MIGRATION AND UTILIZATION OF RESERVE SUBSTANCES DURING FLIGHT IN *APHIS CRACCIVORA* KOCH.

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(One Text-figure.)

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Synopsis.

A histological examination of *A. craccivora* alatae indicated that an important fuel reserve in this species is glycogen. Deposits of fat were also considerable in young adults, although the fat body cells contracted in size to a narrow layer next to the cuticle about one to two days after the final ecdysis and there was an associated mobilization of part of the glycogen from the abdomen to other parts of the body. Glycogen reserves decreased as the aphids aged.

Both glycogen and fat were utilized during starvation and aphids survived a maximum of 4-5 days without food. In moribund aphids the glycogen reserves had almost completely disappeared but appreciable reserves of fat were still evident.

Aphids, suspended from a fine wire in the laboratory and stimulated by a slight air current, vibrated their wings strongly for periods ranging from a few minutes to several hours. However, aphids more than five days old could not be induced to "fly" and there was a narrow age range at which the aphids would readily vibrate their wings and at which the duration of flight was most prolonged. This occurred one to two days after the final ecdysis. Glycogen was progressively reduced in all regions of the body as the duration of flight increased.

Recently arrived alatae collected at Gosford and Sydney on the New South Wales coast in September showed greatly reduced glycogen reserves compared with swarming aphids taken a few days before at Moree, several hundred miles to the north-west. From the appearance of the fat body itself the aphids in each locality were approximately the same age, namely, 0-2 days old. It was concluded that the aphids had depleted their glycogen reserves during migration in upper wind currents from the inland breeding areas to the central coast.

Swarming populations at Moree consisted of several aphid species including *Aphis craccivora* Koch, *Myzus persicae* Sulz., *Lipaphis erysimi* Kalt., and *Capitophorus elaeagni* Del Guer., and these species were represented in roughly the same proportions in the Gosford collections, *M. persicae* being the dominant species. However, collections in the Sydney district showed a majority of *A. craccivora* with very few *M. persicae*.

Introduction.

Black aphids have been reported by the New South Wales Department of Agriculture infesting broad beans and French beans since 1922 but it was not until 1950 that the species was definitely identified as *Aphis craccivora* Koch (synonymous with *A. leguminosae* Theob. and *A. medicaginis* Koch) (Dyce, unpublished thesis, University of Sydney). Johnson (1957) indicated the presence of extensive breeding grounds of this species in north-western inland areas of New South Wales. From observations in the field, correlations with wind data and other sources, he advanced the theory that plagues of *A. craccivora* experienced in some years on the central coast of New South Wales resulted from long distance dispersal of the aphid away from these inland breeding areas.

The appreciable reserves of glycogen present in many insects (Babers, 1941) suggest the general use of this readily available material as an energy source during flight. The duration of flight to exhaustion in *Drosophila* varies with the age of the fly and with the total glycogen content of the insect (Williams *et al.*, 1943). However, it has been shown that the principal source of energy for flight in the Desert Locust,

Schistocerca gregaria Forsk., was fat, the remainder being glycogen (Weis-Fogh, 1952). Fat also provides energy for flight in another migrating insect, *Eutettix tenellus* (Fulton and Romney, 1940). Rough determinations of the distances flown were made from analysis of the chloroform extractive contents of these leafhoppers as they moved along an extended migration route away from the breeding area.

The object of the present paper is to provide evidence for long-range dispersal by histological examination of the distribution of glycogen or fat and their mobilization during sustained flight in alate forms of *A. craccivora* in the laboratory. An analysis of migrant aphids collected in the field is also made to measure differences in reserve substances of aphids obtained from their breeding grounds in the north-west of New South Wales and aphids collected in coastal areas of New South Wales, the latter having presumably been transported by winds from the north-western districts (Johnson, 1957).

Method of Examination of Stored Reserves.

Alate forms of the aphid were used in all experiments, and broad beans in winter and cow peas in summer proved suitable hosts in that the aphid populations increased rapidly on them and produced large numbers of alatae as the plants began to wilt 2–3 weeks after germination. However, in the spring and early summer it was necessary to use scarlet runner beans, medics and clovers to maintain colonies, and these were not as suitable for production of alatae. Fourth instar nymphs with wing buds were removed from stock colonies 24 hours before the adults were required and a large proportion of these became adults the following day. All host plants were grown in earthenware pots covered with perspex tubes and maintained in a glasshouse.

(*a*) *Glycogen.*

Alate viviparous females were fixed in Carnoy's solution and split in halves by a median longitudinal cut with a fragment of a safety razor blade, following the method described by Wigglesworth (1949) for *Drosophila*. The half insects were stained with a saturated solution of light green in 90 per cent. alcohol and the glycogen revealed by the iodine method (Wigglesworth, 1942) and with Best's carmine (Carleton and Leach, 1947). The two methods gave similar results, the glycogen appearing as brilliant red granules with Best's carmine and a mahogany colour with iodine. Microtome sections of Carnoy-fixed material were cut in paraffin and stained with Best's carmine.

(*b*) *Fat.*

The usual method of fixing in Bouin's fluid and staining with Sudan Black B proved unsuitable with the half-aphids and fat was demonstrated by fixing aphids in Baker's formaldehyde-calcium (Baker, 1945) for five days or more. Half-aphids were then prepared as above and washed in several changes of distilled water, transferred to 50 per cent. glycerine, and mounted in glycerol jelly. Baker's formaldehyde-calcium fixes all tissues but leaves fat and, examined under the binocular microscope, it appears as whitish globules in reflected light or bright shining yellow in transmitted light, the rest of the insect tissue having a dark appearance. The aphids were dipped in 50 per cent. alcohol before being immersed in the fixative to overcome difficulty in wetting the insects. The method was useful for contrasting relatively large changes in the appearance of fat reserves but small differences were not readily perceptible.

(*c*) *Comparison of Reserves in Different Aphids.*

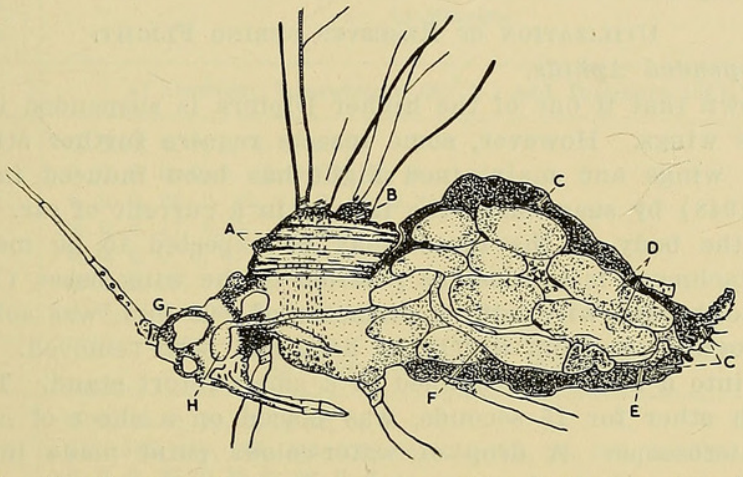
The comparison was put on a roughly quantitative basis by giving each prepared slide of stained half-aphids a serial number and comparing with an appropriate control slide. A score system was used whereby separate parts of the body of each aphid were allotted points with a possible maximum of ten in each case according to the area and intensity of staining in the region. Thus, for example, glycogen in the fat body of the abdomen, embryos, alimentary canal, thoracic muscles, head, etc., was compared in different slides and with a control slide and finally given score ratings.

Stored Reserves in Alate Aphids.

(*a*) *Distribution of Glycogen and Fat.*

The general distribution of glycogen in an adult viviparous female, approximately one day old, is shown in Text-figure 1. The bulk of the glycogen is situated in the fat body, especially that of the abdomen, although appreciable amounts occur along the surface of the indirect flight muscles and their points of insertion in the wall of the thorax. On the whole, glycogen in *A. craccivora* was not as conspicuous as the massive deposits reported in *Drosophila* (Wigglesworth, 1949).

In the fat body, discrete fat droplets were particularly obvious in the caudal region and at the base of the cornicles in the abdomen, in the prothorax, mesothorax and head, and the scutellum of the thorax.



Text-fig. 1.—The distribution of glycogen in an alate viviparous female *Aphis craccivora*, 0-1 days old, shown in a sagittal section. A, Glycogen between muscle bundles and insertions; B, Glycogen in the thoracic fat body; C, Fat body of abdomen with glycogen; D, Embryos with glycogen; E, Rectum; F, Midgut; G, Fat body of head; H, Pharynx.

(*b*) *Changes in Reserves with Age.*

From six to eight aphids, for each of the age groups 0, 1, 2, 3, 4, 7 and 14 days old, were tested for glycogen and a similar number for fat. All aphids used were removed from various stock broad-bean plants as nymphs and maintained on the one plant throughout the experiment, aphids of the required age being taken from the plant at intervals.

In the newly emerged adult what Wigglesworth (1949) termed the "larval fat body" was still evident and filled a large part of the abdomen. The loosely packed fat body cells contained very heavy deposits of fat and an intense staining reaction for glycogen indicated large reserves of the latter. Glycogen was at a relatively low level in the head and thorax but fat was here also present in large amounts as discrete droplets.

During the next 1–2 days the fat body cells in the abdomen contracted in size to a narrow layer next to the cuticle with a consequent reduction in glycogen and fat content. There was, at the same time, an increase in the glycogen content of the fat body and muscles of the thorax, in the head and the alimentary canal. This glycogen level was maintained in all regions up to the fourth day but on the seventh day there was a reduction in all regions except the head and alimentary canal, while on the fourteenth day glycogen had been reduced in all parts of the body to about two-thirds of that observed in a one-day-old aphid. The embryos and alimentary canal maintained a fairly constant level of glycogen throughout the life of the aphid.

No decisive change in fat content was observed as age increased, after the initial major reduction in the size of the fat body cells.

(*c*) *Changes in Reserves during Starvation.*

One-day-old aphids were confined in a small perspex and organdie cage which was placed over a dish of water to maintain an almost saturated atmosphere. The aphids

immediately climbed to the top of the cage towards the light from a window near by and after a brief period of activity remained motionless until death, which occurred after a maximum of five days. Six aphids were placed in fixative at eight-hourly intervals until death and stained half-aphids were compared with control slides of aphids of the same age taken directly from the host plant.

A small drop in glycogen was observed after 24 hours but it was not until after 40 hours that glycogen was reduced significantly in the fat body. In the later stages (90–120 hours) very little glycogen remained in the fat body but appreciable amounts were retained in the thoracic muscles and alimentary canal. Similarly, fat was reduced only slightly until about 40 hours without food, when there was a progressive reduction until death, although, even in the moribund aphid, about half the fat present in a recently-fed insect remained.

Utilization of Reserves during Flight.

(*a*) *Flight in Suspended Aphids.*

It is well known that if one of the higher Diptera is suspended in still air it will readily vibrate its wings. However, some insects require further stimuli before they will vibrate their wings and maintained flight has been induced in *Locusta* species (Kennedy *et al.*, 1948) by suspending the insects in a current of air. The distribution of stress within the body of the insect may be expected to be most natural when the position of attachment is as close as possible to the wing bases (Hollick, 1941).

A fine phosphor-bronze wire with a diameter of 0·08 mm. was soldered to the end of a long entomological pin from which the head had been removed. The point of the pin was inserted into a cork and clamped to a small retort stand. The aphid, lightly anaesthetized with ether for 15 seconds, was placed on a sheet of cork on the stage of a binocular microscope. A drop of water-colour paint made into a thick paste was painted on the tip of the bronze wire and the wire was then held horizontally and symmetrically to the aphid so that the minute paint globule came in contact with the mesothorax when the wire was gently lowered from above and behind the aphid. The paint quickly dried and secured the aphid to the wire without interfering with wing movement. It was essential that the paint be of the correct "gluey" consistency so that it would adhere to the wire in the form of a minute droplet. Aphids required from 10 to 20 minutes to recover fully from the ether. A sharp puff of air then generally resulted in the aphid elevating its wings to the "ready-to-fly" position.

In addition to the removal of the tarsi from contact with any object a further stimulus of air movement was required before the aphids would vibrate their wings. This was provided by an electric fan, blowing through a mosquito netting screen, so that the resultant draught reaching the aphid was not more than 90 feet per minute. Consistent wing movement was not possible at wind speeds greatly above this. A sudden puff of wind from in front and below was then sufficient to induce the aphid to vibrate its wings. Some aphids were very erratic in their response and constant stimulation was required, while others "flew" strongly for hours in the steady air draught. Flight to complete exhaustion was apparently not obtained.

An attempt was made to conduct the experiment at a constant temperature of 80°F. and relative humidity of 85 per cent. in a warm room but only a few aphids responded under these conditions. The results are summarized in Table 1, where duration of flight in the warm room as well as at room temperature is given for the different age groups. A wide range of temperature and humidity conditions was encountered in the latter as the experiment was carried out over an extended period of time, temperature ranging between 50° and 90°F. during the period of testing.

(*b*) *Changes in Stored Reserves during Flight.*

Sixteen aphids, having "flown" for periods ranging from 20 minutes to 540 minutes, were tested for glycogen by the carmine and iodine methods. A progressive reduction of glycogen in all regions of the body except the embryos was observed as the duration of flight increased. In the aphid that had flown continuously for nine hours glycogen was negligible in the thorax but traces remained in the head and fat body of the abdomen. Sixteen aphids were also tested for fat; they had flown for periods

ranging from 7 minutes to 415 minutes and it was only in those aphids that had flown for more than 20 minutes that a slight reduction in fat could be observed when compared with a control slide. There did not appear to be a progressive reduction of fat as the duration of flight increased, although comparisons were difficult as most of the aphids were suspended for several hours at an age when a large reduction was taking place in the size of the fat body cells and no direct conclusion could be made concerning the importance of fat as a fuel during sustained flight.

TABLE 1.

Duration of Aphid Flight in Laboratory.

Aphid Age Groups (in Days).	Number of Aphids Tested, with Duration of Flight in Minutes.
	At Constant Temperature (80° F.) and Humidity (85%).
0–1	0, 0, 0, 0, 0, 0, 6, 0, 0.
1–2	0, 0, 0, 9, 0, 0, 18, 0, 78, 48, 0.
2–3	0, 0, 0, 24, 0, 45, 21, 0, 62, 0, 0, 23.
3–4	23, 0, 0, 17, 0, 0.
4–5	0, 0, 0, 0, 0, 0.
6–7	0, 0, 0, 0.
7–8	0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
	At Room Temperature (Variable).
0–1	0, 0, 16, 0, 14, 0, 0, 12, 0, 0.
1–2	317, 547, 415, 307, 372, 292, 91, 224, 374, 96, 190, 74, 285, 273, 251.
2–3	0, 0, 0, 45, 0, 0, 0, 82, 0, 0.
3–4	124, 0, 0, 45, 0, 0, 0, 82, 0, 0.
4–5	0, 29, 0, 0, 0, 0, 0, 0.
6–7	0, 0, 0, 0, 0.
7–8	0, 0, 0, 0, 0.

OBSERVATIONS ON SWARMING APHID POPULATIONS.

A visit was made to Moree in September, 1952, and aphids collected. On the return trip observations were made at Gosford and again at Sydney a few days later. Only alate aphids were considered in the field collections.

Moree is situated approximately 300 miles north-west of Sydney and it is an area of extensive black soil plains upon which Burr medic, *Medicago hispida* var. *denticulata*, thrives in the spring months. Burr medic is a favoured host of *A. craccivora*. According to local reports there had been a big aphid increase in Moree during August but after a week of rain the aphids almost vanished. There was another increase in numbers during September, when heavy swarming occurred. These swarms were particularly dense on 12th, 13th and 14th September in Moree and several aphid species were thought to be involved. Observations were commenced on 15th September, when there was evidence of previous huge colonies in the very large numbers of cast skins in the old medic stands. The medics and other annual pasture plants were commencing to wilt and a mild swarm of aphids was observed on the afternoons of 15th and 16th September. Samples were collected from various host plants and from swarming aphids in the air. Wasp parasites and predatory Coccinelid beetles were very common amongst the reduced aphid colonies.

It was observed that the main host of *A. craccivora* in the Moree district was the widespread Burr medic. Other aphid species present were *Myzus persicae* Sulzer which thrived on many host plants including the weeds Marshmallow, *Malva parviflora* and milk thistle, *Sonchus oleraceus*, and the turnip aphid, *Lipaphis erysimi* Kalt., which was found on wild turnip, *Brassica campestris*. These weeds were unusually abundant in 1952. Of the aphid species taken in the air, 62 per cent. were *M. persicae*, 22 per cent. *A. craccivora*, 14 per cent. *L. erysimi* and 3 per cent. *Capitophorus elaeagni* Del Guer. from a total sample of 107 aphids collected by net.

At Gosford on the central coast of New South Wales the first report of black aphids on bean crops in 1952 was received by the Government Entomologist on 17th September. On 18th September several farms in the bean-growing area were visited; the aphid situation was similar on all farms, there being 3–4 *A. craccivora* alatae per plant on about 40 per cent. of the bean plants in each crop. Aphids were observed in the air and a sample consisted of 57 per cent. *M. persicae*, 25 per cent. *A. craccivora*, 14 per cent. *L. erysimi*, and 3 per cent. *C. elaeagni* from a total of 173 aphids collected.

On 22nd September, at Sydney, 50 miles south of Gosford, very heavy aphid swarms were observed in several districts. Strong, dry winds kept the aphids dispersed and after a cool southerly change about mid-day they disappeared. However, in the late afternoon, when calm and warmer conditions prevailed, large numbers were observed swarming above grass, etc., as they left unsuitable plants, and specimens were collected. The latter consisted of 90 per cent. *A. craccivora*, 6 per cent. *L. erysimi*, 2 per cent. *M. persicae* and 2 per cent. *C. elaeagni*, from a total sample of 402 aphids.

Stored Reserves in Field–Collected Aphids.

Although several aphid species were collected, only *A. craccivora* were considered in the glycogen and fat determinations. Specimens were placed in fixative when collected and later tested for glycogen and fat in the laboratory. A slight variation in the glycogen technique was necessary, as Carnoy's solution fixes tissues in less than one hour and Bouin's fluid was used as an alternative so that the fixed aphids could be stored for several days before staining.

At Moree 23 alatae were collected from French beans on 15th September. Both glycogen and fat were at a high level in the fat body and thoracic muscles, being comparable with that observed in 0–2-day-old aphids bred in the laboratory on cowpeas. At Gosford 33 alatae were taken from French beans and 20 caught in flight on 18th September. The former had evidently recently settled on the host plants, as no young were present. Glycogen was similar in both groups, being almost negligible in the fat body of the abdomen and thorax and greatly reduced in the thoracic muscles and head. No record of fat content was made. At Sydney 47 alatae were taken in flight and 55 collected from *Melilotus indica* and *Trifolium recumbens* plants on 22nd September. There were no aphids on these plants the previous day. Glycogen in the aphids taken in flight was very much reduced and similar to that observed in aphids collected at Gosford, although the level of glycogen in the fat body of the abdomen was slightly higher. However, the fat body of the aphids appeared to be extensive, particularly in the abdomen, and was similar to that observed in 0–2-day-old aphids bred in the laboratory. The glycogen level in the aphids taken from the legumes was slightly higher than in those taken in flight but was not comparable with the large deposits in aphids collected at Moree. The fat body was greatly reduced in size in all regions of the body.

A further 77 alatae were collected from a plot of *M. indica* at Sydney University on 9th October, 17 days later. These were not new arrivals, as no swarms occurred in Sydney after 23rd September, nor could they have been produced as offspring of previous migrants as alate viviparous females invariably give rise to apterous nymphs. Glycogen in these insects was similar to that in aphids collected at Moree, although the staining reaction was not quite so intense, indicating that, although well fed, these aphids were probably somewhat older than those collected at Moree or at Sydney previously.

Discussion.

The age at which aphids flew most readily in the laboratory and at which the duration of flight was most prolonged coincided with the maximum concentration of glycogen in the body of the aphid. These deposits of glycogen in the fat body of the thorax and abdomen were drawn on extensively by the aphids during starvation and for vibrating the wings while suspended from a fine wire. The extensive use of fat as a fuel during flight was not demonstrated, although the method of examination for fat was not sufficiently precise to observe other than major changes in the

appearance of the fat body and no conclusion was drawn as to the importance of fat as an energy source during flight. It was sufficient to know that the more readily-observed glycogen reserves were progressively depleted during flight and could be used as a pointer to any recent muscle activity in the aphids.

There was a very small range of ages at which aphids could be induced to fly in the laboratory. A sharp peak of response was obtained in aphids 1–2 days old (Table 1) and a period of settling, involving feeding and reproducing on a suitable host plant, had an adverse effect on the ability of the aphids to vibrate their wings. Johnson (1953) indicated that autolysis of the flight muscles occurred in several aphid species and that flight was not possible for more than a few days after the final ecdysis. Thus, not only was it necessary to stimulate special receptors by air movement but it was apparent that the aphid must be in a particular physiological state before flight could begin. Swarming in natural populations could only occur when several conditions, such as the age of the aphids themselves, the state of the host plants, and the related climatic conditions, combined to overcome the natural resistance of aphids to any movement away from their food and shelter habitat.

It was only in very young adults that the fat body occupied extensive areas of the abdomen, after which the cells were reduced in size to form a narrow layer next to the cuticle, and aphids collected from swarming populations at Moree, Gosford and Sydney in September all showed this extensive "larval fat body" and were apparently of approximately the same age.

The flying aphids collected at Gosford and at Sydney showed greatly depleted glycogen reserves compared with aphids in the large swarms at Moree a few days earlier and it is probable that these or similar swarms were transported by winds to the New South Wales coast from their inland breeding areas several hundred miles to the north-west. Migrants collected in Sydney after feeding for 2–3 weeks on a favoured host had their glycogen reserves restored to the normal high level. The aphids, because of their slow speed of flight and the presence of a mountain range between their inland breeding grounds and the coastal plain, could not have travelled unaided and were probably carried by upper prevailing winds as suggested by B. Johnson (1957). However, C. G. Johnson (1951) considered that once active flying ceased the aphids might not remain aloft for long and, under natural conditions, might continue to vibrate their wings and use up available glycogen reserves although directed by prevailing winds.

The host plants of the several aphid species observed at Moree were annuals which commenced to die at about the same time of the year and gave rise simultaneously to conditions favourable for the production of alatae. Thus *M. persicae*, *L. erysimi* and *C. elaeagni*, as well as *A. craccivora*, were observed swarming at Moree and again at Gosford in roughly the same proportions, with *M. persicae* predominating. *L. erysimi*, *C. elaeagni* and *A. craccivora* and only a few *M. persicae* were observed at Sydney. The aphid arrivals at Gosford on 17–19th September may have been the result of a single period of swarming in the Moree region while the migrants observed at Sydney on 22nd September could have originated from a more southerly area such as Gilgandra or Coolah, where the favoured hosts of *M. persicae* may not be so widespread.

Long-range dispersal in favourable years by upper wind currents to the coastal areas of New South Wales may be a feature of several small-bodied, flying insects, particularly aphids, and the climatic conditions of the inland breeding areas allied with the prevailing upper air currents could have an important bearing on the likelihood of infestation by these insects on coastal crop and orchard areas, when even a close study of the local coastal conditions would give no indication of a likely infestation.

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