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Short-term Effects of Ferulic Acid on Ion Uptake and Water Relations in Cucumber Seedlings

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ABSTRACT

Ferulic acid (FA) is commonly found in soils and is considered an allelochemical. Studies have suggested that FA and other phenolic acids decrease plant growth in part by decreasing the absorption of mineral nutrients and water. However, no studies have examined these parameters in a single experimental system to investigate how FA affected both ion uptake and plant-water relations in whole plants. Using intact cucumber (*Cucumis sativus* L. cv. Early Green Cluster) seedlings, we examined short-term effects of FA on ion uptake kinetics, transport promoters and inhibitors, and water relations as indicated by a pressure–volume analysis. We found that after 3 h of treatment, 200 μM FA inhibited net ion uptake, particularly NO_3^-, and promoted net K^+ efflux from seedling roots. The addition of fusicoccin, a K^+ transport promoter, counteracted the inhibitory effect of FA on net K^+ uptake. Concurrent treatment of seedlings with FA and tetraethylammonium, a channel-blocking salt, reduced average K^+ efflux by 66%. Treatment of seedlings with FA also decreased leaf water potential (Ψ_l) and turgor pressure (P_T). However, decreased Ψ_l and P_T were not caused by changes in the osmotic properties of the symplast or stomatal conductance. A decrease in water absorption is a likely explanation for the loss of P_T observed. The results of our experiments indicate that both ion uptake and plant-water relations can be adversely affected by FA.

Key words: *Cucumis sativus*, ferulic acid, allelopathy, ion uptake, water relations.

INTRODUCTION

Phenolic derivatives of benzoic acid and cinnamic acid are commonly found in soils, and many of these compounds are considered allelochemicals (Rice, 1984). Phenolic allelochemicals are released by plants into soils as leaf leachates, root exudates, and by plant tissue decomposition. The concentration of phenolic acids in rhizosphere soil solutions may reach 100 μM (Kuiters, 1990). Studies have shown that plant growth can be rapidly inhibited by some phenolic compounds. For example, the leaf area of cucumber (*Cucumis sativus* L. cv. Early Green Cluster) seedlings 2 d after treatment with 500 μM ferulic acid (FA), a cinnamic acid derivative, was 35% less than control seedlings (Blum, Dalton, and Shann, 1985). This study and others have suggested that FA decreases plant growth in part by decreasing the absorption of mineral nutrients and water (Balke, 1985; Blum *et al.*, 1985; Einhellig, 1986).

Several studies have found a lower mineral content in plants treated with FA and other phenolic acids (Balke, 1985; Kobza and Einhellig, 1987; Mersie and Singh, 1988). More direct evidence for the inhibitory action of FA on nutrient uptake comes from experiments which showed that K^+ and P_i uptake decreased for several hours after excised roots were treated with FA (Glass, 1973, 1974; McClure, Gross, and Jackson, 1978; Harper and Balke, 1981).

Changes in ion permeability may be accompanied by changes in water permeability. The permeability of roots to water is affected by root age and metabolic activity as well as various environmental factors (Kramer, 1983). Decreased leaf water potential (Ψ_l), shoot turgor pressure (P_T) and osmotic potential (Ψ_π) have been observed in soybean (*Glycine max* (L.) Merr.) and sorghum (*Sorghum vulgare* Moench) seedlings after treatment with 250 to 1000 μM FA for 1 d (Patterson, 1981; Einhellig, Muth, and Schon, 1985). Transpiration and water utilization in

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cucumber seedlings decreased after 2 d of treatment with 500 μM FA (Blum *et al.*, 1985). However, Blum *et al.* (1985) noted that cucumber seedlings wilted within 2 h of treatment with 500 μM FA but visibly recovered within 24 h. It was suggested that FA temporarily modified water uptake by the roots, which reduced the inward diffusion of water needed for cell and leaf expansion.

Although decreased water absorption could account for decreased turgor and wilting in FA-treated seedlings, turgor is also regulated by solute transport and cell wall extensibility. A close relationship exists between active ion transport and plant-water relations in which the regulation of turgor is of particular importance (Kramer, 1983). It is thus important to understand how FA affects these interactive physiological processes in a single experimental system to provide a more complete picture of how FA inhibits growth.

To investigate how FA affected ion uptake and plant-water relations, we examined short-term effects of FA on intact cucumber seedlings. By using intact seedlings, the effect of FA on ion uptake would not be confounded by excision and wounding. The study was conducted by measuring how FA affected ion uptake kinetics, transport promoters and transport inhibitors. The transport promoter fusicoccin (FC) and the channel-blocking salt tetraethylammonium (TEA^+) were used to determine how seedling response to FA would be altered by compounds that modified the movement of solutes across the plasmalemma. Fusicoccin is a fungal toxin that promotes ATPase activity, membrane hyperpolarization, and K^+ uptake (Blatt, 1988). Tetraethylammonium blocks K^+ channels in a number of plant and animal tissues (Stanfield, 1983). The effect of FA on plant-water relations was determined by a pressure–volume (P–V) analysis. A P–V analysis provides measurements of Ψ_l, P_T and Ψ_π (Kramer, 1983).

MATERIALS AND METHODS

Plant material

Cucumber seeds were germinated at 28–30 °C in vermiculite moistened with Hoagland's nutrient solution (Hoagland and Arnon, 1950). After 3 d, individual seedlings were transferred to 120 cm^3, foil-wrapped bottles containing nutrient solution (pH 5·5). Nutrient solutions were changed 6 d later. Seedlings were grown for 11–18 d at room temperature under a light bank providing a photosynthetic photon flux density (*PPFD*) of 140 μmol quanta m^{-2} s^{-1} at plant level on a 12 h photoperiod. Nutrient solutions were replaced with 500 μM $CaSO_4$ solutions 1 d before experimental treatments began.

Net ion uptake experiments

The roots of 11–14-d-old seedlings were placed in 50 cm^3, foil-wrapped beakers containing 15 cm^3 of aerated treatment solution (one seedling per beaker). Excised roots were also used in one experiment. The treatment solution consisted of 500 μM $CaSO_4$, 2·0 mM 2(*N*-morpholino)ethanesulphonic acid (MES) and 0–3 mM K_2SO_4. In experiments examining inorganic anion transport, K_2SO_4 was replaced with either 500 μM KNO_3 or KH_2PO_4. Treatment solutions contained 0–2 mM FA. The pH of all treatment solutions was adjusted to 5·5 with NaOH. During the treatment period, seedlings were incubated at room temperature under the light bank for up to 3 h.

In the FC experiment, treatment solutions contained combinations of 10 μM FC and 200 μM FA. The FC was dissolved in 30 mm^3 absolute EtOH, and equal volumes of EtOH were added to control solutions.

In the TEA^+ experiment, plants were first incubated individually in 100 cm^3 of solution containing 2·0 mM MES, 500 μM $CaCl_2$ and 0 or 10 mM TEA-Cl for 30 min. Seedlings were then transferred to 15 cm^3 aerated treatment solutions that contained combinations of 10 mM TEA^+, 0–3 mM KCl and 200 μM FA. In addition, five plants were treated with combinations of 10 mM NaCl and 200 μM FA.

After the incubation period, treatment solutions were restored to the initial 15 cm^3 volume with deionized water and assayed for inorganic ions and phenolic acids. The net uptake or release of solutes by the plant was based on the solute concentrations in the treatment solutions after the incubation period. All treatments were done in triplicate or quadruplicate, and the experiments were conducted twice.

The concentration of K^+ in the treatment solutions was measured by atomic absorption spectrophotometry. The SO_4^{2-} and NO_3^- concentrations were determined with a Dionex 2010-I ion exchange chromatograph equipped with an AS4A anion exchange column with a micromembrane suppressor. Phosphate (P_i) concentration was measured colorimetrically (Taussky and Shorr, 1953). The concentration of FA was determined by reversed-phase HPLC using an automatic injection system and a 4·0 μm spherical Nova/PAK C18 Radial PAK Cartridge. Either an isocratic or gradient solvent system composed of methanol, water, ethyl acetate, and acetic acid was used.

Pressure–volume experiments

Roots of 15–18-d-old intact seedlings were placed in 15 cm^3 aerated treatment solutions containing 0 or 200 μM FA. Seedlings were then incubated at room temperature for 2 h under a 300 W *PAR* incandescent spotlight, which provided a *PPFD* at plant level of 600 μmol quanta m^{-2} s^{-1}. Afterwards, seedlings in their aerated treatment solutions were covered with a plastic bag containing a piece of wet cheesecloth and placed in the dark for 1 h so the plants could rehydrate.

For the P–V assay, the first primary leaf from a plant in each treatment was excised after incubation in the dark and sealed in a pressure bomb containing a wet paper towel to maintain humidity during the procedure. The initial balance pressure was determined in the usual way (Tyree and Hammel, 1972). The pressure in the chamber was then increased by 0·15 MPa, and the volume of sap was measured after 5 min by collecting the expressed liquid on preweighed adsorbent tissue contained inside a plastic cap. After each 5 min period, pressure inside the chamber was reduced by at least 0·5 MPa and the next balance pressure determined. The process was repeated until the balance pressure exceeded 1 MPa. The experiment was conducted five times.

The balance pressure (P^*), like the leaf water potential may be written as the sum of the average osmotic pressure (Π) and the volume averaged turgor (P_T):

$$P^* = \pi - P_T. \quad (1)$$

According to Tyree and Hammel (1972), the first term on the right can be written as:

$$\pi = RTN_s/V, \quad (2)$$

where N_s/V is the average symplastic osmotic concentration of all the cells in the tissue and R and T are the gas constant and temperature in Kelvins. The volume of the symplast (V) may be written $V=V_o-V_e$, where V_o is the symplastic volume at the initial balance pressure, or at full hydration depending on the circumstances, and V_e is the cumulative volume of sap expressed from the leaf.

We found that the turgor pressure, as a function of V_e, is adequately described by the simple exponential expression:

$$P_T = P_{max}\exp(zV_e) \qquad (3)$$

where P_{max} is the initial turgor corresponding to $V_e=0$, and z is a coefficient that indicates the rate of change in turgor with volume (Helkvist, Richards, and Jarvis, 1974). The coefficient z can be related to the maximum volume elastic modulus (ϵ_{max}) of the tissue by the following expression: $\epsilon_{max}=V_oP_{max}z$.

Substituting equations 2 and 3 into equation 1 yields the model for the balance pressure which was used for the analysis:

$$P^* = \frac{RTN_s}{V_o - V_e} - P_{max}\exp(zV_e). \qquad (4)$$

To analyse the data an iterative, regression-based technique was developed. The technique allows extrapolation of the leaf P–V data back to zero xylem pressure potential. This allows estimation of tissue properties in the fully hydrated state without incurring the tissue changes which are reported by Meinzer, Rundel, Sharifi, and Nilsen (1986) to occur on rehydration of excised leaves. Data were first fitted to this model (Eq. 4) by a non-linear least squares program (NLLSQ by CET Research Ltd., Norman, OK) to obtain values for the four coefficients N_s, V_o, P_{max}, and z. Using this set of coefficients, negative increments of V_e were added to V_o until the model yielded a $P^*=0$. The absolute value of the resulting V_e increment was then added to the V_e data, thus creating a new data point. The data, including the extrapolated value, were fitted again and the process repeated until no further improvement in the fit was obtained. The resulting set of coefficients apply over the entire range of leaf water content, and V_o and P_{max} are the symplastic volume and turgor at full hydration.

In cases where there was insufficient data in the region of positive turgor (most of the FA-treated leaves), only the osmotic component of equation 4 was used. The data for $P_T=0$ was fitted by linear regression to the reciprocal balance pressure model of Tyree and Hammel (1972):

$$1/P^* = (V_o/RTN_s) - (1/RTN_s)V_e \qquad (5)$$

where the intercept is V_o/RTN_s (i.e. $1/\pi_o$, the reciprocal of the initial osmotic pressure) and the slope is $1/RTN_s$. The same procedure was also applied to the data previously fitted by the non-linear model to demonstrate the validity of comparing coefficients obtained by these two methods.

Stomatal conductance experiments

Roots of 15–18-d-old intact seedlings were placed in 15 cm^3 aerated treatment solutions containing 0 or 200 μM FA. Seedlings were then incubated under a 300 W *PAR* spotlight for 2 h, placed in plastic bags containing wet cheesecloth and incubated for 1–2 h in the dark. After incubation in the light and dark, stomatal conductance was measured on both the adaxial and abaxial surfaces of the first primary leaf using a Li-Cor model 1600 steady-state porometer. Adaxial and abaxial stomatal conductances were summed to calculate the total stomatal conductance per leaf. In each treatment, three to six plants were measured, and the experiment was conducted four times.

Statistical analysis

Analysis of variance and least squares regression techniques were used to evaluate the data statistically (Sokal and Rohlf, 1981). Mean values are reported ±s.e.

RESULTS

Net K^+ and anion uptake experiments

From incubation solutions containing 250 μM K_2SO_4, the average net K^+ uptake by intact seedlings in the control treatment was 3·84±0·43 μmol K^+ g^{-1} root fr. wt. after 3 h (Fig. 1). Net K^+ uptake by seedlings treated with 20 μM FA for 3 h was not significantly different from that in the control treatment ($P>0·05$). In the 200 μM FA treatment, however, there was a net release of K^+ into the treatment solutions. The average change in net K^+ uptake between the control and the 200 μM FA treatments was 5·18 μmol K^+ g^{-1} root fr. wt. ($P<0·03$). Average net K^+ release in the 2000 μM FA treatment increased over that in the 200 μM FA treatment by only 1·1 μmol g^{-1} root fr. wt. ($P<0·03$), suggesting that whatever mechanism was being affected was nearly saturated by the 200 μM FA treatment.

In an experiment using excised roots, the net uptake of K^+ after 3 h in the control treatment was 1·9±0·4 μmol g^{-1} root fr. wt. Excised roots treated with 200 μM FA released a net 4·0±1·7 μmol K^+ g^{-1} root fr. wt.

In addition to reduced K^+ uptake, the net uptake of three macronutrient anions was inhibited by FA (Fig. 1). The average inhibition of net anion uptake in the 200 and 2000 μM FA treatments was 83, 57 and 53% of the control for NO_3^-, P_i, and SO_4^{2-}, respectively ($P<0·001$). The 20 μM FA treatment significantly affected only net NO_3^- uptake, which decreased by 18% ($P<0·03$).

The amount of FA removed over 3 h from treatment solutions by roots of intact seedlings increased with FA concentration. No FA was detected in the 20 μM treatment solutions at the end of the 3 h incubation period.

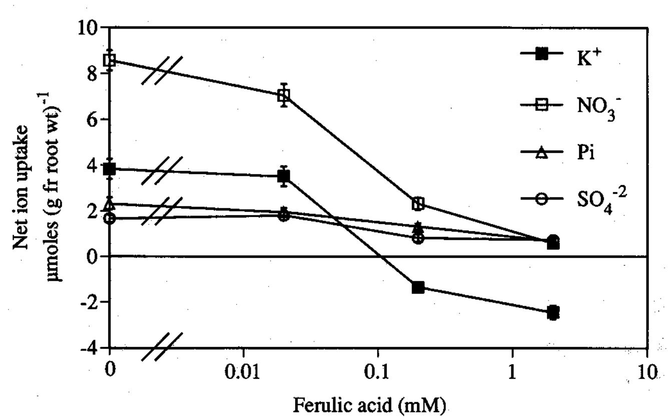

FIG. 1. Mean (±s.e.) net ion uptake from incubation solutions (15 cm^3) initially containing 500 μM K^+ and either 500 μM NO_3^-, P_i or SO_4^{2-}, by roots of intact cucumber seedlings after treatment with 0–2 mM ferulic acid for 3 h.

For the 200 μM treatment, 94% $\pm$ 2% was removed, whereas only 17% of the FA was depleted from the 2000 μM treatment solution. In the excised root experiment, 94 $\pm$ 5% of the 200 μM FA was removed from the treatment solution after 3 h.

Results from the net K^+ uptake-versus-concentration experiment showed that treatment of seedlings with 200 μM FA for 3 h promoted net K^+ efflux (Fig. 2). In incubation solutions initially containing no K^+, an average net release of 0·56 $\pm$ 0·08 μmol K^+ g^{-1} root fr. wt. was measured in the control treatment after 3 h. However, net K^+ release from seedling roots treated with 200 μM FA was, on average, almost eight times greater after 3 h than that from control seedlings ($P < 0{\cdot}001$) (Fig. 2).

The form of the flux-versus-concentration curve for net K^+ uptake also suggested that 200 μM FA promoted net K^+ efflux. In the control treatment, the form of the net K^+ uptake curve was an oblique hyperbola (Fig. 2). In the FA treatment, however, the non-linear increase in net K^+ uptake with increasing K^+ concentration from 0·125 to 0·5 mM was strongly suppressed (Fig. 2). The linear increase in net K^+ uptake from incubation solutions containing 0·5 to 6·0 mM K^+ was also inhibited by treatment with FA, as indicated by linear regression models. The slope of the linear model for the control treatment was 2·62 $\pm$ 0·20, whereas it was 1·87 $\pm$ 0·11 in the FA treatment, a 29% decrease.

Fusicoccin experiment

Net K^+ uptake from treatment solutions containing 250 μM K_2SO_4 varied during the 3 h incubation period (Fig. 3). Little K^+ was removed by seedlings during the first hour of incubation. Rates of average net K^+ uptake for the control seedlings increased during the second and third hours to approximately 1·0 μmol g^{-1} root fr. wt. h^{-1}. Seedlings in the FC treatment took up 2·6 times more K^+ than the control seedlings after the second and third hours of incubation ($P < 0{\cdot}001$) (Fig. 3). For seedlings treated with 200 μM FA, there was a small net uptake of K^+ after the second hour of incubation and net release of K^+ after the third hour (Fig. 3). However, the addition of FC to treatment solutions containing FA increased the average net amount of K^+ taken up after the second and third hours of incubation to levels not significantly different from controls ($P > 0{\cdot}05$) (Fig. 3).

The amount of 200 μM FA taken up per seedling increased with incubation time. On average, 10, 15, 43, and 81% of the FA was depleted from the solutions after 0·5, 1, 2, and 3 h of incubation, respectively. Mean uptake of FA was not significantly affected by FC ($P > 0{\cdot}05$).

TEA experiments

The treatment of seedling roots with 200 μM FA for 3 h in incubation solutions initially containing no KCl increased net K^+ efflux by about nine times compared with controls (Fig. 4). In the FA plus TEA^+ treatment, however, the average net release of K^+ into incubation solutions initially containing no K^+ decreased by 66% ($P < 0{\cdot}002$) (Fig. 4). There were no other significant effects of TEA^+ on net K^+ uptake. The net release of K^+ from seedlings treated with FA and 10 mM NaCl was not significantly different from the net release of K^+ from seedlings treated with FA, suggesting that Cl^- was not involved in the TEA-Cl inhibition of net K^+ efflux in the FA treatment.

Pressure–volume analysis

The mean xylem pressure potential (Ψ_{1x}) of leaves from plants treated 3 h with 200 μM FA was 0·22 MPa lower than that of the controls (Table 1). Because the FA-treated plants failed to rehydrate adequately during their incubation in humid darkness, there was insufficient data in the region of positive turgor to allow for curve fitting by the non-linear model (Eq. 4). Therefore, only the

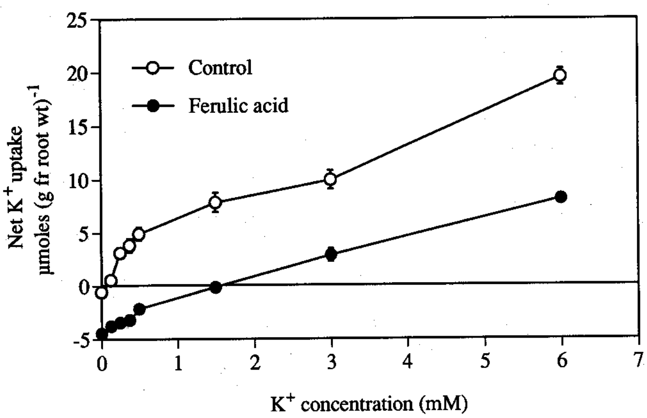

FIG. 2. Mean ($\pm$s.e.) net K^+ uptake from incubation solutions (15 cm^3) initially containing 0–6 mM K^+ by seedling roots after treatment with 0 or 200 μM ferulic acid for 3 h.

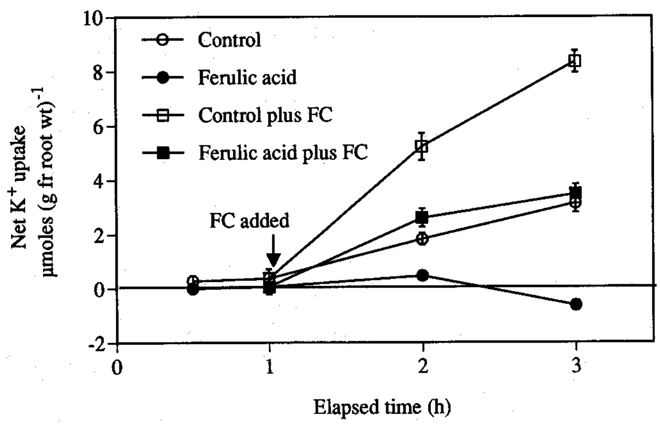

FIG. 3. Mean ($\pm$s.e.) net K^+ uptake from incubation solutions (15 cm^3) initially containing 250 μM K_2SO_4 by seedling roots over 3 h after treatment with combinations of 200 μM ferulic acid and 10 μM fusicoccin (FC). Fusicoccin was added 1 h after seedlings were treated with ferulic acid.

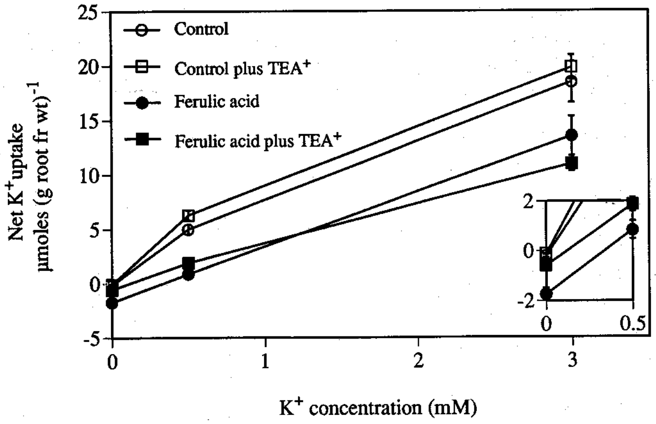

FIG. 4. Mean ($\pm$s.e.) net K^+ uptake from incubation solutions (15 cm^3) initially containing 0–3 mM KCl by seedling roots over 3 h after treatment with combinations of 200 μM ferulic acid and 10 mM tetraethylammonium (TEA^+). Plants were pretreated in incubation solutions (100 cm^3)$\pm$10 mM TEA^+ for 30 min. The inset shows in greater detail the effects of ferulic acid and TEA^+ on net K^+ uptake from incubation solutions initially containing 0 mM KCl.

osmotic component of equation 4 was used, and the data were analysed according to the linear reciprocal balance pressure model (Eq. 5) (Tyree and Hammel, 1972). However, the first step was to establish that there was no difference between coefficients determined by the linear (Eq. 5) method, and those determined from the non-linear method (Eq. 4). Control data were fitted to both equations 4 and 5 and the resulting parameters compared (Table 1). The first two rows of Table 1 show that there were no significant differences between π_o, P_T, V_o, or N_s as determined by either method. It was then possible to compare the controls and treatment coefficients based on the linear analysis of equation 5. When $1/P^*$ was plotted against V_e, the data sets for both the control and the FA-treated plants were similar in the linear portion of the plot where $P_T=0$ (Fig. 5). Linear regressions of the data in this portion of the plot showed no difference in the osmotic

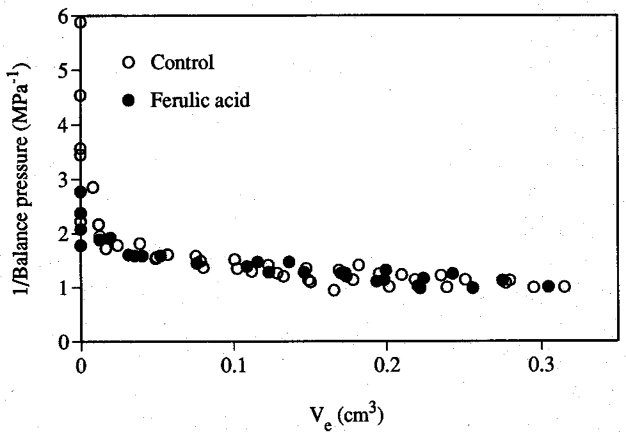

FIG. 5. Plot of the reciprocal of the balance pressure against the expressed sap volume (V_e) obtained from the pressure–volume analysis for seedlings treated with either 0 or 200 μM ferulic acid for 3 h.

pressure between the FA-treated plants and the controls, indicating there was no FA-induced change in the osmotic properties of the leaf symplast. Therefore, the decreased apoplastic water potential in the FA-treated plants caused a significant reduction in symplastic turgor pressure (Table 1).

There were adequate data in the region $P_T>0$ to obtain a non-linear fit in only two of the five FA-treated plants, but both of the z values thus obtained fell well within two standard errors of the control values. Thus, we can tentatively conclude that FA caused no difference in the elastic expansion characteristics of treated tissue.

Stomatal conductance experiments

After a 2 h incubation in the light, the mean stomatal conductance of the first primary leaf in the control and FA-treated plants was $0{\cdot}64\pm0{\cdot}10$ and $0{\cdot}49\pm0{\cdot}09$ cm s^{-1}, respectively ($P>0{\cdot}05$). After an additional 1–2 h incubation in the dark, stomatal conductance decreased on average by 25%, but there was still no statistically significant difference in stomatal conductance between the control and FA-treated plants.

DISCUSSION

We observed that treatment of cucumber seedlings with 200 or 2000 μM FA for 3 h inhibited net K^+, NO_3^-, P_i, and SO_4^{2-} uptake (Fig. 1). Net NO_3^- uptake was also reduced 18% by treatment with 20 μM FA. This suggested that NO_3^- uptake could be inhibited by low concentrations of FA.

Net K^+ efflux was observed when seedlings were treated with 200 μM FA for 3 h in incubation solutions initially containing less than 500 μM K^+ (Figs 1–4). Increased net K^+ efflux in response to 250 μM FA was also reported by Bergmark (1990) for intact roots of corn (*Zea mays* L.) seedlings. In our experiments, net K^+ efflux was substantially reduced in seedlings treated with FA and TEA^+ compared with those treated with FA, suggesting that blockade of K^+ channels with TEA reduced net K^+ efflux (Fig. 4).

Potassium efflux channels have been characterized in guard cells, pulvini motor cells and aleurone layer cells where they allow K^+ efflux upon membrane depolarization (Hedrich and Schroeder, 1989). These channels are important for regulating turgor. Tetraethylammonium has been used to block K^+ channels in wheat (*Triticum aestivum* L.) cells (Moran, Ehrenstein, Iwasa, Bare, and Mischke, 1986), pulvini cells (Moran, Ehrenstein, Iwasa, Mischke, Bare, and Satter, 1988) and giant algal cells (Tester, 1988). The blockade of these channels with TEA^+ would reduce net K^+ efflux, which was observed in the FA plus TEA^+ treatment. However, the observation that TEA^+ had no significant effect on net K^+ uptake by either control or FA-treated seedlings when KCl was present in the incubation solution was unexpected. Kochian,

TABLE 1. *Leaf P–V parameters from both the non-linear (Eq. 4) and linear (Eq. 5) models*

Ψ_{lx} is leaf xylem pressure potential, π is osmotic pressure, P is turgor, V is symplastic volume, N_s is osmotic solute quantity and z is a coefficient that indicates the rate of change in turgor with volume. The subscript o indicates values referenced to the first balance pressure ($V_e = 0$), except for values below the line where the balance pressure was forced to zero by the iterative fitting procedure. For values below the ~~dashed~~ line, $P_o = P_{max}$ and V_o is the symplastic volume at full hydration. Values are means ± s.e. ($n = 10$). $^*P < 0{\cdot}05$.

Treatment and model	Ψ_{lx} (MPa)	π_o (MPa)	P_o (MPa)	V_o (cm^3)	N_s (mmole)	z (cm^{-3})
−FA	−0·282	0·564	0·282	0·5734	0·13	—
Non-linear	±0·042	±0·006	±0·041	±0·0500	±0·01	
−FA	−0·282	0·568	0·286	0·5895	0·14	—
Linear	±0·042	±0·007	±0·038	±0·0620	±0·02	
+0·2 mM FA	−0·498*	0·600	0·104*	0·5905	0·14	—
Linear	±0·049	±0·014	±0·036	±0·0947	±0·02	
Final iteration at full hydration						
−FA	0	0·555	0·555	0·5818	0·13	−95·36
Non-linear		±0·004	±0·004	±0·0494	±0·01	±14·29
+FA	0	0·553	0·553	0·5441	0·12	−78·79
Non-linear		±0·009	±0·009	±0·0103	±0·004	±4·55

Xin-Zhi, and Lucas (1985) reported that 10 mM TEA^+ substantially reduced the slope of the linear component of the K^+ influx isotherm in corn roots. Although TEA^+ is a classic inhibitor of K^+ channels in numerous tissues, the affinity of TEA^+ for K^+ channels varies widely (Stanfield, 1983). In addition, the effect of external K^+ on TEA^+ binding has received little attention (Tester, 1988). However, in most cases, it is reasonable to assume that a TEA^+ block indicates K^+ permeability (Stanfield, 1983). Our experiment in which TEA^+ was replaced with 10 mM NaCl showed that the FA-induced net K^+ efflux was not reduced by Cl^-, the anion present in the TEA-Cl treatments.

Ferulic acid may also have inhibited ion carriers or secondary, gradient-coupled transport systems (Figs 1, 2). Although the uptake mechanisms for K^+, NO_3^-, SO_4^{2-}, and P_i are not fully understood, evidence from a variety of plant species suggests that uptake occurs by an active, voltage-dependent cotransport mechanism (symporter) (McClure, Kochian, Spanswick, and Shaff, 1990; Sanders, 1990). In our experiment, the flux-versus-concentration relationship for net K^+ uptake suggested that the inhibition of net K^+ uptake by FA was greater than net K^+ efflux, as determined with seedlings treated with FA in incubation solutions initially containing no K^+ (Fig. 2). In addition, previous studies found that treatment of excised roots with 250–500 μM FA substantially reduced both K^+ [$^{86}Rb^+$] and P_i influx (Glass, 1973, 1974; McClure *et al.*, 1978; Harper and Balke, 1981). Bergmark (1990) found that 250 μM FA rapidly restricted $^{15}NO_3^-$ influx by roots of intact corn seedlings while having little effect on $^{14}NO_3^-$ efflux. It was suggested that FA interfered with carrier proteins or operation of a cotransport system essential to induced NO_3^- uptake.

Although no direct biochemical mechanisms for the mode of action of FA were identified in our experiments, the results did suggest some explanations for the decreased net ion uptake observed in the FA treatments. The results of our FC and TEA^+ experiments showed that FA-inhibited net K^+ uptake could be countered by these treatments (Figs 3, 4), presumably from stimulated K^+-ATPase activity, membrane hyperpolarization, and the blockade of K^+ efflux channels (Stanfield, 1983; Blatt, 1988). It is possible that the effects of FC and TEA^+ on net K^+ uptake were simply additive to the effects of FA and did not necessarily act at the same site. However, Glass and Dunlop (1974) found that treatment of excised barley (*Hordeum vulgare* L.) roots with FA depolarized the transmembrane electrical potential. It was proposed this resulted from non-specific increases in cell membrane permeability while the phenolic acid was present.

The results of the P–V analysis showed that seedlings treated with 200 μM FA for 3 h had decreased Ψ_l and P_T, but similar Ψ_π, compared to seedlings in the control treatment. These results contrasted somewhat with those reported by Einhellig *et al.* (1985) for sorghum seedlings treated with 250 μM FA for 1–6 d, in which values for Ψ_l, P_T, and Ψ_π in FA-treated plants were all lower than those in control plants. Our results clearly showed that Ψ_π in cucumber seedling leaves was unchanged by treatment with 200 μM FA for 3 h. Thus, the failure of the FA-treated plants to rehydrate as fully as the controls was due to causes other than a disruption of the tissue symplastic equilibrium water relations or cell wall elastic properties.

Measurements of stomatal conductance in the control and 200 μM FA treatments were not significantly different, which indicated that the decreased Ψ_l and P_T in the FA-treated plants were not caused by unrestricted water loss through open stomates. The slightly lower average conductance in FA-treated plants was probably due to turgor loss and partial passive closure of the stomata. Therefore, a likely explanation for decreased P_T in seedlings treated with FA was decreased water absorption.

Within the context of allelopathy, the results of our experiments demonstrated that both ion uptake mechanisms and water relations of plants can be adversely affected by FA. The uptake of mineral nutrients, particularly NO_3^-, could be suppressed by micromolar quantities of FA. However, decreased acquisition of mineral nutrients may not be the primary factor limiting growth of cucumber seedlings treated with FA. Klein and Blum (1990) found that the inhibitory effects of FA on cucumber leaf expansion could not be overcome with increasing soil N levels. Decreased hydraulic conductivity and an inadequate influx of water necessary for cell expansion thus appear to be important factors limiting short-term growth of cucumber seedlings treated with FA.

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