THE EFFECTS OF FLOWERING AND FRUIT FORMATION ON THE SUPPLY OF PHOTOSYNTHETIC ASSIMILATES TO THE NODULES OF *PISUM SATIVUM* L. IN RELATION TO THE FIXATION OF NITROGEN

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SUMMARY

Both nitrogenase activity and the accumulation of $^{14}$C-labelled photosynthates in the nodules of pea plants in nitrogen-free culture reached maxima shortly before flowering and fruit development. During the period from flowering to fruiting, nitrogenase activity and accumulation of $^{14}$C-photosynthates in the nodules declined by 60%, whereas the photosynthesis of the plant doubled. Studies of the translocation of the photosynthates within the plant during this period suggested that this might be due to an increase from 17% to 50% of the photosynthates which accumulated at the shoot apex. Consistent increases in nitrogenase activity over the 24 hours following removal of the shoot apex were only achieved in fruiting plants, however, although vegetative, flowering and fruiting plants all showed increased accumulation of photosynthates in the nodules. Continuous removal of flowers as they were formed over a 2-week period resulted in a large increase in accumulation of photosynthates in the nodules and in nitrogenase activity.

Other factors which may regulate nitrogenase activity in these plants are discussed.

INTRODUCTION

During the growth of pea plants in nitrogen-free media, a marked correlation exists between the accumulation of labelled photosynthates in the nodules and the rate of nitrogen fixation, as measured by acetylene reduction (Lawrie and Wheeler, 1973). Nitrogenase activity, the total fresh weight of active nodules and their accumulation of radioactive photosynthates were all at a maximum just prior to flowering, when there was a rapid decline in both nitrogenase activity and accumulation of photosynthates in the nodules. These results suggest that the nutritional demands of the reproductive processes may starve the nodules of assimilates necessary to support optimum levels of nitrogenase activity. Similar conclusions have been drawn for some other species, e.g. Pate (1958a) showed that *Vicia sativa* root nodules were most active in fixation just prior to flowering and that removal of the flower buds resulted in continued nodule development on secondary roots. Likewise, Roponen and Virtanen (1968) observed a delay in nodule senescence in *Pisum sativum* on removal of the apical flower bud, resulting in the production of abnormally large, active nodules.

Not all nitrogen-fixing plants show a decrease in nitrogen fixation with the onset of

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flowering, however. For example, Bond (1936) found that nodule efficiency in soybeans grown in nitrogen-free culture rose to a maximum during flowering. A subsequent decrease in efficiency during fruiting was attributed either to a reduction in the supply of carbohydrates to the nodules or to the presence of an increasing number of inactive bacteria. By contrast, in field-grown soybeans, Hardy et al. (1971) and Mague and Burris (1972) found that more than 90% of the total nitrogen fixed, as assayed by acetylene reduction, was due to activity during fruit formation and maturation. Removal of the maturing pods prevented the increase in nitrogenase activity and nodule weight which normally occurred during fruit development in soybean and it was suggested that the increase in nitrogenase activity reflected the plant demand for nitrogen (Hardy et al., 1968). Studies with tropical perennial legumes (Whiteman, 1970) did not reveal any close relationship between the development of reproductive organs and nodules and, in Desmodium spp., maximum nodule yield preceded onset of flowering by 1–3 months.

The reasons for these variations in the effect of flowering and fruit formation on the nitrogen-fixing process, both between species and in the same species under different conditions of growth, are not known. In this study, the extent to which photosynthesis of the plant can satisfy the competition for assimilates between the different metabolic 'sinks' has been studied in relation to changes in nitrogenase activity and accumulation of photosynthates in the nodules during flowering and fruit formation and on detachment of the apices.

Materials and Methods

The culture of plants of Pisum sativum L. cv. 'Alaska', assays of acetylene reduction and estimation of radioactivity of plant parts at intervals following exposure of the shoots to $^{14}$CO$_2$ for 15 or 30 min have been described previously (Lawrie and Wheeler, 1973). As before, the plants used were of small stature and had a short life cycle, probably due not only to the culture techniques employed but also to the high temperatures experienced in the glasshouse on sunny days, when temperatures of about 30°C were often attained for short periods.

Where the short-term effects of removing the shoot apices were investigated, plant shoots were exposed to $^{14}$CO$_2$ for 30 min immediately before excision of the apices. Measurements of radioactivity of nodules of both treated and intact plants were made immediately and at intervals during the 24-h period after excision. Acetylene reduction was measured simultaneously on plants treated similarly but incubated in a non-radioactive atmosphere.

The long-term effects of removing the shoot apices of flowering plants were investigated by excising the existing flowering apices of 3-week-old plants and those newly formed until the plants were 5 weeks old. Groups of both treated and untreated plants were then exposed to $^{14}$CO$_2$ for 30 min and harvested 10 h after exposure.

Results

Acetylene reduction and accumulation of $^{14}$C-labelled photosynthates during plant growth.

Table 1 shows that acetylene reduction reached a maximum in vegetative plants 3 weeks old and then declined rapidly with the onset of flowering at this age and during subsequent fruit development. The total radioactivity of these plants, determined at weekly intervals during growth, after they had photosynthesized in an atmosphere
Table 1. Changes in fresh weight, acetylene reduction and radioactivity during growth. Plants were exposed to $^{14}$CO$_2$ for 15 min and radioactivity assessed after 6 h 'cold-chase'. The standard error of the mean was always less than 10%.

<table>
<thead>
<tr>
<th>Age of plant (weeks)</th>
<th>Whole plant</th>
<th>Nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh weight (mg)</td>
<td>nmoles C$_2$H$_2$ reduced plant$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>1</td>
<td>591</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1294</td>
<td>1562</td>
</tr>
<tr>
<td>3</td>
<td>1549</td>
<td>8695</td>
</tr>
<tr>
<td>4</td>
<td>1570</td>
<td>3384</td>
</tr>
<tr>
<td>5</td>
<td>2005</td>
<td>504</td>
</tr>
<tr>
<td>6</td>
<td>1844</td>
<td>0</td>
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containing $^{14}$CO$_2$ doubled between weeks 3 and 4 to reach a maximum in plants 4 weeks old, before declining. By contrast, the total fresh weight of the plants increased until week 5. Maximum radioactivity in the nodules coincided with maximum nitrogenase activity at week 3, while the fresh weight of nodules per plant was at a maximum between week 3 and 4.

**Distribution of $^{14}$C-labelled photosynthates in relation to plant age**

In 2-week-old vegetative plants, leaves 3 and 4 were most heavily labelled immediately after exposure of the shoots to $^{14}$CO$_2$ for 15 min and were probably the most photosynthetically active leaves (Fig. 1a). Older leaves (1 and 2) showed less activity and the shoot apex (shown as L$_5$), roots and nodules showed only negligible activity. After a ‘cold-chase’ of 6 h the activity of the nodules, root and shoot apex rose to 0.3%, 30% and 17% respectively of the total radioactivity of the plant and, after 25 h, was 0.2%, 39% and 25%.

In 3-week-old plants, the leaves (3–6) most active in photosynthesis were highly labelled immediately after exposure of the shoot to $^{14}$CO$_2$ and the flowers accumulated
6% of the total radioactivity at this time (Fig. 1b). The activity of the leaves declined by half over the next 25 h, whereas the activity of the root and flower rose to 23% and 33% respectively of the total radioactivity of the plant. By contrast, the radioactivity of the nodules fell from 3% of the total activity at 6 h to 1% at 25 h after feeding. Although the total radioactivity accumulated in the nodules never exceeded 3% of that of the whole plant, the specific activity of the nodules exceeded that of the most photosynthetically active leaves only 4 h after exposure to $^{14}$CO$_2$ and was greater than any other plant part except the apex.

In 4-week-old fruiting plants, most of the radioactivity was again contained initially in the most photosynthetically active leaves (3–7) immediately after feeding, but the fruit also accumulated 7% of the total radioactivity of the plant. Twenty-five hours after feeding, the activity of these leaves had declined by half and the nodules and roots contained only 0.8% and 12% respectively of the total radioactivity, whereas the fruit contained 50% of the total plant activity.
The most striking changes in the accumulation of radioactive photosynthates as the plants aged from 2 to 4 weeks were therefore the three-fold increase in the percentage accumulation by the shoot tip as it changed from a vegetative to a reproductive state and the accompanying three-fold decrease in the percentage accumulation in the roots. The activity accumulated by the nodules expressed as a percentage of the total plant radioactivity increased twenty-fold as plants aged from 2 to 3 weeks but declined to only one-tenth of this in 4-week-old plants. The nodules nearly always showed the highest specific activity of any plant part, however, with the exception of those leaves most active in photosynthesis.

**Effect of removal of apex on the accumulation of ¹⁴C-assimilates and acetylene reduction of the nodules**

Rather variable results were obtained in experiments designed to study the short-term effects of detachment of the vegetative, flowering or fruit-bearing apices of pea plants on the acetylene reduction activity and accumulation of radioactive photosynthates.

Table 2. *Effect of continuous removal of flowers on the decline of acetylene-reducing activity during ageing of pea plants from 3 to 5 weeks old. (Flowers were removed on formation from 3-week-old plants and the plants were harvested for analysis when 5 weeks old. An untreated set of plants of the same age was harvested simultaneously)*

<table>
<thead>
<tr>
<th></th>
<th>Treated plants</th>
<th>Intact plants</th>
<th>t value of difference between means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh weight of nodules per plant (mg)</td>
<td>36.70 ± 6.18</td>
<td>7.80 ± 1.87</td>
<td>3.687**</td>
</tr>
<tr>
<td>Acetylene-reducing activity of nodules (n moles C₂H₂ reduced mg⁻¹ fresh weight nodules h⁻¹)</td>
<td>126.49 ± 28.03</td>
<td>42.84 ± 9.14</td>
<td>2.837*</td>
</tr>
<tr>
<td>Radioactivity accumulated by nodules dpm mg⁻¹ fresh weight nodules)</td>
<td>98.0 ± 19.4</td>
<td>9.3 ± 1.6</td>
<td>4.785***</td>
</tr>
</tbody>
</table>

in the nodules. In 2-week-old vegetative plants, the accumulation of ¹⁴C-labelled photosynthates was usually slightly greater in the nodules of treated than intact plants, although only the differences between activities 4 and 12 h after treatment attained statistical significance (Fig. 2a). By contrast, the effect of excision on the acetylene-reducing activity of the nodules was much less clear, with the activity of treated plants significantly greater than that of intact plants 8 h after treatment, but significantly less after 24 h (Fig. 2b).

In 3-week-old flowering plants, significant increases in the accumulation of labelled photosynthates by the nodules of treated plants were observed at all time of harvest up to 24 h after detachment of the apex (Fig. 2c). The acetylene-reducing activity of treated plants was always slightly lower than that of intact plants but the differences were not significant (Fig. 2d).

In 4-week-old fruiting plants, the accumulation of ¹⁴C-labelled photosynthates in the nodules of treated plants was always greater than in intact plants (Fig. 2e), but increases were significant only 8 and 24 h after detachment of the fruit. The acetylene-reducing activity of the nodules of treated plants was at least three times that of the intact plants (Fig. 2f), but replicate experiments showed considerable variability in the size of this
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difference, although significant increases in activity 4 h after treatment were found in all experiments.

Removal of the flower buds as they were formed on plants 3–5 weeks old prevented the usual decrease in acetylene reducing activity (Table 2). Both the acetylene-reducing activity and the nodule yield of 5-week-old treated plants were three times those of intact plants of similar age, while the specific activity of the nodules of the treated plants 10 h after exposure of the shoots to $^{14}$CO$_2$ was ten times that of untreated, fruit-bearing plants.

**DISCUSSION**

The close relationship between plant development and nitrogenase activity (Table 1) suggests that *Pisum sativum*, grown in nitrogen-free culture, behaves similarly to those species whose reproductive growth is associated with inhibition of nitrogen fixation (see Introduction). The possibility that a reduction in the supply of photosynthates to the nodules is a major cause of the reduction in nitrogenase activity is supported by the 60% decrease in specific radioactivity of the nodules, following photosynthesis of the shoots in an atmosphere containing $^{14}$CO$_2$, as the plants changed from the vegetative (3-week-old plants) to the reproductive (4-week-old) condition. This occurred despite a two-fold increase over this period in the total photosynthetic capacity of the plant, as measured by the accumulation of $^{14}$C-photosynthates (Table 1). Photosynthates which would otherwise be available for root and nodule metabolism are apparently diverted to the shoot tip, as shown by the two-fold and four-fold increases in accumulation of photosynthates in this region when plants flowered and fruited respectively (Fig. 1).

If the diversion of photosynthates away from the root system to the shoot apices is the critical factor in the reduction in nitrogenase activity which accompanies reproductive growth in these plants, then detachment of vegetative and floral apices would be expected to stimulate nitrogenase activity less than the detachment of fruiting apices, which provide a stronger 'sink' for photosynthates. Short-term experiments, carried out over 24 h following excision of the apices, partly support this hypothesis since only in fruiting plants were the increases in accumulation of $^{14}$C-labelled photosynthates, which occurred in the nodules of vegetative, flowering and fruiting plants on excision of the apices, associated with increased nitrogenase activity (Fig. 2). However, in replicate experiments, nitrogenase activity in flowering plants consistently showed a decline after detachment of the flower, which was almost significant statistically, while nitrogenase activity in vegetative plants increased 8 h after detachment of the apices and then decreased after 24 h. These results show that an increase in the supply of photosynthates to the nodules does not necessarily result in increased nitrogenase activity.

Lovell, Oo and Sagar (1972) reported that removal of the shoot apices of pea plants reduced export of assimilates from the leaves, which they suggested was governed mainly by the demand of the metabolic ‘sinks’ of the plant. It is possible, therefore, that, in the plants used in the present studies, not all of the photosynthates which would normally be translocated to the shoot apex are available for translocation to the nodules after excision of the apex and the amount redirected may vary with the development of the plant. In addition, Hardy *et al.* (1968) found that, in field-grown soybean plants, nitrogenase activity increased as the plant demand for nitrogen increased during flowering and fruiting. Removal of the apex of vegetative and reproductive pea plants might, therefore, result in a fall in nitrogenase activity by reducing the plant demand for nitrogen.
but the detachment of the fruits should have reduced activity even further if this is a major mechanism for the control of nitrogenase activity. The lower specific radioactivity of the nodules of 4-week-old fruit-bearing plants compared with 3-week and 2-week-old plants, following photosynthesis in a labelled atmosphere (Fig. 2), suggests that there may be a greater carbohydrate deficiency in the older nodules, relative to their potential nitrogenase activity, despite the greater photosynthetic capacity of 4-week-old plants (see above). This deficiency could arise from the massive diversion of photosynthates to the developing fruits in 4-week-old plants (Fig. 1c) and may result in the greater increase in nitrogenase activity when the supply of photosynthates to the nodules is increased compared with younger plants.

In long-term experiments in which the effect of the continuous removal of flowers on nitrogenase activity during ageing of pea plants from 3 to 5 weeks was studied, there was a three-fold increase in nitrogenase activity and a ten-fold increase in accumulation of $^{14}\text{C}$-photosynthates in the nodules of treated plants. Nodule yield was also increased, as reported by Roponen and Virtanen (1968). These results again show how, under some circumstances, increased nitrogenase activity and increased accumulation of $^{14}\text{C}$-photosynthates may be correlated.

The production of abnormally thick stems and leaf blades and the growth of axillary buds which occurred during the long-term experiments suggest, as would be expected, that there is a marked alteration in the hormonal balance within the plant over the 2 weeks of these experiments. This in turn may have profound effects upon the distribution of assimilates between different metabolic 'sinks' in the plants. The question arises whether changes in the hormone balance within the plant, which must result from decapitation, may influence the distribution of photosynthates during the more short-term experiments described above. Seth and Wareing (1967) noted that the effect of indole acetic acid on $^{32}\text{P}$ accumulation in decapitated bean internodes could be detected within 2 h of $^{32}\text{P}$ application 8–10 cm from the top of the decapitated plant, although gibberellic-acid- and kinetin-treated plants showed little additional activity over control plants even after 12 h. These experiments suggested that auxin might have an effect on long-distance transport over a relatively short period of time, but that the effects of gibberellin and kinetin over similar periods were minimal. The effects of hormonal changes on nitrogenase activity have not been investigated thoroughly, although high levels of free auxins and gibberellic acid have been reported in nodules of $P.\ arvense$ (Pate, 1958b), alder (Dullaart, 1970b) and lupin (Dullaart, 1970a; Dullaart and Duba, 1973) and at least part of the auxin in alder nodules is produced by the plant in response to infection (Dullaart, 1970b). Short-term changes in hormonal content in plants bearing nitrogen-fixing root nodules have not been studied, but Thimann (1963) noted that nodule growth was associated with the presence of excess auxin in a free, active form in several species and that the auxin content of the nodules fell towards the end of plant life. Pate (1958b) observed fluctuations in the hormone content of $P.\ arvense$ nodules during plant growth and found that the nodule content of two growth-promoting substances decreased towards the end of nodule life. Dullaart (1970b), however, found no significant changes in auxin levels of the nodules of the perennial alder plant during 1 year's growth.

It is not possible, therefore, at the present time to deduce the precise effects which changes in hormonal balance in the experimental plants may have had on the results reported here. They could be a major factor in determining the effects of flowering and fruit formation on nitrogen fixation in different species, perhaps by controlling the com-
petition for photosynthates between the different metabolic ‘sinks’ in the plant. The possibility of hormone-directed translocation of assimilates affecting nitrogenase activity in plants bearing nitrogen-fixing root nodules merits detailed study.

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REFERENCES


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