A STUDY OF ROOT EXUDATES BY THE FOG-BOX TECHNIQUE

By M. F. CLAYTON*† and J. A. LAMBERTON*

Summary

The fog-box technique has been used to grow plants of Tagetes species and Albizia lebophanta Benth. for root exudate studies. The quantity of organic matter detected in the root drip was extremely small. The results are discussed and compared with findings previously reported in the literature.

I. INTRODUCTION

The study of root exudates has assumed considerable importance because of the many claims that such exudates influence soil fertility, that in some cases they may exert a toxic action against other plants and thus affect the competition between plant species, and that they may determine the type and population density of microorganisms in the rhizosphere. The possibility that organic substances from living roots may have marked effects on the growth of the same or of a neighbouring organism has been widely discussed, and has been reviewed in detail by Borner (1960), Woods (1960), Garb (1961), and Rovira (1964). On the whole the claims for direct toxic or other action against plants have been only speculative, unsupported by systematic study or proof that the compounds thought to be active would have any effect in the soil.

Borner (1960) concludes that "Massive secretion of organic materials from intact uninjured roots probably does not occur in most species of plants. Such materials as do diffuse from roots probably are restricted to the immediate rhizosphere and do not exercise a direct influence on other plants. These materials do affect the microorganisms of the rhizosphere and may indirectly affect the health and nutrition of the plants." The evidence for the effect on microorganisms is well founded and has been discussed by Rovira (1964). A notable example is the substance present in root exudate from potato and tomato roots which stimulated hatching of potato root eelworm, and which is active in solutions at dilution greater than $1 \times 10^{-7}$ (Callam, Todd, and Waring 1949; Jones 1962).

Great experimental difficulties arise in the isolation of exudates from plant roots without contamination by substances derived from the soil and associated microorganisms, and without loss of exuded material through adsorption on soil particles, or decomposition by microorganisms. Of the more important investigations carried out under carefully controlled conditions in which a liquid nutrient medium without soil was used, Rovira (1969), Rovira and Harris (1961), and Scheffer, Kickuth, and Visser (1962a, 1962b) have been concerned with small plants in the first few weeks of growth. In these studies precautions were taken to maintain sterile conditions, but with large plants this is difficult to achieve. Stotzky, Culbreth, and Mish (1962) have described a method of doing this satisfactorily with a single

† Present address: Horticultural Research Section, CSIRO, Merbein, Vic.
plant, but the apparatus required is complex for use with greater numbers or with larger plants.

II. Fog-box Technique

In the present work the fog-box technique described by Went (1957, p. 81) was used. As shown in Figures 1 and 2, the plants were grown on the removable lid of a box fitted with two spray nozzles through which a fog of nutrient solution was injected, by means of compressed air, onto the roots. Root drip falling from the roots was collected in a centrally placed tray. The method has the advantage of avoiding the use of soil, and allowing collection of root drip without disturbing the roots, but it would be difficult to keep the roots sterile, and this was not attempted. However, the roots were clean in appearance and free from obvious fungal contamination. Because the conditions were not sterile it was decided that specific compounds, or types of compounds, should be sought in the root drip and that no significance should be attached to the detection of commonly occurring substances (e.g. common amino acids or sugars) which might derive from the roots or from bacteria on the surface of the roots. By observing the methods described in Section V it was possible to maintain large plants in a healthy condition in the fog box. The plants flowered and were otherwise normal in appearance, and had well-developed

Fig. 1.—(a) Tagetes erecta growing in a fog box. (b) Root development of T. erecta plants grown in the fog box. The lid of the fog box has been removed.
root systems. With large plants (as in Figs. 1 and 2) supplied with nutrient spray made up from a slightly modified Hoagland solution as recommended by Went (1957, p. 79), the root drip showed a pH higher by up to 1.3 units than the initial pH (6.20) of the spray solution.

![Fig. 2.](image)

Fig. 2.—(a) Albizzia lophantha growing in a fog box. (b) Roots of A. lophantha plants.

III. PROBLEMS INVESTIGATED AND RESULTS

Two plant species were chosen for investigation, namely Tagetes spp. (Compositae) and Albizzia lophantha Benth. (Leguminosae), because there was evidence that their roots contained unusual constituents which could easily be detected, and some suggestion that these might be exuded into the soil.

(a) Compounds in the Roots of Tagetes Species

The roots of several Tagetes species contain two unusual thiophene compounds, α-terthienyl (I) and 5-(3-buten-1-ynyl)-2,2'-bithienyl (II) (Uhlenbroek and Bijloo 1958, 1959; Horn and Lamberton 1963) which have been shown to have a high in
vitro toxicity to nematodes (minimum lethal dose for I, 0·1–0·2 p.p.m. to *Heterodora rostockiensis*, 0·5 p.p.m. to *Anguina tritica*). The relative freedom of *Tagetes* from nematode attack has been attributed to these compounds.

![Chemical structures](image)

The thiophenes I and II show an intense blue fluorescence in very dilute solutions in light petroleum, and they are very easily detected and estimated because of their intense ultraviolet absorption. For this reason they seemed suitable compounds to search for in the fog box root drip. The roots of *T. erecta* (horticultural ‘African marigolds’) and *T. minuta* (syn. *T. glandulifera*) which were grown satisfactorily in the fog box (Fig. 1) contained compounds I and II, but *T. erecta* roots contained relatively more of the more stable compound I, and this species was considered more suitable for experimental purposes.

Although the compounds I and II were easily estimated in extracts from small pieces of root and the roots of small seedlings, no trace could be obtained by extracting large volumes of root drip with low-boiling light petroleum, and it seems clear that under fog-box conditions I and II are not exuded from the roots. Recent biological experiments (Hesling, Pawelska, and Shepherd 1961; Omidvar 1961) lend support to these findings. Exudates collected from the roots of *Tagetes* spp. have been shown to have no appreciable nematocidal effect, and eggs of potato eelworms hatch as freely in root diffusate from *Tagetes* spp. as in soil water. As suggested below, different conditions may apply in the soil, but there are now reports which strongly suggest that the nematocidal action of I and II is limited to those animals which feed upon the plant, and does not extend to the surrounding soil (Jones 1962). By processing a large volume of collected root drip the only substances shown with certainty to be present were traces of amino acids and sugars. Stigmasterol occurs in the roots, but no trace could be detected in the root drip.

The roots of *T. erecta* grown in aerated nutrient solution contained aspartic acid, asparagine, glutamic acid, glycine, serine, alanine, probably valine, and one other unidentified acid, but extracts from crushed roots of older plants, which had been grown in a flower-bed and had completed flowering, contained a wider range of amino acids (aspartic acid, glutamic acid, serine, glycine, asparagine, alanine, lysine, histidine (?), proline, valine, tryptophan (?), and phenylalanine), and it was particularly noticeable that there were more amino acids having higher *R*<sub>p</sub> values in both the solvent systems used. In examining root drip it was very difficult to get satisfactory paper chromatograms of the amino acids which had been separated on Dowex 50 (H<sup>+</sup>). Concentrations were too low, and when two-dimensional chromatograms were run the spots developed with ninhydrin were too diffuse and faint for detection. For this reason chromatograms were run in one direction only in n-butanol–acetic acid–water.

Root drip was collected from plants at the flowering stage over an interval of 2 weeks, and the amino acids were concentrated in a volume of 1 ml. Paper chromatograms were then obtained showing resolution of the amino acids into 11 reasonably
discrete spots, but most of these were very faint, and only three were sufficiently intense and characteristic in colour for identification, with some certainty, as proline, aspartic acid, and asparagine. The occurrence of proline may be noted: it was also found in the roots of the older soil-grown Tagetes, but not in the roots of younger plants grown in nutrient solution. Although the identification of the amino acids was unsatisfactory it can be said that in the concentrate from the root drip there was a mixture of ninhydrin-positive compounds having \( R_f \) values ranging up to 0.7 in the solvent system chosen, and that these were mostly on the limits of detectability by the methods used.

(b) Compounds in the Roots of Albizzia lophontha

The seeds of \( A. \) lophontha are a source of an unusual sulphur-containing amino acid, djenkolic acid (III) (Gmelin, Hasenmaier, and Strauss 1957), and the ureido amino acid albizzine (IV) (Gmelin, Strauss, and Hasenmaier 1958; Kjaer and Larsen 1959). Both of these amino acids occur only in a very limited number of plants belonging to the Mimosaceae. When unripe seeds of \( A. \) lophontha are crushed, or older seeds are ground up and moistened, a powerful and unpleasant odour of the mercaptan is produced because djenkolic acid undergoes enzymatic decomposition releasing methane dithiol (V), and this in turn decomposes slowly to hydrogen sulphide and thioformaldehyde.

\[
\begin{align*}
\text{SCH}_2\text{CHCOOH} & \xrightarrow{\text{C-S-Lyase}} \text{CH}_2\text{SH} + 2\text{CH}_3\text{COOH} + 2\text{NH}_3 \\
\text{NH}_2 & \\
(\text{III}) & \\
\text{SCH}_2\text{CHCOOH} & \\
\text{NH}_2 & \\
\text{H}_2\text{NCONHCH}_2\text{CHCOOH} & (\text{IV})
\end{align*}
\]

It has now been found by paper chromatography that albiziine and djenkolic acid both occur in the roots of small seedlings and older plants of \( A. \) lophontha, but in much lower concentrations than in the seeds. A fainter but characteristic odour of methane dithiol develops when the roots are crushed or when soil is washed from them with water. The detection of methane dithiol by odour is extremely sensitive, and it may be detected in this way at concentrations below the threshold for chemical detection. Both of the amino acids III and IV were easily detected in the roots of the \( A. \) lophontha plants growing in the fog box, and they were also shown to be present, although in very low concentration, in the collected root drip. As in the case of the Tagetes spp., when the root drip obtained over a 2-week period was processed, the spots developed on paper chromatograms were very faint.
No odour of methane dithiol could be detected in the fog box while the plants were undisturbed, nor could any dithiol be detected by chemical means in air drawn across the intact roots, but if the roots were agitated gently or handled the odour of methane dithiol was immediately noticeable although no damage to the roots was visible. This suggests that in the soil abrasion and pressure on the roots would be likely to lead to production of some methane dithiol, the release of which implies the exudation of both djenkolic acid and the specific enzyme (a C-S-lyase) which decomposes it. Evolution of the dithiol seems to be associated with damage to the tissue: it is possible to germinate Albizia seeds without an unpleasant odour, but handling seeds which have imbied water immediately produces an odour.

In addition to djenkolic acid and albizzine, the roots of older soil-grown A. lophantha plants were found to contain aspartic acid, glutamic acid, serine (?), alanine, valine, proline, and leucine, while the seeds of A. lophantha and the roots of seedlings grown in nutrient solution gave a similar pattern, but some of the amino acids of higher RF (in both solvent systems used) gave faint spots or were absent. It was possible to detect djenkolic acid and albizzine in the root drip amino acids by using specific spray reagents and running the seed amino acids simultaneously as a reference standard. Although the root drip material gave a range of ninhydrin-positive spots ranging up to $RF \cdot 0.7$ in n-butanol-acetic acid–water, these were faint and difficult to detect in two-dimensional chromatograms. Aspartic acid, proline, and sulphur-containing compounds of low RF were found to be present. To obtain better resolution, and because both djenkolic acid and albizziine have low RF values, descending paper chromatograms were run in n-butanol-acetic acid–water and developed after 72 hr with the chloroplatinic acid reagent which is used to detect sulphur-containing compounds. This showed a spot which also gave a positive ninhydrin test, and corresponded in position with djenkolic acid in the seed amino acids used as a marker. A similar chromatogram treated with Ehrlich's reagent developed three spots, one of which corresponded in position to the ureido acid in the control, and the other two had lower RF values. It has therefore been shown that the root drip contains both djenkolic acid and albizzine although the amounts present are very small.

Albizia species commonly contain saponins (Varshney and Khan 1962) and the saponins from A. lebbeck, A. odoratissima, A. anthelmintica, and A. procera consist of complex glycosides of oleicolic, echinocystic, and machaerinic acids. The dust from milled A. lophantha root bark has the unpleasant sternutatory action observed in a number of plants rich in saponins, and aqueous extracts of the roots foamed strongly, but the saponins from this species have not been characterized chemically. After acid hydrolysis of the aqueous extracts a sapogenin giving a strong Liebermann–Burchard test may be extracted. When the roots of small Albizia seedlings were washed with water to free them from sand particles foaming was particularly noticeable. Although the saponin, as evidenced by the foam produced, was leached from the roots by water after only gentle abrasion, there was no sign of saponin in the fog box root drip. No foaming was observed during concentration of the root drip, and acid hydrolysis followed by extraction failed to give anything producing a positive Liebermann–Burchard test.
ROOT EXUDATES

It was thought at first that Albizzia root saponins might have some effect on seed germination in the soil, but failure to detect any in the root drip suggested that the amount derived from intact roots would be low. Saponin contained in the winged bracts and seed coats has been shown to regulate dormancy in the saltbush (Atriplex canescens), and solutions of saltbush saponins have a strong inhibitory effect on seed germination (Nord and Van Atta 1960). This agrees with the observation that saponins from the Indian species Albizzia lebbeck stimulate seed germination in very dilute solution, but are inhibitory at higher concentration (Varshney and Farooq 1953). Poor germination and growth of cotton plants grown in rotation with alfalfa have been attributed to saponins leached from alfalfa roots (Mishustin and Naumova 1955), but this derives from decaying roots, and is not a root exudate. The gas evolved from crushed moistened A. lopanthera seeds shows a marked inhibitory effect on the germination of radish seeds. Some radish seeds were placed on moistened filter paper around moistened crushed A. lopanthera seeds on a small watchglass contained in a petri dish and the whole covered with a glass plate. Germination and growth of the radish seedlings were found to be markedly inhibited, but several crushed seeds were needed to fully inhibit growth, and it seems improbable that similar concentrations of evolved gas would be reached in the soil. As thiols are normally germination stimulators rather than inhibitors (Toole et al. 1956) it seems likely that the inhibition is produced by ammonia, which has been shown to be evolved from the crushed seeds also. Inhibition of germination by the gas from germinating beet seeds has been attributed to ammonia (Stout and Tolman 1941a, 1941b).

IV. DISCUSSION

The amount of organic matter isolated from root drip was very small: thiophenes I and II, known to be present in the roots, could not be detected in the root drip from Tagetes species, and only amino acids and sugars in very small amounts were detected with certainty. A. lopanthera root drip likewise contained trace amounts of amino acids, including djenkolic acid and albizzine. The observation that methane dithiol, and by implication djenkolic acid and the specific C-S-lyase which acts upon it, are released from the roots when they are touched makes it seem likely that the effects of abrasion and pressure will lead to greater amounts of exudation in the soil. A further example of the effect of light abrasion is the release of saponins on gentle handling of the roots of Albizzia grown in sand. Although these are not exudates in the strict plant physiological sense (Rovira 1964) they would be important under natural conditions. From this point of view it must be stressed that the results obtained with the fog box should not be assumed to apply in the soil.

It is likely that drying out and wetting of the soil will lead to considerable physical stress at the root surface, especially where there is close contact between the soil colloids and the mucilaginous root cap area. Wilting conditions are difficult to control in fog-box experiments: although the plants recover when spraying is interrupted for a time and then resumed, the root system may be extensively damaged and further growth of the plant will depend on the formation of more rootlets. It was considered that dead rootlets might encourage the growth of saprophytic fungi and bacteria, which would lead to contamination. Katznelson, Rouatt, and Payne
(1955) demonstrated that greater amounts of exudate, notably amino acids, are produced when plants are allowed to wilt, and this finding is supported by the work of Martin (1957) who studied the exudation of scopoletin from roots of oat seedlings. Under favourable conditions the amount of scopoletin was very small and was estimated at 3·8 µg from 100 plants, but under unfavourable conditions the amount detected was increased. In distilled water 121·9 µg was detected after 86 hr, and wilting also produced more scopoletin. Borner (1960) noted that “the liberation of scopoletin can be considered a way of measuring the physiological condition of the root cells”.

Compared with the amounts of amino acids detected by Rovira (1959) when growing small seedlings in nutrient solution under sterile conditions, the amount found in root drip was very small. Stotzky, Culbreth, and Mish (1962) likewise reported considerably greater exudation of amino acids and sugars from older plants under sterile conditions. The lower yield in the fog box may be partly caused by the presence of bacterial contaminants which consume part, at least, of the exuded material at the root surface. A further suggestion is that the penetration of nutrient fog through the mat of suspended roots may be non-uniform, and that the effective surface area might be much less than the root size would indicate. Investigations by Pearson and Parkinson (1961) and Scherth and Snyder (1961, 1962) show that most amino acids originate from the root tip region. In the fog-box experiments the bulk of the root material would be mature and possibly suberized. The amounts isolated are more in accord with the results of Scheffer, Kickuth, and Visser (1962b), who used nutrient solution under sterile conditions, and from the roots of 10,000 seedlings of Eragrostis curvula isolated only 16·1 mg of exudate composed of 10 common amino acids, malic acid, and catechol; and of Martin (1957) whose work on scopoletin is quoted above. Comparison is difficult because Rovira and also Scheffer and co-workers used only small plants. Rovira found that the amount of amino acid isolated in the first and second weeks respectively differed little on a “per plant” basis, and it was concluded that “the exudation from the seedlings diminished with time on a unit weight of tissue basis”. Extrapolation from results obtained with such small plants is difficult, but considering the low yields reported by Martin and by Scheffer and co-workers it seems that the present fog-box study may have been carried out with far too few plants for the collection of significant amounts of exudate.

A difficulty arises in working with very small plants, especially if the estimation of free amino acids is to be used as a measure of exudation from roots: the ungerminated seeds initially may contain up to 4–5% of free amino acids, and during germination the seed diffusate has a high content of amino acids. Although the very small seedling contains much less free amino acid than the seed, the proportion still seems to be high when compared with older samples of root, and it may be inadvisable to start sampling nutrient solution for root exudate at an early stage if the detection of amino acids is used as a criterion.

It seems probable that exudation takes place only, or mainly, in the root hair region near the growing tip: support for this view comes from the study of micro-organisms in the rhizosphere, where attack occurs at root hairs and in the actively growing portion. Rovira (1964) suggests that in the soil exudation may be stimulated
ROOT EXUDATES

by the microorganisms themselves. Possibly initial abrasion leading to leakage from damaged areas of roots is followed by attack by microorganisms, which then proceed to stimulate further excretion. The ease with which damaged roots give “exudates” emphasizes the need for care about techniques if meaningful results are to be obtained. The amount of damage which occurs in removing plants from sand and washing the roots is so great that relatively large amounts of “exudate” are found. The amount of organic matter in fog box root drip was so low that a few very small pieces of crushed root would give rise to much greater amounts. It could be argued that the fog-box conditions are too artificial and give no information about events in the soil: on the other hand, unless care is exercised the amount isolated may be only a measure of the abrasion that has occurred in handling the plant. This raises the question of defining root exudates, a problem which is discussed by Rovira (1964). In many of the earlier studies on root exudates (see the reviews listed in the Introduction) an uncritical approach to the problem has been adopted, and anything which can be extracted from roots, even under drastic conditions, has in some cases been termed an exudate.

The fog-box technique has undoubtedly limitations, chiefly the difficulty of maintaining sterile working conditions and working with a limited number of plants, and the most promising technique would appear to be along the lines adopted by Rovira (1959) and Stotzky, Culbreth, and Mish (1962), but applying radioactive labelling methods. The fog box has some advantages: there is a continuous change of nutrient solution and any exuded material should be washed away from the roots before much reabsorption has taken place. Amino acids for example are known to be taken up by plant roots (Wright 1962). A point not considered in most studies of root exudates in which liquid media have been used is the need for checking the pH of the solution round the roots. Especially with larger plants an unfavourable pH may be reached quite quickly, but this does not occur in the fog box because the nutrient solution is changed continuously.

V. Experimental

(a) Description of the Fog Box

The fog box (45 cm square base, 100 cm deep) was made from an opaque sheet of polyvinyl chloride, divided 30 cm from the base into two parts for ease of cleaning and collection of root drip. The upper section had nine evenly spaced holes (12.5 cm square) at the top, and was covered by a sheet of white polystyrene plastic which held the plants with their roots hanging down into the box. A frame of light rods was attached to the top of the box to support the larger plants. The box was kept dark inside by covering the top with aluminium foil and black plastic tape, and a clear plastic window (30 cm square) in one wall was covered by an opaque shutter. Two atomizers were set in opposite walls, 12 cm from the bottom of the box and 15 cm from diagonally opposite corners.

The nutrient solution, a Hoagland solution slightly modified according to Went (1957, p. 79), was made up every 2-3 days in stainless steel drums from which it was pumped through opaque polyvinyl chloride tubing to a filter combined with a constant-level device controlling the head of liquid maintained at the atomizers so that it just ceased to flow. Compressed air, freed of dust and oil droplets by passage through an industrial filter, reached the atomizers at 5 lb/sq in. pressure. As described by Went, the spray was controlled by solenoid valves, allowing the spray to operate for a selected number of seconds in every minute according to the requirements of the plants, and with a time clock to allow different rates for day and night. For convenience small seedlings were grown in aerated nutrient solution until they reached a height of several inches, and then transferred to the box, because larger plants required less spray than small seedlings.
(b) Collection of Root Drip

Root drip was collected from the mature plants at the rate of about 2–2·5 l/day. Compared with the nutrient solution being used, the root drip showed an increase in pH from the initial value of 6–20 by up to 1·3 units [e.g. with Albizzia plants, as in Fig. 2(b)]. With large plants growing in aerated nutrient solution in pots, greater changes in pH were observed: unless the nutrient solution was changed frequently the pH soon approached a value of 8–0. This change is probably associated with rapid uptake of NO$_3^-$ from the solution, and might be prevented by using an alternative nitrogen source.

Tagetes root drip was extracted with low-boiling light petroleum (30–40°C boiling range) before concentration because the thiophenes I and II may be appreciably volatile; otherwise the accumulated root drip was collected daily and stored at 0–5°C. Batches of root drip collected over a period of 2 weeks were concentrated under reduced pressure at a temperature not exceeding 35°C to a volume of approximately 2 litres. The solution was partially desalted by adding an equal volume of ethanol. The precipitated salt was filtered off and the aqueous alcoholic filtrate reduced to a volume of 300 ml; sufficient ethanol was then added to bring the alcohol concentration up to 80%, causing the precipitation of further quantities of inorganic salts, which were removed by filtration and washed with a little 80% aqueous ethanol. The combined filtrates and washings were evaporated to dryness under vacuum keeping the temperature below 20°C, and the residue dissolved in a small volume of water. This solution was added to a freshly washed column of Dowex 50 (H$^+$) or Zeocarb 225 (H$^+$), and the column washed with distilled water. Amino acids were recovered from the column by elution with dilute ammonia solution (0·5N at first, later 0·1N), until the eluate contained ammonia. The eluted material was recovered by evaporating at 20°C under high vacuum. It was necessary to use large columns of ion-exchange resin because of the amount of inorganic salts, and because of the resulting contamination from dissolved material from the column the actual weight of material eluted was not regarded as significant. Similar quantities of residue were obtained in control experiments in which nutrient solution was added to similar amounts of resin and the same washing procedure followed. Larger quantities of amino acids were not recovered when other desalting procedures, as described by Rovira (1956), were followed.

Having removed the amino acids the acidic aqueous solution obtained from the Dowex 50 (H$^+$) column was partially concentrated in vacuo and added to a column of Dowex FF (HCO$_3^-$) form to remove acids. The neutral eluate and washings were examined for the presence of phenolic compounds, sugars, and other neutral compounds. In the case of Albizzia plants the aqueous solution was extracted with ether, hydrolysed with acid, and then re-extracted with ether to test for the presence of sapogenins. None could be detected by a Liebermann–Burchard test on the faint trace of residuum recovered from the ether extraction.

(c) Estimation of Compounds in the Roots of Tagetes and Albizzia

(i) Thiophenes I and II in Tagetes Roots.—As an example, the roots of eight small seedlings of T. erecta (age c. 2 weeks) grown in aerated nutrient solution were macerated in aqueous ethanol in a Waring Blender. The solution was filtered and extracted with light petroleum (120 ml). The light petroleum solution showed an intense blue fluorescence in ultraviolet light, and a 1 in 25 dilution had $\lambda_{max}$ 340 m$\mu$. $E_{580}$ 0·34. When the residue from evaporating this solution was chromatographed on alumina (activity IV on the Brockmann scale) light petroleum eluted II, mixed with glycides of fatty acids, and the fraction eluted by light petroleum–10% ether contained I. Comparison of the ultraviolet spectra of these fractions with the spectra of pure I and II (Uhlenbrook and Bijloo 1958, 1959) showed that the roots contained I and II in almost equal proportions, the total amounting to approximately 10·2 mg.

The later fractions from the chromatographic separation above yielded crystalline material which melted at 184–185°C after recrystallization from ethanol. After purification as its acetate this was identified as stigmastanol, not previously reported to occur in Tagetes roots. The roots of T. minuta contain eight times as much II and I (Horn and Lambert 1963).

(ii) Compounds in Albizzia Roots.—Djenkolic acid and albizziiene were identified in paper chromatograms of the seed amino acids by comparing with the data published by Gmelin,
Hasenmaier, and Strauss (1957) and Gmelin, Strauss, and Hasenmaier (1958), and in paper chromatograms run with synthetic djenkolic acid. The other commonly occurring amino acids identified are mentioned in Section III. For analysis by paper chromatography the seed amino acids were used as a reference standard, and these were compared with the amino acids from the roots of Albizzia seedlings growing in the fog box, and with the root drip amino acids.

The saponins were not characterized, but their presence was shown as follows: an aqueous alcoholic extract of the roots was repeatedly extracted with ether, concentrated hydrochloric acid (1 part per 10 parts solution) was added, and after heating on a steam-bath for 1 hr the solution was again extracted with ether. This yielded a crude saponin fraction which gave a very strong Liebermann–Burchard test (red→blue→deep green).

(d) Identification of Amino Acids

Amino acids were detected by paper chromatography, the techniques described by Smith (1958) being used. For one-dimensional chromatograms the solvent system n-butanol-acetic acid–water (4 : 1 : 1 v/v) was used, for two-dimensional chromatograms the same mixture was used in the first direction, followed by phenol–water (4 : 1 v/v) containing ammonia (sp. gr. 0·880, 0·5% by volume). Spray reagents were:

(i) ninhydrin (0·2% in acetone);
(ii) ninhydrin in acetone plus cyclohexylamine (0·5%);
(iii) ninhydrin in acetone plus di cyclohexylamine (0·5%);
(iv) Ehrlich's reagent to detect ureido compounds (yellow colour positive);
(v) \( \text{H}_3\text{PO}_4\cdot\text{H}_2\text{O} \text{ (0·0033m) + KI (0·066m) to detect sulphur-containing compounds.} \)

The amino acids from Tagetes and Albizzia roots were separated on Dowex 50 (H\(^+\)), and eluted by dilute ammonia.

VI. Acknowledgments

The authors wish to thank Dr. A. D. Rovira for discussions and comments on this work, and to acknowledge the helpful advice of Professor N. A. Sorenson and Dr. J. M. Swan in the choice of plant species for investigation. They are also indebted to Mr. J. B. Ross for his help in problems arising in the design and maintenance of the fog box and associated equipment.

VII. References


Rovira, A. D., and Harris, J. R. (1961).—Root excretion in relation to rhizosphere effect. V. Plant & Soil 14: 119. (References to earlier work of Rovira are cited herein.)


Went, F. W. (1957).—"The Experimental Control of Plant Growth." pp. 79, 81. (Chronica Botanica Co.: Waltham, Mass.)
