INFLUENCE OF EPICUTICULAR WAXES ON FOLIAR ABSORPTION OF NITRATE IONS BY APRICOT LEAF DISKS*

By D. R. LEECE†‡ and A. L. KENWORTHY†

The poor response of *Prunus* spp. to foliar nitrate sprays under field conditions (Leece and Kenworthy 1971) is not caused by an inability of *Prunus* leaves to metabolize nitrate ions (Leece, Dilley, and Kenworthy 1972). This is analogous to the response of *Prunus* spp. to urea sprays (Dilley and Walker 1961*a*). Dilley and Walker (1961*b*) demonstrated that pretreatment of peach leaf disks by immersion in acetone to dissolve lipid components of the cuticle increased subsequent urea absorption. Bukovac (1965) found that lightly brushing the upper and lower surfaces of peach leaves with a camel-hair brush enhanced uptake of 3-chlorophenoxy-α-propionic acid by 22 and 34% respectively. Brushing was assumed to have partially removed or rearranged the epicuticular waxes.

This paper reports that the uptake of nitrate ions by apricot leaf disks, via the astomatous cuticle, was enhanced by partial removal or disruption of the epicuticular waxes.

Materials and Methods

Fully expanded, mid-shoot leaves were obtained from 5-year-old apricot trees (*Prunus armeniaca* L. cv. Curtis) growing at East Lansing during the summer of 1970. The leaves were transferred to the laboratory in polyethylene bags at 0–4°C.

Nitrate ion uptake by apricot leaves was assayed by measuring the induction of nitrate reductase in excised leaf disks, following cuticular penetration. Apricot leaf disks were prepared for the uptake studies using the technique of Sargent and Blackman (1962) as modified by Greene and Bukovac (1971). Glass cylinders (height 10 mm; external diameter 24 mm; internal diameter 21 mm) were attached to the upper, astomatous surface of leaf disks (diameter 25 mm) using RTV 11 liquid silicone rubber (General Electric Company, Waterford, New York) hardened with Harter T 1 catalyst (Wacher Chemie GMBH Company, Munich, Germany). The disks were placed in 9-cm Petri dishes lined with moist filter paper, and 1·0 ml of 0·4M KCl (control) or 0·4M KNO_3 (enzyme induction solution) was pipetted into each cylinder. Both solutions contained 0·1% (v/v) X 77 surfactant (alkylarylpolyoxyethylene glycols, free fatty acids, and isopropanol—Chevron Chemical Company, Ortho Division, San Francisco, California). The dishes were incubated in a water-bath at 25°C for 15 hr and at a light intensity (from fluorescent tubes) of 8600 lux at leaf level. Following incubation, the glass cylinders were peeled off the disks, the disks were rinsed with distilled water, blotted dry, then assayed for nitrate reductase as previously reported (Leece, Dilley, and Kenworthy 1972).

Where required, disruption and partial removal of epicuticular waxes was achieved either by brushing (10 strokes in one direction with a camel-hair brush), or with 80% (v/v) aqueous acetone (leaf wiped with acetone-treated tissue paper then dried immediately with untreated paper). Chloroform proved unsuitable for wax removal as it produced leaf senescence within 3 hr.

* Manuscript received 13 December 1971. This work formed part of a thesis presented by the senior author to Michigan State University for the degree of Ph.D. (1970).

† Department of Horticulture, Michigan State University, East Lansing, Michigan, 48823.

‡ Present address: N.S.W. Department of Agriculture, Biological and Chemical Research Institute, Rydalmere, N.S.W. 2116.

Four treatments were compared. Four disks were obtained from the same leaf without including major veins, and one treatment was allocated randomly to each disk. The experiment was replicated four times and the replicates of each treatment were bulked for enzyme assay. The experiment was conducted on five separate occasions and each occasion was treated as a replicate for statistical analysis.

Results and Discussion

Nitrate reductase was induced when leaf disks were treated with 0·4M KNO_3 for 15 hr as shown in the following tabulation. This indicates that nitrate ion uptake had occurred via the cuticle. Where epicuticular waxes were undisturbed, enzyme activity induced by KNO_3 was about threefold that of the KCl control; this increase, however, was not significant at $P = 0{\cdot}05$. Where epicuticular waxes had been disrupted, either by brushing the leaf surface or by wiping the surface with an acetone-soaked tissue, enzyme activity was about twice that of the KNO_3–undisturbed wax system, and about sixfold that of the KCl control.

Induction System	Nitrate Reductase Activity (nmoles nitrite per g fresh wt. per hr)*
0·4M KCl (control)	217 a
0·4M KNO_3	
Leaves undisturbed	629 ab
Leaves brushed	1246 bc
Leaves acetone-washed	1454 c

* Means followed by the same letter do not differ significantly at $P < 0{\cdot}05$ (Tukey's ω-procedure, 1953—see Steel and Torrie 1960).

During uptake in the leaf-disk assay, the nitrate ions would have to: (1) diffuse through the lipophilic cuticle; (2) penetrate the hydrophilic cell walls and enter and diffuse through the apoplast; (3) be transported from the apoplast across the plasmalemma—possibly an active step mediated by a nitrate permease—and be deposited in the cytoplasm; (4) by concentrating in the cytoplasm, induce enzyme synthesis. The enzyme activity of the disks immediately after the 15-hr uptake period would be directly related to the nitrate uptake rate during the preceding 15 hr, probably with (1), (3), or (4) as the rate-limiting step.

In an earlier study (Leece, Dilley, and Kenworthy 1972), nitrate uptake via the petiole was measured, using the nitrate reductase assay. In that system, steps (2), (3), and (4) were operating as above, but step (1) became petiole uptake, which was controlled by the rate of leaf transpiration. That experiment has been repeated employing the same leaves as used in the present study. For these leaves, optimum induction conditions were: substrate, 0·01M KNO_3; induction period 9 hr. Resulting enzyme activity was 2892 nmoles nitrite formed per gram fresh weight per hour. The differences in optimum induction conditions and enzyme levels between the petiole-uptake study and the cuticle-uptake study (see tabulation) must have been due, in large part, to rate differences between transpiration-assisted uptake via the petiole and diffusion through the cuticle (though conditions for enzyme induction were not strictly comparable for the two systems). Thus, one implication is that the rate-limiting step in foliar uptake of nitrate may be associated with permeability properties of the cuticle.

The present study demonstrated that nitrate uptake occurred via the cuticle, even when the epicuticular waxes were undisturbed. However, partial removal or disruption of these waxes doubled the rate of uptake. Thus, we conclude that the epicuticular waxes were a factor in reducing the nitrate uptake rate, probably acting by reducing the rate of cuticular penetration. These waxes may play a role in the poor response of *Prunus* spp. to foliar nitrate sprays.

Acknowledgments

We are grateful to Professor M. J. Bukovac for his interest in this work and his constructive criticisms of the manuscript; and to Mr. J. Flore for his assistance in developing the leaf-disk technique.

References

Bukovac, M. J. (1965).—Some factors affecting the absorption of 3-chlorophenoxy-α-propionic acid by leaves of the peach. *Proc. Am. Soc. hort. Sci.* **87**, 131–8.

Dilley, D. R., and Walker, D. R. (1961*a*).—Assimilation of C^{14}, N^{15} labeled urea by excised apple and peach leaves. *Pl. Physiol., Lancaster* **36**, 757–61.

Dilley, D. R., and Walker, D. R. (1961*b*).—Urease activity of peach and apple leaves. *Proc. Am. Soc. hort. Sci.* **77**, 121–34.

Greene, D. W., and Bukovac, M. J. (1971).—Factors influencing the penetration of naphthaleneacetamide into leaves of pear (*Pyrus communis* L.). *J. Am. Soc. hort. Sci.* **96**, 240–6.

Leece, D. R., Dilley, D. R., and Kenworthy, A. L. (1972).—The occurrence of nitrate reductase in leaves of *Prunus* spp. *Pl. Physiol., Lancaster.* (In press.)

Leece, D. R., and Kenworthy, A. L. (1971).—Effect of potassium nitrate foliar sprays on leaf nitrogen concentration and growth of peach trees. *HortScience* **6**, 171–3.

Sargent, J. A., and Blackman, G. E. (1962).—Studies on foliar penetration. 1. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. *J. exp. Bot.* **13**, 348–68.

Steel, R. D. G., and Torrie, J. H. (1960).—"Principles and Procedures of Statistics." p. 109. (McGraw–Hill Book Company, Inc.: New York.)