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AMINO-ACIDS IN THE BLEEDING SAP OF FRUITING TOMATO PLANTS

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Abstract

1. Growth and amino-acid content of the bleeding sap and roots of flowering and fruiting tomato plants were compared with those of deflowered plants.

2. The vegetative growth of fruiting plants decreased.

3. The amino-acid concentration of the bleeding sap increased at first with the age of the plants. After flowering it decreased in the flowering and fruiting plants, and was about constant in the deflowered plants.
Roots of fruiting plants contained less amino-acid.

4. Although the bleeding sap of some roots contained hardly any amino-acids, the amino-acid content of the roots was about normal.
It is concluded that a pool of free amino-acids is present in the root, which is not directly available for the bleeding sap.

Introduction

Some years ago, amino-acids were found to be present in the bleeding sap of most plants. (e.g. Kursanov, 1957, Van Die, 1958, Mothes and Engelbrecht, 1952) and since then there have been many investigations into the nature of these amino-acids.

Van Die (1958, 1959, 1960, 1961) analysed the bleeding sap of the tomato, finding that glutamine was the major component.

It was found in some experiments by the present author that bleeding sap of young tomato plants contained less amino-acid than that of older ones, and the question then arose whether this might be correlated with flowering and fruiting.

Leonard (1962) gave a review of the literature on the interrelations of vegetative and reproductive growth of plants, but no data were given about the transport of amino-acids from root to shoot. The experiments of Murneek (1926) have already shown that there is a retardation of vegetative growth and a decrease in nitrogen content of the vegetative parts of fruiting tomato plants, as compared with those of defruited plants. Hudson (1960) observed that after this retardation of vegetative growth, there was an increase in root growth when the first fruits had been picked. It was found by Bollard (1957) that the nitrogen content of the tracheal sap of the apple increased during flowering and then decreased to the original level. Potapov and Cseh (1955) gave some data about the nitrogen content of the bleeding sap of maize during its growth. The organic nitrogen content was at a maximum in the bleeding sap of 48-day-old plants, and at a minimum in that of 54-day-old plants, but it is not clear at what time flowering and fruiting started. KURSANOV (1960) suggests that some of the amino-acid, synthesized in the root, rises with the transpiration stream and is used in the growing regions of the shoot and in the developing fruit.

In the following experiments we tried to obtain some information about the amino-acid concentration of the bleeding sap of tomato plants, both with and without fruits.

MATERIAL AND METHODS

Plants. Tomato plants (cultivars Moneymaker and Florissante) were grown in a nutrient solution in containers, each of which held four plants in eighteen litres of solution. The nutrient solution was pumped from a big stock vessel into the containers, and from there back into the stock vessel, at a rate of 40 litres/hour. On its way it absorbed enough oxygen for the aeration of the roots.

Both ammonium sulphate and calcium and potassium nitrate were used as sources of nitrogen.

The minimum temperature at night in the greenhouse was about 15° C, the maximum temperature in the daytime varied between 22° C and 35° C.

Bleeding. Bleeding experiments were carried out in a dark room at a constant temperature of 23° C. The bleeding sap was collected in tubes cooled by ice.

Root extract. Roots were dried between filter paper and stored at — 25° C.

The frozen roots were powdered in liquid nitrogen, and the powder stored at — 25° C. It was later shaken with 96 % alcohol (final concentration 80 %) to obtain an extract.

Dry weight. The frozen root powder was dried at 95–100° C.

Amino-acids. Free amino-acids were determined in the bleeding sap and root extract after VAN DIE (1959).

One dimensional chromatograms were made with a tert. butanol-formic-acid-water mixture (695–10–295) on Whatman No. 1 filter paper. The paper was sprayed with a solution of ninhydrin in acetone (0.2 %).

EXPERIMENTS AND RESULTS

Exp. 1. In this experiment about one hundred tomato plants were used (cultivar Moneymaker). All flowerbuds were removed from half of these plants. Samples were taken at fortnightly intervals.

Four plants were decapitated and the bleeding sap was collected. When the plants flowered, four flowering (fruiting) plants and four deflowered plants were used. From a second group of plants, two

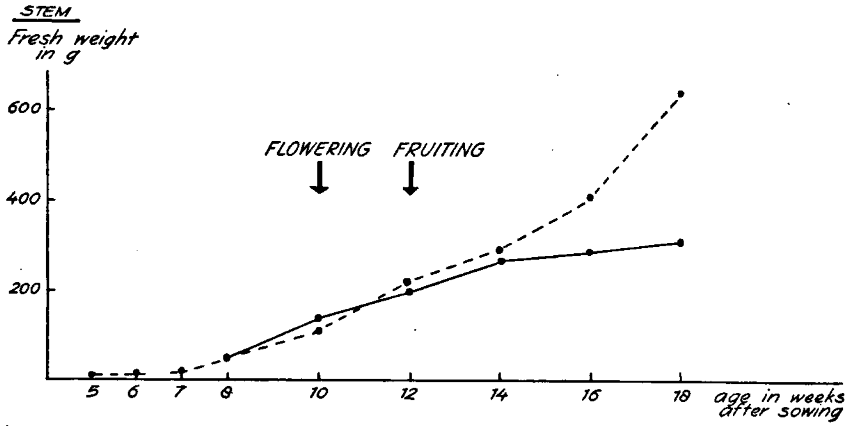


Fig. 1

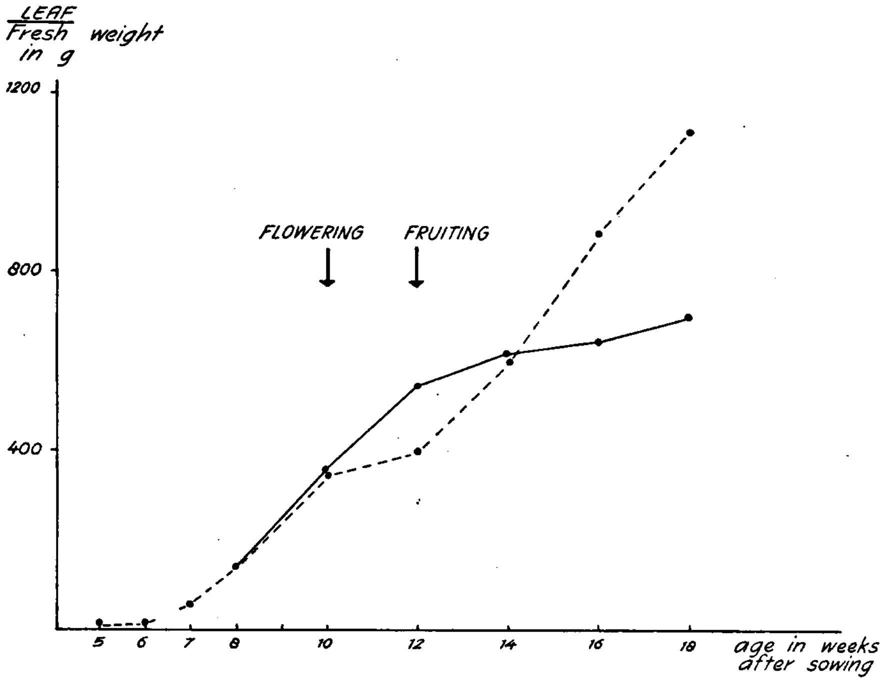


Fig. 2

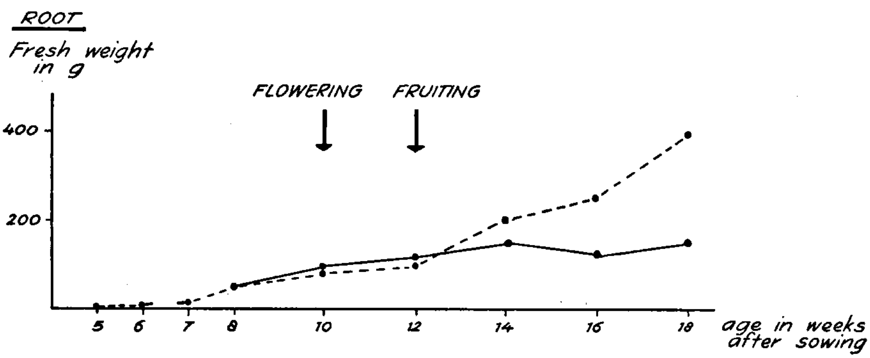


Fig. 3

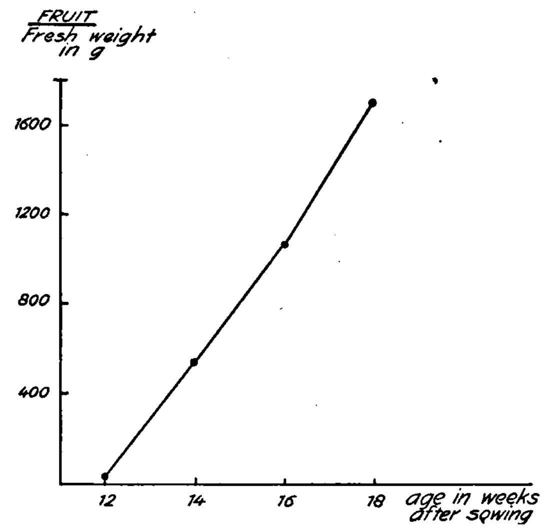


Fig. 4

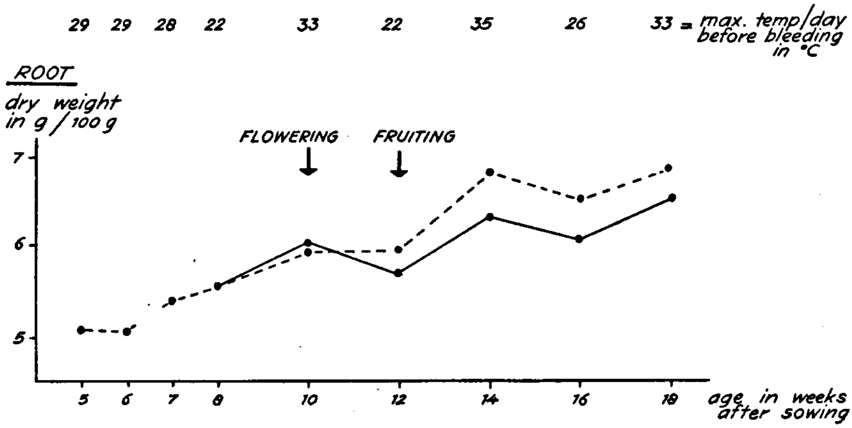


Fig. 5

Fig. 1, 2, 3, 4, 5. Fresh weight of stem, leaves, root and fruits, and dry weight of the root of tomato plants, measured at one or two weeks intervals during growth. The continuous line represents the plants with flowers and fruits, the broken line those without. The average weight of four plants is given.

series were analysed to obtain further information about small plants (five and six weeks old). The plants were placed in a fresh nutrient solution one day before the bleeding experiment and again after decapitation. The plants were decapitated between 8.30 and 9.00 a.m. Fresh weight of stem, leaves and fruits was measured after the decapitation; bleeding sap was collected from 9.30 a.m. till 5.30 p.m. in two hour fractions. At 5.30 p.m. the roots were dried, weighed and stored at — 25° C.

From the results shown in Fig. 1, 2, 3, and 4, it is clear that the vegetative growth in fruiting plants is decreased. Considerably more starch was found in the leaves and stem of the deflowered 18-week-old plants than in the same parts of the fruiting plants. The dry-weight of the leaves and the lower part of the stem was higher in the deflowered 18-week-old plants than in the fruiting plants (Table 1).

TABLE 1

Dry-weight of leaves and stem of 18-week-old plants as a % of fresh weight

	Deflowered plants	Flowering and fruiting plants
Lower stem	18.3	9.8
Upper stem	8.4	10.4
Old leaves	10.9	7.9
Young leaves	15.1	6.9

The results, given in Fig. 5, show an increase in dry-weight of the roots, as plants were growing. The dry-weight of the roots of plants without flowers and fruits was higher than that of plants with flowers and fruits; the fluctuations in root dry-weight corresponded with the fluctuations in the maximum temperature in the greenhouse on the day before the experiment (Fig. 5).

In Fig. 6 the amino-acid concentration of the bleeding sap is given for the first two-hour fraction. This gives, according to PATE (1962), the best picture of what is going on in the intact plant. Several changes, for example a decrease in respiration, may occur in the root after a longer time of bleeding. The total amount of amino-acids in the bleeding sap of the first two-hour fraction is given in Fig. 7.

Fig. 6 and 7 show that the amino-acid concentration and the total amount of amino-acid in the bleeding sap increased with the age of the plants. In bleeding sap of deflowered plants of 8 weeks and older the amino-acid concentration was more or less constant. The root dry-weight and the amino-acid concentration of the bleeding sap oscillated in the opposite direction, as high (low) dry-root weight corresponded with a low (high) amino-acid concentration of the bleeding sap. However, the total amount in the bleeding sap of these plants increased during growth. In the bleeding sap of plants with flowers and fruits there was a peak in amino-acid concentration and

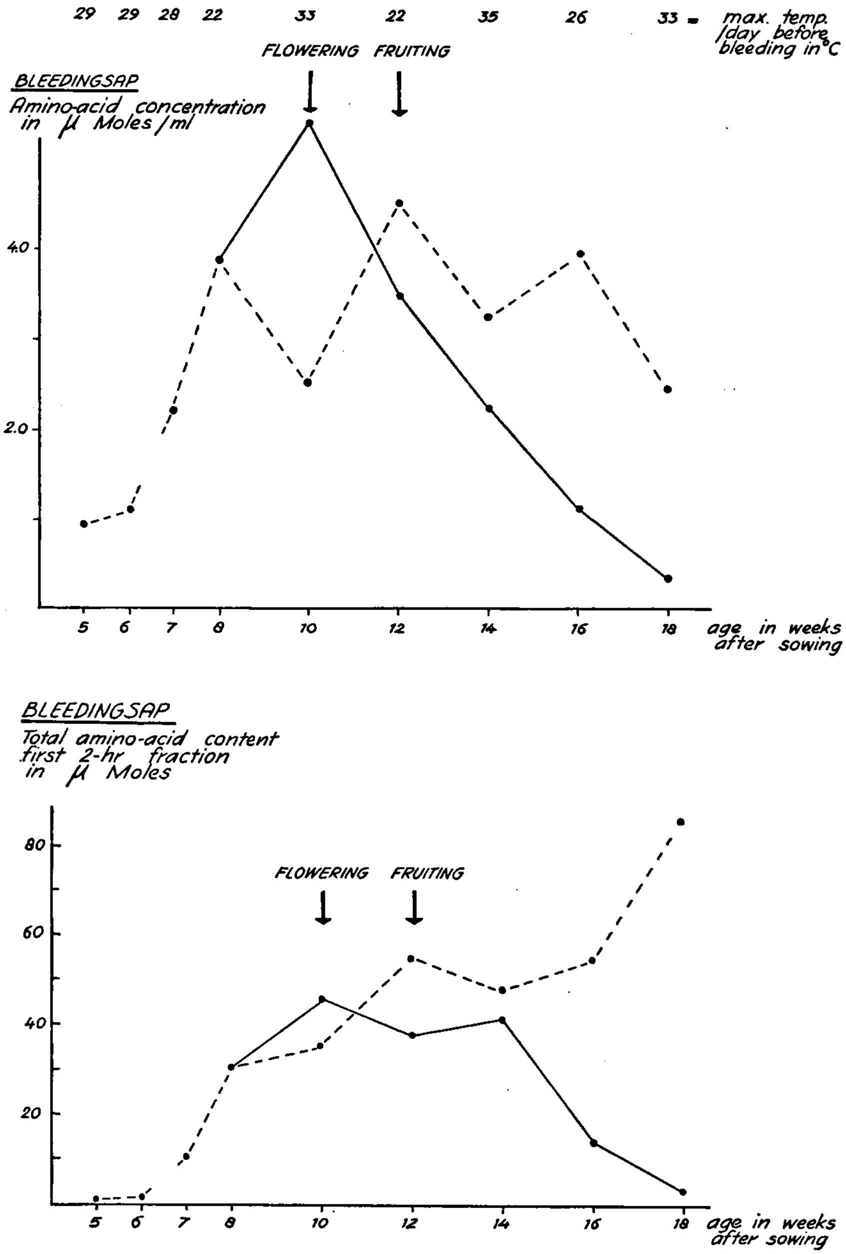
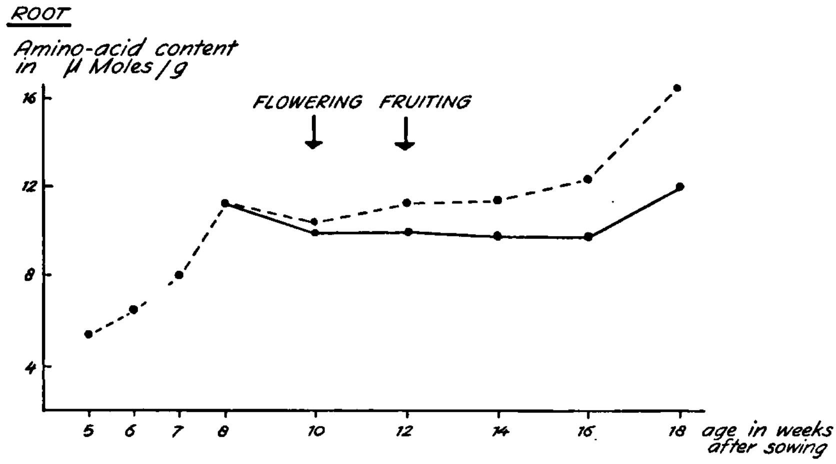


Fig. 6 and 7. Amino-acid concentration and total amino-acid content of the first two-hour bleeding sap fraction of tomato plants, determined at one or two-week intervals during growth. The continuous line represents the plants with flowers and fruits, the broken line those without. Average values of four plants are given.



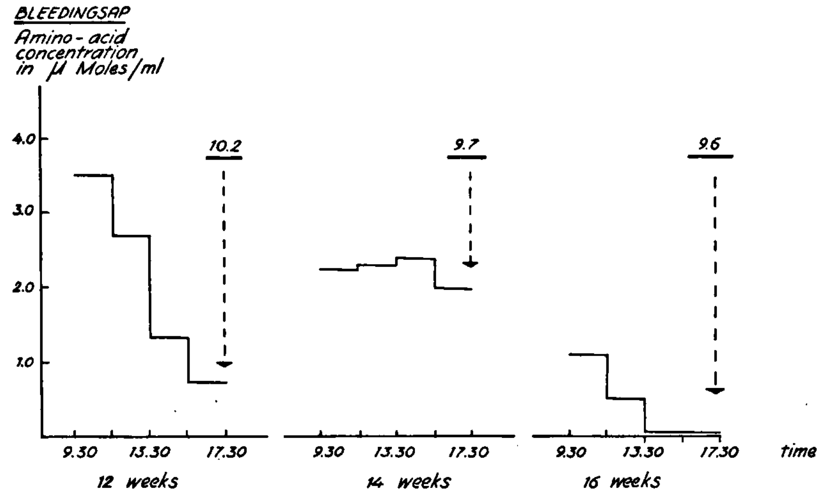


Fig. 8. Amino-acid content of the root of tomato plants (in μMoles/g fresh weight), determined at one or two week intervals during growth. The continuous line represents the plants with flowers and fruits, the broken line those without. Average values of four plants are given.

Fig. 9. Amino-acid concentration of four bleeding sap fractions of tomato plants at the age of 12, 14, and 16 weeks. The underlined data refer to the amino-acid content of the roots (in μMoles/g fresh weight), determined after the bleeding experiment. Average values of four plants are given.

total amount of amino-acids during first flowering, and afterwards a sharp decline during fruiting.

The amino-acid content of the root also increased during growth and it was higher in plants without flowers and fruits than in plants with flowers and fruits (Fig. 8).

In 16-week-old fruiting plants, the bleeding sap contained hardly any amino-acids. However, when the amino-acid content of the roots was determined after the bleeding experiment, it proved to be normal (Fig. 9), and the same was found in some other experiments described below.

Exp. 2. Tomato plants (cultivar Florissante), which were about three months old, were used. Two plants were placed in a fresh nutrient solution four days before the experiment. The plants were decapitated at 8.30 a.m. and bleeding sap was collected for two days in two-hourly fractions.

The results, given in Fig. 10, show that hardly any amino-acids were found in the bleeding sap on the second day. The roots, which were analysed after bleeding, contained about the usual amount of amino-acids.

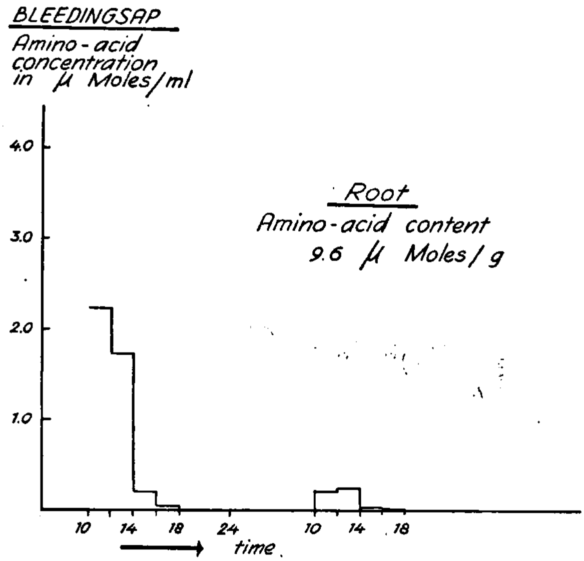
Exp. 3. About 3-month-old tomato plants were used (cultivar Florissante).

Five days before the experiment four plants were placed in a fresh nutrient solution. The plants were decapitated at 8.30 a.m. and the roots of two plants were placed in a nutrient solution without ammonium sulphate (ammonium sulphate was replaced by potassium sulphate). The roots of the other two plants were placed in the normal nutrient solution. Bleeding sap was collected in two-hourly fractions for two days. A difference in amino-acid concentration is already apparent in the first fraction of the bleeding sap, as is shown in Fig. 11, and it was shown by means of chromatography that the decrease in amino-acid concentration was largely accounted for by a drop in the glutamine content. The ammonium concentration in this bleeding sap fraction of the roots, which did not get any ammonium, was considerably lower than that in the bleeding sap of the roots, supplied with ammonium (32 μg/ml and 72 μg/ml).

Discussion

Experiment 1 shows clearly that there is a decrease in vegetative growth of tomato plants with flowers and fruits, compared with the growth of plants without flowers and fruits. The root growth increased after the first ripe fruits were picked. New white roots were visible. These findings are in close agreement with the results of Murneek (1926) and Hudson (1960).

The fluctuations in the dry-weight of the root correspond with



↑ Fig. 10. Amino-acid concentration of two hour bleeding sap fractions of tomato plants compared with the amino-acid content of the root, determined after the bleeding experiment. Average values of two plants are given.

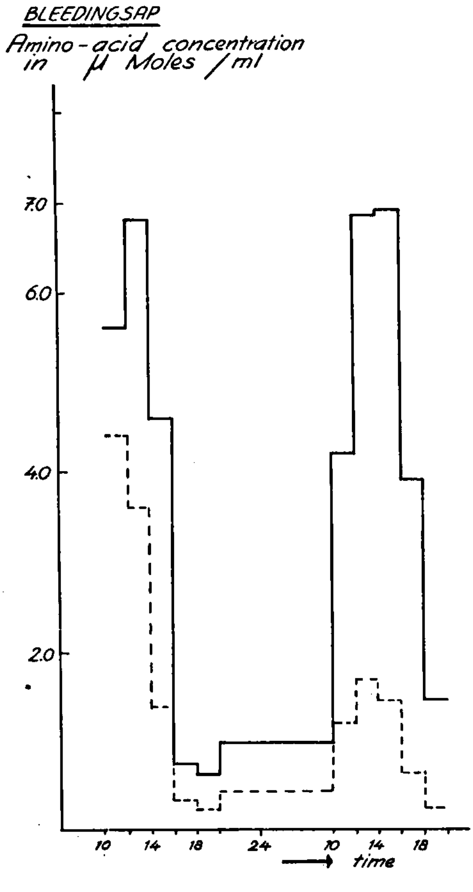


Fig. 11. Amino-acid concentration of two hour bleeding sap fractions of tomato plants, placed in a nutrient solution with ammonium (continuous) and without ammonium (broken line). Average values of two plants are given. ←

the variations in temperature on the day before the plants were set bleeding. This might have a parallel with the data given by RICHARDSON (1953). He stated that the root growth in *Acer* responds within 12 hours to changes in the temperature of the shoot. On hot days, when there is more light, photosynthesis is higher, and this might result in a bigger transport of photosynthates from shoot to root.

In the bleeding sap of fruiting plants the total amount of amino-acids decreased during the development of the fruits. In deflowered plants, however, it increased during this time. According to HUDSON (1960) the fruit is able to monopolize the food resources of the plant at the expense of other organs. He even found a rapid death of part of the root system as the first fruits were swelling. It might, therefore, have been possible that in the bleeding sap of fruiting plants the amino-acids originate from a breakdown of root protein, as is found in some parts of the shoot of nitrogen deficient plants. There is a transport of amino-acids, following a breakdown of protein, from the older to the younger leaves. From the present experiment it seems unlikely that amino-acids, originating from a breakdown of root protein, are transported from root to shoot, as there is a sharp decline in amino-acid concentration of the bleeding sap (Fig. 6). It is, however, possible that, following a decrease in carbohydrate transport from shoot to root (HUDSON, 1960), the amino-acid synthesis in the root decreases. This may result in a decrease in root growth as well as in a decrease in amino-acid transport from root to shoot.

It is not clear where the amino-acids in the bleeding sap come from. KURSANOV (1960) said that amino-acids present in the bleeding sap are synthesized in the root. VAN DIE (1960) supposed that the amino-acids synthesized in the root enter a pool, where they are mixed with amino-acids from breakdown and resynthesis of proteins. From this pool they arrive in the bleeding sap. According to him it is possible that amino-acids in the bleeding sap of older plants come from a breakdown of proteins.

The results of Experiment 1 and 2 showed that the amino-acid content of the root can be considerable, whereas the bleeding sap contains hardly any amino-acids. There seems to be an amino-acid pool, from which the amino-acids cannot reach the bleeding sap directly. One may suppose that some of the amino-acids, synthesized in the root, enter the bleeding sap directly. The rest of these amino-acids enter the pool and might be used in protein synthesis. A second indication for this assumption is shown in Experiment 3. Here it was possible to affect the amino-acid concentration of bleeding sap within two hours by altering the ammonium supply in the nutrient solution.

In some way, the situation is reminiscent of results obtained by R. J. Helder (personal communication). He found that some of the halide ions, taken up by barley roots, were transported directly to the shoot, whereas the remainder entered a pool, from where they could not reach the xylem vessels.

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