Growth and wilting of radish seedlings, *Raphanus sativus*, infested with the aphid, *Myzus persicae*

**By J. M. S. Forrest, A. Hussain and A. F. G. Dixon**

Zoology Department, The University, Glasgow, G12 8QQ

(Accepted 11 April 1973)

**Summary**

After 10 days of infestation by the aphid *Myzus persicae* growth of radish seedlings was reduced by 38%, and the volume of sap bleeding from the stumps of cut stems of infested seedlings was 4% of that exuding from the stumps of uninfested seedlings of the same age. Diminished bleeding could not be accounted for by the 52% reduction in the weight of the roots of infested seedlings. After 14 days of infestation, necrotic patches developed along the veins of the cotyledons which yellowed noticeably. If freed of aphids the seedlings subsequently resumed growth and there was an increase in the volume of bleeding sap produced by their cut stems. Symptoms associated with aphid infestation cannot be attributed either to virus disease or initially to exhaustion of the seedling's carbohydrate and nitrogen reserves. Possible causes of the symptoms described are discussed.

**Introduction**

Insects and nematodes which feed by sucking sap from plants can cause wilting and reduce growth in their host plants (Kunkel, 1933; Hildebrand, 1938; Sloan, 1938; West, 1946; Carter, 1952; Ortman & Painter, 1960; Mittler & Sylvester, 1961; O'Bannon & Reynolds, 1965; Van Emden, Eastop, Hughes & Way, 1969; Dixon, 1971a, b). Aphid infestation can result in a plant having poorly developed roots (West, 1946; Ortman & Painter, 1960; Dixon, 1971b), and this may lead to wilting, especially in drought conditions (Van Emden et al. 1969).

In the present paper the effect of aphid infestation on growth and wilting of radish seedlings is described.

**Materials and Methods**

*Plants.* Radish seedlings cv. Cherry Belle were obtained by sowing 25 g of radish seeds in trays (60 × 32 × 8 cm) containing John Innes compost no. 1, 6 cm deep. The trays of seedlings were kept at 18 °C with a photoperiod of 17 h and were watered daily. Seedlings were also grown in water culture (Mitchell & Livingston, 1968), the seeds being sown, at the same density as in soil, on to muslin supported on a wire frame just at the surface of the nutrient solution.

*Infestation with aphids.* When the seedlings were 5 days old c. 1.5 g of aphids were sprinkled over each tray. The duration of infestation varied in different experiments.
as indicated. Seedlings were freed of aphids by exposing them to dichlorvos vapour from a proprietary resin-impregnated strip. Uninfested control seedlings were kept under the same conditions and given the same insecticide treatment.

**Aphid damage to plants.** After 10 days of aphid attack infested and uninfested radish seedlings were examined. After 14 days of infestation cotyledons were cleared in formalin:acetic acid:water (18:1:1, v/v) and were sectioned at 10 μm and stained with safranin and fast green.

**Rooting of excised stems and cotyledons.** The ability of excised stems and cotyledons to develop roots was determined by placing them on moist tissue paper in a covered plastic tray for 7-8 days, after which they were examined for root growth.

**Collection of bleeding sap.** To obtain 'bleeding sap' the stems of radish seedlings were cut through with a razor blade 2 cm above soil level, and the liquid which exuded from the stumps was collected with a Pasteur pipette over a period of 24 h and its volume measured. Between collections, to maintain a high humidity and prevent drying out, the stumps were covered with a plastic canopy.

**Collection of roots and tops for analysis.** The roots of radish seedlings in water culture grew down through the muslin into the nutrient solution and were collected by cutting them as close as possible to the underside of the wire support. Tops were cut as indicated for the collection of bleeding sap. Both tops and roots were washed in running water before determining their carbohydrate and nitrogen content.

**Estimation of dry weight, soluble carbohydrate and total and soluble nitrogen.** Roots and stems plus cotyledons of infested and uninfested 15-day-old plants were weighed after drying for 24 h at 100°C. The soluble carbohydrate content was determined by the anthrone method of Yemm & Willis (1954). The total and soluble nitrogen were extracted from freeze-dried radish tops and roots and the amounts determined with a Coleman nitrogen analyser.

**RESULTS**

*External appearance and growth of aphid infested seedlings*

The appearance of 15-day-old seedlings infested with *M. persicae* is shown in the Plate, fig. 1. Aphid-infested seedlings were stunted and flaccid. The roots had fewer lateral branches and root hairs and were flaccid and ivory coloured rather than white as in uninfested seedlings (Plate, fig. 2). Growth of the first leaves was arrested and the cotyledons were also smaller. Over 70% of the stomata were closed on the cotyledons of both uninfested and infested 15-day-old seedlings. Fifteen-day-old uninfested seedlings had produced their first leaves and more than 50% of the stomata on these were open. On infested seedlings, however, the first leaves were not developed and the only stomata, those on the cotyledons, were mostly closed.

The fresh weight of the above-ground parts of infested seedlings was 34% less and that of the roots 52% less than in uninfested plants (Table 1). Aphid infested seedlings eventually died if not freed of aphids.

*Injury to veins associated with aphid infestations*

*M. persicae* generally feeds on the phloem elements of its host plants (Fenjves, 1945; Pollard, 1971). We observed the aphid to settle initially on the midrib at the base of
radish cotyledons and the offspring distributed themselves radially along the veins towards the edges of the lamina. If the infestation lasted for 14 days the cotyledons yellowed and the veins turned brown. The dark coloration of the veins was visible to the naked eye but was especially obvious when the cotyledons were cleared (Plate, Table I.

Table 1. The effect of aphid infestation on the fresh weight of tops, the fresh and dry weight of roots, and the volume of bleeding sap collected over a period of 24 h from the cut stems of 15-day-old radish seedlings grown from 25 g of seed

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Sap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tops</td>
<td>Roots</td>
<td>Ratio uninfested</td>
</tr>
<tr>
<td>Infested</td>
<td>87.4 (2)†</td>
<td>18.7 (4)</td>
<td>0.97 (2)</td>
</tr>
<tr>
<td>Uninfested</td>
<td>132.7 (2)</td>
<td>39.2 (4)</td>
<td>1.49 (2)</td>
</tr>
</tbody>
</table>

Degrees of freedom 1 1 1 1

**, *** Significance at 1% and 0.1% probability respectively.
† Number of trays of seedlings recorded in parentheses.

Fig. 1. Diagram of a vein and surrounding tissues of a cotyledon from an infested (a) and uninfested (b) radish seedling.

The veins of the cotyledons of uninfested seedlings were translucent and protruded from the underside of the cotyledons, whereas in infested seedlings they were brown and recessed within the tissue of the cotyledon. Microscopical examination of the veins revealed that the brown coloration was due to dead cells immediately around the stylet sheaths of the aphids (Text-fig. 1). Cells also died where this aphid fed on the mature leaves of a radish (Plate, fig. 4).
Effect of aphid infestation on the rooting of excised stems and cotyledons

The cotyledons of aphid infested seedlings approached senescence more slowly if the aphids were removed 10 days after infestation than if aphids were allowed to remain. However, although the cotyledons showed no visible damage, significantly fewer excised cotyledons from plants infested for 10 days produced roots \( (\chi^2 = 34 \text{ d.f.} = 1, P < 0.001, \text{Table 2}). \) Aphid-free excised stems with or without cotyledons from both infested and uninfested seedlings produced roots and resumed growth (Table 2). Rooted explants, even from previously infested seedlings, grew normally when planted in compost.

Table 2. The effect of aphid infestation on the rooting of explants from radish seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Stem plus cotyledons</th>
<th>Stem minus cotyledons</th>
<th>Cotyledons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. rooted</td>
<td>%</td>
</tr>
<tr>
<td>Aphid-infested for 10 days</td>
<td>50</td>
<td>49</td>
<td>98</td>
</tr>
<tr>
<td>Uninfested</td>
<td>45</td>
<td>45</td>
<td>100</td>
</tr>
</tbody>
</table>

*** Significance at \( 0.1 \% \) probability.

Table 3. The effect of aphid infestation on the average fresh to dry weight ratio of stems plus cotyledons, and the volume of bleeding sap collected over 24 h from the cut stems of seedlings grown from 25 g of seed, \( 0 \) and 7 days after 10 days of aphid infestation

<table>
<thead>
<tr>
<th>Days from end of aphid infestation...</th>
<th>0</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vol. of bleeding sap (ml)</td>
<td>Vol. of bleeding sap (ml)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Fresh/dry wt</td>
<td>Fresh/dry wt</td>
</tr>
<tr>
<td>Infested</td>
<td>14.75 (4)†</td>
<td>0.37 (10)</td>
</tr>
<tr>
<td>Uninfested</td>
<td>15.69 (4)†</td>
<td>9.32 (10)</td>
</tr>
<tr>
<td>( t = 3.29^{<em>} ) ( d = 9.99^{</em>**} )</td>
<td>( t = 0.38 ) ( d.f. = 8.9 )</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

† Number of trays of plants recorded in parentheses.

**Effect of aphid infestation on the quantity of sap exuding from cut stems**

After 10 days of infestation the volume of bleeding sap was only 4% of that which could be collected from uninfested seedlings. If cleared of aphids after 10 days of infestation and kept free for a further 7 days, the previously infested seedlings exuded a significantly greater volume of sap \( (d = 9.24, P < 0.001, \text{Fisher-Behrens test}), \) but this was still only 39% of that produced by the uninfested seedlings (Table 3).

It is unlikely that the 96% reduction in volume of bleeding sap collected from seedlings infested for 10 days can be accounted for by the smaller roots of aphid-infested seedlings (Table 1). However, it could partly account for the smaller volume of sap collected from previously infested seedlings which were allowed to recover for 7 days.
The fresh to dry weight ratio of the above ground parts of infested seedlings was significantly less than that of uninfested seedlings (Table 3).

**Effect of aphid infestation on the quantity of carbohydrate and nitrogen present in radish seedlings**

Whilst they are feeding on plants, aphids remove carbohydrates and amino nitrogen. This drain on the plant's food reserves could result in a reduction in growth. After 10 days of infestation the level of soluble carbohydrates in the roots of infested seedlings was not significantly lower than in uninfested seedlings. Likewise, 10 days of aphid infestation had no significant effect on the total and soluble nitrogen content of radish seedlings (Table 4).

### Table 4. The effect of aphid infestation for 10 days on the percentage (dry weight) soluble carbohydrates, and total and soluble nitrogen in the tops and roots of radish seedlings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soluble carbohydrate (%)</th>
<th>Nitrogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tops</td>
<td>Roots</td>
</tr>
<tr>
<td>Infested for 10 days</td>
<td>2.6 (3)*</td>
<td>1.6 (3)</td>
</tr>
<tr>
<td>Uninfested</td>
<td>3.0 (3)</td>
<td>2.2 (3)</td>
</tr>
<tr>
<td>t</td>
<td>1.728</td>
<td>2.35</td>
</tr>
</tbody>
</table>

* Figures in parentheses are number of samples taken from two trays each of infested or control seedlings.

**DISCUSSION**

After 10 days of aphid infestation, growth of the tops and roots of radish seedlings was greatly reduced. West (1946) described similar symptoms in tomatoes being attacked by the aphid * Macrosiphum solanifolii*. However, if freed of aphids after 10-14 days, both whole radish seedlings and isolated parts of seedlings can resume growth. After 14 days of aphid infestation irreparable damage had been done to the phloem of the cotyledons on which the aphids fed, and subsequently the cotyledons became senescent.

Aphids on seedlings that had been infested for 10 days became restless and this might be associated with the ability of these seedlings to produce only a small quantity of bleeding sap. Many aphids are dependent on turgor pressure to force phloem sap through their stylets (Mittler, 1957), and reduced turgor pressure could be responsible for the restlessness of the aphids. Restlessness of aphids on heavily infested plants has also been observed by Orman & Painter (1960) and Mittler & Sylvester (1961).

The reduction in bleeding sap occurred even though adequate soil moisture was available to the roots. Wilting in other aphid/plant interactions has been attributed to reduced root development in infested plants (Van Emden et al. 1969). Reduction in root growth which results when the aerial parts of a plant are attacked by plant sucking insects and fungi is rapid and dramatic (Carter, 1948; Orman & Painter, 1960; Last,
This has been attributed to the depletion of the plant’s carbohydrate reserves (Last, 1962; Van Edmen et al. 1969). However, after 10 days of infestation there was an insignificant reduction in the level of soluble carbohydrate. While it is unwise to draw a firm conclusion from this experiment it does appear that the carbohydrate reserves of infested seedlings are not exhausted.

Damage to infested seedlings could also have resulted from the removal by aphids of metabolites including plant hormones (Maxwell & Painter, 1962), and possibly from the injection of growth inhibitors (Dixon, 1971a; Hussain, Forrest & Dixon, 1973). In probing the tissues of radish seedlings *Myzus persicae* may induce a wounding response which could result in the death of the cells in the immediate vicinity of the stylet sheath. It is also possible that the extensive wounding inflicted by the aphid could induce more extensive physiological changes in seedlings. The relative importance of the various factors is unknown. Virus, however, can be excluded as a cause of the symptoms described here, as rooted, aphid-free explants of previously infested seedlings were grown for four months without recurrence of the symptoms associated with aphid attack.

The most obvious physiological differences between infested and uninfested radish seedlings were changes in growth and aspects of water balance which are said to be directly or indirectly under hormonal control (Wright & Hiron, 1969; Jones & Mansfield, 1970, 1972; Mizrahi, Blumenfeld, Bittner & Richmond, 1971; Tal & Imber, 1970, 1972; Glinka & Reinhold, 1971, 1972; Most, 1971; Zeevart, 1971; Kuiper, 1972). The influence of aphids on the hormonal balance of radish seedlings will be described in a second paper.

We are grateful for financial assistance from the Agricultural Research Council, the Science Research Council and the Royal Society. Thanks are also due to Mrs E. M. Scott for her excellent technical assistance and Dr T. J. Dixon for her helpful criticism of the manuscript.

REFERENCES


Growth and wilting of aphid-infested radish seedlings


EXPLANATION OF PLATE

Fig. 1. Fifteen-day-old radish seedlings. Left: infested with aphids for 10 days. Right: uninfested.

Fig. 2. Details of roots of 15-day-old radish seedlings. Left: infested with aphids. Right: uninfested.

Figs. 3, 4. Cleared cotyledons and leaves of radish plants to show necrotic areas along veins caused by feeding aphids. (with controls).
Plate 1

J. M. S. Forrest, A. Hussain and A. F. G. Dixon

(Facing p. 274)