19.5	21.0	+ 5.91	+ 3.84
3	11.5	8.0	- 1.68	- 2.84
4	9.5	5.5	- 1.85	- 3.72
5	25.0	26.5	+ 7.30	+ 7.86
6	28.5	21.0	+ 6.63	+ 7.75

Note: All the plant-groups were compared within the same experiment rather than separately, as was done in the experiments of series 1.

Leaves of the various plant-groups were compared with the help of multi-leaf cages.

Number of replicates = 9.

Table 11. Feeding-response studies: development of aphid colonies on the plant-groups under various treatments. The aphid were not starved as against experiments of the 1st series

Plant-group No.	Total number of aphid per plant	Percentage of adults per plant	Percentage of winged females per plant
1	234.6	23.75	3.37
2	151.83	37.75	3.48
3	67.27	17.49	35.04
4	34.75	11.11	63.34
5	204.95	53.74	4.38
6	122.45	40.12	5.08

Note: Observations were extended up to a period of 21 days.

Number of replicates = 9.

starved aphid fed on these plants because of hunger alone. This implies that hunger also acts as a feeding stimulant.

However, it will be noted that whether the aphid individuals were fully-fed or starved, they showed relatively more, though insignificant at all the statistical levels used, preference for plant-groups 5 and 6 than for plant-groups 1 and 2. This difference, because it occurred so consistently, could not be attributed to experimental errors. Thus it appears that these differences are due to some other nutritional components of the plant cell-sap which also act as feeding stimulants along with vitamin C and sucrose. According to Alikhan (in Litt.), Beck and

Hanec (c.f. 47), Kennedy (27), Maltais and Auclair (34), Mittler (37), Rasmussen (c.f. 47), and Thorsteinson (45, 47), these are compounds belonging to vitamin B-complex and nitrogen groups. A thorough discussion of the role played by these compounds has been published by Thorsteinson (47). According to him thiamine and alpha-tocopherol (members of the vitamin B-complex group), peptone, amides, betaine, asparagine, monosodium glutamate, alanine, serine and gamma-aminobutyric acid (members of the amino acid group) have been found to stimulate feeding by *Chorthippus longicornis* (Latrielle), *Tribolium confusum* (Duvall), *Hylotrupes bajalus* (Linn.), *Pyrausta nubilalis* (Hübner), and *Leptinotarsa decemlineata* Say by Beck and Hanec (c.f. 47), Losciavo and Thorsteinson (in Litt.), Rasmussen (c.f. 47), Thorsteinson (45) and Thorsteinson et al (in Litt.). Unfortunately, because of the time-limit, it was impossible to study the individual effects of these chemicals on the feeding behaviour of the aphid; to provide direction for future work the indirect combined effects of nitrogen compounds upon the feeding responses of aphid were paid some consideration. The following subsection deals with this side of the project.

#### IV AN EXPERIMENTAL STUDY OF THE INFLUENCE OF THE QUANTITY OF NITROGEN ON THE FEEDING BEHAVIOUR OF APHIS.

These experiments were more or less of the same type as those carried out by Nourteva (38) with *Aphrophora alni* and *Empoasca* sp.

The test plants, e.g. *Vicia faba* L. English Tick beans variety „Niki 7”, were raised in the same way as in the previous experiments, but here there were three types of treatment only, i. e.

- I. Plants raised on normal soil and watered with water alone,
- II. Plants raised in sterile sand and watered with 10 gm. of egg-albumin daily, and
- III. Plants raised in sterile sand and watered daily with 10% solution of ammonium sulphate.

Once a week all these plant-groups were watered with normal water (e. g. tap water).

These treatments were carried out for a total period of 13 days, after which the leaves of the plants were offered to the insects in the same way as in the previous experiments, e. g. in multi-leaf cages. The results of these behaviour experiments are tabulated in Table 13.

The various plant-groups, according to the treatment they were receiving, varied considerably in their external appearance and obviously

Table 12. The influence of the host-plant, subjected to various treatments, upon the rate of honeydew deposition by *Aphis fabae* Scopoli after 24-hours of feeding period. As against series 1 experiments, the aphids were not deprived of food prior to feeding

Plant-group number	Average No. of droplets per aphid	Treatments on left significantly different from <sup>1)</sup>					
		Plant-groups number					
		1	2	3	4	5	6
1	4.66		n.s	x	x	n.s	n.s
2	4.30			x	x	n.s	n.s
3	9.30				n.s	x	x
4	12.30					x	x
5	4.90						n.s
6	5.25						

Note: <sup>1)</sup> Number of replications = 12

n. s. = Not significant; x = Significant at 1% level.

Table 13. Preference of adults of *Aphis fabae* Scop. for leaves of various plant-groups receiving various treatments. The aphid were not deprived of food prior to feeding on these plants

	Percentage of aphid settled on	Percentage of restless aphids	Percentage of weight gained after 24 hours feeding
1. Control	23.3	30.0	9.04 (3)
2. Albumin	40.0	6.6	14.61 (4)
3. Ammon. Sulphate	33.3	13.3	16.32 (4)

Note: Number of replicates = 6.

The percentage loss or gain in weight was calculated on the basis of weight lost by a representative sample of aphid individuals kept in glass vials without food or water for the duration of the experiment.

Figures in parenthesis indicate the number of aphid individuals weighed to arrive at the percentage weight lost or gained.

in their physiological status too. Thus the control plants appeared yellower than the treated plants. The treated plants, on the other hand, were not only the greenest but also were taller and bushy, and although the control plants were vigorously flowering and podding by the end of the experimental period, not a single flower opened on the treated plants.

It will be noted from the foregoing table that although the majority of the adults settled on albumin-treated plants, more weight was gained by the aphid which fed on the ammonium sulphate-treated plants. This implies that among treated plants, as far as the selection of a particular host was concerned, albumin-treated plants were preferred, while the cell-sap of ammonium sulphate-treated plants were

more fed upon. This means, as Kennedy (27) has pointed out before, that the aphid exerts two types of preferences, (1) behavioural, and (2) nutritional, the former depending upon the olfactory and the latter on the gustatory stimuli provided by the host. The first type of behaviour, therefore, can be called the „botanical preference” and the second the „nutritional preference”. An excellent explanation and discussion of these two types of behaviour has been given by Kennedy (27).

Having thus established experimentally that the host-selection in aphid is brought about by a combination of various factors, of which the primary plant substances play a major role, the next obvious step was to see of what practical value these findings are. From the point of view of an economic zoologist the testing field is that of evolving resistant plant varieties. Ultimately studies were carried out at this institute, during 1960, and the results achieved are reported in the following section.

#### V THE FACTORS GOVERNING THE RESISTANCE OF BEANS TO BLACK BEAN APHIS.

Seeds of *Rastatter* (R) and *Schlanstedter* (S), two German varieties of *Vicia faba* L., were obtained from the Institut für Pflanzenzüchtung, Quedlinburg, Germany, by courtesy of Professor Hans J. Müller, and seeds of Niki 7 (T), a commercial variety of English Tick beans, were kindly supplied by Dr. N. H. E. Gibson, the University of Leeds, England. These were sown together with the seeds of *Phaseolus vulgaris*, variety „The Prince” (N, below), and variety „The Snap-pod” (P) — supplied by the Institute of Agricultural Botany, Cambridge, England — on April 14th, 1960, at the Botanical Garden, Lublin, in the layout shown below.

T S N R P T S N R P  
S N R P T S N R P T  
N R P T S N R P T S  
R P T S N R P T S N  
P T S N R P T S N R

24 centimeters were left between each sowing position and the next to allow access to the plants for recording purposes. Most of the *Phaseolus vulgaris* plants failed to germinate; records from this variety, therefore, were discontinued, to permit concentration of attention on the three varieties of *Vicia faba* L. Five seeds of each variety were sown close together at each position to give more plant mass for aphids when the plants were young. On April 25th the plants were thinned to leave two vigorous specimens at each position. Growth recording and the counting of aphid were started on April 29th.

The type of growth record made was determined largely by aphidological considerations. The height (length of longest shoot) of each plant was recorded as well as the number and combined length of shoots. The compact bunch of buds and unexpanded leaves at each growing shoot-tip was counted as one unit, including developing inflorescences consisting of closely adpressed floret buds between which the aphids could not crawl. The nodes with expanded leaves were counted as individual units and classified according to the stage of the flowers and fruits, if any. Nodes with loosening floret buds between which aphids could crawl were distinguished from nodes at which the flowers had opened, and these in turn from nodes at which pods were visible. Nodes were counted as flower or pod nodes only so long as at least one flower or pod remained there, after which they were counted simply as expanded leaf nodes. The last growth records were made on July 10th; on August 28th the remaining plants were harvested (Fig. 4).

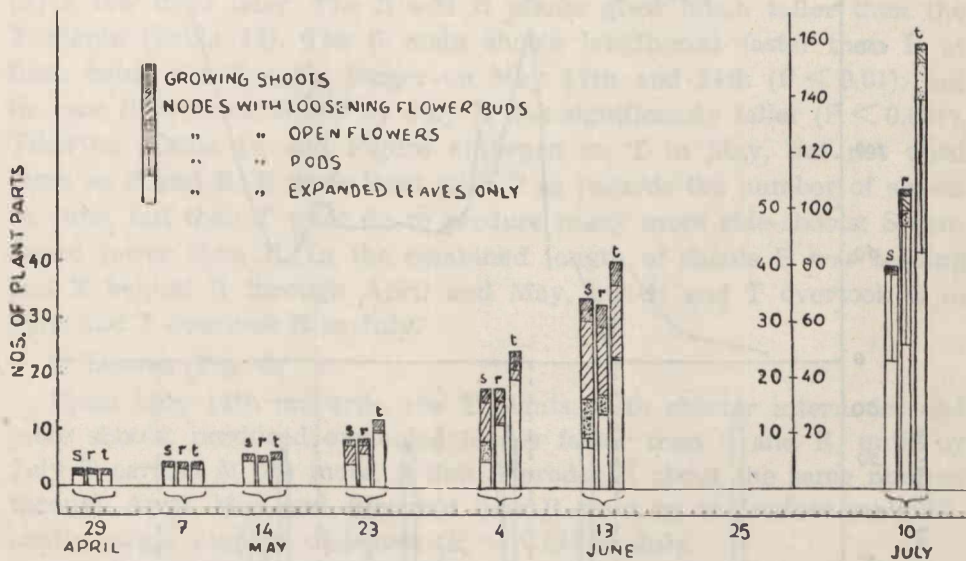


Fig. 4. Growth, flowering and fruiting of *Schlanstedter* (s), *Rastatter*, (r) and Tick beans (t). Mean numbers of the various plant parts developed per plant, based on 50 plants of each variety for the first three dates and 5-6 thereafter

From May 14th to June 25th the winged *Aphis fabae* were counted on these plants. Only those alatae which were settled and apparently feeding were counted; many of these were in or between the furled leaves at the shoot-tips, or between the loosening floret-buds, which were gently parted to find them. In an attempt to obtain some information on their reproduction as well as their incidence in the plants,

the alatae were not removed at every count, but they and their progeny were all killed and washed off with Wofatox (liquid) spray (supplied by the District Quarantine and Plant Protection Station, Lublin) at intervals (Fig. 5), to prevent the production of second generation progeny and minimize damage to the plants. On each of eleven days in June the alatae were counted separately on the various organs of the plants and removed at each count. Neither these removals nor the periodic sprayings made any obvious difference to the daily numbers of alatae found (Fig. 5). It appeared that most of those counted each day had left again by the next and most counted each day were new arrivals.

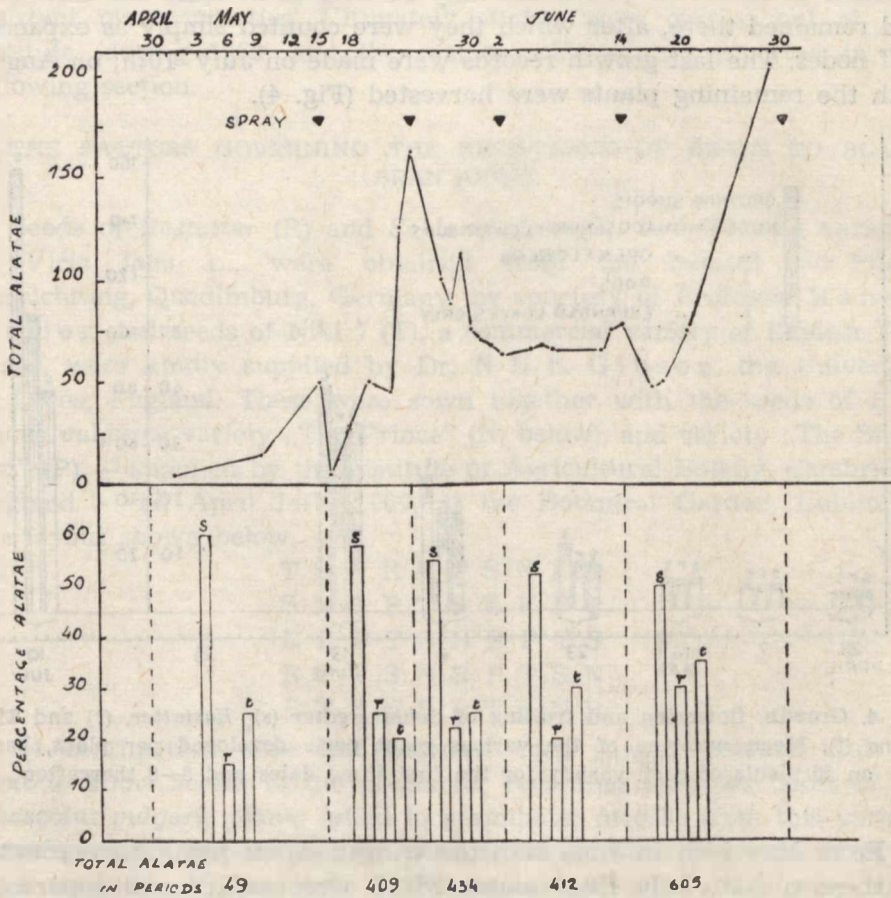


Fig. 5. Alatae aphids counted on the plants. Above: combined totals on all plants on individual days. Open columns — *Aphis fabae*; Solid column — Aphids other than black bean aphid. Below: mean daily percentage of *Aphis fabae* on each variety in five successive periods.

For chemical analysis, the plant-samples consisted of the terminal growth (i. e.) from the terminal bud down to the base of the fourth internode), and of the middle growth (i. e. from the 5th node to the base of the 6th internode), each sample being taken from 6 plants of each of the three replicates.

Analysis of total and soluble nitrogens and invert sugar was carried out by the reduction methods of Kjeldahl and Fehling respectively, while for vitamin C analysis the visual titration method of Farmer and Abt (1936) was used. All these methods have been described fully by Paech and Tracey (1956) in their five-volumed book „Moderne Methoden der Pflanzenanalyse“: Berlin, Springer-Verlag (vol. 1—4).

## Plant Growth

### I Shoots

*Schlanstedter* (S) and *Rastatter* (R) emerged together and the *Niki 7* (T) a few days later. The S and R plants grew much taller than the T plants (Table 14). The S main shoots lengthened faster than R at first, being significantly longer on May 17th and 24th ( $P < 0.01$ ); but in June R overtook S and by July R was significantly taller ( $P < 0.001$ ). Tillering (Table 14 and Figure 6) began on T in May, but not until June on S and R. R drew level with T as regards the number of shoots in June, but then T went on to produce many more side-shoots; S produced fewer than R. In the combined length of shoots S was leading and T behind R through April and May, but R and T overtook S in June and T overtook R in July.

### II Leaves (Fig. 4).

From May 14th onwards, the T plants, with shorter internodes and more shoots, produced expanded leaves faster than S and R, until by July T carried 50.0% more. R and S produced about the same number through April, May and June but then R went on to produce a significantly larger number of leaves ( $P < 0.01$ ) in July.

### III Flowers and Fruits (Fig. 4).

The first flower buds developed at about the 8th node (above soil level) on the main shoots of S, about the 10th of R and about the 12th of T. Hence the „loose” flower buds became available earliest on S and latest on T. Nodes with such buds were significantly more numerous on S and R in the last week of May ( $P < 0.01$ ) and first week of June ( $P < 0.001$ ). Through June and July, as the S flowers opened and the pods formed, while the shoot growth slowed in relation to R, the number of nodes bearing „loose” flower buds on R equalled and then surpassed those on S. Flower opening and pod formation on R showed

a corresponding initial lag behind S before overtaking. T lagged still more, starting to flower only in mid-June but finally producing more flowers and pods than R.

The later produced units classed as „growing shoot tips”, and „loose” flower-buds on any one shoot were naturally less vigorous, containing fewer, smaller and less bunched growing leaves or buds than the earlier

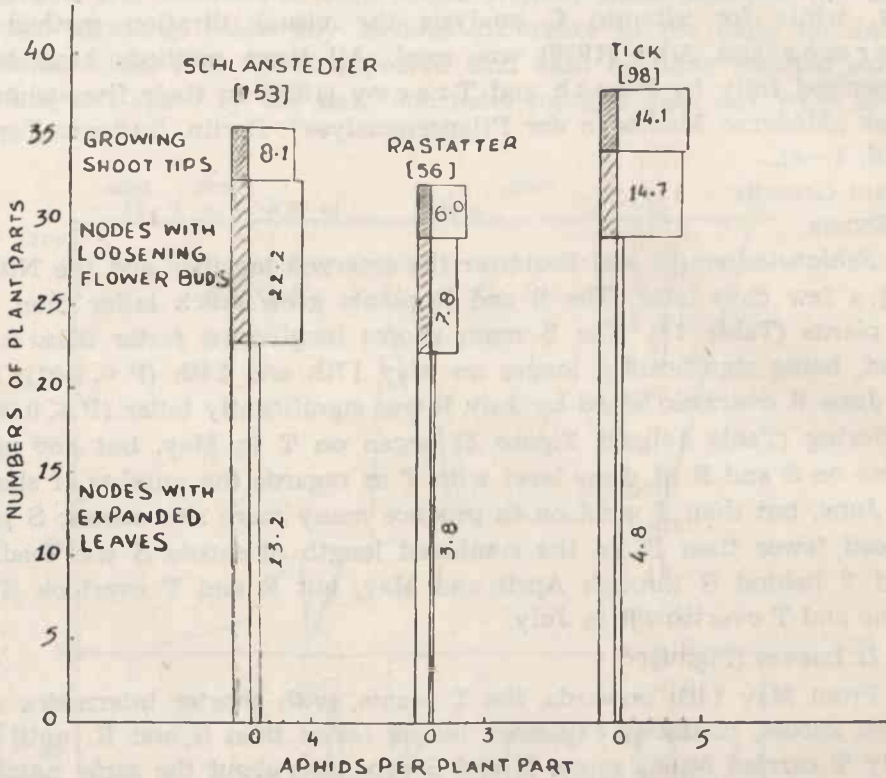


Fig. 6. Distribution of alatae *Aphis fabae* on the plants, June 5th—12th (seven counts). Mean numbers of plant parts available through this period shown in the vertical columns. Mean daily percentage aphid incidence among parts and varieties shown by the areas of the blocks against the right side of the columns, the width of each block indicating the relative density of aphids on the parts. Numbers in brackets under varietal names are total aphids for the period.

ones; later pods were not filled and later flowers fell without opening. That is to say, the more numerous leaves and inflorescence produced in late June-July by R, and still more by T, were „starved” by comparison with the organs similarly classified but produced earlier by S. On the other hand, the shoot tips of T became the most vigorous in June as the S and then the R slowed down.



Thus the growth pattern of *Rastatter* fell between that of *Schlanstedter* and *Niki 7* in this trial; R flowered and ripened later than S, but earlier than T. The early vigorous growth of S was more concentrated in fruits and that of T in shoots, while that of R was divided more equally between fruits and shoots. Aphid incidence.

(a). Total *Aphis fabae* on the plants

Counts of alatae were made on a total of 29 days and they were fewer on R than on S on every day except one. There were fewer alatae on R than on T also, except for 5 days in mid-May and 3 days in mid-June; and there were fewer on T than on S on all but 2 days. These counts are summarized in Fig. 5 where the daily percentage of all alatae found on the three varieties is shown as a mean for each period between sprayings. When the alatae began to arrive at the end of April and the beginning of May there was an average of 3 to 4 alatae on S for every

Table 14. Shoot growth on the three varieties: *Schlanstedter* (S), *Rastatter* (R) and *Tick* (T). Means per plant.

Date	No. shoots			Length of longest shoot, cm.			Combined length of shoots, cm.		
	S	R	T	S	R	T	S	R	T
29/4	1	1	1	5.9	5.6	3.6	5.9	5.6	3.6
7/5	1	1	1.1	12.0	10.2	6.5	12.0	10.2	6.3
14/5	1	1	1.3	19.3	16.3	10.5	19.3	16.3	10.9
23/5	1	1	2.0	31.8	30.2	19.3	31.8	30.2	28.6
4/6	2.5	2.4	3.3	52.7	53.6	33.9	68.8	72.8	75.9
13/6	3.4	4.0	4.0	70.1	76.7	48.4	133.6	148.1	139.6
10/7	4.1	5.5	9.0	103.4	125.0	88.9	247.1	435.5	521.0

Note: These figures are based on 50 plants of each variety for the first three dates and 5 to 6 thereafter, since a large number of plants of each variety were harvested for analysis purposes.

one on R or T, but this ratio fell steadily thereafter and was about 4:3 after mid-June. Accordingly, the differences observed on single days between S on the one hand, and R or T on the other, were statistically significant on most days in May but not in June. The progeny counts (Table 15) show a similar fading of the previous clear varietal differences by late June, when all the plants were maturing.

Among plants of one variety there was no correlation between plant height and the number of alatae collected; but there was an „edge-effect” in that the plants along the more exposed west and north borders of the plots collected more alatae than the others.

(b). Distribution of *Aphis fabae* on the plants

Table 15. Total *Aphis fabae* progeny on the three varieties of beans

Date	<i>Schlanstedter</i>		<i>Rastatter</i>		<i>Niki 7</i>	
	Total No.	Percentage	Total No.	Percentage	Total No.	Percentage
14.5	287	41.3	101	14.4	306	44.2
23.5	451	58.3	135	17.6	184	23.9
25.6	2523	33.2	2155	28.2	2931	38.6

Vigorously growing organs were preferred as they became accessible on all the varieties, the furled leaves at the shoot-tips and the loosening flower buds below. Most settled alatae had crawled into crevices but the exposed ones too were commoner on growing shoot tips and flower buds than on expanded leaves, flowers, pods or stem. The progeny were still more concentrated into crevices, feeding particularly on bud bases, pedicels and the adjacent stem. Thus on each variety the aphid were first concentrated at the shoot tips, then in later counts an increasing proportion appeared among the flower buds, and finally they were found more or less evenly distributed up the plant as it matured. Because S flowered first, then R and then T, these distribution changes occurred „out of phase” in that order. The drop in the total number of alatae (Fig. 5) and progeny (Table 15) on T in relation to S and R in May was clearly due to the loosening and colonisation of the flower buds on S and then on R, before T (Fig. 4). The proportion of alatae on the flower buds rose to a high level and remained high for longer on S than on R before it fell again with approaching maturity; on T the proportion on the flower buds never rose much higher than that on the shoot tips.

Table 16 and Figures 8 and 9 illustrate these differences. For the construction of Figure 8 the numbers of plant parts present on each separate day were estimated by interpolation between the counts made on June 4th and 13th (Fig. 4) to obtain more accurate estimates of the aphid numbers per part (densities), which provide the most direct measure of the aphid preferences. Later in June the number of loosening flower buds declined and the records for that period are inadequate to estimate the numbers of plant parts available for colonisation. Figure 9, therefore, shows simply the percentage of alatae on the three classes of plant parts.

Early in June the alatae were about three times denser (horizontal axes in Fig. 6) on the shoot tips, and on the flower buds, than on the expanded leaves ( $P < 0.001$ ), and the 3 varieties did not differ significantly in this respect. But the mean density of alatae on all parts combined was about 40.0% higher on S and T than on R ( $P < 0.01$ ).

The smaller total number of alatae (Table 16, and areas in Fig. 6) on R than on S was mainly due to the smaller number settling among the loosening flower buds of R ( $P < 0.001$ ). This in turn was due partly to the lesser density of alatae on the individual R buds and partly to the fact that at this time there still were fewer nodes with

Table 16. Mean daily number of alatae of *Aphis fabae* (expressed as square roots) on the shoot tips, flower buds and expanded leaves of the three varieties on seven days (June 5th to 12th) and four days (June 14th to 25th)

	June 5th to 12th			June 15th to 25th		
	S	R	T	S	R	T
Shoot-tips	1.9	1.6	2.3	4.1	2.5	3.3
Flower-buds	3.5	1.9	2.6	3.9	4.3	3.8
Expanded leaves	2.4	1.3	1.4	4.0	3.2	2.7
Least significant difference at						
5%		0.7			0.9	
1%		0.9				
0.1%		1.2				

such buds available on R than on S (vertical axes in Fig. 6). The shoot tips of R were rather more numerous (if less vigorous), so that the alatae here, although less dense, were in all hardly fewer than on S. The total number of alatae on the expanded leaves was significantly lower on R than on S ( $P < 0.01$ ).

The difference between the total numbers of alatae on R and T was not made up in the same way, for in this case the shoot tips contributed as much to the differences as did the flower buds ( $P < 0.05$  for both), and the expanded leaves contributed nothing. The higher density of alatae on the flower buds of T more than compensated for the larger number of such buds on the R plants at this time. Earlier in May and the first days of June, when there were few flower buds on T, these naturally contributed less to the difference in the total numbers; the shoot tips then carried the majority of alatae on T and were almost entirely responsible for the overall difference between T and R.

The situation changed considerably after mid-June (Fig. 7 and Table 16). Some of the alatae were now found on the pedicels of open flowers and these have been counted in with those on expanded leaves. On the expanded leaves themselves, some preference now appeared for the lowest, which were showing signs of incipient senescence. With leaf and inflorescence growth coming to an end on S, the alatae were no

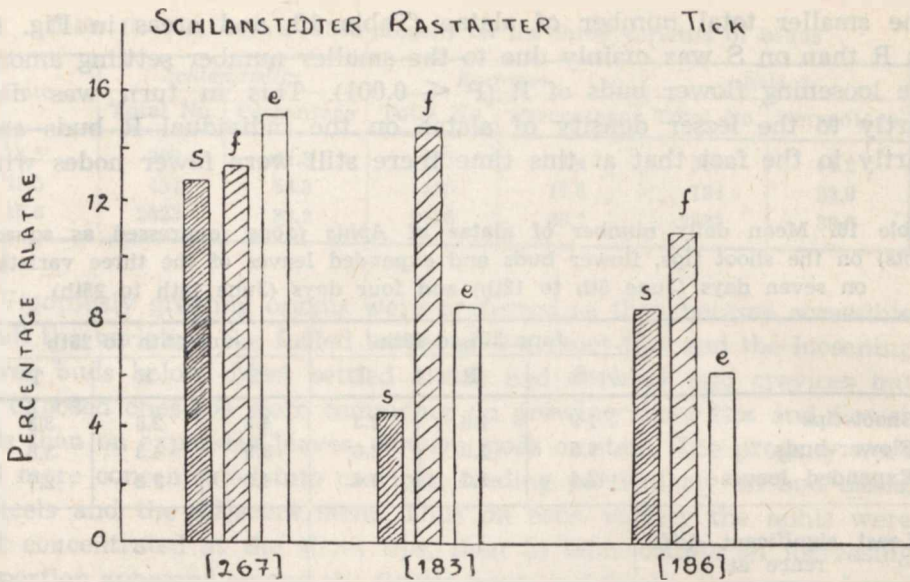


Fig. 7. Distribution of alatae *Aphis fabae* on the plants on June 14th—25th (four counts). Mean daily percentage incidence of aphids among parts and varieties with percentage on growing shoot tips (s) nodes with loosening flower buds (f) and nodes with expanded leaves (e) shaded to correspond with figure 8. Numbers in brackets are total aphids for the period.

Table 17. Nitrogen and sugar contents (percentage of dry matter) and sugar-nitrogen ratios of the terminal growth of three varieties of Broad beans at two different stages of their growth

	<i>Niki 7</i>	<i>Schlanstedter</i>	<i>Rastatter</i>
Total Nitrogen			
Early growth	6.02	5.74	4.48
First blossom	4.69	5.04	3.92
Soluble Nitrogen			
Early growth	3.05	2.63	1.89
First blossom	1.89	2.10	1.68
Invert Sugar			
Early growth	6.7	11.2	17.4
First blossom	20.4	19.4	23.6
Ratio of sugar to total nitrogen			
Early growth	1.1	1.9	3.9
First blossom	4.4	3.8	6.0
Ratio of sugar to soluble nitrogen			
Early growth	2.2	4.3	9.2
First blossom	10.8	9.2	14.1

Table 18. Nitrogen and sugar contents (percentage of dry matter) and sugar nitrogen ratios of the middle growth of the three varieties of Broad beans at three different stages of their growth

	Niki 7	Schlanstedter	Rastatter
Total Nitrogen			
Early growth <sup>1</sup>	5.60	5.88	4.69
First blossom <sup>2</sup>	5.74*	6.03	5.25
Full bloom <sup>3</sup>	5.12	5.54	3.71
Soluble Nitrogen			
Early growth	2.10	2.52	1.89
First blossom	3.47	2.94	2.63
Full bloom	2.31	2.21	1.68
Invert Sugar			
Early growth	12.6	9.6	13.1
First blossom	4.4*	4.4	6.2
Full bloom	16.4	13.4	22.6
Ratio of sugar to total nitrogen			
Early growth	2.2	1.6	2.8
First blossom	0.8	0.7	1.2
Full bloom	3.2	2.4	6.1
Ratio of sugar to soluble nitrogen			
Early growth	6.0	3.8	6.9
First blossom	1.3	1.5	2.4
Full bloom	7.1	6.1	13.5

Note: 1 30-days' old plants.

2 40-days' old plants.

3 58—60 days' old plants.

\* Since the majority of the leaves on each plant variety were in the early senescing stages, these sudden changes were presumably brought about by a change in the internal oxygen tension of these leaves.

longer most numerous on flower buds. But the R plants were still producing relatively vigorous flower buds and now entered the phase when these buds carried more alatae than the other parts. The R buds were now collecting as many alatae as those on S; although the other parts of S were still collecting more and in all S bore some 20.0 per cent more alatae than R or T ( $P < 0.05$ ). There was little change in the distribution of alatae among the parts of T, but in all the T plants were no longer collecting more than R.

The variety *Rastatter* thus appeared to be somewhat resistant and, consequently, was less preferred by the *Aphis fabae* alatae than *Schlanstedter* and *Niki 7*. However, this resistance and rejection of *Rastatter* was dependent on the physiological status of the plants of this

variety. Thus the preference was most marked when the plants were young and diminished progressively as growth slowed and maturity approached. The alatae settled and reproduced preferentially on the young vigorously growing organs (i. e. those destined to grow most afterwards) of all the three varieties: the shoot tips and especially the young inflorescences. Among these, they preferred the less vigorous ones. Thus their preference for the earlier flower buds (as indicated by their density as compared with other organs on the same plant) was strongest on *Schlanstedter*, which produced the fewest and individually most vigorous ones, and weakest on *Niki 7* which produced the greatest number and individually least vigorous ones. The relative incidence of alatae among organs and varieties varied continuously according to the changing availability of vigorously growing organs, which was in turn governed by the different developmental patterns of the varieties. The overall resistance of *Rastatter* was associated with its „compromise” type of developmental pattern in which shoot and fruit growth were more evenly balanced (growth less concentrated in either) than in the other two varieties during the critical weeks of May and early June. The growth of the earliest variety, *Schlanstedter*, was more vigorous than that of *Rastatter*, for a short time, in the shoots, and more especially in the inflorescences, and it was these two types of organ in the same sequence and timing which collected most of the alatae making up the larger total numbers of *Schlanstedter* in those weeks. The growth of the latest variety, *Niki 7*, on the other hand, was more vigorous in the shoots (leaf-production) than that of *Rastatter* through those weeks, and these organs collected more alatae than the shoot tips and flower buds of *Rastatter* combined. When the *Niki 7* inflorescences eventually developed *Rastatter* was producing its later weaker ones, and here too the *Niki 7* now collected more alatae. A comparison of *Schlanstedter* and *Niki 7* completes the circle. The growth pattern of the *Niki 7* differed from that of *Schlanstedter* in the same way as did that of *Rastatter*, but more so, since *Niki 7* was to flower still later with inflorescences still individually weaker at their best. Correspondingly, the difference in susceptibility between *Schlanstedter* and *Niki 7* was, to an even greater extent than that between *Schlanstedter* and *Rastatter*, due to more alatae being collected by the *Schlanstedter* inflorescences, the shoot tips contributing nothing.

The results of chemical analyses are reproduced in Tables 17, 18 and 19, which clearly show that whereas these plant varieties did not differ very much from each other in their vitamin C contents, the phloem-sap supplying young vigorously growing organs of *Schlanstedter* and *Niki 7* was definitely richer in organic nitrogen (though to a lesser

extent). On the other hand, however, the cell-sap of *Rastatter* was richer in organic sugar contents. This implies that whereas none of these varieties lacked olfactory stimuli, the sap available for aphids on *Rastatter* was less nutritious than on the other two varieties throughout the critical periods of active growth from April to mid-June.

These results not only confirm the results obtained in the preliminary laboratory and greenhouse experiments but also confirm the facts postulated by Alikhan (1) in his „sugar-nitrogen ratio index” hypothesis.

## VI DISCUSSION

1. *Aphis* behaviour. The relative olfactory and gustatory stimulatory effects of vitamin C, saccharides and organic nitrogen were studied both in terms of settling preferences and of average feeding rates, in the laboratory and the greenhouse, both on artificial diets and living plants. The multi-leaf cages and the use of the olfactometer provided clear evidence that *Aphis fabae* selects its food-plants with the help of the smell emitted by vitamin C, and accepts those plants, or plant organs, which are rich in vitamin C and amino-nitrogen. The plants, or plant organs, which are rich in sugars are, however, not accepted.

The results of these laboratory and greenhouse experiments were confirmed in the field by studying the resistance and susceptibility of two German varieties of *Vicia faba* L., i.e. *Rastatter* and *Schlanstedter*, and one English Tick beans variety *Niki 7*. Since these varieties did not differ significantly from each other in their vitamin C contents at any stage of their growth they were equally attractive to the aphid *alatae*. However, as these varieties differed from each other in their nitrogen (the life-giving substance, and the actual nutrition for the aphid), as well as sugar (which is not necessary for aphid metabolism, and acts as repellent or toxin on the feeding behaviour of the insect) contents, they consequently differed in their susceptibility to aphid attack.

2. Review of antecedent theories. It has been adequately established that the feeding behaviour of phytophagous insects, and therefore, food-plant selection, is regulated for the most part by taste and smell. There seem to be two schools of thought concerning what plant constituents provide the stimuli that determine food-plant preferences. In several papers (17, 18, 32), the thesis is pronounced by Fraenkel that food-plant selection is determined solely by the „odd” or secondary chemicals, which plants synthesize for no apparent metabolic purpose. A similar view is advanced by Dethier (11). There is, as pointed out before, in fact, little doubt that many such substances do act as repellents

or inhibitors of oviposition and feeding, and this fact alone would establish them as very significant determinants of food-plant selection.

A very few of these substances, as cited elsewhere, have been shown to function in reverse, stimulating oviposition, aiding in finding food, and stimulating feeding by insects specific to the food-plants that contain them. Although a number of attempts to extend the list of such examples has proved unfruitful or indecisive, Fraenkel (18) and Lipke and Fraenkel (32) have stoutly argued that secondary chemicals are solely responsible for guiding phytophagous insects in general to their preferred food-plants and providing the chemical stimuli required to induce feeding. Dethier (11, 12) states that the causal role of nutrients in food-plant selection is an open question.

Let us consider the statement of Lipke and Fraenkel (32, p. 32).

„The most fundamental aspect of host-selection in leaf eating insects inquires whether the selection is governed (a) by the nutritional superiority of the plant or region of the plant serving as food for the insect, or (b) by the presence or absence of attractants or repellents in plants of more or less uniform food value to which the parasitic species has become adapted. The latter view implies that the token stimuli, i.e. the substances which are responsible for the acceptance or rejection of the host, have no nutritional value for the insect in the sense that they are metabolized and incorporated into the host-tissue or biochemically involved in tissue synthesis.”

An examination of this quotation raises several questions about the validity and appropriateness of these propositions, including:

- a. Is the „nutritional superiority” of the preferred food-plants supposed to regulate (if it does so) the selection of food of insects through behavioural mechanism? Certainly, any other kind of mechanism could operate only through a process of natural selection and is not relevant in the present context. If then the mechanism has a behavioural basis, we must agree that it is improbable that insects can recognize the wholesomeness of a leaf — nutritiousness as such can scarcely constitute a feeding stimulus.
- b. But, is there any reason why some of the nutrient substances in leaves cannot supply chemotactic stimuli that elicit feeding by insects not repelled by any of the other constituents of the leaves?
- c. In proposition (b), why does the postulate, that leaves contain attractants and repellents, imply that all these substances are token stimuli of no metabolic use to the insects? (This is a restatement of point (b) which, however, is related to proposition (a)).

This summary exclusion of nutrients as potential feeding stimulants and regulators of food-plant selection is surprising in view of the fact



that one the most common nutritious constituents of plants (sucrose and vitamin C) has been known for many years to influence gustation in various leaf-eating insects (13).

Even in insects that do not seem to respond to pure sugars or vitamin C, food-selection behaviour may be influenced by the gustatory summation effects of saccharides, or vitamins, with other substances (14, 15). That insects do, by and large, select food-plants which are nutritious is attested by their successful survival, but it is not at all necessary that they sense the taste or smell of every essential nutrient, Sucrose or vitamin C, for example, occur in nature in substrates (leaves or other plant tissues) that, generally speaking, also contain all of the other nutrients required by insects. Oddly enough, it is this same relative uniformity in leaf constitution that led Fraenkel (17) to infer that it must be only „odd” chemicals that regulate food selection. Contributing to this conclusion is the apparent impression that nutrients occur in plants only as proteins, complex carbohydrates, and fats which, in the pure state, are presumed to have no distinctive smell (18). It is, however, generally known that simple carbohydrates (sugars), amino acids and amides, organic acids, vitamins and various other metabolic products occur in plants in a free form. Because many of these substances initiate and sustain feeding in insects, they have been designated as „sapid” nutrients and their role as feeding stimulants has been discussed by Alikhan (in litt.) and Thorsteinson (45, 46, 47). Of course, not all of the nutrients essential to insects occur in a free form in plants, and not all those that do are necessarily essential in the metabolic sense. However, it is quite clear that some of the nutrients in plants act as gustatory indicators of a suitable food substrate. From this point of view there is little reason why we should not include such nutrients with secondary substances in the class of token stimulants.

There remains the fact that some secondary substances (mustard-oil glucosides) are essential to the food-selection behaviour of at least three insect species (44, 51), although even here the sapid nutrients are equally essential to food-selection. It would be tempting to generalise from this that secondary substances are necessary feeding stimulants at least for insects highly selective in their food-plant preferences. The Colorado beetle, *Leptinotarsa decemlineata* Say, is highly selective, yet the evidence indicates that no secondary substance is involved as a feeding stimulant or attractant. We can alternatively explain its food preferences in terms of avoidance of feeding inhibitors in plants outside its food-plant range (c. f. 47). Removal of palpi results in acceptance of leaves refused by the normal larvae, an indication of the significance of inhibitors (6). Which of these two mechanisms will explain the

majority of food-plant specificities among oligophagous insects can be established only after a great many more cases have been successfully investigated.

Discoveries of new dimensions in food-plant selection, not predictable at the present time, may be expected. It can already be foreseen that various permutations are possible when one considers that oviposition behaviour as well as feeding response mechanisms contribute to the overall regulation of food-plant selection in many insect species. In aphids, food-selection patterns appear to be somewhat more complex, considering the phenomenon of host-plant alternation that occurs in this group. This circumstance prompted Kennedy (27) to propose the „dual discrimination” hypothesis. Since the same species of aphid is sensitive at one period to differences in leaf-age or quality and at another time to host-plant species, there is apparently a duality of behaviour, but this is hardly less puzzling than the neurophysiological reversal of behaviour mechanisms relating to feeding and dispersal that Kennedy (26) himself later elucidated. Secondary chemicals may be involved in recognition of food-plant species, as Lipke and Fraenkel (32) argue, although I have not been able to find any such secondary substance during my work reported in the previous pages, but it is probable, as I have found, that nutrients contribute significantly to the regulation of both of these food selection patterns.

In a recent paper, Fraenkel (18) has suggested that plant parasites, especially insects, constitute an evolutionary cause for the development of odd chemicals in plants. This, however, is not compatible with his other claim that secondary substances are the sole attractants in plants that regulate food-plant selection. It is essential to realize that „selection” of a food plant is equivalent to accepting it, and real acceptance cannot occur in the absence of some kind of chemotactic feeding stimulus. If insects „caused” plants to develop secondary chemicals, the plant must have been subject to insect attack before and, therefore, must have contained feeding stimulants from the beginning. Since these stimulants could not (by this theory) have been secondary chemicals, they must have been nutrients. If nutrients regulated feeding behaviour before plants developed secondary chemicals, it is hardly conceivable that they do not significantly influence feeding behaviour and, hence, food-plant selection today.

3. A revived catenary theory of host-plant selection. Dethier (11) has summed up the whole problem of food plant selection in two questions: „1) how is the preference implemented? 2) what is the genetic basis and evolutionary history of specific plant preferences?” Regarding the second question he (11) has applied

his extensive erudition with a success quite beyond my capacity to emulate, much less excel. Perhaps one should, however, question the concept of „passive selection” on the grounds that all selection is passive, in that inhibitors and repellents exercise a veto in the food-plant selection of all insects, and, on the other hand, all food-selection is active in that the insect responds to effective feeding and oviposition stimulants when it encounters them. The evolutionary acquisition of responsiveness to new stimuli, if obligatory, does not make the selection more active (it is at least as likely to restrict it); although, by way of compensation, the new response, if olfactory, may aid the insect in finding the food-plants within its reduced botanical range. The exclusion of nutritional requirements as a causative evolutionary factor is also open to question in the light of the later findings of Beck (c.f. 47) and others.

Turning to the first primary question of how the preference is implemented, the two precepts advanced by Dethier (11) can be re-estimated as; a). Although physical stimuli define the environment for oviposition and feeding, the decisive act of host-selection is regulated predominantly by the chemical sense. (This has been discussed at length by Thorsteinson (47), and b). The behaviour of phytophagous insects towards their food-plants is best understood when analysed into a series of component phases. Dethier (11) lists three such components: a) orientation to the food, b) biting response, and c) continued feeding. This series becomes more complete and acquires a regenerating path if we add d) dispersal. The necessity of regarding dispersal as reciprocally antagonistic to the first components in the cycle has already been discussed fully by Thorsteinson (47) and others, including Kennedy (26).

It is now necessary to recall the older concept of stimulus-response chains, reviewed, for example, by Wigglesworth (53) and more recently by Nourteva (38). In the light of more recent studies, however, we must recognize not only the principle that a given link (reflex arc) in the chain facilitates the manifestation of the following link, but also that each unit response is likely to require the simultaneous influence of more than one eliciting stimulus. This latter principle applied not only to the interaction of two different types of stimuli, e. g. colour and odour, but also to the summation effects of several stimuli of the same general type, e. g. chemotactic (say, for example, sinigrin and sapid nutrients). The link in the chain of stimuli is, therefore, to be regarded as occurring as a parallel as well as in a sequence. Recognition of this principle will help to fend off the human temptation

to exaggerate the significance of an individual, isolated stimulus, however intriguing it may appear.

It is not stretching the simile too far to regard these circular chains as larger links of greater branching chains in an evolutionary system. The whole problem of food-plant selection, certainly in the evolutionary perspective and even on the relatively simpler contemporary scale, is best understood in terms of a concatenation of unit effects. While these unit phenomena require to be studied one or two at a time, their significance can only be fully appraised in the aggregate.

**Recapitulation.** In an attempt to provide a fresh basis for the development of our understanding of food-plant selection, the following symbolic statement, modified from Thorsteinson (46), is offered:

$$F \xrightleftharpoons{E_{sn}} -I - D + E_{sn} (E_p)$$

An optimal feeding response,  $F$ , implies that the substrate is devoid of feeding inhibitors,  $I$ , or deterrents,  $D$ , and contains the chemotactic stimulants,  $E$ , essential to elicit feeding. The essential feeding stimulants of general botanical distribution, to the best of our present knowledge, are sapid nutrients,  $E_{sn}$ . In order to encompass in this general statement the behaviour of any insect species that require special feeding stimulants of limited botanical distribution and presumably of no strictly metabolic consequence, we include the removable term ( $E_p$ ). This represents, in anthropocentric terms, a „piquant” stimulus; the objective rationalization of this designation follows.

In the only definitive, established illustrations of essential feeding stimuli of limited botanical distribution, where the substances have been identified (44, 51), neither the nutrients (in this case only potentially sapid) nor the metabolically neutral, piquant stimuli are sufficient independently to elicit an optimal feeding response. In view of this and of the fact that piquant substances are not essential to feeding activity and food-plant selection in many insects, including some with a very restricted food-plant range, it seems appropriate to regard these special stimuli as agents that prime receptivity to sapid nutrients (lower response thresholds) much as light perceived by the dorsal ocelli is said to prime receptivity in other nervous circuits (53).

This account is applicable with no essential modification to the adult as well as the larvae in species that depend on the same food in both feeding stages, although the stimuli required to elicit oviposition or even feeding in the adult may not coincide precisely with those related to feeding by the larvae. In *Lepidoptera* and other insects in which the adult female either feeds on quite different plants, or perhaps does not feed at all, there does not seem to be any question of nutrients

(directly important to the adult) influencing her selection of food-plants for her off-spring (10, 11). However, this does not altogether exclude the possibility that oviposition may be influenced by chemotactic stimuli derived from substances of some nutritional use to the larvae. Dethier (10) mentions an early report by Hancock of a grasshopper that briefly tastes the plant before ovipositing on it. Perhaps the ovipositor of some phytophagous insects may be equipped to perceive chemotactic stimuli, as has been reported for hymenopterous parasites by Dethier (10).

4. Insect resistance in plants. Food-plant selection, in the sense that it is equivalent to food-plant acceptance, is related to susceptibility of plants to insects. On the other hand, the feeding behaviour of insects could not stimulate a „selection” characteristic unless some plants are rejected. This platitude relates food-plant selection to insect resistance in plants.

In the context of food-plant selection behaviour only the „nonpreference” type of resistance is of importance, not the other two types, „antibiosis” and „tolerance” recognized by Painter (40). Antibiosis, however, must play some indirect, evolutionary role in food-plant selection. The class of plants acceptable to insects is the sum of the classes „susceptible” and „tolerance”; these two groups are distinguished only for practical purposes and do not represent different basic behavioural mechanisms in insects. It is of special interest that the possibilities are as great for finding plant resistance to polyphagous as to oligophagous insects (40); this seems to be related to the fact that the chemotactic sense seems to be as highly developed in one group as the other (45).

Morphological resistance is rarely, if ever, independent of other types of resistance, and it is to be noted that Painter (40) does not recognize morphological resistance as one of the primary. Phenological resistance is probably of considerable significance in nature, but it is a consequence of the dissynchronization of growth patterns of insects and plants and, from the point of view of feeding behaviour, is only accidental.

The experience of the investigators of insect resistance in plants is a rich source of inspiration for the experimental study of feeding behaviour in phytophagous insects (40, 50). It is gratifying to observe that the more critical experimental studies of feeding behaviour are shedding light on insect resistance and susceptibility in plants. There is, perhaps, no field of experimental investigation of phytophagous insects in which the discovery of new fundamental biological relationships and potentially useful findings overlap to a greater degree.

## VII CONCLUSION

The extraordinary variety of insect-plant relationships is likely to be based on more diverse mechanisms than can be foreseen at present. The promise of rewards is rich for investigators who approach the problem free of deeply rooted bias, and they will be able to improve on this account before long.

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## STRESZCZENIE

1. *Aphis fabae* Scop., podobnie jak inne polifagi, znajduje atrakcyjne dla siebie rośliny dzięki zapachowi, jaki posiada sok komórkowy w związku z obecnością w nim witaminu C. Liście innych roślin, które zazwyczaj nie są gospodarzami tego owada, przyciągały go również, jeśli posmarowano je roztworem kwasu askorbinowego.

2. Sporządzono odpowiednią pożywkę do badań nad smakiem u *Aphis fabae* wyrażającym się w reakcjach pokarmowych. Liście roślin badano w postaci wyciągów. Wyciągi te, podobnie jak i inne substancje testowe, np. witamin C i sacharoza, podawano w agarze. Ilość pobranego pokarmu oceniono z ilości pokarmu pobranego przez dorosłe owady na podstawie ich ubywania i przybywania na wadze.

3. Stwierdzono, że stężenie wszystkich składników pokarmowych wywiera wpływ na reakcje pokarmowe mszyc, ale nie wydaje się, by jon wodorowy miał tu jakieś znaczenie. Optimum reakcji pokarmowej osiągnięto przy pH 5,1.

4. Wyniki tych badań laboratoryjnych znalazły potwierdzenie w doświadczeniach przeprowadzonych w cieplarni z użyciem żywych roślin, do których sztucznie wprowadzono aminokwasy (za pomocą albuminu jajka), cukier (podlewając rośliny roztworem sacharozy) i witaminu C (podlewając rośliny roztworem witaminu C). Za kryterium reakcji owada uznano:



- (a) ilość pobranego pożywienia
- (b) szybkość pojawiania się nimf
- (c) procent pojawiających się form bezskrzydłych
- (d) okres dojrzewania nimf.

Obserwacje w tym kierunku potwierdziły wyniki laboratoryjne i wykazały również, że wyższe ilości cukrów w soku komórkowym działają odpychająco na reakcje pokarmowe mszyc.

5. Działanie wyższych wartości cukrów badano również na wydzielinie substancji miodowej mszyc. Wyniki tych badań bardzo trudno jest ująć statystycznie z powodu dużej śmiertelności owadów karmionych roślinami z dużą zawartością cukru. Jednakże przy pominięciu czynnika śmiertelności stwierdzono, że szybkość wydzielania substancji miodowej jest proporcjonalna do zawartości cukru w soku komórkowym gospodarza.

6. Po stwierdzeniu w laboratorium chemotaktycznej roli witaminu C i cukrów w wyborze rośliny przez mszyce, dalszym krokiem było oczywiście stwierdzenie praktycznej wartości tego odkrycia. Dwie niemieckie odmiany bobu — *Rastatter* i *Schlanstedter* i jedna angielska Tick „*Niki 7*” zostały wzięte z ogrodu botanicznego UMCS i przeniesiono na nie mszyce. Z punktu widzenia wrażliwości mszycy, badano nie tylko poszczególne odmiany, lecz nawet poszczególne organizmy roślin. Jednocześnie przeprowadzono analizę chemiczną zawartości azotu, cukrów i witaminu C. Następnie zestawiono wyniki tych badań. Wszystko to potwierdziło moje badania, dokonane w pracowni, mianowicie, że dana odmiana roślinna jest proporcjonalna do zawartości aminokwasów i witaminu C, a odwrotnie proporcjonalna do zawartości cukru w soku komórkowym tej rośliny. Na podstawie tych badań wywnioskowałem, że polifagi, takie jak mszyce, są bardzo ograniczone co do wyboru roślin żywicielskich, do których się przystosowują.

## РЕЗЮМЕ

1. *Aphis fabae* Scop., подобно другим полифагам находят нужные им желательные растения по запаху, который выделяет клеточный сок в связи с содержанием в нем витамина С. Листья других растений, которые обычно не являются хозяевами этого насекомого будучи смазаны раствором аскорбиновой кислоты привлекают его.

2. Был приготовлен соответствующий питательный раствор, употребляемый в исследованиях по вкусу у *Aphis fabae* отражающимся

в питательных реакциях. Листья растений были исследованы в виде гомогенатов. Эти гомогенаты, так же как и другие испытуемые вещества напр. витамин С и сахароза давались в агаре. Количество потребленной пищи определялось по количеству пищи потребленной взрослыми насекомыми на основании их привеса.

3. Установлено, что повышение концентрации всех питательных компонентов оказывает влияние на пищевые реакции у тлей, но кажется невероятным чтобы какое-либо значение имел при этом водородный ион. Оптимальное значение рН было 5,1.

4. Результаты этих лабораторных исследований были подтверждены опытами, проведенными в теплице на живых растениях, в которые вводились искусственным путем аминокислоты (альбумин яйца), сахара, витамин С, (растения подливались раствором сахарозы и витамина С). В основе критерий реакции насекомого легли следующие показатели:

- а) количество потребленной пищи,
- б) время до появления личинок,
- в) процент появляющихся бескрылых форм,
- г) период созревания личинок.

Наблюдения, проведенные в этом направлении показали также, что более высокое содержание сахаров в клеточном соке оказывает отрицательное влияние на пищевые реакции тлей.

5. Исследовалось также влияние повышенного количества сахаров на выделение медового вещества у тлей. Однако, при статистической обработке результатов этих опытов обнаруживаются большие затруднения ввиду большой смертности у насекомых, питающихся растениями с большим содержанием сахара. Все же даже пренебрегая этим фактом было установлено, что скорость выделения медового вещества пропорциональна содержанию сахара в клеточном соке хозяина.

6. После установления в лабораторных условиях хемотактической роли витамина С и сахаров в выборе растения тлями, дальнейшим шагом было, естественно, изучение практического значения этого открытия. Два немецких сорта бобов Раштаттер и Шланштедтер и один английский Тикк „Найки 7” были взяты из ботанического сада университета и затем на них переносились тли. Исследования касались чувствительности тлей не только на сорта но также и на отдельные растения. Одновременно проводились определения азота, сахаров и витамина С. После этого была произведена сводка этих исследований.

Всё это подтвердило результаты лабораторных наблюдений, а именно что адаптации этого насекомого к данному сорту благоприятствует наличие витамина С, и отрицательно влияет содержание сахара в клеточном соку растения.

На основании этих исследований автор пришел к заключению, что полифаги подобные тлям очень ограничены в отношении выбора растения-хозяина, к которому они приспособляются.

