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DISTRIBUTION OF GLYCINE-BETAINE AND PROLINE IN WATER STRESSED AND UNSTRESSED BARLEY LEAVES

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Key Word Index—*Hordeum*; Gramineae; *Schizaphis graminum*; aphid; greenbug; proline; glycine-betaine; water stress.

Abstract—The concentration of proline was found to be similar in the epidermis, the vascular bundles and mesophyll parenchyma protoplasts of barley seedlings. In stressed leaves, proline was accumulated mainly in the vascular bundles and the epidermis. Glycine-betaine was *ca* 15 times more concentrated in the epidermis and the vascular bundles than in mesophyll parenchyma protoplasts of non-stressed leaves. In stressed leaves, glycine-betaine accumulated preferentially in the vascular bundles and the epidermis. The feeding behaviour of the greenbug *Schizaphis graminum* on barley leaves was the same on stressed and non-stressed seedlings.

INTRODUCTION

Plant metabolites may affect plant–insect interactions. For example, phenolic compounds [1], hydroxamic acids [2] and indole alkaloids [3] have been suggested as resistance factors of several Gramineae against aphids. Small nitrogenous compounds such as glycine-betaine and proline are known to accumulate in barley under water stress [4]. It has been suggested that glycine-betaine increases the susceptibility of wheat to rust [5] and of water stressed barley to *Schizaphis graminum* [6]. The possible role of proline in plant–insect interactions has also been discussed [6–8]. *Schizaphis graminum* biotype C feeds preferentially from the vascular tissues in sorghum [9] and in barley [10]. Therefore, the location of compounds in tissues may determine the performance of aphids on the plants. In this paper, we report on the distribution of glycine-betaine and proline in leaf tissue and on the feeding behaviour of *S. graminum* on stressed and unstressed barley seedlings.

RESULTS AND DISCUSSION

Distribution of compounds

The content of proline was similar in the different tissues of unstressed seedlings (Table 1). Under water stress, proline accumulated mainly in the vascular bundles and the epidermis. Glycine-betaine was heterogeneously distributed among the tissues of barley leaves. The highest concentration of this compound was found in the vascular bundles and the epidermis of leaves from unstressed seedlings. In leaves from water stressed seedlings, glycine-betaine accumulated mainly in the vascular bundles. Accumulation of both compounds in mesophyll parenchyma protoplasts was low.

Feeding behaviour of aphids

Glycine-betaine and proline reduced the feeding time of aphids on artificial diets (Fig. 1). Proline, however, was

Table 1. Distribution of proline and glycine-betaine in stressed and unstressed tissues of barley leaves

Tissue	Proline (mmol/kg dry wt)		Glycine-betaine (mmol/kg dry wt)	
	Normal	Stressed	Normal	Stressed
Whole leaf	5.1	13	32	46
Epidermis	7.3	73	158	224
Vascular bundles	6.6	93	120	242
Mesophyll protoplasts	5.4	9	9	11

Four-day-old barley seedlings were exposed to water stress. Six days later the content of proline and glycine-betaine was determined. The water potential was −5.5 and −9.8 bars in unstressed and stressed plants, respectively. Standard errors were lower than 5% and are omitted for simplicity.

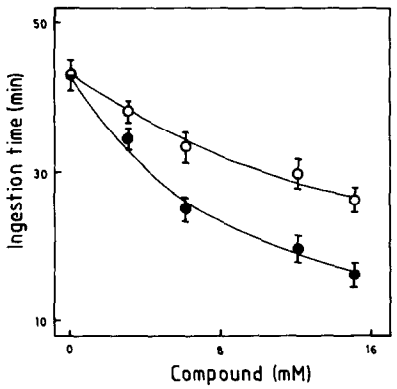

Fig. 1. Effect of glycine-betaine and proline on the feeding behaviour of *S. graminum* on artificial diets. Feeding was electronically monitored for 2 hr. The diets contained glycine-betaine (○) or proline (●). Values are means of eight individuals ±s.e.

the better antifeedant. The concentration range of both compounds used in this experiment was similar to that found in plants. The feeding behaviour of *S. graminum* on seedlings was also studied (Table 2). *Schizaphis graminum* ingested preferentially from phloem, with short ingestion periods from non-vascular tissues. There were no significant differences between ingestion times in stressed and non-stressed seedlings. Contrary to the observations made with artificial diets, accumulation of glycine-betaine and proline in the leaves did not affect the feeding behaviour of the aphids. Changes in the content of sugars, amino acids and other compounds, which are known to occur under water stress [11], may also affect the feeding behaviour of *S. graminum* on seedlings.

If proline plays a role in interactions between barley or other plants and insects it is not readily apparent. Contradictory reports exist over its role in plant resistance to insects. It has been suggested that plants with a high content of proline are more susceptible to insects [7]. The content of proline, however, did not affect the susceptibility of barley [8]. Moreover, high concentrations of proline decreased the reproduction of *S. graminum* feeding on artificial diets [6]. The reduction of ingestion of diets caused by proline and glycine-betaine may be due to the high concentration of nitrogen in the diet. Insects that feed on low-nitrogen diets consume more food than those that feed on high-nitrogen diets [12].

Glycine-betaine accumulated mostly in the vascular bundles and did not change the feeding behaviour of aphids on the plants. It is therefore likely that aphids feeding on stressed plants ingested higher amounts of glycine-betaine than those feeding on normal plants. Glycine-betaine increases reproduction of aphids reared on artificial diets or on barley seedlings [6, 13].. It also decreases the toxic effects of gramine, a barley resistance factor against herbivvores [13]. The mechanism of action of glycine-betaine on aphids is not known. Glycine-betaine stimulates respiration of *Pseudomonas syringae* and reduces the effects of gramine on the bacterium [14]. Barley cultivars that accumulate high amounts of glycine-betaine, although more resistant to drought or high salt concentration, may be more susceptible to pathogens or insects.

Table 2. Feeding behaviour of *S. graminum* on stressed and unstressed barley seedlings

Feeding behaviour	Stressed (min)	Unstressed (min)
Ingestion from non-phloem	18±2	16±3
Ingestion from phloem	43±4	47±7
Salivation	36±5	34±7
Non-ingestion	89±9	83±6

Adult aphids were fed on stressed and unstressed barley seedlings for 3 hr. Plants were stressed as explained in the Experimental. Each value is the mean of eight samples ±s.e. No significant differences were found between treatments.

EXPERIMENTAL

Plants and stress treatment. Two groups of 4-day-old seedlings cv. F. Union were watered daily with different amounts of water for six days. The water potential of the leaves was measured at this stage [15]. These plants were used to isolate tissues and protoplasts as described [16].

Analyses of compounds. Proline was extracted by homogenizing leaves in 3% aq. sulphosalicylic acid and quantified as described in ref. [17]. Glycine-betaine was extracted and quantified as described in ref. [18].

Aphid assays. Adult nymphs of *Schizaphis graminum* biotype C were used. Aphid probing behaviour in plants was electronically monitored as described [9, 10]. Aphids were starved for 2 hr and then tethered to a 40 μm copper wire and placed on stressed and unstressed barley seedlings. A second electrode was placed in the soil. The probing behaviour of aphids was also studied on an artificial diet (pH 6) containing either proline or glycine-betaine, and 30% sucrose, aminoacids and mineral salts [19]. The waveforms generated by aphids were interpreted as described [9, 20, 21].

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REFERENCES

1. Dreyer, D. L. and Jones, K. C. (1981) *Phytochemistry* **20**, 2489.
2. Argandoña, V. H., Corcuera, L. J., Niemeyer, H. M. and Campbell, B. C. (1983) *Entomol. Exp. Appl.* **34**, 134.
3. Corcuera, L. J. (1984) *Phytochemistry* **23**, 539.
4. Hanson, A. D. (1980) *Hort. Sci.* **15**, 623.
5. Strange, R. N., Mayer, J. R. and Smith, H. (1974) *Physiol. Plant Pathol.* **4**, 277.
6. Zúñiga, G. E. and Corcuera, L. J. (1987) *Phytochemistry* **26**, 367.
7. Haglund, B. M. (1980) *Nature* **228**, 697.
8. Bright, S. W. J., Lea, P. J., Kueh, J. S. H., Woodcock, C., Holloman, D. W. and Scott, G. C. (1982) *Nature* **295**, 592.
9. Campbell, B. C., Mc. Lean, D. L., Kinsey, M. G., Jones, K. C. and Dreyer, D. L. (19) *Entomol. Exp. Appl.* **31**, 140.
10. Zúñiga, G. E., Varanda, E. M. and Corcuera, L. J. (1988) *Entomol. Exp. Appl.* **47**, 161.
11. Hanson, A. D. and Hitz, W. D. (1982) *Ann. Rev. Plant Physiol.* **33**, 163.
12. Mattson, W. J. (1980) *Ann. Rev. Ecol. Syst.* **11**, 119.
13. Zúñiga G. E. and Corcuera, L. J. (1987) *Phytochemistry* **26**, 3197.
14. Zúñiga, G. E. Sepúlveda, B. A. and Corcuera, L. J. (1986) VI Reunión Nacional de Botánica, Valdivia, Chile.
15. Scholander, P. F., Hammel, H. T., Hemmingsen, E. S. and Brandstreet, E. D. (1964) *Proc. Nat Acad. Sci. U.S.A.* **39**, 119.
16. Argandoña, V. H., Zúñiga, G. E. and Corcuera, L. J. (1987) *Phytochemistry* **26**, 1917.
17. Bates, L. S., Waldren, R. P. and Teare, I. D. (1973) *Plant Soil* **39**, 205.
18. Grieve, C. M. and Grattan, S. R. (1983) *Plant Soil* **70**, 303.
19. Argandoña, V. H., Peña, G. F., Niemeyer, H. M. and Corcuera, L. J. (1982) *Phytochemistry* **21**, 1573.
20. McLean, D. L. and Kinsey, M. G. (1964) *Nature* **202**, 1358.
21. McLean, D. L. (1970) *Ann. Entomol. Soc. Am.* **64**, 499.