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Uptake and Phloem Transport of Glucose-Fipronil Conjugate in *Ricinus communis* Involve a Carrier-Mediated Mechanism

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ABSTRACT: Some compounds containing glucose are absorbed via the monosaccharide transporters of the plasma membrane. A glucose-fipronil conjugate, *N*-[3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazol-5-yl]-1-(*β*-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-methanamine (GTF), has been synthesized in our previous work. GTF exhibits moderate phloem mobility in *Ricinus communis*. In the current paper, we demonstrate that the uptake of GTF by *Ricinus* seedling cotyledon discs is partly mediated by an active carrier system (K_m1 = 0.17 mM; V_{max}1 = 2.2 nmol cm^{-2} h^{-1}). Four compounds [D-glucose, sucrose, phloridzin, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)] were examined for their effect on GTF uptake. Phloridzin as well as CCCP markedly inhibit GTF uptake, and D-glucose weakly competes with it. The phloem transport of GTF in *Ricinus* seedlings is found to involve an active carrier-mediated mechanism that effectively contributes to the GTF phloem loading. The results prove that adding a glucose core is a reasonable and feasible approach to confer phloem mobility to fipronil by utilizing plant monosaccharide transporters.

KEYWORDS: *glucose, fipronil, uptake, phloem transport, monosaccharide transporter, Ricinus communis*

INTRODUCTION

Phloem mobility is one of the attributes of new pesticides that can be desirable and beneficial under various circumstances.[1] For example, the effective way to control piercing and sucking insects is using phloem mobile insecticides, especially when the pests are settled in the roots or inside leaf deformations and galls.[2] Similarly, the development of phloem-mobile or ambimobile fungicides that can be applied to foliage to control root or vascular system pathogens has long been highly desired.[3] Therefore, phloem-mobile pesticides need to be developed for pest control efficacy.

Among phloem-mobile pesticides, three herbicides are well-known to be transported by identified carrier systems, namely, auxin herbicides (e.g., 2,4-D), which involves an active process mediated by a carrier;[4] paraquat, which enters plant cells via a diamine carrier;[5] and glyphosate, which exhibits carrier-mediated uptake via a phosphate transporter.[6] Several studies have explored the potential of nutrient carrier systems in terms of xenobiotic phloem mobility.[7,8] For instance, *ϵ*-(2,4-dichlorophenoxyacetic acid)-L-lysine (2,4-D-Lys), a conjugate with an *α*-amino acid function, exhibits high phloem mobility. The uptake of 2,4-D-Lys has been proven to be mediated by an active carrier system.[9] A study on hydroxymethyloxamyl glucuronide (JR522) indicates that glucuronidation is indeed a good way to confer phloem mobility to immobile or poorly mobile pesticides.[10] Therefore, the addition of an amino acid or sugar to the parent compounds of existing nonphloem-mobile insecticides to make them cell permeant should be an alternative efficient strategy to develop a novel insecticide with phloem mobility properties.

D-Glucose, a preferred carbon and energy source, is transported across cell membranes by some distinct plant monosaccharide transporters,[11] which play key roles in source–sink interactions. The transport of 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose (2-NBDG), a conjugate containing a D-glucose group and 7-nitrobenz-2-oxa-1,3-diazole (NBD) moiety, has been proven to be mediated via a plant plasmalemma-bound hexose transporter.[12] *N*-{3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-iodo-1*H*-pyrazol-5-yl}-*N*-{[1-(*β*-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-yl]methyl}-*N*-{[1-((*N*-(7-nitrobenz-2-oxa-1,3-diazole-4-amine))-propyl)-1*H*-1,2,3-triazole-4-yl]methyl}amine (IPGN) is a novel fluorescent conjugate containing glucose and NBD. Uptake experiments on tobacco cells have proven that the glucose moiety can guide IPGN into tobacco cells via hexose transporters.[13] These previous studies indicate that glucose moieties may guide its conjugates into plant cells, tissues, or organs.

In a recent paper,[14] our group has synthesized a novel conjugate of the insecticide fipronil, *N*-[3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1*H*-pyrazol-5-yl]-1-(*β*-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-methanamine (GTF) (Figure 1), containing a glucose moiety. The phloem mobility of GTF in *Ricinus communis* seedlings as well as its long-distance transport and metabolism have been studied. Contrary to fipronil, GTF exhibits moderate phloem mobility, and there are two uptake plateaus in time–course experiment curve. However, the diffusion of this conjugate through the membranes must be very low due to its physicochemical properties [molecular mass (MW) = 680 D, polar surface area (PSA) = 211 Å^2, and number of hydrogen bonds acceptors (HBA) = 13].[14] A long-distance transport

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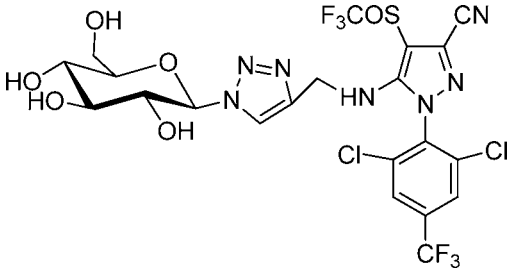

Figure 1. Chemical structure of GTF.

experiment on an adult castor bean has indicated that GTF is transported to the root system and cleaved to release the parent compound fipronil. The higher concentration of fipronil in the roots than in the "lower stem" is also incompatible with a diffusion mechanism. These experimental results may be the basis of the inference that the uptake and phloem transport of glucose−fipronil conjugate in *R. communis* involves a carrier-mediated mechanism. The purpose of this work is to test this inference and assess the contribution of the carrier-mediated mechanism to the phloem mobility of GTF. In the present paper, the uptake of GTF is demonstrated to be active carrier mediated. Information on the effect of D-glucose, sucrose, and phloridzin on GTF phloem transport is also provided.

MATERIALS AND METHODS

Chemicals. GTF was prepared according to our previously described.[14] The compounds to be added to incubation medium were purchased from Alfa-Aesar [carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)] and from Sigma-Aldrich (Phloridzin).

Plant Materials. Castor beans seeds (*R. communis*), obtained from the Agricultural Science Academy of Zibo Shandong China, were placed in humid cotton for 48 h at 27 °C prior to sowing in wet vermiculite. Seedlings were grown as previously described.[3] Six days after sowing, average sized seedlings were selected for the experiments.

Uptake in Cotyledon Discs. Discs (1.13 cm^2 surface) were obtained using a 12 mm diameter cork borer from *Ricinus* cotyledons according to a previously described method.[15] The discs were floated on preincubation medium containing 20 mM MES as buffer (pH 5.5), 250 mM mannitol, 0.25 mM $MgCl_2$, and 0.5 mM $CaCl_2$. After a 30 min preincubation period, the discs were incubated in the same buffer solution containing different concentrations (0.025−1.0 mM) of GTF with or without other substances (D-glucose, sucrose, phloridzin, or CCCP). The discs were gently and constantly oscillated on a reciprocal shaker at 28 °C for 1 h. At the end of incubation, the discs were rinsed (three times for 2 min each) in a solution of pure water/acetone (9/1, v/v), ensuring that all GTF on the cotyledon surface was washed away. The discs (12 discs for each treatment) were macerated with portions of acetone in a glass mortar and pestle, transferred to volumetric flasks (10 mL), and ultrasonically treated for 15 min with 10 mL of acetone. The extracts were filtered, and the filtrate residues were re-extracted twice. The combined extracts were rotary evaporated to dryness in a bath with temperature not exceeding 40 °C, and the residues were dissolved in 1 mL of methanol. The resulting solutions were analyzed on an Agilent Technologies 1100 HPLC system equipped with a vacuum degasser, a quaternary pump, an autosampler, and a UV−visible detector. An Agilent C18 reversed-phase column (5 μm, 250 mm × 4.6 mm i.d.) was used and maintained at 25 °C. The flow rate was 1 mL min^{-1}, and the injection volume was 10 μL. The mobile phase consisted of acetonitrile and water (50/50, v/v). The absorbance wavelength was 210 nm. A series of standard solutions of GTF (0.5, 1, 5, 10, 25, and 50 μM) were prepared in methanol to obtain a calibration curve. The linear equation of GTF was $y = 20.31x - 1.11$ ($r = 0.9993$). Recovery studies were developed at the three spiking levels of 0.1, 0.5, and 1 mg/kg, and the individual mean recovery rates for GTF were 98.6, 99.3, and 101.2%. Lower limits of detection calculated as a signal/noise ratio = 3 for GTF was 0.034 mg/L. Duncan's multiple range tests at a 5% probability level were used to determine statistical differences among treatments.

GTF in the discs was identified using a Waters Acquity ultraperformance liquid chromatography (UPLC) system equipped with a Waters Quattro Premier XE mass spectrometer using electron spray ionization (ESI). Chromatographic separations were run on an ACQUITY UPLCTM BEH C18 column (1.7 μm, 2.1 mm × 100 mm; Waters). The column was maintained at 40 °C, and the samples were eluted with an isocratic elution of acetonitrile and water (60/40, v/v). The eluent flow rate was 0.25 mL min^{-1}, and the injection volume was 3 μL. The mass spectrometer was operated in the positive electrospray ionization mode (ESI^+) using the multiple reaction monitoring (MRM) mode. The capillary voltage was 3000 V in the positive mode. The sample cone voltage was 30 V. The desolvation and source temperatures were set to 450 and 105 °C, respectively. The desolvation gas flow rate was 650 L/h. GTF fragmentation was performed using argon as the collision gas at a collision gas flow of 0.2 mL min^{-1} and collision energy of 20 V. Mass spectrometry (MS) was first conducted in full-scan mode to analyze the parent ion. Then, the daughter ion scans of the parent ion were performed by optimizing the collision energy to obtain a maximum response and define fragmentation pathways. Finally, ion m/z 680 of the highest signal intensity was selected as the parent ion, and the fragment ion at m/z 518 was selected as the daughter ion for MRM analysis (Figure 2).

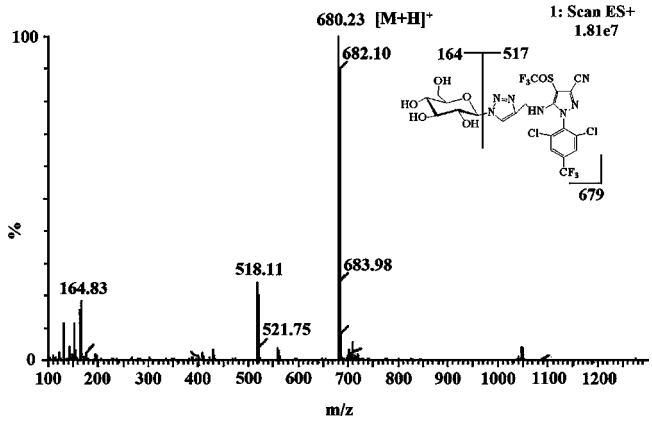

Figure 2. Positive ion ESI mass spectrum of GTF with fragmentation patterns marked. Finally, ion m/z 680 of highest signal intensity was selected as the parent ion, and the fragment ion at m/z 518 was selected as the daughter ion for MRM analysis.

Phloem Sap Collection and Analysis. The phloem sap collection method from the upper part of the hypocotyl was similar to that recently described.[14,16] The cotyledons were incubated in buffered solution containing 20 mM MES, 0.25 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 0.1 mM GTF without (control) or with other substances (D-glucose, sucrose, or phloridzin) at pH 5.5. After 1 h of incubation, the hypocotyl was severed in the hook region for phloem exudation, and the collected sap was stored in ice until analysis. The phloem sap was analyzed by HPLC after dilution with pure water (phloem sap/pure water, 1/5, v/v) using the aforementioned system.

Physicochemical Properties. Physicochemical properties of GTF and fipronil were predicted using ACD/Laboratories version 12.02 software. This package of programs calculates log K_{ow} (the pH-independent 1-octanol−water partition coefficient) and pK_a (the ionization constant in aqueous solution).

RESULTS AND DISCUSSION

Predicting the Phloem Mobility of GTF Using the Kleier Model. The Kleier model has been developed for the plant vascular system to enable the prediction of the phloem systemicity of a compound based on physicochemical proper-

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ties (log K_{ow} and pK_a) of molecules.[17] Both nonionized and acidic compounds are well accounted for by this passive diffusion model with the exception of a few xenobiotics, whose transport is carrier mediated.[18,19] This model was used to predict the phloem mobility of GTF and fipronil in the current study. According to the calculations of log K_{ow} and pK_a made by the ACD/Laboratories version 12.02 software (Table 1),

Table 1. Predicted log K_{ow} and pK_a Values of GTF and Fipronil[a]

name	log K_{ow}	pK_a	log C_f[c]
fipronil	3.98 ± 1.52	14.0[b]	←100
GTF	2.80 ± 1.59	14.80	←100

[a]All parameters were calculated using ACD/Labs version 12.02 software. [b]Assumed nonacidic and assigned pK_a of 14. [c]Log C_f values were calculated for a short plant.[17] Plant parameters for these calculations are L = 0.15 m, l = 0.05 m, $r = 5 \times 10^{-6}$ m, $v = 3.0 \times 10^{-4}$ m s^{-1}, pH (phloem) = 8, and pH (apoplast) = 6. The permeability of the membrane was calculated using log P = 1.2 (log K_{ow}) − 7.5.

both GTF and fipronil were in the nonmobile molecule area (Figure 3). The prediction result conformed with the

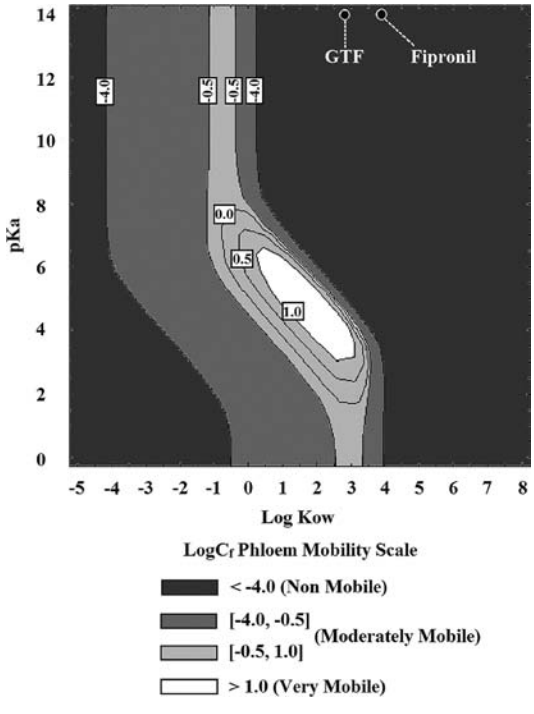

Figure 3. Prediction of phloem mobility of GTF and fipronil using Kleier map (log C_f as a function of log K_{ow} and pK_a).[17] Log K_{ow} and pK_a were calculated by ACD/Labs version 12.02 software, and plant parameters are for a short plant (see Table 1). The permeability of the membrane was calculated using log P = 1.2 (log K_{ow}) − 7.5.

experimental data of fipronil but not with the fact that GTF exhibits moderate phloem mobility in *Ricinus* seedlings based on our previous study.[14] The main purpose of this model is to provide useful information on the systemic ability of compounds rather than accurate predictions of mobility.[20] Strong discrepancies between predictions and experimental data may indicate the involvement of an active transport mechanism instead of diffusion through the plasma membrane.[15] By analysis of the key properties (MW = 680 Da, PSA = 211 Å^2, and HBA = 13) of GTF[14] and based on the well-accepted "rule of five",[21] the diffusion of this conjugate through membranes was most likely to be very low. The poor absorption of GTF predicted by its physicochemical properties may be overcome by a carrier-mediated mechanism. These results backed up our inference that the uptake and transport of glucose−fipronil conjugate involve a carrier-mediated mechanism. Consequently, further experiments were carried out to obtain direct physiological evidence from *Ricinus* seedlings.

Concentration Dependence of GTF Uptake. The GTF uptake by discs from *Ricinus* cotyledons was measured after 1 h of incubation for concentrations ranging from 0.025 to 1 mM. Given the limited water solubility of the conjugate, 1 mM was set as the highest concentration. As shown in Figure 4B, there

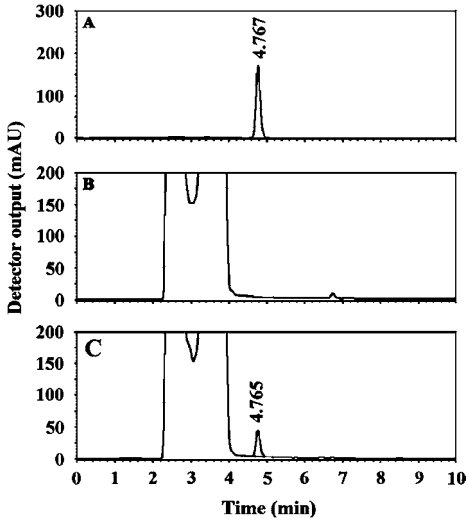

Figure 4. HPLC chromatograms of the standard solution of GTF and the extract of discs from *Ricinus* cotyledons. (A) Standard solution, retention time of GTF (RT, 4.77 min). (B) Control, cotyledon discs were incubated in buffer solution containing GTF at 0.2 mM for 1 min, and then, they were rinsed (three times for 2 min each) in a solution (pure water/acetone, 9/1, v/v); no GTF residue was detected. (C) Treated set, the discs were incubated in buffer solution containing GTF for 1 h.

was no GTF residue on the surface of the cotyledon according to our extraction procedures. Therefore, the detected GTF was from the interior of the cotyledon tissues (Figure 4C). GTF in the extraction solution was further identified by UPLC-tandem MS. The precursor and major product ions of GTF (m/z 680/518) were monitored in the MRM mode (Figure 2), and this method is highly selective and specific. It proved the existence of GTF in plant sample extraction according to retention time of 1.34 min in positive ion mode (Figure 5).

Concentration-dependent experiments of GTF indicated that GTF uptake involved two components: a saturable component at lower concentrations (0.025−0.2 mM) and a nonsaturable component at higher concentrations (Figure 6A). The apparent kinetic parameters (Figure 7A) obtained using Lineweaver−Burk plots were K_m = 0.41 mM and V_m = 9.4 nmol cm^{-2} h^{-1}. Numerous transport studies have indicated that several organic solute transport modes are mediated by the simultaneous operation of saturable and nonsaturable (diffusion-like) uptake.[9,22,23] For example, the nonsaturable transport of glucose has been reported in olive cells.[24] Therefore, we suggest that our experimental data may result from the

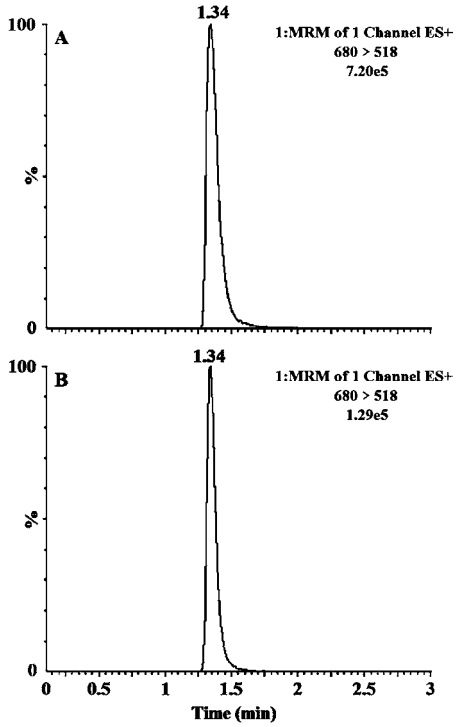

Figure 5. MRM chromatograms of GTF: (A) standard solution (RT, 1.34 min) and (B) plant sample extraction solution of discs from *Ricinus* cotyledons.

coexistence of at least two transport systems allowing the uptake of GTF. CCCP, which dissipates the proton motive force, has been widely utilized in the study of transmembrane transport.[25] When used at 50 μM, this protonophore inhibited GTF uptake, especially the saturable component (Figure 6B).

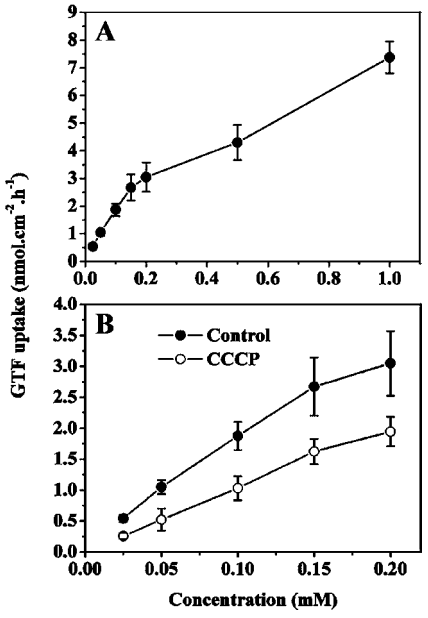

Figure 6. Concentration-dependent uptake of GTF by discs from *Ricinus* cotyledons. Discs were preincubated in a buffered solution at pH 5.5 for 30 min and then transferred to the incubation medium containing GTF for 1 h [A, concentration range from 0.025 to 1.0 mM; B, complementary set, without (control,●) or with 50 μM CCCP (○)]. Each point was the mean of three sets of 12 discs ± SE.

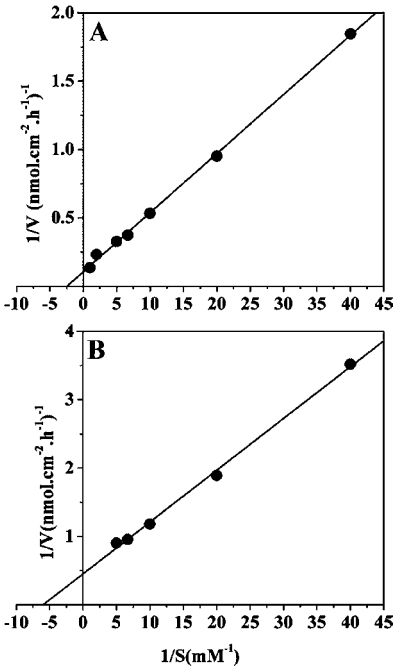

Figure 7. Lineweaver–Burk plots of GTF uptake. The kinetic data were calculated from the slope and the intercept of the Lineweaver–Burk plot. (A) Data from Figure 6A, the line yields a K_m = 0.41 mM and V_{max} = 9.4 nmol cm^{-2} h^{-1}, and the points corresponding to the highest concentrations (0.5 and 1.0 mM) parallel the vertical axis. (B) Data from Figure 6B after subtracting the CCCP-insensitive component, the line yields a K_m = 0.17 mM and V_{max} = 2.2 nmol cm^{-2} h^{-1}.

This is consistent with an H^+-dependent active transport as described for sugars.[22] After subtracting the passive component from the total uptake of GTF, the apparent kinetic parameters (Figure 7B) were K_m1 = 0.17 mM and $V_{max}1$ = 2.2 nmol cm^{-2} h^{-1}. K_m1 was at least 1 order of magnitude lower than that of the saturable component of sugar uptake.[26,27]

On the basis of the concentration dependence of GTF uptake by cotyledon discs and the inhibitory effects of CCCP (Figures 6 and 7), GTF uptake can be concluded to be involved an active uptake system. The active uptake component represents 52.4% of the total GTF uptake at 0.025 mM, 45.0% at 0.1 mM, and 36.2% at 0.2 mM (Figure 6B). These results displayed an obvious decreasing trend with gradually increased external concentrations. The carrier-mediated uptake of glyphosate has been reported in the leaf protoplasts of broad bean via a phosphate transporter.[6] However, Bromilow and Chamberlain[28] have suggested that this effect is observed only at concentrations up to 5 μM, beyond which diffusion processes may predominate even if carrier processes are operated at lower concentrations. Our experimental data corresponded to the analysis of glyphosate uptake. To examine further active uptake properties of GTF by cotyledon tissues, the next stage of the current study was operated at a moderate concentration of 0.1 mM.

Effect of Various Substances on GTF Uptake by *Ricinus* Cotyledons. D-Glucose and sucrose, the most important monosaccharide and disaccharide, were selected to determine the effect on 0.1 mM GTF uptake by cotyledon discs. The uptake of GTF exhibited weak competition with D-glucose at a concentration of 1 mM. In contrast, 1 mM sucrose did not affect the conjugate uptake under the experimental

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conditions (Figure 8). In cases of the CCCP-sensitive component of the conjugate influx being less than the one-

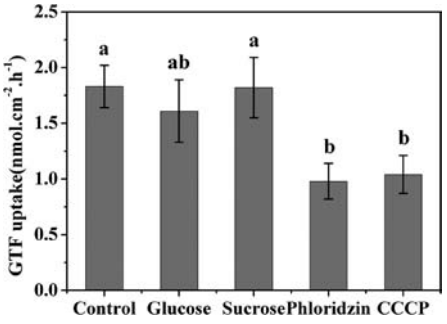

Figure 8. Effect of glucose, sucrose, phloridzin, and CCCP, respectively, on 0.1 mM GTF uptake by discs from *Ricinus* cotyledons. Discs were preincubated in a buffered solution at pH 5.5 for 30 min and then transferred to the incubation medium containing 0.1 mM GTF for 1 h without (control) or with 1 mM glucose, 1 mM sucrose, 2 mM phloridzin, or 50 μM CCCP, respectively. The figure indicates that the data (mean of three sets of 12 discs ± SE) within a column followed by the same letter are not significantly different in Duncan's multiple range test ($P > 0.05$).

half of the total uptake (Figure 8, control), D-glucose inhibited the total absorption of GTF by 12% and the CCCP-sensitive component by 28%. Phloridzin, the hexose transport inhibitor, was used to investigate active glucose uptake in plant cells.[29] Two millimolar phloridzin significantly inhibited 0.1 mM GTF uptake by 46.4% (Figure 8). The foregoing results indicated that the uptake of glucose−fipronil conjugate by *Ricinus* cotyledons involves an active carrier-mediated mechanism.

According to previous studies, the active uptake of substances mainly occurs in the leaf veins. Autoradiographs of leaf tissues show that [^{3}H]2,4-D-Lys is localized in mesophyll and veins,[9] and a similar picture is noted for amino acid uptake in broad bean foliar discs.[7] Sucrose is also accumulated more intensively in the veins.[27] In combination with our previous study that GTF exhibited moderate phloem mobility in *Ricinus* seedlings, there are reasons for believing that GTF could mainly accumulate in the veins. Therefore, further inhibition test and competition experiments were carried out to investigate whether carrier-mediated mechanism would effectively contribute to the phloem loading of GTF during this process.

Effects of Various Substances on GTF Accumulation in the Phloem Sap of *Ricinus* Seedlings. The effects of D-glucose, sucrose, and phloridzin on GTF accumulation in phloem sap using the *Ricinus* system were studied. The *Ricinus* system is widely employed to evaluate the phloem mobility of nutrients and xenobiotics.[3,9] This model is reliable only if the xenobiotics tested are nonphytotoxic in short-term experiments, that is, if they do not depolarize the transmembrane potential difference (PD). Our previous study has indicated that GTF has no depolarizing effect on the transmembrane PD.[14] Phloridzin (5 mM) induced a marginal depolarization of the normal PD of mesophyll cells.[30]

Under our experimental conditions, GTF displayed a moderate capacity of accumulation in phloem sap at an external concentration of 0.1 mM. Time−course experiments indicated that the GTF concentration in the phloem sap increased for 3 h before reaching the first plateau and then reached the second plateau at 6 h (Figure 9, control). Its concentration in the phloem sap was approximately 0.27- and

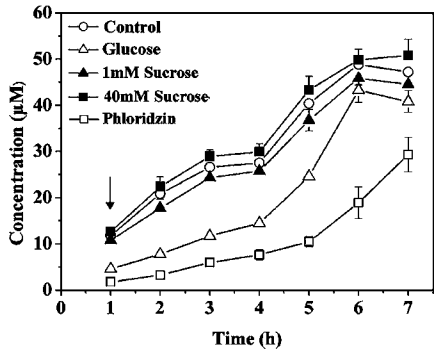

Figure 9. Time course of GTF concentration in phloem sap of *R. communis*. The cotyledons were incubated in the buffered solution containing 0.1 mM GTF without (control, ○) or with 1 mM glucose (△), 1 mM sucrose (▲), 40 mM sucrose (■), or 2 mM phloridzin (□), respectively, at pH 5.5. The hook was severed at time 1 h (arrow), and then, the sap was collected every 1 h during 7 h. Mean of three sets of 10 plants ± SE. Vertical bars were not shown when smaller than the symbols or covered by the symbols.

0.47-fold those of the incubated solution at 3 and 6 h, respectively. When *Ricinus* cotyledons were incubated in the same incubation medium containing 1 mM D-glucose, the GTF concentration in the first-plateau period (3−4 h) decreased by almost half (Figure 9). Similarly, the GTF concentration in the first-plateau period (3−4 h) decreased by nearly three-quarters in the presence of 2 mM phloridzin. However, 1 mM sucrose had little effect on the conjugate phloem mobility under the experimental conditions. The inhibitory effect of D-glucose and phloridzin weakened in the second-plateau (6−7 h). The experiment clearly showed that glucose is a stronger competitor of GTF than sucrose at the early stage of the phloem loading of GTF (Figure 9). No experiments have been carried out to compare the effect of phloridzin on glucose and sucrose uptake by cotyledons discs of *R. communis*. Sucrose is the main plant osmoticum translocated in the *Ricinus* phloem. If sucrose loading is inhibited by phloridzin, then the long distance transport of phloem sap and its various solutes, GTF included, could be affected. To test whether the inhibitory effect on GTF was directly or indirectly caused by phloridzin, the experiments were also conducted at higher concentration of sucrose (40 mM). As the concentration of sucrose increases 40-fold, the GTF concentration in the phloem sap had no significant change (Figure 9), which indicates that sucrose carriers are not involved in long-distance transport of GTF. Hence, we suggest that the inhibition of the hexose carrier is the main factor of the inhibition of GTF accumulation in the first-plateau period.

In summary, on the basis of the significant inhibitory effect of the uncoupled CCCP and the sugar transport inhibitor phloridzin, the experimental results of GTF uptake by cotyledon tissues demonstrate that the uptake of a glucose−fipronil conjugate was found to involve an active carrier-mediated mechanism. Monosaccharide transporters may transport this glucose−fipronil conjugate because D-glucose obviously affected the uptake of GTF, but sucrose did not. The clear inhibition of 1 mM glucose and 2 mM phloridzin on long-distance transport of GTF indicates that a similar carrier-mediated mechanism effectively contributes to the GTF phloem loading. A possible explanation for the weakening of the competition effect of 1 mM glucose on GTF at the end of long-term experiments (Figure 9) is that it may be caused by

aging or a more or less complete uptake of glucose from the incubation medium.

The identification and functional characterization of a transporter are not simple, especially in higher organisms.[31] Thirteen clusters have been recognized in the monosaccharide transporter superfamily, with 66 and 22 putative monosaccharide transporters (MSTs) in the *Arabidopsis* and rice genomes, respectively.[32] The full understanding of such complex sugar uptake kinetics becomes laborious in plants, let alone that of GTF. Further investigations of the phloem loading and unloading of GTF are required. The current paper demonstrated that the uptake and phloem transport of a glucose−fipronil conjugate involved a plant sugar carriers system. Hence, information on new strategies for pesticide discovery, development, and targeting is provided.

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ABBREVIATIONS USED

GTF, *N*-[3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl) sulfinyl]-1*H*-pyrazol-5-yl]-1-(β-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-methanamine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; 2-NBDG, 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose; NBD, 7-nitrobenz-2-oxa-1,3-diazole; MW, molecular mass; PSA, polar surface area; HBA, number of hydrogen bonds acceptors; log K_{ow}, the pH-independent 1-octanol−water partition coefficient; pK_a, the ionization constant in aqueous solution; MRM, multiple reaction monitoring; PD, potential difference

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