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PLANT ROOT EXCRETIONS IN RELATION TO THE RHIZOSPHERE EFFECT

V. THE EXUDATION OF B-GROUP VITAMINS

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INTRODUCTION

In spite of the considerable amount of evidence for the exudation of many organic substances from the roots of plants, there is relatively little information on growth factors present in root exudates. The only report of measured exudation of identified growth factors is that of West [18] who reported significant quantities of biotin and thiamine from flax roots grown aseptically in nutrient solution. The validity of these thiamine levels is now in doubt because the *Staphylococcus aureus* assay method of West and Wilson [19] also responds to the thiazole plus pyrimidine components of thiamine (Schopfer [13]). Bhuvaneswari and Sulochana [4] have reported the presence of substances having "thiamine-biotin replacement value" in the exudates from the roots of a number of plants but no distinction was made between these two vitamins. The exudation from tomato and pine roots of unidentified growth factors which promoted the growth of mycorrhizal fungi in the presence of salts, sugars and B-vitamins was reported by Melin [9]. Subsequently, Melin and Ramas Das [10] reported that this substance ("M" factor) was exuded by a range of plants.

The present investigation was undertaken with the aim of quantitatively assessing the amount of the various members of the B-group vitamins which are exuded by the roots of a number of plants species. The effects of different environmental conditions upon this exudation were also studied.

MATERIALS AND METHODS

(a) *Preparation of exudate*

(i) Plants. Ten plants were used, *viz*: – Tomato, (*Lycopersicon esculentum* Mill.) var. "South Australian Early Dwarf", field pea (*Pisum arvense* L.) var. "White Brunswick", subterranean clover (*Trifolium subterraneum* L.) var. "Bacchus Marsh", lucerne (*Medicago sativa* L.) var. "Hunter River", white clover (*Trifolium repens* L.) var. Ladino (U.S.A.), berseem clover (*Trifolium alexandrinum* L.), clustered clover (*Trifolium glomeratum*, L.), crimson clover (*Trifolium incarnatum* L.), strawberry clover (*Trifolium fragiferum* L.), phalaris (*Phalaris tuberosa* L.).

(ii) Seed sterilization and plant culture. The seeds were surface-sterilized by shaking with a 7% calcium hypochlorite filtrate for two hours for peas and one hour for the remaining seeds. Germination of the seeds was carried out on tapwater agar so that the medium could not supplement the vitamin content of the germinated seeds and influence the composition of the exudate. The plants were grown by the method described by Rovira [11] except that distilled water replaced the plant-nutrient solution in all experiments other than that in which the effect of plant nutrition on exudation was studied. In this case a 1-in-4 dilution of Hoagland and Arnon [7] solution was used. The use of distilled water or dilute plant-nutrient solution obviated the need to desalt the solution following concentration prior to assay. The solutions supporting the roots were changed at the intervals, as shown in Tables 1 to 9, and concentrated at 30°C in a rotary film evaporator (Craig *et al.*[5]). The final volume of the concentrate was adjusted to provide the equivalent of exudate from four root systems per millilitre.

The effect of light intensity upon plant growth and exudation was studied by shading plants with perforated metal cages (Rovira [11]). In this way incident daylight was reduced to 40% in the low light-intensity treatment. In experiments upon effects of temperature, limited control was achieved by growing plants in ventilated glass boxes 90 × 60 × 60 cm, fitted with supplementary heaters. The temperature ranges were:– "low" treatment, 16°C (Min.) to 30°C (Max.) and "high" treatment, 24°C (Min.) to 36°C (Max.). These correspond to the "low" and "medium" temperatures of previous experiments described by Rovira [11].

(b) *Bioassay of vitamins*

The vitamin content of root-exudate concentrates was determined by bioassay methods using a test organism with essential growth-factor requirement grown in medium free of that factor. Standard response curves were obtained by adding the factor in a range of specified concentrations; the growth response of the same organism to root exudate was then compared to the standard. The growth response of the test organism was measured both acidimetrically and turbidimetrically for bacteria, and gravimetrically for the fungus.

The assay media was reconstituted from Difco dehydrated culture media.

Stock cultures of Lactobacilli were carried in stabs of Micro Assay Culture Agar (B319, Difco Manual [6]); the inoculum was prepared from growth for 24 hours at 35°C in Micro Inoculum Broth (B320), centrifuged and resuspended in isotonic saline. Stock cultures of *Neurospora sitophila* were carried on slants of *Neurospora* culture agar (B321), and a spore suspension in isotonic saline from 48 hour culture used as inoculum.

Bioassays using bacteria were done in 20 × 125-mm Kimble screw-cap culture tubes containing 10-ml aliquots of media. Turbidimetric determinations were made on each tube immediately after inoculation and after incubation at 35°C for 16 to 20 hours by measuring the percentage transmission at wavelength 660 mμ using a Unicam SP400 D.C. absorptiometer with modified tube-holder. After 72 hours (except for thiamine) acidimetric determinations were made by titrating the contents of each tube with 0.1 *N* sodium hydroxide using bromothymol blue as indicator. Bioassay by *Neurospora* was carried out in 50-ml erlenmeyer flasks plugged with cotton and containing 10-ml aliquots of medium. After 5 days growth at 30°C, the flasks were steamed, mycelial mats harvested, squeezed dry between tissue, rolled into pellets and dried on a spotting tile at 100°C for two hours in a vacuum oven, and mycelium weighed to constant weight.

(i) Biotin. The test organism used was *Lactobacillus plantarum* (*syn. L. arabinosus* 17–5) ATCC 8014 in Biotin Assay Medium (B419, Difco Manual [6]) modified from Skeggs and Wright [14]. Standard biotin tubes were prepared from a solution of crystalline biotin (free acid) diluted to give a range of 0.0 to 1.0 μmg per 10-ml aliquot medium in 12 steps. A standard curve for each assay was prepared by plotting mean values of percentage transmission and mean values of titration readings expressed in ml 0.1 *N* sodium hydroxide for each step of the range. The most effective assay range lay between 0.025 and 0.50 μmg per tube. Biotin content of exudate was estimated by matching response at suitable dilution to the standard curve.

(ii) Pantothenic acid. The test organism used was *Lactobacillus plantarum* (*syn. L. arabinosus* 17–5) ATCC 8014 in Pantothenate Assay Medium (B323, Difco Manual [6]), modified from Skeggs and Wright [14]. Standard pantothenic acid tubes were prepared from calcium pantothenate dissolved in 0.02 *N* acetic acid—sodium acetate (A.O.A.C.[1]), and diluted to give a range of 0.0 to 0.10 μg per tube in steps of 0.01 μg. Standard curves were prepared for tubidimetric and acidimetric readings. The most effective assay range was within 0.02 to 0.09 μg pantothenic acid per tube.

(iii) Niacin. The test organised was *Lactobacillus plantarum* (*syn. L. arabinosus* 17–5) ATCC 8014 in Niacin Assay Medium (B322, Difco Manual [6]), the medium of Snell and Wright [16] as modified by Barton-Wright [2] and adopted by A.O.A.C.[1]. Standard niacin tubes were prepared from an aqueous solution of nicotinic acid diluted to give a range of 0.0 to 0.50 μg per tube in steps of 0.05 μg. Standard curves were prepared for turbidimetric and acidimetric readings. The most effective assay range lay between 0.05 and 0.40 μg per tube.

(iv) Riboflavin (vitamin B_2). The test organism was *Lactobacillus casei* e ATCC 7469 in Riboflavin Assay Medium (B325, Difco Manual [6]) modified

from Snell and Strong [15] by reduction of dextrose to 2%. Standard riboflavin tubes were prepared from riboflavin dissolved in 0.02 *N* acetic acid and diluted with water to give a range of 0 to 250 μmg per tube in steps of 25 μmg. Standard curves were prepared for turbidimetric and acidimetric readings. The most effective assay range lay between 25 and 200 μmg per tube.

(v) Thiamine (vitamin B_1). The test organism was *Lactobacillus fermentum* 36 ATCC 9338 in Thiamine Assay Medium (B326 Difco Manual,[6]) of Sarett and Cheldelin [12]. Standard tubes were prepared from an aqueous solution of thiamine hydrochloride diluted to give a range of 0.0 to 0.05 μg per tube in steps of 0.005 μg. A standard curve for each assay was prepared from turbidimetric measurements after 16 to 18 hours growth at 35°C; acidimetric determinations cannot be used. The most effective assay range lay between 0.005 and 0.03 μg per tube.

(vi) Pyridoxine (vitamin B_6). The test organism was *Neurospora sitophila* 299 ATCC 9276 in Pyridoxine Assay Medium (Difco Manual, B324 [6]), the medium of Stokes *et al.*[17] as modified by Barton-Wright [3]. Standard flasks were prepared from an aqueous solution of pyridoxine hydrochloride to give a range of 0.0 to 1.0 μg per flask in steps of 0.1 μg. A standard curve was prepared by plotting mean dry weights of mycelium for each step of the range. The most effective assay range lay between 0.1 and 0.8 μg per flask.

RESULTS

(*a*) *Vitamin content of exudate*

The assessment of the role of accessory growth factors in influencing growth of micro-organisms in the rhizosphere, was made in relation to magnitude of response by test organisms rather than in absolute terms. On this basis, both the quantity and the numbers of vitamins appearing in the exudate were low. The results summarised in Table 1 show that the most active substance was biotin, although the absolute amount exuded was very small indeed; this was exuded in largest amounts by field pea, moderate amounts by lucerne, small amounts by white clover and tomato, and less by the grass *Phalaris tuberosa*. Each plant species tested showed amounts in the exudate sufficient to give significant growth responses by micro-organisms unable to synthesise biotin. Pantothenate was next in order of activity, but only in field pea, lucerne, and tomato were the amounts of a significant order. Very little niacin was found in the concentrate and the amount was significant only for field pea. Traces of riboflavin and thiamine were found in field-pea exudate, but for the others the bioassay was negative. Pyridoxine was absent at assayable levels in all concentrates.

TABLE 1

B-group vitamins in root exudates (concentrated to 4 plants per ml) from 5 plant species at two harvest times

Exudate	Vitamin and effective assay range					
Age in weeks	Biotin (0.025–0.50) μmg/ml	Thiamine (0.005–0.40) μg/ml	Riboflavin (0.025–0.20) μg/ml	Niacin (0.05–0.40) μg/ml	Panto-thenate (0.01–0.09) μg/ml	Pyrid-oxine (0.10–0.90) μg/ml
Lucerne						
0–1	0.75	Trace (?)	—	⩽ 0.1	0.045	—
1–2	0.65	—	—	0.06	0.048	—
White clover						
0–1	0.125	—	—	—	< 0.010	—
1–2	0.37	—	—	0.01	0.010	—
Field pea						
0–1	10	Trace (?)	—	0.4	0.100	—
1–2	16	< 0.0075	Trace (?)	1.6	0.320	—
Tomato						
0–1	0.25	—	—	⩽ 0.1	0.055	—
1–2	0.225	—	—	0.03	0.024	—
Phalaris						
0–1	0.10	—	—	⩽ 0.1	⩽ 0.010	—
1–2	0.125	—	—	0.02	0.017	—

Table 1 may be summarised by saying that other than biotin, and to a lesser extent pantothenate and niacin, the vitamin-B complex was not found to be present in appreciable quantities in root exudate harvested under the conditions of the experiment.

(*b*) *Plant species*

The data in Table 1 also show that pronounced differences occur from plant to plant. Field pea produced by far the greatest amounts of each of the B-group vitamins, and was the only plant species to show traces of thiamine and riboflavin, although devoid of pyridoxine. It was the largest plant used in the test series and made appreciable growth during the experiment (Table 2). The other leguminous plants, lucerne and white clover, also gave moderate yields, but it is dangerous to draw generalisations at the family level when tomato yielded as much biotin and more pantothenate and niacin than did white clover. Of the species under test, the grass *Phalaris tuberosa* gave the lowest yields and was the smallest plant in the test series (Table 2). Whilst it is likely that patterns exist in the way the B-group vitamins are exuded, all that can be said is that

TABLE 2

Fresh weights of five plant species at two harvest times (mg per plant)

Plant species	Age 7 days		Age 14 days	
	Tops	Roots	Tops	Roots
Lucerne	34.5	5.3	20.6	15.0
White clover	6.1	2.7	5.9	4.2
Field pea	626	230	764	396
Tomato	25.0	8.9	33.5	11.6
Phalaris	8.9	3.0	11.4	8.9

individual plant species show differences which could not easily be correlated with size, *e.g.* lucerne and tomato of comparable size (Table 2) gave large differences in biotin exuded (Table 1).

(*c*) *Age of plant*

The data of Table 1 show that the exudation of vitamins during the first week after germination differs very little from that of the second week on a per plant basis. The harvested fresh weights of plants show considerable differences from the first to the second week. This is particularly reflected in the growth of roots (Table 2), and therefore it would seem that exudation from the seedlings diminished with time on a unit weight of tissue basis.

(*d*) *Light intensity*

It can be seen in Table 3 that a reduction in light intensity did not produce large or clear-cut influences on total vitamin exudation of several plant species; trends found for one vitamin did not necessarily hold for others and different reactions were given by different plant species.

Tomato showed little effect of reduced light intensity on either vitamin content (Table 3) or plant weight (Table 4). There were no appreciable differences in the amounts of biotin; traces of niacin were found only at the lower light intensity. Subterranean clover exuded more biotin and a trace of thiamine at high light intensities; there were no differences in fresh weights of plants. Lucerne exuded more biotin and traces of riboflavin and pantothenate at low light intensities; there were no differences in fresh weights of plants.

Field pea showed better growth response to high light intensities

TABLE 3

Influence of age of plant and light intensity upon exudation of B-group vitamins by four plant species

Exudate		Vitamin and effective assay range					
Age in weeks	Light intensity, %	Biotin (0.025–0.50) μmg/ml	Thiamine (0.005–0.40) μg/ml	Riboflavin (0.025–0.20) μg/ml	Niacin (0.05–0.40) μg/ml	Panto-thenate (0.01–0.09) μg/ml	Pyrid-oxine (0.1–0.9) μg/ml
Tomato							
0–1	100	0.019	—	—	—	—	—
	40	0.019	—	—	0.010	—	—
1–2	100	0.012	—	—	—	—	—
	40	0.015	—	—	0.010	—	—
Subterranean clover							
0–1	100	0.045	0.019	—	0.010	—	—
	40	0.025	—	—	0.015	0.004	—
1–2	100	0.045	0.010	—	—	0.002	—
	40	0.014	—	—	—	—	—
Lucerne							
0–1	100	0.014	—	—	0.010	—	—
	40	0.170	—	< 0.005	0.010	—	—
1–2	100	0.025	—	—	—	—	—
	40	0.035	—	—	—	0.001	—
Field pea							
0–1	100	0.20	—	< 0.005	0.025	0.001	—
	40	0.36	—	—	0.025	0.012	—
1–2	100	1.42	—	—	0.037	—	—
	40	0.40	—	—	0.140	0.0025	—

as the harvest weights of tops and roots show in Table 4, but this cannot be correlated with the trends in exudation of vitamins shown in Table 3 where biotin was increased and a trace of riboflavin found at high light intensities but more niacin and pantothenate were exuded at low light intensities.

(*e*) *Temperature*

Tomato showed greater exudation of vitamins at lower temperatures. This is shown in the data of Table 5 for biotin and for the trace

TABLE 4

Fresh weights of four plant species under two light intensities at two harvest times (mg per plant)

Plant species and percentage light intensity		Age 7 days		Age 14 days	
		Tops	Roots	Tops	Roots
Tomato	100%	18.7	7.0	24.5	10.0
	40%	16.8	7.3	25.4	8.8
Subterranean clover	100%	74	19.0	78	25
	40%	78	18.5	78	18
Lucerne	100%	12.7	8.3	17.8	7.5
	40%	15.6	8.1	17.5	8.1
Field pea	100%	540	223	755	421
	40%	587	193	645	257

TABLE 5

Influence of temperature and age of plant upon exudation of B-group vitamins by tomato and subterranean clover seedlings

Exudate		Vitamin and effective assay range					
Age in weeks	Growth temperature *	Biotin (0.025–0.50) μmg/ml	Thiamine (0.005–0.40) μg/ml	Riboflavin (0.025–0.20) μg/ml	Niacin (0.05–0.40) μg/ml	Pantothenate (0.01–0.09) μg/ml	Pyridoxine (0.1–0.9) μg/ml
Tomato							
0–1	Low	0.033	0.010	< 0.005	—	0.001	—
	High	0.025	—	—	—	—	—
1–2	Low	0.025	—	—	—	—	—
	High	0.010	—	—	—	—	—
Subterranean clover							
0–1	Low	0.118	0.010	0.010	0.015	0.010	—
	High	0.089	—	—	0.016	0.006	—
1–2	Low	0.083	0.010	—	0.015	0.013	—
	High	0.250	0.009	< 0.005	0.025	0.011	—

* Low temperature = 16°C (Min.) to 30°C (Max.).
High temperature = 24°C (Min.) to 36°C (Max.).

TABLE 6

Fresh weights of seedlings of tomato and subterranean clover grown under two temperature regimes and at two harvest times (mg/plant)

Plant species and growth temperature	Age 7 days		Age 14 days	
	Tops	Roots	Tops	Roots
Tomato				
Low	23.6	8.7	23.1	10.5
High	24.3	12.9	26.3	7.3
Subterranean clover				
Low	83.5	24.1	67.2	25.7
High	68.0	23.5	82.9	22.9

quantities of thiamine, riboflavin and pantothenate; no niacin was found. Subterranean clover on the other hand exuded more biotin and more niacin at higher temperatures, but at lower temperatures there was more pantothenate, more riboflavin and traces of thiamine The fresh-weight data for the plants (Table 6) shows that there was little appreciable influence of temperature except tomato tended to show more rapid growth at higher temperature.

(f) Sterility

The results of the previous experiments had been obtained using surface-sterilized seeds grown in test tubes under ordinary aseptic precautions. In order to assess the influence of a normal microflora derived from the seed-coat, about one hundred seeds were washed in 10 ml sterile distilled water to provide an inoculum from which 0.1 ml of suspension was added to each test tube at time of transplanting. The sterile set received an aliquot of the same seed-washings sterilized by autoclaving.

From Table 7 it can be seen that tomato and subterranean clover each showed maximal vitamin contents in exudates from the sterilized series, although fresh weights of plants did not indicate effects upon plant growth. Under non-sterile conditions, the reduction in biotin was dramatic. In the case of subterranean clover where there was appreciable exudation, the amount was reduced to one third; tomato with very little biotin exuded showed complete loss due to micro-organisms. Similar trends were shown for pantothenate.

TABLE 7

Influence of rhizosphere micro-organisms and age of plant upon exudation of vitamins and harvest weights of seedlings of tomato and subterranean clover

Age in weeks	Presence of rhizosphere micro-organisms	Fresh weight of plants		Vitamin and effective assay range		
		Tops mg/plant	Roots mg/plant	Biotin (0.025–0.5) μmg/ml	Niacin (0.05–0.4) μg/ml	Pantothenate (0.01–0.09) μg/ml
Subterranean clover						
0–1	Sterile	76.0	19.6	0.045	< 0.1	0.013
	Non-sterile	84.5	21.0	0.012	< 0.1	—
1–2	Sterile	75.0	19.0	0.044	—	< 0.005
	Non-sterile	76.0	23.5	0.015	—	—
Tomato						
0–1	Sterile	19.7	8.6	0.015	—	< 0.005
	Non-sterile	18.0	7.5	—	< 0.01	—
1–2	Sterile	24.5	12.0	0.012	< 0.1	< 0.005
	Non-sterile	21.5	12.6	—	< 0.1	—

TABLE 8

Influence of mineral nutrition and age of plant upon exudation of vitamins and harvest weights of seedlings of tomato and subterranean clover

Age in weeks	Growth solution	Fresh weights of plants		Vitamin and effective assay range		
		Tops mg/plant	Roots mg/plant	Biotin (0.025–0.5) μmg/ml	Niacin (0.05–0.4) μg/ml	Pantothenate (0.01–0.09) μg/ml
Subterranean clover						
0–1	Nutrient solution	109	21.3	0.04	0.1	< 0.005
	Distilled water	95.9	19.3	—	< 0.1	0.01
1–2	Nutrient solution	134	30.0	0.04	0.2	< 0.005
	Distilled water	90.7	22.6	—	< 0.1	0.0075
Tomato						
0–1	Nutrient solution	43.8	10.7	—	< 0.1	< 0.005
	Distilled water	19.2	2.3	—	< 0.1	< 0.005
1–2	Nutrient solution	69.1	14.2	—	0.15	< 0.005
	Distilled water	23.7	10.8	—	< 0.1	< 0.005

(*g*) *Mineral nutrition*

All previous experiments had been conducted with distilled water as the liquid in the test tubes in which plants were grown. In order to ascertain whether mineral nutrition of the plant influenced the exudation of vitamins, the mineral solution of Hoagland and Arnon [7] was substituted as a plant nutrient solution, but to reduce salting out during concentration, it was used at one-quarter strength.

The data in Table 8 shows that improved supply of plant nutrients increased the exudation of vitamins as well as plant growth. In this experiment tomato was a poor source of any vitamin except niacin; this may have been due to possible contamination of the plants during this experiment. Subterranean-clover exudates contained more biotin and more niacin in the nutrient solution series, but less pantothenate. The only explanation for this latter observation is that the glasshouse programme was carried out during the winter months giving the low-temperature effect coupled with less total growth in distilled water; under these conditions the exudation of pantothenate would be increased.

(*h*) *Seed size and clover species*

Previous experiments had shown that the exudation of vitamins was greatest for seedling of large size, *viz* field pea, and least for that of small size, *viz Phalaris tuberosa*, subterranean clover exuded most vitamins under conditions of maximal plant growth *i.e.* for plants grown in plant-nutrient solution. In order to resolve the importance of seed size and plant size upon exudation, and in order to show relationships between species within the same genus, an experiment was conducted with five species of clover, *viz Trifolium subterraneum* L. (subterranean clover), *T. alexandrinum* L. (berseem), *T. fragiferum* L. (strawberry clover), *T. glomeratum* L. (clustered clover) and *T. incarnatum* L. (crimson clover). Within this group, the subterranean clover seed was graded into two sizes by passing through screens to give samples with diameter less than 1.9 mm (small seed) and greater than 2.6 mm (large seed). After germination on agar, the seedlings were transferred to tubes containing one-quarter strength plant-nutrient solution, and the exudate harvested after ten and twenty days.

Subterranean clover from seed of different size gave rise to plants

TABLE 9

Influence of seed size and plant size upon exudation by five species of clover

Clover	Age at harvest, days	Seed size	Fresh weights of plants		Vitamin and effective assay range		
			Tops mg/plant	Roots mg/plant	Biotin (0.025–0.5) μmg/ml	Niacin (0.05–0.4) μg/ml	Pantothenate (0.01–0.09) μg/ml
Subterranean clover	0–10	small	85	17.4	0.42	< 0.1	0.030
		large	153	26.0	0.30	< 0.1	< 0.005
Subterranean clover	11–20	small	99	19.9	0.17	< 0.1	0.011
		large	175	32.4	0.26	0.125	0.018
Strawberry clover	0–10		19.0	5.1	0.15	< 0.1	0.047
	11–20		50.8	7.1	0.12	< 0.1	0.023
Berseem	0–10		47.0	12.9	0.20	< 0.1	0.020
	11–20		50.2	17.3	0.26	0.14	0.019
Crimson clover	0–10		53.0	9.2	0.12	< 0.1	0.012
	11–20		50.2	17.3	0.09	< 0.1	0.008
Clustered clover	0–10		11.4	3.5	0.05	< 0.1	0.006
	11–20		51.0	7.1	0.04	< 0.1	0.006

of different fresh weights, the large seeds giving seedlings of greater weights of both tops and roots sustained over the period of the experiment (Table 9). Total biotin exudation was the same for plants from both small and large seeds although small seed plants produced more biotin in the first ten days than in the second, and produced more pantothenate than did the large. Niacin exudation was small, but more was produced from large seed plants.

The other four clovers produced seedlings of comparable top weights after twenty days, but root growth by clustered clover and strawberry clover was much less than that of berseem and crimson clover (Table 9). Despite the close botanical and size relationships of these plants, vitamin exudation showed variations quite unrelated to seed size or plant size.

(i) Seasonal trends in vitamin exudation

The much lower amounts of vitamins exuded by tomato and subterranean clover in some experiments (Tables 3, 7, and 8) than in others (Tables 1 and 9) may have been due to the former experiments being conducted during winter whereas the experiments in which high yields were recorded were carried out in summer and early autumn. However, further experiments with controlled-

environment growth cabinets would be necessary to establish the importance of day length, temperature and light intensity in this apparent seasonal trend in the exudation of vitamins by roots.

DISCUSSION

Of the numbers of the B-vitamin group for which assay were made, biotin was the only vitamin which consistently appeared in the exudate in significant quantities. Pantothenate and niacin were often present but were generally in quite low amounts, riboflavin and thiamine occurred infrequently and then only in trace amounts, while pyridoxine could not be detected.

According to Schopfer [13], plant roots synthesize biotin, but not thiamine, pantothenate, pyridoxine or niacin. It is not surprising then that biotin was consistently present in amounts significant to influence microbiological activity. These amounts are of the micro-milligram order due to the high biological activity of biotin. In view of Schopfer's report it is surprising that the less sensitive bioassays for microgram amounts of pantothenate and niacin so often gave positive responses and for amounts which in absolute terms but not in terms of microbiological activity, exceeded that of biotin.

The results have indicated that different plant species exude different amounts of the vitamins but no distinctive general patterns emerged. The variations in biotin and pantothenate in the exudates from the various clover species illustrate the inconsistency; *e.g.* from biotin exudation, subterranean > berseem > crimson = strawberry > cluster, while for pantothenate, strawberry > subterranean = berseem > crimson > cluster. This species difference was not related to seed size or plant size but appears to be a characteristic of plant species. In the case of extremes in seed size *e.g.* pea and phalaris it is not surprising that the large-seeded plant which produced a large root system released greater amounts of growth factors. During the initial seedling stages of growth at which these experiments were conducted there appeared to be little, if any, effect of age on vitamin exudation.

A study of the influence of various environmental conditions failed to reveal consistent effects of light intensity or temperature except for slight increases in pantothenate release at low light intensity and low temperature. This finding is in contrast to that in which it was

shown that amino acid exudation was markedly increased at high light intensity and high temperature (Rovira [11]).

Improved nutrition of tomato and subterranean clover plants were accompanied by increased exudation of biotin and niacin, but not of pantothenate. This may be the direct effect of improved plant growth, *e.g.* with plant-nutrient solution compared to distilled water, and in such cases the exudation of pantothenate was generally found to increase under less favourable growing conditions.

The marked reduction of biotin and pantothenate in the root exudate of subterranean clover and tomato when micro-organisms are present in the root environment illustrates the importance of maintaining complete asepsis in any critical root-exudate studies. The organisms introduced into the rhizosphere from the seed inoculum were capable of utilizing biotin and, to a lesser degree, pantothenate, and so exhausted it. There appeared to be no synthesis and release into the medium of these or others of the B-vitamins by the microflora, but possibly the duration of the experiment was insufficient to detect release of microbially synthesised vitamins. Older plants, supporting higher rhizosphere populations, may have an appreciable turn-over of growth factors. In the soil where intense microbial competition occurs with consequent rapid shifts in populations, synthesis and release of growth factors could be quite important. Lochhead [8] found that about one half of the soil isolates and somewhat more than half of the rhizosphere isolates synthesized one or more of the following growth factors, thiamine, biotin, riboflavin, vitamin B_{12} and "terregens" factor. In the rhizosphere this may mean that micro-organisms themselves supply the bulk of the growth factors produced thus supplementing the often meagre amounts released by the plant roots.

SUMMARY

The root exudates from seedlings of ten plant species grown under conditions of controlled environment and nutrition were biassayed for six vitamins of the B-group. Biotin was consistently present in the exudates in amounts sufficient to influence the growth of rhizosphere micro-organisms. Pantothenate and niacin were generally present, but usually at low levels unlikely to influence the microflora; riboflavin and thiamine were occasionally found in traces; pyridoxine was not detected in any root exudate.

The vitamin content of the exudate varied with plant species. Field pea

released large quantities of biotin, pantothenate, and niacin, but other plants including legumes, produced exudates medium to low in vitamin content and varying in relative amounts of each. Subterranean clover produced moderate amounts of vitamins, and from seed samples of graded size exuded vitamins in quantities unrelated to seed size. A comparison of five species of clover showed distinct differences in patterns of exudation in closely related plant species.

Raising temperature and reducing light intensity by shading, produced only small effects upon vitamin exudation. Improved nutrient status produced marked increases in plant growth, but only small increases in amount of vitamin exuded, with pantothenate an exception tending to be released in greater amounts under unfavourable growing conditions. The presence of a root microflora caused sharp reduction in vitamin concentration of the culture solution.

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