Exogenously-Applied Glycinebeyaine is Not Rapidly Re-Translocated in Cotton

J. Gorham[1], J. Bridges[1], K. Jokinen[2] and K. Tiihonen[3]
[1]Centre for Arid Zone Studies, University of Wales, Bangor, LL57 2UW, U.K.;
[2]Cultor Corp., Finnsugar Bioproducts, P.O. Box 105, FIN-00241 Helsinki, Finland;
[3]Cultor Corp., Technology Centre, FIN-02460 Kantvik, Finland.

ABSTRACT

The redistribution of methyl-^{14}C-glycinebetaine was examined after local application to young cotton plants at first flowering. Labelled glycinebetaine in a solution containing Tween-20 (0.05%) was applied to the surface of young or old leaves of cotton (cv. CIM-443) using a syringe. In some cases a self-sealing plastic bag was used to maintain high humidity around the fed leaf. Plants were subsequently grown for 7-10 days in a greenhouse or in a controlled-environment room. Fed leaves were carefully washed prior to sampling. Movement of label was detected in three ways. Plants dried 5 days after treatment and subjected to autoradiography for 5 days showed concentrated labelling in the fed leaf, with lower activity in the attached petiole and the adjacent stem. Long-distance transport of label (as happens in rapeseed etc.) was not observed. More quantitative data were obtained by dividing the plants into stem, petioles, leaves and sympodial branches, drying the samples and burning them in a sample oxidizer. This confirmed that there was little movement of label to other main-stem leaves or to the shoot apex, but some movement into the adjacent main stem and sympodial structures. In these experiments it is assumed that glycinebetaine is not easily metabolized in cotton, and remains in solution. Extraction of sap from different parts of the plants produced a similar pattern of label distribution to sample oxidation. Only when methyl-^{14}C-glycinebetaine was directly injected into a petiole was there substantial movement of label - mostly into the attached leaf and the adjacent stem.

Introduction

Glycinebetaine (betaine) sprayed onto the leaves of crop plants can increase yields in stressed and unstressed environments (Agboma, 1997; Gorham, Jokinen, Malik and Khan, this volume). Cotton also contains high concentrations of endogenous glycinebetaine (Gorham, 1996), and experiments with exogenous topical applications of labelled glycinebetaine in other species have demonstrated rapid translocation to other parts of the plants (Ladyman, *et al.*, 1980; Mäkelä *et al.*, 1996). We report here on the extent of translocation of labelled glycinebetaine in cotton.

Materials and Methods

Experiment 1

Plants of CIM-443 were grown in a greenhouse to first flowering. Methyl-14C-glycinebetaine (0.36 μCi in 15 μl of 0.05% Tween 20) was applied in the evening, when the atmosphere had high relative humidity, to a young mature leaf of Gossypium hirsutum cv. CIM-443 using a syringe. The label was applied to the middle lobe of the leaf only, and distributed as evenly as possible over this area. Samples were taken (destructive harvests of different plants) at 3,6,12 and 24 hours, and at 3,5 and 7 days. Fed leaves were carefully washed prior to sampling. Since earlier harvests showed no significant translocation, only data from the last harvests will be presented. Some samples were dried and oxidized (Harvey Model OX400 Biological Sample Oxidizer, using 'Oxosol C14' (National Diagnostics) as scintillant) to give total label distribution on a dry weight basis. In other cases leaf samples were frozen and used to extract leaf sap. Since betaine is found mainly in the soluble fraction it was thought that leaf sap would provide a quicker and cheaper alternative to sample oxidation. Aliquots of leaf sap were counted in 'Aquasol' (Du Pont) scintillant. Other plants were used for autoradiography.

Experiment 2

The second experiment was conducted in a controlled-environment growth room. The change was necessary because of demolition of the greenhouse used in the previous experiment. The growth room was operated at 30 ^{o}C and the light intensity was about 700-mmol m-2 s-1 PAR. Label was fed to different plant parts (see below), and the fed tissue was enclosed in a self-sealing plastic bag containing a little water to ensure that the solution of label did not dry out until most of the betaine had been absorbed. Only sap samples were analyzed in this experiment.

Results

Experiment 1

The data presented below are from four separate plants. Samples from the first plant were subjected to sample oxidation. Sap samples were analyzed from the second plant, while the remaining two plants were dried and autoradiographed.

Sample oxidation

Labelled glycinebetaine was applied to the central lobe of the main stem leaf at node 7 (Table 1, arrow). In these experiments nodes are numbered from the top down, i.e. node 1 is the youngest node

The data shown below are from both the fed portion of the leaf and the other lobes of this leaf, and from both younger and older nodes. At node 7 the activity was found mainly in the fed lobe, but also in the petiole, unfed leaf tissue, the sympodial stem and the subtending internode (stem). In older tissues the activity was mainly confined to the stem. This was also true for node 6, but there was more radioactivity in the youngest leaves and petioles than in the young stem sections. Some activity was also detected in the young sympodial branches. There was less than 2 % in any tissue sample, expressed as a percentage of that applied (not including the fed portion of leaf), showing that little of the applied glycinebetaine had been translocated, even to other tissue at the fed node. Uptake into the fed portion of the leaf was > 90 %.

Sap samples

In this case the labelled glycinebetaine was applied to the main stem leaf at node 4 (Figure 1, arrow). Again the highest activity (other than in the fed leaf lobe) was found in the petiole, unfed leaf and subtending stem at leaf 4. At node 3 and the older nodes (5 to 12) most of the activity was in the stem tissue, with relatively little in younger portions of the stem or most of the leaves and petioles.

Distribution of label in the sympodial branches was examined in more detail in this plant (Figure 2). At nodes 4 and 5 there was activity mainly in the sympodial stem and in the bracts, while in younger sympodia (with very young squares) the leaves + petioles and the young squares contained more radioactivity than the stems.

Autoradiography

Autoradiographs were made from several plants harvested at different times after 14C-glycinebetaine was applied, but only the plants taken 5 and 7 days after labelling showed any sign of translocation. After 5 days radioactivity was detected principally in the petiole and stem below the fed leaf, in the other (unfed) lobes of the fed leaf and in a sympodial branch two nodes below the fed leaf. No radioactivity was apparent in younger tissues after 1 week of exposure of the autoradiograph. The plant harvested 7 days after labelling with 14C-glycinebetaine showed even less translocation of betaine from the fed lobe. Again there was no detectable radioactivity in the younger tissues, and in the older tissues the label was visible only in petiole and stem tissue, except for the main veins in the unfed portion of the fed leaf.

Experiment 2

The second experiment was conducted in a controlled-environment growth room. Label was fed to different plant parts (see below), and the data are presented in concise form in Table 2.

Uptake into the plants was high (>90%) in this experiment because of the precautions taken to keep the applied 14C-glycinebetaine in solution until it had been absorbed (the fed tissue was enclosed in a self-sealing plastic bag containing a little water). Most of the label applied to the leaves remained in the fed leaves, particularly in the old mainstem leaf (column 1). Where translocation did occur, this was mainly to the adjacent petiole, sympodial structures and stem. Little label reached the apex or the youngest main stem leaves when 14C-glycinebetaine was fed to main stem or sympodial leaves.

In contrast, when 14C-glycinebetaine was directly injected into the petiole, there was movement of radioactivity mainly into the attached leaf, but also to the apex and to the internode subtending the fed petiole. Without further experiments it is not possible, however, to attribute these results to differences in the tissues (leaf versus petiole) or to differences in feeding procedure (surface application versus direct injection).

Conclusions

Exogenously applied glycinebetaine is not rapidly translocated in cotton, at least from leaf tissue. Direct injection into a petiole resulted in some translocation, but further experiments are needed to clarify the circumstances under which translocation can occur.

References

Agboma, P.C. (1997): Improving drought tolerance of field crops by foliar application of glycinebetaine. Ph.D. thesis. Publ. No. 49, University of Helsinki, Department of Plant Production, Section Crop Husbandry. 50 pp.

Gorham, J. (1996): Glycinebetaine is a major nitrogen-containing solute in the Malvaceae. Phytochemistry 43:367-369.

Ladyman, J.A.R., W.D. Hitz and A.D. Hanson. (1980): Translocation and metabolism of glycine betaine by barley plants in relation to water stress. Planta 150:191-196.

Mäkelä, P., P. Peltonen-Sainio, J. Jokinen, E. Pehu, K. Setälä, R. Hinkkanen and S. Somesalo. (1996): Uptake and translocation of foliar-applied glycinebetaine in crop plants. Plant Science 121:221-230.

Table 1. Distribution of radioactivity (Bq) at different nodes after feeding methyl-^{14}C-glycinebetaine to the middle lobe of the mainstem leaf at node 7 (arrow).

Node	Leaf	Petiole	Stem	Sympodial branch
1	117	117	22	0
2	179	179	49	60
3	67	67	53	104
4	148	148	66	205
5	8	23	69	179
6	30	14	259	74
7 (fed) ➡	34883			
7	580	663	345	460
8	19	10	193	56
9	26	10	180	0
10	10	8	139	0
11	7	5	122	0
12	4	6	57	0

Table 2. Summary of distribution of ^{14}C-glycinebetaine (% applied) when fed to different tissues (arrows) of cotton plants (CIM-443). Uptake was > 90% of the amount applied.

	Part Fed with ^{14}C-Glycinebetaine			
Part	Old Leaf	Young Leaf	Sympodial Leaf	Young Petiole
apex	0.3	0.8	0.2	6.3
younger leaf	0.5	0.7	0.1	
younger petiole	0.1	0.4	0.1	
younger sympodial			0.2	
stem above	0.6	0.8	0.2	1.7
leaf	⇒ 81.9	⇒ 71.0	0.1	35.5
petiole	2.5	5.7	0.1	⇒ 30.0
sympodial				
leaf		5.0	⇒ 74.7	0.5
petiole			5.3	0.6
square		4.0	2.3	4.5
stem			2.3	7.1
stem below	0.5	1.4	0.2	5.1
older leaf		0.3	0.1	
older petiole		0.2	0.1	

Figure 1. Experiment 1. saps

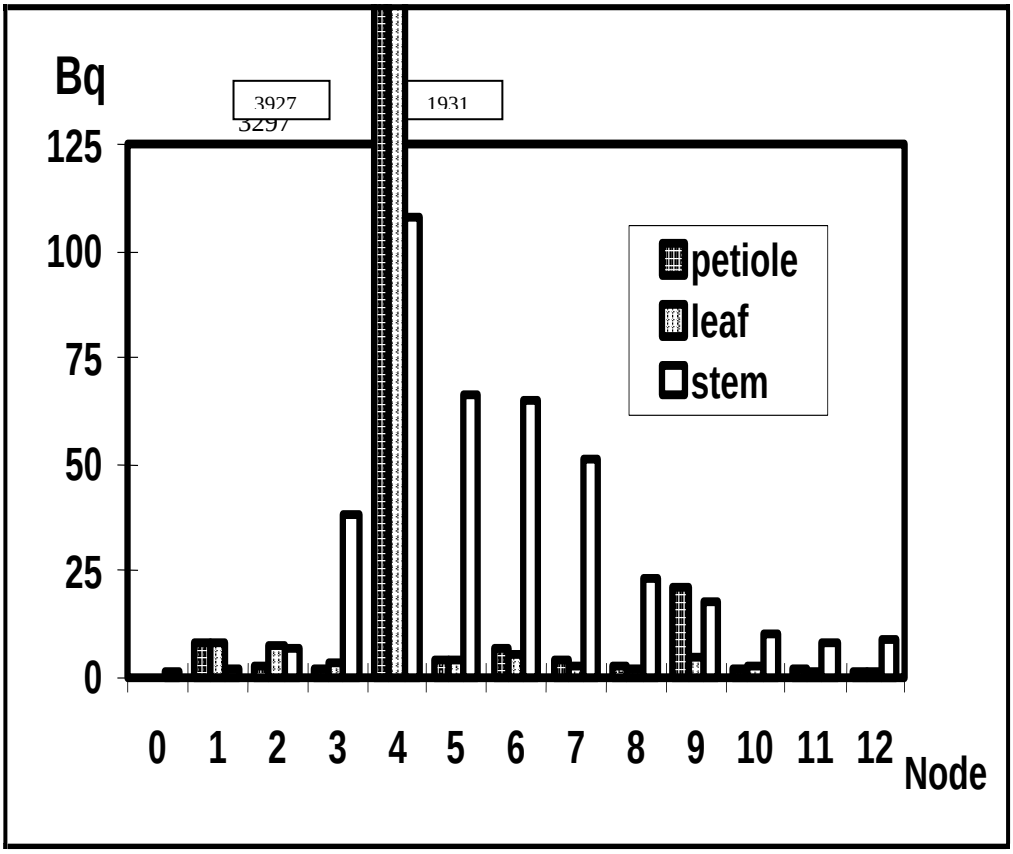

Figure 2. Distribution of label in the sympodial branches.