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Uptake and Xylem Transport of Fipronil in Sunflower

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The phenylpyrazole insecticide, fipronil, is used in seed coating against *Agriotes* larvae, which infest mainly corn and sunflower. Coating the seeds of the cultivated plants with fipronil has proven its effectiveness against *Agriotes* populations. In the case of sunflower or even corn, the possible root uptake of this insecticide may lead to a toxic effect against pollinators such as honeybees. In the present report, the uptake and transport of fipronil inside the sunflower seedling was studied in the laboratory. In a first study, sunflower was cultivated on an aqueous medium containing fipronil. An intense root uptake of fipronil occurred, leading to a transport into leaves depending upon transpiration. In a second study, plants were cultivated on a soil in which fipronil was uniformly distributed. Under our soil conditions (20% organic carbon), the partition coefficient between soil and water (K_d) was found to be equal to 386 ± 30. The average rate of fipronil transfer from soil water to seedlings was from 2 to 2.6 times lower than water transfer. During the 3 week experiment, 55% of recovered labeled compounds was in the parent form and 35% had been converted to lipophilic metabolites, with either a 4-CF_3-SO_2 or 4-CF_3-S substituant, which are also very potent lipophilic insecticides. This paper suggests that the possible uptake of fipronil by sunflower seedlings under agronomic conditions is mainly controlled by the physicochemical characteristics of the seed-coating mixture.

KEYWORDS: Fipronil; sunflower; *Agriotes*; seed coating; plant uptake; transpiration; xylem

INTRODUCTION

The intensive monoculture of such plants as corn, wheat, sunflower, or potatoes requires a chemical control of several soil predatory insects, especially the *Agriotes* genus (*1*). *Agriotes* larvae are represented by several species that live for several years inside the soil and whose food is mostly constituted of storage seed compounds.

This control has been previously achieved with full soil treatments using long residual insecticides such as lindane (*2*) or other organochlorine derivatives, now banned in Europe, and more recently with carbamates (*3*).

Over the past decades, upon replacement of the banned chemicals, two new chemical insecticide families were used, the neonicotinoid family (*4*) (e.g., imidacloprid) and the fiprole family (e.g., fipronil, **Figure 1**). Fipronil acts on the γ-amino butyric acid (GABA)-gated chloride channel, mostly in insects, with low mammalian toxicity. The main change introduced by these new active ingredients was that an efficient control of pests such as *Agriotes* was obtained through a seed-coating strategy. In this case, solid insecticide particles were stuck on the resting seeds of the cultivated plants with the appropriate

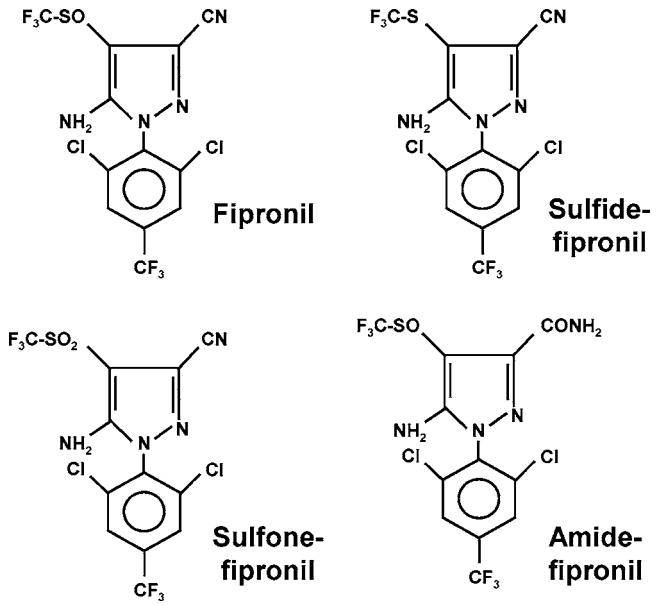

Figure 1. Chemical structures of fipronil and its first metabolites. Sulfide–fipronil, 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl)phenyl-4-trifluoromethylsulfide pyrazole; sulfone–fipronil, 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl)phenyl-4-trifluoromethylsulfone pyrazole; amide–fipronil, 5-amino-3-amide-1-(2,6-dichloro-4-trifluoromethyl)phenyl-4-trifluoromethylsulfinyl pyrazole.

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additives. The ecological advantage of such a strategy was to limit the insecticidal pressure to a small space surrounding the seed (*5*).

The possible absorption of the active ingredient by the cultivated plant itself still needs to be further demonstrated because it might represent a risk for beekeeping (*6*). The objective of this study was to examine ^{14}C-fipronil uptake and translocation by sunflower seedlings under experimental conditions.

MATERIALS AND METHODS

Chemicals. Fipronil [5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl)phenyl-4-trifluoromethylsulfinyl pyrazole (*7*)] was used (99.3% purity, Sigma). The ^{14}C molecule was uniformly labeled on the aromatic ring with a 741 Bq nmol^{-1} specific activity (Rhône-Poulenc, Ongar, U.K.). Sulfide−fipronil (98.8% purity), amide−fipronil (99.8% purity), and sulfone−fipronil (99.7% purity) were synthesized by Rhône-Poulenc, Ongar, U.K.

Sunflower Cultures. A total of 10 hydrated sunflower seeds were placed on humidified filter paper at 25 °C in the dark. Under these conditions, first roots and cotyledons began to develop after 4−5 days. After this stage of development, the seedlings were cultivated either in a liquid medium or on soil.

For the hydroponic culture, seedlings were inserted into a block of polystyrene and then placed in a container with 100 mL of aqueous Hoagland's medium, upon which the plants floated with the roots immersed in the medium.

For the soil cultures, young seedlings grew in pots containing 400 g of a humus−clay (1:1, w/w) mixture humidified with 200 mL of water with 0.17 nmol of ^{14}C-fipronil g^{-1} of soil.

The photoperiod for this culture was 16 h in the light and 8 h in the dark. The temperature was fixed at 18 °C during the night and 28 °C during the day. The relative humidity was maintained between 65 and 80%, allowing for a high transpiratory rate of the seedlings.

The amounts of ^{14}C-fipronil added in each of these experiments were always under the solubility limit in water (2 mg L^{-1} at 20 °C).

Biometric Measurements. At 0, 6, 13, and 18 days, two plants were picked up for biometric measurements. Their fresh and dry weights were measured as well as the length of the stems and roots. Furthermore, the leaves were counted and weighed, and their surface was measured.

Transpiration Rate. The weight of each pot containing one plant was measured every day, compared to the weight of a pot without a plant, and then the loss of water corresponding to the plant transpiration was calculated. The transpiration rate was evaluated as the water loss per leaf surface unit.

Soil−Water Partition. The fipronil solution (200 mL) containing 272 pmol mL^{-1} (210 Bq mL^{-1}) was thoroughly mixed with 200 g of a mixture of humus and clay (1:1, w/w).

This mixture was poured into a filtration funnel number 1. At regular intervals, a small amount of the solution was obtained by suction and its radioactivity was measured. The radioactivity of dry soil samples was also periodically measured after mineralization.

After 4 days (**Figure 2**), a stable equilibrium was reached for a fipronil concentration in the soil equal to 270 ± 10 pmol g^{-1} and a concentration in the water decreasing to 0.7 ± 0.2 pmol mL^{-1}. At this stage, the partition coefficient soil/water was 386 ± 30. When the high carbon content (20%) of the chosen soil was taken into account, this value of the partition coefficient was shown to be the same order of magnitude as in Tingle et al. (*6*) and Ying and Kookana (*8*) (K_d from 5 to 14 with 0.5−2% organic carbon).

Mineralization and Radioactivity Counting. Plant (roots, leaves, and stem separately) and soil fresh samples were submitted to three successive extractions with acetone. The residual powders were shown to be deprived of radioactivity.

The acetonic solutions were evaporated to dryness. An aliquote of the dry residue was dissolved with 10 mL of mineralization mixture, H_2O_2/perchloric acid/H_2O (1:1:1, v/v/v), in closed polyethylene flasks. After 5−6 days of mineralization at 25 °C, the radioactivity of the mineralization mixture was measured through scintillation counting (1414 Winspectral EG&G Wallac). During the mineralization step, the absence of $^{14}CO_2$ leaching from the flasks was controlled, using a KOH trap.

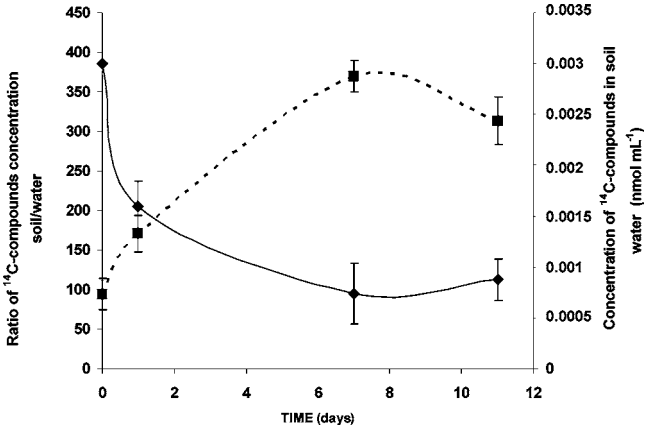

Figure 2. Changes of the fipronil concentration in soil water and evaluation of the soil/water partition coefficient. (■) [Radioactivity]$_{soil}$/[radioactivity]$_{water}$; (◆) [radioactivity] in soil water; Three replicates ± SE.

Each measurement was repeated 10 times. The average value and standard error (SE) were calculated as shown in all of the figures.

Analyses of the Acetonic Extracts. Fipronil degradates from harvested plant tissues and soil extracts were analyzed using thin-layer chromatography (TLC) and gas chromatography−mass spectrometry (GC−MS).

TLC was carried out using silica gel plates (F_{256} Merck) with two successive solvents, dichloroethane/acetic acid (19:1, v/v) and petrol ether (bp 40−60 °C)/dichloroethane/acetic acid (20:19:1, v/v/v) as a second solvent (*9*). The quantitative estimation of the different labeled compounds was established by using a [^{14}C] thin-layer chromatoscanner (Berthold). Under these conditions, the measured R_f values were 0.35 for fipronil, 0.80 for sulfide−fipronil, 0.68 for sulfone−fipronil, and 0.064 for amide−fipronil as shown with the use of pure derivative references (*10*).

GC−MS measurements were carried out on a HP6840/HP5973 apparatus (Agilent Technologies, Les Ulis, France) equipped with a MDN-12 fused silica capillary column (30 m, 0.25 mm internal diameter, 0.25 μm film; Supelco). The injector was used in the split mode, with a split ratio of 50:1 and an injection volume of 2.5 μL. The oven temperature was held at 70 °C for 4.5 min, then increased to 240 °C at a rate of 50 °C min^{-1}, and held for a further 20 min. To detect fipronil and its standard metabolites, samples were analyzed in the full-scan mode (50−550 mass range). The following retention times were determined for standard chemicals (**Figure 1**): sulfide−fipronil (11.17 min), fipronil (11.29 min), sulfone−fipronil (13.11 min), and amide−fipronil (16.32 min).

Autoradiography. Autoradiographies of dry plant material were performed with Kodak films (DEF 5) in the dark.

RESULTS

Fipronil Root Uptake by Sunflower Seedlings. Sunflower resting seeds immersed in a solution of ^{14}C-fipronil were shown to be unable to absorb any significant amount of the insecticide. The same was previously observed with numerous other pesticides and several different types of seeds (*11, 12*).

In marked contrast with this situation, after the beginning of germination and growth of the seedlings, the exchanges between the aqueous medium and the living material generally show an intense rate (*13−15*). It was therefore necessary to demonstrate whether the fipronil insecticide had the necessary structural features to be transported into the seedling and to measure its rate of transport.

For this purpose, a simple experiment was carried out, where sunflower seedlings were cultivated on an aqueous medium, to which ^{14}C-fipronil was added at a 480 nM concentration.

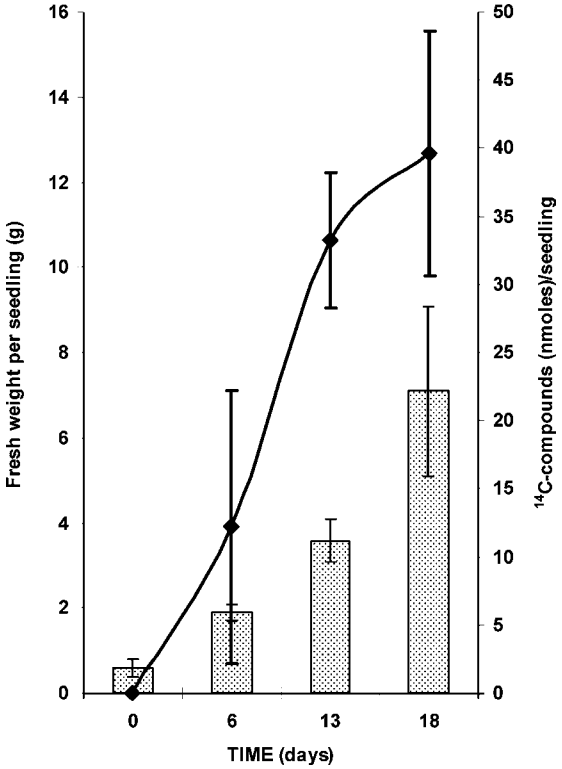

Figure 3. Growth parameters and fipronil uptake for sunflower seedlings cultivated on an aqueous medium with fipronil (480 nM). (Dotted bars) Fresh weight per seedling (g); (◆) radiolabeled content (nmol/seedling); 10 replicates ± SE.

Seedlings floating on the medium developed their roots inside it. During the experimental period (18 days), 10 new leaves were successively formed after the development of the cotyledons.

The weight of fresh matter of one seedling was 0.7 ± 0.2 g at day 0 and increased to 7 ± 2 g at day 18 (**Figure 3**).

The whole amount of fipronil found inside one seedling progressively increased from 0 at the beginning of the experiment to 39 ± 9 nmol per plant at the end.

Parts **A** and **B** of **Figure 4** show the distribution of the ^{14}C active ingredient inside the seedlings.

As shown in **Figure 4A**, more than 74% of the radiolabeled compound was absorbed by the plant after 18 days, most of which (44%) were accumulated inside the leaves.

The stem content was comparatively very low. The root content, important at first, decreased during the second part of the experiment, when the medium concentration was lower, showing that the labeled compound was transferred from the roots to the aerial parts.

Figure 4B shows that the fipronil concentration increased steadily in the leaves but remained lower than the root concentration until the last step of the experiment, when the leaf and root concentrations were approximately identical.

As a whole, these results demonstrate that an intense root uptake of fipronil occurred, from an actual water solution to the seedlings. Moreover, most of the labeled product was transported into the aerial parts, suggesting a xylem transfer associated with transpiration, as shown by the leaf autoradiography during the first stage of penetration (**Figure 5A**).

Fipronil Distribution Inside the Different Leaves of the Seedlings. Figure 5B shows the amount of ^{14}C-labeled compounds accumulated in each type of leaf after 6, 13, and 18 days. The amount of fipronil cm^{-2} rose steadily inside each

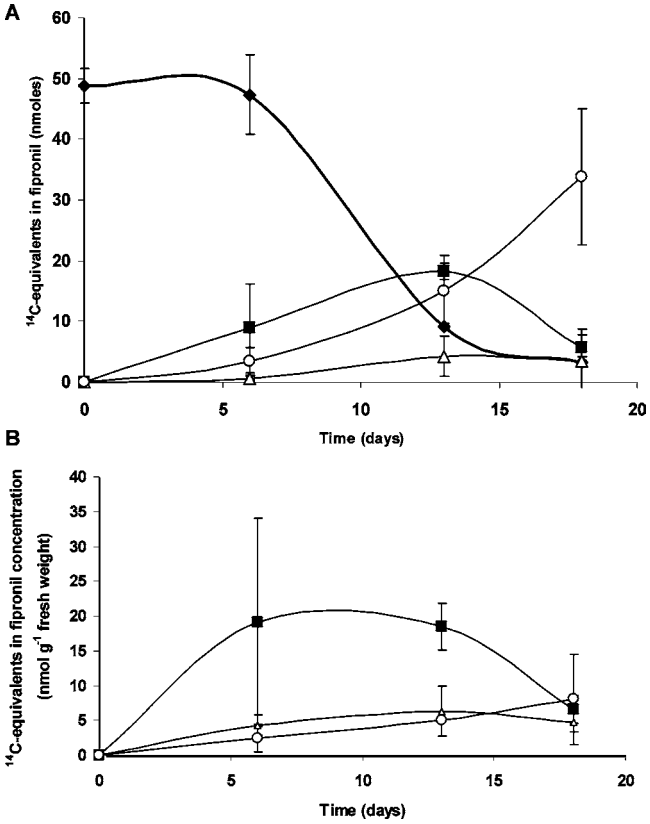

Figure 4. Kinetics of radiolabeled compound uptake into the different organs of sunflower seedling cultivated on liquid medium (fipronil, 480 nM). (A) Content of ^{14}C compounds in the medium and in one seedling, as expressed in nanomoles per type of organ. (B) ^{14}C concentration changes in the different organs of the seedlings. (◆) Medium; (■) roots; (△) stem; (○) leaves; 10 replicates ± SE.

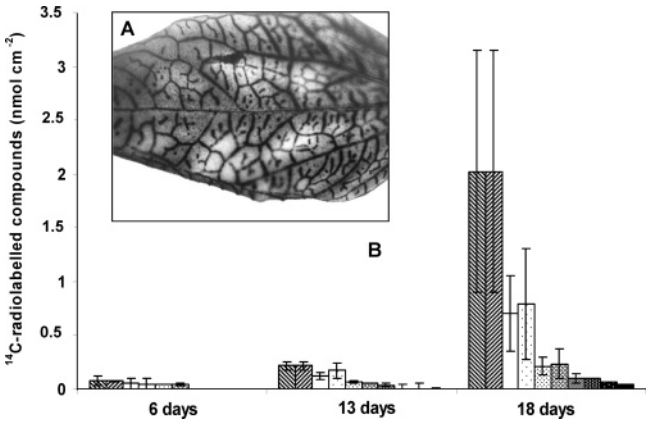

Figure 5. ^{14}C-Fipronil amount accumulated in each leaf (L) of sunflower plants cultivated on a hydroponic solution (fipronil, 480 nM). (A) Autoradiography of a leaf (leaf 4) after 6 days. (B) Amounts of fipronil equivalents measured in each type of leaf and as a function of time. Bars represent from left to right: cotyledon 1, cotyledon 2, leaf 1, leaf 2, leaf 3, leaf 4, leaf 5, leaf 6, leaf 7, and leaf 8; 10 replicates ± SE.

organ, but this accumulation reached its maximum in the cotyledons and in the first two leaves.

This can be understood because these organs had the longest life duration and remained physiologically active during the whole experiment. Furthermore, during the first days of the experiment, the concentration of the absorbed solution was at its maximum.

Table 1. Estimation of the Ratio between the ^{14}C Concentration in the Medium and the Calculated Average Concentration in the Whole Seedling Xylem Sap (10 Replicates ± SE)

time period (days)	C_m[a] (nmol mL^{-1})	Q'[b] (nmol)	V[c] (mL)	C_s[d] $= Q'/V$ (nmol mL^{-1})	C_m/C_s
0–6	0.67 ± 0.03	13 ± 10	49 ± 6	0.26	2.6
6–13	0.54 ± 0.02	24 ± 9	113 ± 13	0.21	2.6
13–18	0.18 ± 0.06	5 ± 1	76 ± 13	0.07	2.6

[a] C_m = average concentration of ^{14}C-fipronil in the hydroponic solution (in nanomoles/milliliter). [b] Q' = quantity (in nanomoles) of ^{14}C compounds absorbed by seedlings over a time period between T_n and T_{n+1}, where T_{n_0}, T_{n_1}, T_{n_2}, and T_{n_3} were 0, 6, 13, and 18 days, respectively. [c] V = transpired water volume per plant (in milliliters). [d] C_s = concentration of ^{14}C compounds in the xylem sap (in nanomoles/milliliter).

Table 2. Estimation of the Ratio between the ^{14}C Concentration in the Medium and the Average Concentration in the Foliar Xylem Sap (10 Replicates ± SE)

time period (days)	Q_L[a] (nmol)	C_L[b] $= Q_L/V$[c] (nmol mL^{-1})	C_m/C_L
0–6	3 ± 1	0.06	11
6–13	12 ± 4	0.10	5
13–18	19 ± 6	0.25	0.72

[a] Q_L = quantity (in nanomoles) of ^{14}C compounds reaching the foliage over a time period between T_n and T_{n+1}, where T_{n_0}, T_{n_1}, T_{n_2}, and T_{n_3} were 0, 6, 13, and 18 days, respectively. [b] C_L = concentration of ^{14}C compounds in the leaf xylem sap (in nanomoles/milliliter). [c] V = transpired water volume per plant (in milliliters).

In contrast, the last leaf numbers 6−8 were only young leaves at the end of the culture, with a limited size, and at this stage, they received a xylem sap with a low fipronil concentration.

Relationships between the ^{14}C Compound Concentration in the Medium and the Calculated Concentration in the Xylem Sap. In this experiment, most of ^{14}C-fipronil uptake appeared to depend upon the transpiration flux of the leaves, in agreement with what was observed elsewhere for xylem-mobile herbicides (*14*).

The increase in the ^{14}C content of the seedlings (Q') and the amount of water lost through leaf transpiration (V) were concurrently measured at three steps during the experiment (**Table 1**).

The ratio Q'/V represents the insecticide average concentration (C_s) in the xylem sap (**Table 1**). The ratios between the medium concentration and the calculated xylem sap concentration C_s remained constant throughout the experiment and reached a value of 2.6. If we consider the increase in the ^{14}C content of the leaves (Q_L), the ratio Q_L/V expresses the average xylem sap concentration at the level of the leaves (**Table 2**). The values of $C_s = Q'/V$ and $C_L = Q_L/V$ clearly differ because exchanges certainly occur between root and sap as a consequence of adsorption/desorption equilibrium changes (*12*). During the experiment, fipronil adsorption on the roots reached a high level at the beginning of the experiment, inducing a 4-fold decrease in the sap concentration, from the roots to the leaves. At the end of the experiment, root desorption of the ^{14}C compounds increased, inducing the opposite situation: the xylem sap concentration in the leaves was 4 times higher than the apparent concentration in the water absorbed by the roots.

As a whole, as shown by **Table 1**, the average concentration in the xylem sap of the seedling was 2.6 times lower than that in the medium, showing that fipronil uptake was slower than water uptake. For plants grown in soil, it was assumed that this relation between the fipronil concentration in soil water and average xylem sap was maintained (*16*).

Table 3. Relation between the Fipronil Concentration in the Soil Water and the Average Xylem Sap (10 Replicates ± SE)

C_w[a] (nmol mL^{-1})	Q'[b] (nmol)	V[c] (mL)	C_s[d] $= Q'/V$ (nmol mL^{-1})	C_w/C_s
$5 \times 10^{-4} \pm 0.8 \times 10^{-4}$	0.13 ± 0.05	500 ± 6	2.6×10^{-4}	2

[a] C_w = average concentration of ^{14}C-fipronil in the soil water (in nanomoles/milliliter). [b] Q' = quantity (in nanomoles) of ^{14}C compounds absorbed by seedling over the time period (25 days). [c] V = transpired water volume per plant (in milliliters). [d] C_s = concentration of ^{14}C compounds in the xylem sap (in nanomoles/milliliter).

Fipronil Uptake from Soil. To verify this relation, it was necessary to conceive an experimental device where fipronil was uniformly distributed inside the soil and where the value of the partition coefficient between the clay−humus complex (CHC) and water could be repetitively measured without disturbing the system.

In **Table 3**, as in **Table 1**, this concentration in soil water was compared to the average sap concentration deduced from Q'/V, which is the relation between the total fipronil amount per plant and the transpired volume. The results show that the average concentration in the xylem sap was approximately half of the concentration in the soil water. The concentration in the soil water was maintained at a constant value because of the adsorption equilibrium with the CHC. In this experiment, the concentration equilibrium between external water and sap, reaching a value of 2, was closed to that obtained with the aqueous medium (2.6).

Figure 6A shows the distribution of the ^{14}C content in the different parts of the plant. In this case, most of the ^{14}C content of plants after 2−25 days of culture was present in the leaves, showing that the strong ^{14}C accumulation found in the roots of plants cultivated on the aqueous medium (**Figure 4**) was certainly due to adsorption. Root adsorption became negligible in the case of soil-grown plants (**Figure 6A**) because adsorption on the CHC was the major phenomenon.

Figure 6B shows the ^{14}C content in the different leaves of the seedlings grown on soil (25 days). This content was between 5 and 25 pmol/leaf, with a maximum accumulated in leaf numbers 1−4. This difference with the experiment on water agrees with the fact that, in soil-grown plants, the water ^{14}C concentration was maintained constant throughout the experiment, as the result of CHC regulation. Then, the ^{14}C accumulation was highest in the leaves having the largest surface and longest life and, therefore, able to have been submitted to the highest transfer of water through transpiration.

Nature of the Labeled Compounds. As previously mentioned, acetonic extraction allowed us to obtain all of the radioactive components present in plant and soil material.

This demonstrated that no bound residue was formed under our conditions, in contrast with what occurred with others compounds (*17*, *18*). Furthermore, 90% of the labeled compounds could be transferred from the hydroacetonic solution to petrol ether through partition, showing that the ^{14}C compounds were lipophilic. The measurement of the insecticidal properties of this mixture on *Aedes aegypti* larvae showed a LC_{50} (24 h) slightly lower than for fipronil itself, which is close to 24 nM as shown by Chaton et al. (*5*), Aajoud et al. (*9*), and Raveton et al. (*10*).

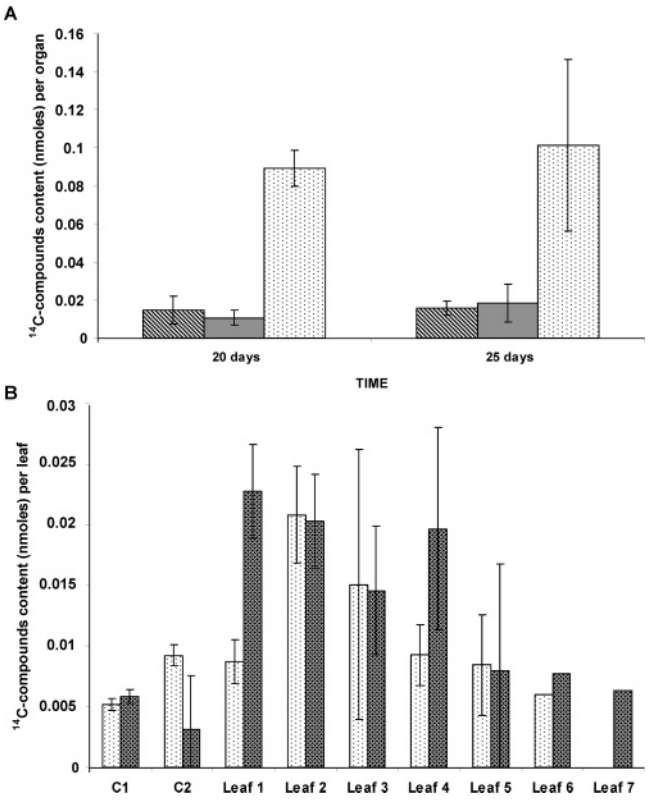

Figure 6. ^{14}C compound distribution in sunflower plants cultivated in soil treated with fipronil (168 nmol kg^{-1}) at 20 and 25 days (10 replicates ± SE). (A) ^{14}C compound contents in the different parts of the plant. Bars represent from left to right: roots, stem, and leaves. (B) ^{14}C compound contents in the different leaves. Bars represent from left to right: 20 and 25 days. (C1) Cotyledon 1 and (C2) cotyledon 2.

GC−MS analyses of the different hydroacetonic extracts showed the presence of fipronil (55%), 5-NH_2-4-CF_3SO_2-3-CN-1-[2,6-dichloro-4-(trifluoromethyl)phenyl] pyrazole (4-CF_3-SO_2 derivative) (25%), and 5-NH_2-4-CF_3S-3-CN-1-[2,6-dichloro-4-(trifluoromethyl)phenyl] pyrazole (4-CF_3-S derivative) (10%). All of these compounds have powerful insecticidal activity (*9*). The 5-NH_2-4-CF_3SO-3-$CONH_2$-1-[2,6-dichloro-4-(trifluoromethyl)phenyl] pyrazole (3-$CONH_2$ derivative), which is more hydrophilic and which has only a low insecticidal activity was poorly represented. The structure of all of these derivatives was established as described in Raveton et al. (*10*).

DISCUSSION

In sunflower under our conditions, fipronil uptake and transport were associated with an intense water movement inside the xylem toward the leaves because of a high transpiratory rate, as is generally the case in natura (*19*, *20*).

Translocation of the insecticide toward the leaves and accumulation inside these organs during the course of the experiment demonstrate that xylem transport was the major phenomenon. A source−sink phloem transfer was also considered in other experiments (results not shown), but its rate was negligible as compared to the xylem translocation. Fipronil uptake from a water solution (**Figure 3**) was shown to be easy, and the transport from roots to the leaves occurred also at a fairly high rate. However, a specific root accumulation was clearly observable during the first part of the experiment, which decreased later when the medium concentration was lower, demonstrating that a reversible partition equilibrium tended to be reached inside roots, possibly involving membrane lipids and/or vessel lignin.

As shown in **Table 1**, the average concentration in the xylem sap inside the whole seedling reached a value that was 2.6 times lower than the medium concentration. This illustrates that the different steps of fipronil uptake by the roots (diffusion, partition with membrane lipids, desorption, and diffusion in xylem vessel sap) occurred at a lower rate than water transfer. Consequently, the actual diffusion/partition equilibrium between the medium solution and inner sap was never reached.

Furthermore, as shown in **Table 2**, the average ^{14}C concentration of the xylem sap at the level of the leaves was much lower than the average value at the level of the whole plant. This demonstrated that the xylem sap concentration decreased during its movement from roots to leaves, probably because of adsorption on the xylem wall compounds (e.g., lignin) (*12*).

Moreover, when the concentration in the medium decreased, a desorption stream from the roots to the medium may be suggested, in the same way as it occurred from the roots to the xylem sap (**Figure 4**).

As a whole (**Figure 4**), fipronil transport in the xylem sap induces a final leaf accumulation involving a concentration increase inside membrane lipids, as is the case of photosystem II (PS II) classical inhibitors such as atrazine or substituted ureas (*19*, *21*−*23*).

Thus, this type of distribution agrees with a passive uptake transport scenario of compounds of medium lipophilicity (*13*, *14*). However, the contrasting values of log K_{OW} found under different conditions (*7*) require further studies: the value of 2.8 found in our laboratory seems to agree with that of atrazine (2.6), explaining the similar fairly good movement inside plants. The 4 log K_{OW} value mentioned in the patent for fipronil would explain the relatively low water solubility (2 mg L^{-1}). Under such an ambiguous situation, it might be supposed that the simple and normalized partition coefficient (K_{OW}) between *n*-octanol and water was not representative of the distribution rule between living components and water for the sulfurated phenylpyrazole series (*24*).

When the fipronil uptake was studied in the presence of soil (**Figures 2** and **6**), an equilibrium was obtained after 3 days between the amounts of fipronil adsorbed to the CHC and those dissolved in water. The ratio between the two values was close to 386 with the chosen soil containing 20% of organic carbon and agreed fairly well with the values of K_{OC} published elsewhere (*6*, *8*).

As a result, the water concentration in the soil remained unchanged until the end of the experiment. In good agreement with the preceding experiment, the average concentration in the xylem sap of the whole plant was 2 times lower than that in the soil water and a final accumulation of ^{14}C was obtained inside the leaves. This accumulation was at its highest level in the first leaves (highest amount of transpired water) and decreased only slowly in the younger leaves (lower amount of transpired water).

The initial root accumulation observed in the first experiment was much weaker in this case, suggesting that it resulted from superficial adsorption (*25*, *26*), which was largely reduced by the presence of the CHC.

As a whole, fipronil uptake and transport in sunflower seedlings seem to be submitted to the same rules as the numerous PS II inhibitors used in preplant strategies.

However, the parent compound itself might have been metabolized during the 3−4 weeks of experiments.

The ^{14}C content of sunflower leaves at the end of the experiment showed the presence of a large amount of fipronil and metabolites, mainly the 4-CF_3-S and 4-CF_3-SO_2 derivatives, which were demonstrated to remain very active insecticides.

When comparing these results to the seed-coated sunflower plants in natura, one can suggest that the important step for plant uptake is fipronil solubilization in soil water, which is not required for insecticidal activity against *Agriotes* larvae because they acquire the insecticide through ingestion in a solid state (*5*).

As a consequence, it seems possible to look for changes in the composition of the formulating mixture for seed coating with fipronil perhaps using slow release strategies (*27*, *28*) (1) to maintain the efficiency against *Agriotes* larvae and (2) to limit uptake and transport into sunflower plants, which occur easily enough, as demonstrated here. Such a purpose might require us to maintain the release rate into soil water below the rate of fipronil metabolization in the soil volume surrounding the seed.

On a more general point of view, considering the French context characterized by a big controversy dealing with a toxic risk for beekeeping resulting of seeds coating, this study demonstrates that a xylem transfer of this insecticide from soil water to leaves is possible in sunflower plants. This point allows us to consider a second physiological step, the possible translocation from leaves to sinks, which is currently under study.

ABBREVIATIONS USED

4-CF_3-S derivative (sulfide−fipronil), 5-NH_2-4-CF_3S-3-CN-1-[2,6-dichloro-4-(trifluoromethyl)phenyl] pyrazole; 4-CF_3-SO_2 derivative (sulfone−fipronil), 5-NH_2-4-CF_3SO_2-3-CN-1-[2,6-dichloro-4-(trifluoromethyl)phenyl] pyrazole; 3-$CONH_2$ derivative (amide−fipronil), 5-NH_2-4-CF_3SO-3-$CONH_2$-1-[2,6-dichloro-4-(trifluoromethyl)phenyl] pyrazole; GABA, γ-amino butyric acid; CHC, clay−humus complex.

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