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ANALYSE ET MODELISATION DU TRANSPORT ET DE LA DISSIPATION DANS LES SOLS DES MOLECULES PHYTOSANITAIRES APPLIQUEES EN TRAITEMENT DE SEMENCES

CAS DU TRITICONAZOLE

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Analyse et Modélisation du Transport et de la Dissipation dans les Sols des Molécules Phytosanitaires Appliquées en Traitement de Semences

Cas du triticonazole

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INTRODUCTION GENERALE

Traitements de semences

Le traitement de semences est une technologie qui s'est considérablement développée récemment, avec l'introduction de nouvelles molécules phytosanitaires systémiques, notamment fongicides, qui permettent de combiner une désinfection de la semence avec une protection à long terme, jusqu'à des stades avancés de développement. Cette technique permet d'économiser la matière active en réduisant considérablement les doses employées grâce à une localisation extrême des dépôts sur la cible, ce qui en fait actuellement un des moyens de lutte parmi les plus économiques et les moins dommageables pour l'environnement.

La protection phytosanitaire à long terme à l'aide de molécules systémiques suppose le maintien dans la plante d'une concentration en matière active suffisamment élevée pour assurer un contrôle satisfaisant des pathogènes. L'activité biologique des molécules phytosanitaires étant directement reliée à la concentration en matière active disponible au niveau des différents organes cibles de la plante (semence, pied et feuilles), l'efficacité du traitement pour une protection à long terme dépendra des interactions entre la molécule phytosanitaire, sa formulation, le sol et la plante.

Les principaux processus à considérer sont alors:

- le relargage de la matière active à partir de la semence (lessivage, cinétiques de désorption);
- le comportement de la matière active dans le sol, transport, dégradation et biodisponibilité;
- la pénétration de la matière active dans la plante (absorption par la semence, les téguments et les racines);
- le transport et la redistribution dans la plante (chargement des parties aériennes).

Le processus de **relargage** à partir de la graine dépend des conditions hydriques du milieu. Il est essentiellement contrôlé par le type de traitement considéré, traitement de semences classique, pelliculage ou enrobages, ainsi que par les agents de formulation employés, favorisant ou non le lessivage de la matière active et sa dissolution dans la solution du sol.

Au niveau de la capacité d'**absorption de la plante** deux paramètres clés sont à considérer: la concentration dans la solution externe et la lipophilie des molécules organiques phytosanitaires. La pénétration dans la plante est directement liée à la biodisponibilité de la matière active, soit essentiellement la quantité présente dans la solution du sol ou susceptible d'y passer. La lipophilie est déterminante pour la pénétration et le transport dans la plante. En effet, le chargement dans les cellules de la plante, aussi bien au niveau de la semence que des téguments ou des racines est principalement un processus de transport passif par diffusion. L'absorption dépend alors de la capacité à franchir les barrières membranaires lipidiques de la plante, donc de la lipophilie de la molécule. Le transport dans la plante vers les parties aériennes est aussi lié à la lipophilie du composé actif, avec un optimum considéré pour un coefficient de partage octanol/eau de 60 (lipophilie moyenne). Par conséquent, l'activité biologique des molécules phytosanitaires systémiques est généralement supérieure pour des molécules lipophiles et de nombreuses molécules phytosanitaires ont un caractère hydrophobe marqué.

Le transport et la dissipation des molécules phytosanitaires dans les sols sont aussi liés à la lipophilie des molécules. Les molécules hydrophobes sont généralement moins mobiles et moins disponibles (présentes en quantités moindres dans la solution du sol). Ainsi, la migration dans le sol et/ou la biodisponibilité pouvant devenir limitantes pour l'efficacité de certaines matières actives, une bonne connaissance du comportement de la molécule dans le sol et de sa répartition spatiale en fonction du temps apparaît primordiale.

Dans la double optique d'évaluer les risques environnementaux liés à l'introduction de molécules organiques xénobiotiques dans les sols (contamination des sols et des nappes) et de connaître la fraction de matière active susceptible d'être absorbée par la plante, il devient nécessaire d'évaluer le comportement du point de vue de la mobilité dans les sols et de la localisation par rapport au développement du système absorbant de la plante, mais aussi de celui de la biodisponibilité des molécules systémiques dans le sol.

Transport et dissipation des molécules phytosanitaires dans les sols

Le comportement des molécules organiques dans les sols dépend des diverses interactions physico-chimiques, biologiques et physiques auxquelles elles sont soumises. La mobilité des molécules résulte ainsi des processus de transferts convectif-dispersif et diffusif, limités par les processus de rétention et de dégradation.

L'adsorption est définie strictement comme une accumulation de la molécule phytosanitaire à l'interface de deux phases du sol, en général les phases solide et liquide. En pratique, on emploie le terme de sorption considérant tout transfert de produit de la solution vers la phase solide du sol.

La dégradation proprement dite (modification de structure chimique) des molécules organiques xénobiotiques dans les sols est principalement attribuable à l'action des organismes vivants du sol (assimilation). Il faut ici dissocier la métabolisation (dégradation partielle) de la minéralisation (dégradation complète) des molécules phytosanitaires. La biodégradation est

généralement limitée à la phase liquide du sol et concerne essentiellement les molécules dissoutes.

Le transport des solutés résulte des transferts convectifs-dispersifs dans la solution du sol. La convection correspond à l'entraînement du soluté dans un fluide vecteur se déplaçant dans le milieu poreux. La dispersion hydrodynamique correspond à l'étalement du front de migration du soluté induit d'une part par les processus de diffusion moléculaire, d'autre part par la distribution hétérogène des vitesses du fluide. Les molécules entraînées par convection suivent alors une trajectoire plus ou moins erratique autour d'un mouvement de dérive moyen. La diffusion moléculaire résulte de mouvements thermiques spontanés dont l'intensité et la direction dépendent du gradient de concentration. L'hétérogénéité du champ des vitesses est due à l'existence d'un gradient de vitesse à l'échelle de chaque pore, aux variations de dimensions des pores et, enfin, à la fluctuation des lignes de courant par rapport à l'écoulement principal, fonction de la géométrie désordonnée du milieu.

Ces processus majeurs sont étroitement liés. Ainsi, les molécules phytosanitaires introduites dans le sol se déplaceront par entraînement vers le bas lors d'écoulements hydriques ou vers le haut lors de remontées capillaires, mais aussi plus lentement par diffusion moléculaire (halo de diffusion).

Influence des agents de formulation

Lors des traitements de semences, qu'il s'agisse des traitements classiques, des pelliculages ou des enrobages, la matière active est incorporée dans une formulation solide ou liquide comprenant divers adjuvants, tensioactifs, matières de charge, adhésifs, colorants, co-solvant... Or, si l'on connaît relativement bien maintenant le comportement global des matières actives dans le sol, l'influence des agents de formulation reste encore à étudier plus précisément. Les divers adjuvants introduits ont pour principal but de maintenir une quantité importante de matière active en solution aqueuse, afin d'obtenir une formulation stable et adaptée à la technique de traitement de semences. Il est cependant concevable d'introduire dans

les formulations des adjuvants spécifiquement destinés à améliorer le comportement des matières actives dans le sol.

Triticonazole

Le triticonazole est un nouveau fongicide actif par contact et systémie, découvert par Rhône-Poulenc Agro en 1988 (voir formule p. 48). Comme tous les triazoles, c'est un inhibiteur de la déméthylation en C_{14} dans la séquence de biosynthèse des stérols des champignons actif contre de nombreux champignons phytopathogènes. N'étant pas phytotoxique vis-à-vis des céréales, le triticonazole peut être appliqué en traitement de semences à des doses importantes. Il permet de protéger efficacement contre les maladies transmises par la semence et le sol (fusariose, carie), ainsi que contre les principales maladies du pied et des feuilles (piétin verse, oïdium, rouille) habituellement traitées par traitement foliaire. Le triticonazole est utilisé principalement en traitement de semences du blé et de l'orge, mais aussi pour la protection du maïs. Il peut également être employé en traitement de sol ou encore foliaire. Appliqué en traitement de semences, il est lentement absorbé par la semence, les téguments et les racines, puis redistribué dans les parties aériennes de la plante par voie apoplastique.

La formulation commerciale préconisée pour le traitement des semences de céréales, REAL, se présente sous la forme d'une suspension aqueuse concentrée comprenant, outre le triticonazole à 200 g L^{-1}, de nombreux agents de formulation, tensioactifs mouillants et dispersants, polymère structurant, agents collants et agents de charge, ainsi qu'un répulsif corvifuge, l'anthraquinone, à 84 g L^{-1}. Le traitement de semences classique consiste à appliquer directement sur les semences la formulation qui est ensuite séchée. On obtient ainsi le dépôt d'un film de formulation sur les semences.

Objectifs des travaux de recherche

L'objectif à long terme consiste à être en mesure de prédire l'efficacité d'un traitement phytosanitaire systémique appliqué au sol en fonction des caractéristiques physico-chimiques de la matière active et du choix des adjuvants de formulation. Ceci devrait permettre d'optimiser la dose de matière active appliquée de manière à réduire les coûts de traitement et limiter les risques environnementaux.

Dans cette optique, les deux objectifs principaux sont:

1/ de comprendre et de modéliser le comportement du triticonazole dans le sol, en élucidant les processus clés et les principaux facteurs de variation concernant son transport et sa biodisponibilité;

2/ d'évaluer l'influence de la formulation sur ces processus et les possibilités d'améliorer l'efficacité des traitements en modifiant la formulation.

Articulation de la thèse

Nous avons, dans un premier temps, dissocié les processus physico-chimiques biologiques et physiques impliqués, afin d'étudier spécifiquement les mécanismes mis en jeu, les principaux paramètres de variation, l'influence de la formulation, et de tenter de les formuler mathématiquement. Nous avons ensuite couplé les différents processus afin d'évaluer leur importance relative et de tenter de décrire le comportement global à l'aide de modèles simples. Toutes les études ont été réalisées sur un seul et même sol choisi comme étant caractéristique des sols cultivés en céréales dans la région parisienne. Ce sol provient d'une parcelle utilisée en monoculture de blé depuis de nombreuses années.

Le memoire de thèse est constitué de cinq chapitres présentés sous la forme d'articles publiés ou soumis à des revues à comité de lecture international.

Les chapitres 1 et 2 traitent de l'influence des agents de formulation sur l'adsorption du triticonazole. Ces études s'appuient sur des mesures d'équilibres d'adsorption permettant une analyse comparative de la quantité adsorbée et des mécanismes mis en jeu. Le chapitre 1 aborde de manière spécifique les mécanismes d'interaction sol (sorbant)/tensioactif/molécule organique. Le chapitre 2 concerne l'influence des adjuvants des formulations commerciales de traitement de semences sur la solubilisation et la sorption du triticonazole.

L'aspect cinétique à long terme de l'adsorption est abordé dans les chapitres 3 et 4, concernant des études menées lors d'incubations sur des temps prolongés. Le chapitre 3 traite de la dissipation de la molécule dans le sol et en particulier de la biodégradation microbienne de la molécule. L'influence des agents de formulation sur les processus biologiques y est aussi étudiée. Le chapitre 4 considère le couplage des processus de sorption avec le processus de transfert diffusif.

Le chapitre 5 concerne le transport convectif-dispersif du composé et sa description à l'aide d'un modèle couplant les différents processus impliqués. L'aspect cinétique rapide de l'adsorption y est abordé. Il permet de faire un bilan et de conclure sur l'importance relative des processus impliqués et la validité des paramètres mesurés pour leur caractérisation.

CHAPITRE 1

Mécanismes d'interactions physico-chimiques dans les systèmes sol-tensioactif-triticonazole

Sorption of Low Levels of Nonionic and Anionic Surfactants on Soil: Effects on Sorption of Triticonazole Fungicide

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Sorption of Low Levels of Nonionic and Anionic Surfactants on Soil: Effects on Sorption of Triticonazole Fungicide

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ABSTRACT

Surfactants are widely used in the commercial formulations of pesticides. Their influence on the fate of systemic pesticides in soil needs to be known for a successfull utilization. We evaluated the sorption of 2 anionic surfactants and a series of 7 nonionic alkylphenolethoxylate surfactants of increasing hydrophilic/lipophilic balance (HLB) in a loamy clay soil. We investigated the effect of low doses of these surfactants on the sorption characteristics of triticonazole fungicide [(1RS)-(E)-5-(4-chlorophenyl-methylen)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol]. The critical micellar concentration (CMC) of the surfactants in pure water and soil-water systems, and surfactant sorption were estimated by surface tension measurements using a classical batch equilibration technique. Triticonazole sorption, alone and in the presence of low doses of surfactants, was also measured by batch equilibration. CMC of the alkylphenol surfactants increased with their HLB. Surfactant sorption onto soil was positively related to their hydrophobicity, and much higher doses were necessary to attain CMC in the soil-water systems. Sorption of considerable amounts of the most lipophilic alkylphenol surfactants significantly increased triticonazole sorption. Proposed mechanisms are modifications of soil surface properties, and increase of soil organic carbon (OC) content. Sorption of the other nonionic and anionic surfactants only resulted in sub-CMC, monomeric concentrations in pore water, which did not alter triticonazole sorption.

Key words: sorption, surfactant, triticonazole, soil organic-matter, hydrophilic-lipophilic balance

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Sorption of Low Levels of Nonionic and Anionic Surfactants on Soil: Effects on Sorption of Triticonazole Fungicide

1 INTRODUCTION

Triticonazole is a new triazole systemic fungicide developed by Rhône-Poulenc Agro and used for cereal seed treatment. The efficacy of systemic pesticides applied in seed treatments depends closely on their dissipation and localization in soil in relation to the uptake by the plant root system. The transport and fate of these molecules in soils is therefore of crucial interest for an optimal utilization.

Sorption of pesticides to the soil is a key process governing their behavior in soils. It is particularly important for poorly water soluble compounds that are strongly retained to soil. Sorption of pesticides significantly reduces their mobility in soils,[1,2] and their bioavailability to plants,[3,4] and microorganisms.[5,6] Soil sorption isotherms of hydrophobic pesticides at diluted concentrations are usually linear,[7] and soil/solution partition coefficients (K_d) may be used to characterize pesticide sorption on a particular soil. Strong correlation of K_d with soil organic carbon (OC) content has been frequently reported,[8,9] which allows for normalization in K_{OC}. Proposed mecanisms are nonspecific interactions with soil organic constituents like London Van der Waals hydrophobic interactions, or partitioning onto soil organic matter (OM).[10] However, sorption of organic chemicals may be modified by the presence of cosolutes. Surfactants are commonly used in the commercial formulation of pesticides, as humectants, dispersants or spreaders. Surfactants are highly active compounds as they have the unique properties of reducing the interfacial tension and form aggregates or micelles above a certain concentration, the critical micellar concentration (CMC). It is thus essential to determine how the sorption of the pesticide is altered by the presence of surfactant adjuvants when both coexist in soil. Numerous studies have dealt with the influence of surfactants on the solubilization and sorption of poorly soluble contaminants in soil-water systems, and mobilization of strongly sorbed chemicals is often reported.[11-13] However, in most cases, high amounts of surfactant were used, which may be applied for decontamination of polluted soils,

but would not be representative of the amounts applied in pesticide formulations. Indeed, only limited amounts of formulation additives are applied with the active ingredient for treatment, which will further dissolve and be diluted in the soil solution. The impact of low, diluted doses of surfactants needs thus to be considered more thoroughly.

Solubilization of hydrophobic compounds is essentially a micellar process, usually attributed to partitioning of the pesticide in the surfactant micellar pseudophase.[14-16] However, it can also occur at surfactant concentrations below the CMC.[17] In soil/aqueous systems, surfactant-pesticides interactions are very complex, and surfactant sorption as well as pesticide sorption need to be considered. Sorption of the surfactant on soil surfaces may result in much higher total surfactant doses being necessary to attain the CMC in soil solution. Sorbed surfactant molecules may also increase pesticide sorption onto soil by dispersing the soil colloids, modifying the soil surface properties, and increasing the soil fractional OM content.[18-20]

The influence of the chemical nature of the surfactant is particularly relevant.[21,22] Nonionic surfactants are usually more efficient at solubilizing hydrophobic compounds than cationic and anionic compounds because they tend to form micelles at lower concentrations.[23] On the other hand, cationic and nonionic surfactants would sorb onto soil to a greater extent than anionic surfactants.[13] Alkylphenol ethoxylate nonionic surfactants (noted C_xPE_y, where x is the number of carbon of the alkyl chain, and y is the number of ethoxylate units) have been extensively studied, and were reported to solubilize high amounts of pesticides,[19] and to significantly reduce their sorption.[24-26] The hydrophobicity of these surfactants vary with their alkylphenol hydrophobic moieties, and with the number of ethoxylate hydrophilic moieties. It is characterized by the hydrophilic-lipophilic balance (HLB), which is basically calculated from the percentage of hydrophilic ethoxylate units reported to the total mass of the surfactant. The number of ethoxylate units and the HLB of these surfactants have been related to their sorption onto soil,[27] and to their ability at solubilizing hydrophobic compounds.[12,22,28]

This study was aimed at (1) evaluating the influence of the HLB of selected nonionic alkylphenol surfactants on their micellisation in water and soil-water systems, and on their sorption onto soil, and (2) evaluating the impact of low doses of these surfactants and of 2

anionic surfactants used in the commercial formulation of triticonazole fungicide on its sorption onto soil.

2 MATERIALS AND METHODS

2.1 Chemical

^{14}C-U-benzyl-labeled triticonazole (specific activity: 1184 MBq mmol^{-1}; radiopurity > 98 %) was provided by Rhône-Poulenc Agrochemicals Company (Lyon, France). Triticonazole water solubility is 8.4 mg L^{-1} at 20°C, vapour pressure is < 10^{-8} hPa at 50°C and distribution coefficient between octanol and water is 1950. Water solutions of ^{14}C-triticonazole were prepared at 5 different concentrations ranging from 0.5 to 5 mg L^{-1} by diluting a saturated water solution of triticonazole (analytical standard, purity > 92 %), adding some µL of the ^{14}C-triticonazole methanol stock solution, and adjusting the concentration with MilliQ water (Millipore, Saint-Quentin, France). Triticonazole concentrations were measured at 262.5 nm with a U.V.-visible spectrophotometer Lambda V (Perkin-Elmer, Überlingen, Germany). The radioactivity was measured by liquid scintillation counting (LSC) using a Kontron Betamatic V counter (Kontron Ins, Montigny le Bretonneux, France).

2.2 Surfactants

Two anionic surfactants present in the commercial formulation of triticonazole for wheat seed treatment (REAL aqueous suspension concentrate), Soprophor FLK and Supragil MNS90, and 7 alkylphenolethoxylates nonionic micellar surfactants were studied. Soprophor FLK is a phosphate tristyrylphonolethoxylate anionic micellar surfactant, and Supragil MNS90 is a sodiumalkylnaphtalensulfate anionic nonmicellar surfactant. Triton X100 is an octylphenolethoxylate that was chosen for its good solubilization properties and its reported ability to reduce sorption of poorly soluble organic compounds. Triton N101 (BC9) and the Igepal BC5, BC8, BC14, BC17, and BC40 surfactants are nonylphenolethoxylates of varying number of ethoxylate functional units (indicated by the BC number). They were chosen in order to test the influence of increasing the polarity of the surfactant, and hence its HLB, by

increasing the length of the ethoxylate polar chain. Triton surfactants were purchased from Sigma-Aldrich Chimie (St. Quentin, France), all other surfactants were furnished by Rhône-Poulenc Agro. All surfactants were used technical grade. The surfactant solutions were prepared by solubilizing the surfactants in MilliQ water under ultrasound.

2.3 Soil

The soil (typic Eutrochrept) was sampled in the surface layer (0-20 cm) of a continuous wheat experimental plot located at Grignon (France). It had a pH in water of 8.2, with (g kg^{-1} of dry soil): 291 of clay, 540 of silt, 145 of sand, 24 of lime and 10.4 of organic C. Soil samples were air dried and passed through a 2-mm sieve. Soil residual gravimetric water content was of 3.6%.

2.4 Triticonazole sorption isotherms

A classical batch equilibrium procedure was used to obtain triticonazole sorption isotherms at 22 ± 1°C. Ten milliliters of ^{14}C-triticonazole water solutions were added to 5 g of soil in 20-ml glass centrifuge tubes with Teflon caps. After 24 h shaking, the samples were centrifuged at 8000 g for 15 min and triticonazole concentration in solution at equilibrium, C_e was calculated from LSC measurement of the radioactivity in the supernatant. The amount of triticonazole sorbed was determined by difference between the initial and equilibrium concentrations in solution. The whole experiment was replicated twice. The same procedure was used to measure triticonazole sorption isotherms in the presence of 80 mg L^{-1} Soprophor FLK or Supragil MNS90 surfactants.

The sorption isotherms were plotted as the amount of pesticide sorbed (x/m, mg per kg of dry soil) versus the equilibrium concentration C_e (mg L^{-1}). Freundlich empirical parameters K_f and n_f were estimated using a least-square nonlinear regression method (S-Plus version 3.2, StatSci, Seattle, WA, USA), from the Freundlich equation:

$$x / m = K_f C_e^{n_f} \qquad [1]$$

The linear sorption partition coefficient K_d was calculated by linear curve fitting from the equation:

$$x/m = K_d C_e \quad [2]$$

and the corresponding K_{OC} was calculated from the relationship:

$$K_{OC} = K_d \text{ (fraction of soil organic C)}^{-1} \quad [3]$$

2.5 Determination of surfactant CMC

The initiation of micelle formation in solution is indicated by the minimal surfactant concentration at which the surface tension ceases to decline. Measurement of the CMC in water was performed using a Krüss Processor Tensiometer Model K12 (Krüss Gmbh, Hamburg, Germany) with a platinum plate (wetting length 40.0 mm, length 19.9 mm, thickness 0.10 mm). Surface tension was first measured in water, and then concentration of the surfactant in solution was increased logarithmically by adding appropriate amounts of a concentrated surfactant stock solution. Measurements were made untill the increase in surfactant concentration did not cause further decrease in the surface tension. Surfactant stock solution concentrations were 200 mg L^{-1} for BC5, 500 mg L^{-1} for BC8, 1000 mg L^{-1} for X100, N101 and FLK, 2000 mg L^{-1} for BC14 and BC17, and 10000 mg L^{-1} for BC40. Plots of the surface tension as a function of the logarithm of surfactant concentration allowed for the graphical determination of the C.M.C. of the surfactants.

2.6 Surfactant sorption on soil

A classical batch method was used to evaluate the sorption of the surfactants onto soil. 30 g of soil and 60 mL of surfactant solutions with concentrations ranging from 0.1 to 50000 mg L^{-1} were mixed in 250 mL centrifuge tubes, and shaken for 24 h at 22 ± 1°C and then centrifuged at 8000 g for 15 min. 50 mL of the supernatant were pipetted, and surface tension was measured with the Krüss K12 tensiometer. The minimal surfactant concentration required to initiate micelle formation in the soil-water system was graphically determined as previously described.

The amount of surfactant sorbed on soil, x/m, can be assessed by surface tension measurements for surfactant concentrations less than that required to attain the CMC in the soil water-system. The concentration in solution at equilibrium (C_e) for a given initial

concentration (C_i) can be determined graphically from the curves of surface tension versus concentration in the soil-water systems by reporting the surface tension measured at C_i on the soil-aqueous curve, and measuring the corresponding concentration C_e remaining after adsorption. As described for triticonazole sorption, the amount of surfactant sorbed was determined by difference between the initial and equilibrium concentrations in solution, and the surfactant K_d values were estimated by linear fitting from the sorption isotherms.

2.7 Triticonazole sorption in presence of surfactants

Triticonazole sorption studies in the presence of surfactant additives were performed using the same batch equilibrium procedure as previously described, with a single initial concentration of triticonazole of 5 mg L^{-1}. The initial concentrations of the surfactants were chosen in function of their CMC in water, in order to obtain sub- and supra-CMC concentrations while remaining at doses representative of formulation field application rates. As an exception, the concentrations of the two most hydrophobic nonylphenolethoxylates were chosen an order of magnitude higher. Soprophor FLK and Supragil MNS90 concentrations ranged from 0.1 to 200 mg L^{-1}. Igepal BC5 and BC8 concentrations were 1000 and 10000 mg L^{-1}, while Igepal BC14, BC17 and BC40 concentrations were 100 and 1000 mg L^{-1}. Finally, Triton X100 and N101 concentrations ranged from 2.5 to 2500 mg L^{-1}. The results were expressed as the percentage of sorbed triticonazole in the presence of the surfactants relative to triticonazole sorption without surfactant.

The change in soil OC content resulting from the surfactant sorption on soil was calculated from the estimates of the amount of sorbed surfactant, converted in amount of carbon sorbed. The new value of soil fractionnal OC content was then used to calculate the corresponding K_{OC} for triticonazole sorption in the presence of surfactant.

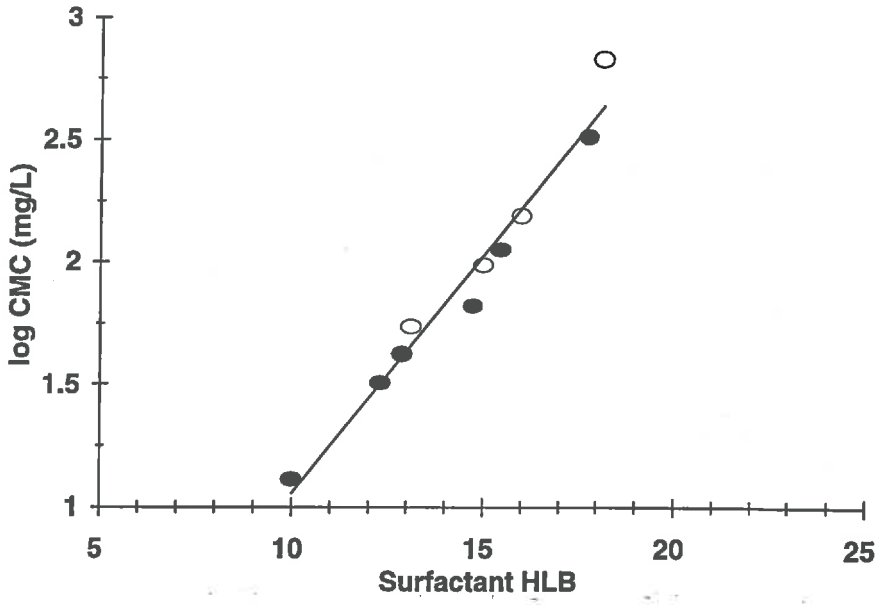

Fig. 1. Relation between measured water CMC (plain symbols) and water CMC reported by Schick (1987) (empty symbols), in log, and HLB of the nonylphenolethoxylate surfactants. Correlation as log CMC = 0.019 HLB -0.09 is represented in plain line.

3 RESULTS AND DISCUSSION

3.1 Surfactant aqueous CMC

The aqueous CMC of the selected micellar surfactants in soil-free systems determined from surface tension measurements, along with molecular weights and HLB of the surfactants, are listed in Table 1. Triton X100 CMC of 151 mg L^{-1} (242 µmol L^{-1}) is in the range of reported values for this surfactant ranging from 170 to 310 µmol L^{-1}.[19,23-25] CMC of the nonylphenolethoxylates ranged from 13 to 324 mg L^{-1} (30 to 164 µmol L^{-1}), and were inversely related to the hydrophobicity of the surfactants. It increased with the number of polar ethoxylates groups, hence with increasing HLB. This was not surprising since surfactants with lower HLB are in general more efficient at micelle formation.[13] Plot of the nonylphenolethoxylate surfactants CMC versus surfactant HLB (Fig. 1), including our results along with reported values for BC9.5, BC15, BC20, and BC50 nonylphenolethoxylates,[29] show that our results are consistent with other published results concerning the same surfactant family, with a strong, positive correlation between the of CMC and HLB of the surfactants (log CMC = 0.19 HLB - 0.9). Finally, Soprophor FLK CMC was measured at 48 mg L^{-1}.

Surfactant	*Triton X100*	*Igepal BC5*	*Igepal BC8*	*Triton N101*	*Igepal BC14*	*Igepal BC17*	*Igepal BC40*	*Soprophor FLK*
Symbol	$C_8PE_{9.5}$	C_9PE_5	C_9PE_8	C_9PE_9	C_9PE_{14}	C_9PE_{17}	C_9PE_{40}	
PM (g mol^{-1})	624	440	572	638	836	968	1980	1350
HLB		10	12.3	12.8	14.9	15.8	17.8	16
CMC (mg L^{-1})	151	13	32	42	66	112	324	48
CMC (µmol L^{-1})	242	30	56	68	79	116	164	36

Table 1. Water CMC of Triton X100 octylphenolethoxylate, Triton N101 (BC9), Igepal BC5, BC8, BC17 and BC40 nonylphenolethoxylates, and Soprophor FLK anionic surfactant. CMC values are graphically determined from the surface tension / log concentration curves.

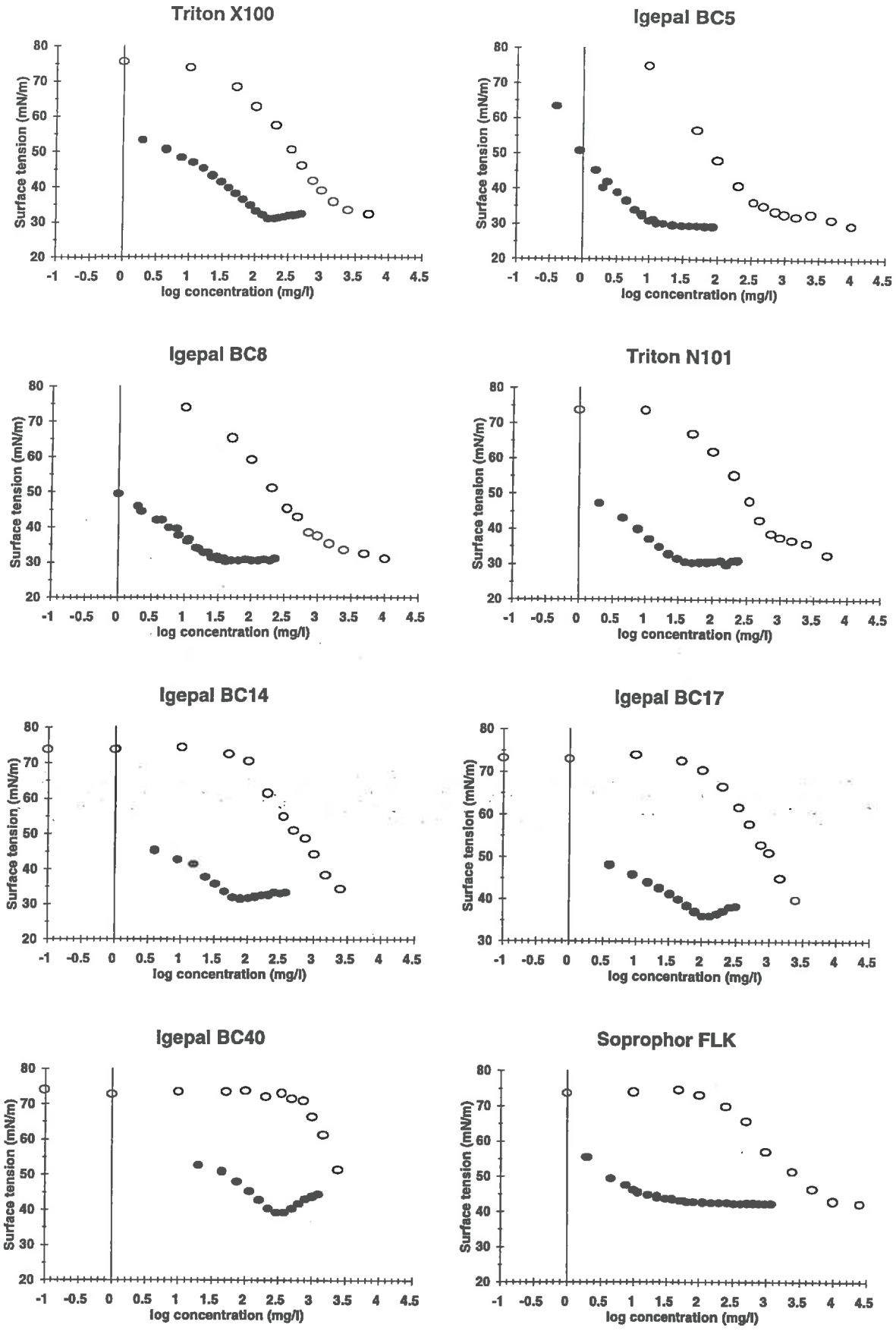

Fig. 2. Surface tension measurements for increasing concentrations of the nonionic and anionic micellar surfactants in water (plain symbols) and soil-water systems (empty symbols).

3.2 Surfactant sorption

The breaks in the surface-tension curves of the soil-water systems were not as marked as in the soil-free systems, with more transitionnal changes in the surface tension of the aqueous phase (Fig. 2), and consequently, measurements of the apparent CMC of the surfactants in soil-water systems were less accurate. This may be attributed to the presence of soluble soil components affecting the surface tension of these systems.[26] However, the right shift of the surface tension curves of the surfactants in the soil-water systems compared to the soil-free systems indicates high sorption on soil of all the surfactants, and show that the concentrations necessary to initiate micelle formation would be considerably higher in the soil-water systems. Similar strong sorption of Triton X100, Igepal CA-720 (C_8PE_{12}), Tergipol NP-10 ($C_9PE_{10.5}$), and other alkylphenolethoxylates and alkylethoxylates on soil has been reported.[24,30] Estimated range of values for the apparent CMC in soil together with estimated averaged K_d of the surfactants are listed in Table 2. The highest CMC and K_d of the nonionic surfactants were found for the most hydrophobic nonylphenolethoxylates, i.e. Igepal BC5 and BC8, and Triton N101 (BC9), suggesting that sorption of the anionic surfactants would probably be related to hydrophobic interactions of the surfactant hydrophobic moiety (alkyl chain) with soil OM. Other results of nonylphenolethoxylate C_9PE_{10} sorption on three soils of varying OM content also indicate that sorption of the alkylphenolethoxylates is associated with organic-matter.[31]

Surfactant	*Triton X100*	*Igepal BC5*	*Igepal BC8*	*Triton N101*	*Igepal BC14*	*Igepal BC17*	*Igepal BC40*	*Soprophor FLK*
CMC ($mg\ L^{-1}$)	2500-3500	9000	7000-11000	12500	3200-4000	3500-4000	> 5000	10000
K_d ($L\ kg^{-1}$)	11±1	88±10	199±23	213±37	86±5	37±6	17±3	216±20

Table 2. Estimated range for the CMC in soil-water systems, and averaged sorption K_d of Triton X100 octylphenolethoxylate, Triton N101 (BC9), and Igepal BC5, BC8, BC17 and BC40 nonylphenolethoxylates, and Soprophor FLK anionic surfactant. C.M.C. values are graphically determined from the surface tension / log concentration curves.

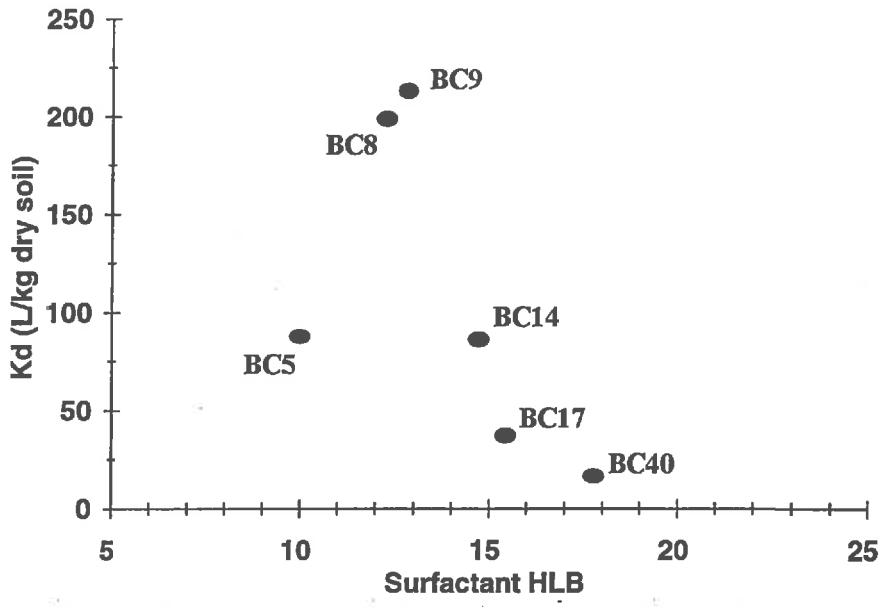

Fig. 3. Relation between estimated average K_d for sorption of the nonylphenol surfactants on Grignon soil and HLB of the surfactants.

Plot of the nonylphenolethoxylates K_d versus HLB of the surfactants (Fig. 3) shows an increase in K_d with surfactant HLB untill a maximum sorption value is attained with Triton N101 (HLB = 12.9), followed by a continuous decrease of the K_d for higher HLB and increasing number of ethoxylate units. Thus, for this series of nonylphenolethoxylate surfactants, a maximum of affinity for interactions with the soil organic surfaces would be attained around a HLB of 13. The decrease in sorption that is observed when the HLB is further increased shows that the affinity of the surfactant for the soil surfaces decreases when the hydrophilic chain of the surfactant increases, which further supports the hypothesis of a sorption of the nonionic surfactants governed by hydrophobic interactions with soil OM. Under this condition, the relatively low K_d estimated for sorption on soil of Igepal BC5 and BC8 most hydrophobic surfactants is not expected. The aggregation number (N_a) of the surfactants increases when HLB decreases.[28] The N_a reflects the affinity between monomers of the surfactant, and thus the ability for self-association and formation of micelles. A high N_a for Igepal BC5, and at a lesser extent for Igepal BC8 may result in preferential surfactant-surfactant interactions rather than surfactant-soil surface interactions, and formation of micelles under the CMC.[15] Similar trends in the influence of the HLB of a series alkylphenolethoxylate were reported for the solubilization of Non Aqueous Phase Liquids Contaminents (NAPL).[22,28] It is thus suggested that surfactant self-affinity, as indicated by the N_a, and number of ethoxylate units are major parameters when hydrophobic interactions are considered, with soil surfaces (sorption) or with hydrophobic compounds (solubilization). In such systems, a maximum of affinity at an optimal HLB value may be expected for a series of alkylphenolethoxylate surfactants.

Surprisingly, Soprophor FLK anionic surfactant sorption was also extremely important, and its apparent CMC in the soil-water system would be of approximately 10000 mg L^{-1}, more than 200 fold its water CMC. Anionic surfactant sorption on the negatively charged soil surface is usually expected to be minimal. A study concerning the anionic surfactant sodium dodecyl sulfate (SDS) showed a less than three fold increase of the CMC of the surfactant in the presence of soil.[32] Other authors reported very weak sorption of linear alkylbenzene sulfonate (LAS) on four soils at surfactant concentrations below 100 mg L^{-1}, and they

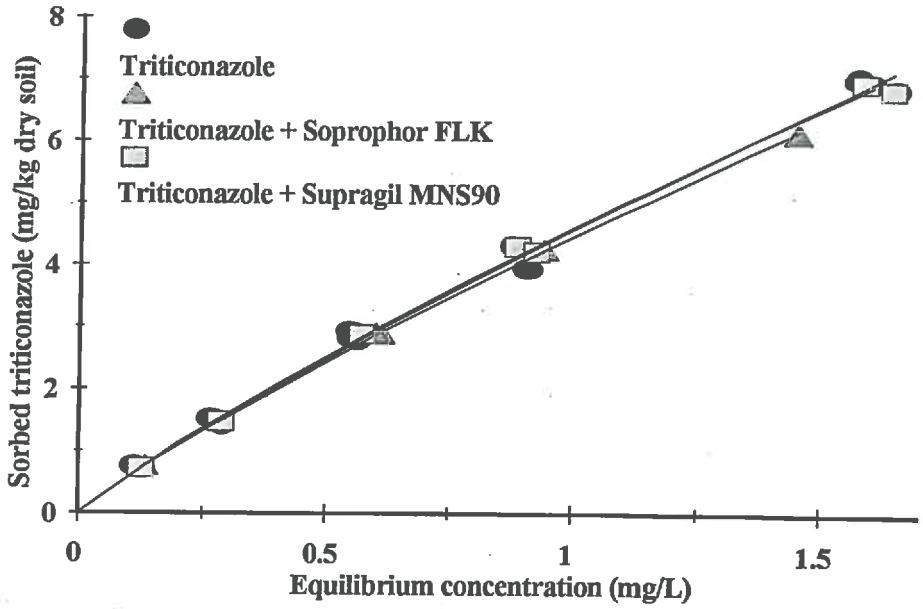

Fig. 4. Equilibrium sorption isotherms of ^{14}C-triticonazole as influenced by the presence of Soprophor FLK and Supragil MNS90 anionic surfactants at 80 mg L^{-1}.

concluded to specific site surface interactions by the sulfonate hydrophilic head on clay minerals.[33] However, these authors also reported exponentially increasing sorption at high LAS concentrations, which they attributed to sorption of hemimicelles (admicellization) onto soil through hydrophobic interactions. The same kind of process may explain Soprophor FLK strong sorption at high concentrations.

3.3 Surfactant influence on triticonazole sorption

Triticonazole sorption isotherms on Grignon soil, alone and in the presence of surfactants, are presented in Fig.4. The sorption isotherm of triticonazole is characterized by a high K_f of 4.61 ± 0.20 indicating strong sorption and a n_f of 0.87 ± 0.02, indicating dominating non specific interaction mechanisms, which would be most likely hydrophobic bonding or solute partitioning on soil organic matter. Consequently linearization of the isotherm and use of the linear partition coefficient K_d to characterize the equilibrium sorption of triticonazole is justified. An averaged K_d value of 4.35 ± 0.08 L kg^{-1} was calculated, which corresponds to a K_{OC} of 418 L kg^{-1}. Similar K_{OC} values were obtained for the sorption of triticonazole on different English soils (Rhône-Poulenc Agro, personal communication).

Triticonazole sorption was not significantly modified by the presence of the anionic surfactants used in REAL formulation, as evidenced in Fig. 4 and 5. Triticonazole sorption isotherms in systems containing 80 mg L^{-1} of these surfactants were identical to the isotherm in surfactant-free system. K_f and n_f Freundlich values of respectively 4.48 ± 0.11 and 0.88 ± 0.01 for Soprophor FLK addition, and 4.60 ± 0.13 and 0.89 ± 0.01 for Supragil MNS90 were extremely close to those for triticonazole alone. Modification of HOC sorption by surfactants usually occurs at supra-CMC concentrations, when a surfactant micellar pseudophase is considered. Hence, our results are not surprising for Supragil MNS90 surfactant since it does not form micelles. In the case of Soprophor FLK, the concentrations added to the system ranged from sub-CMC concentrations of 0.1, 1, 5 and 20 mg L^{-1} to supra-CMC concentrations of 80 and 200 mg L^{-1}. However, strong sorption of the surfactant on soil, as previously evidenced, increased significantly the concentration required to achieve micellisation in the soil solution. Thus, all the initial concentration of Soprophor FLK tested resulted in sub-CMC

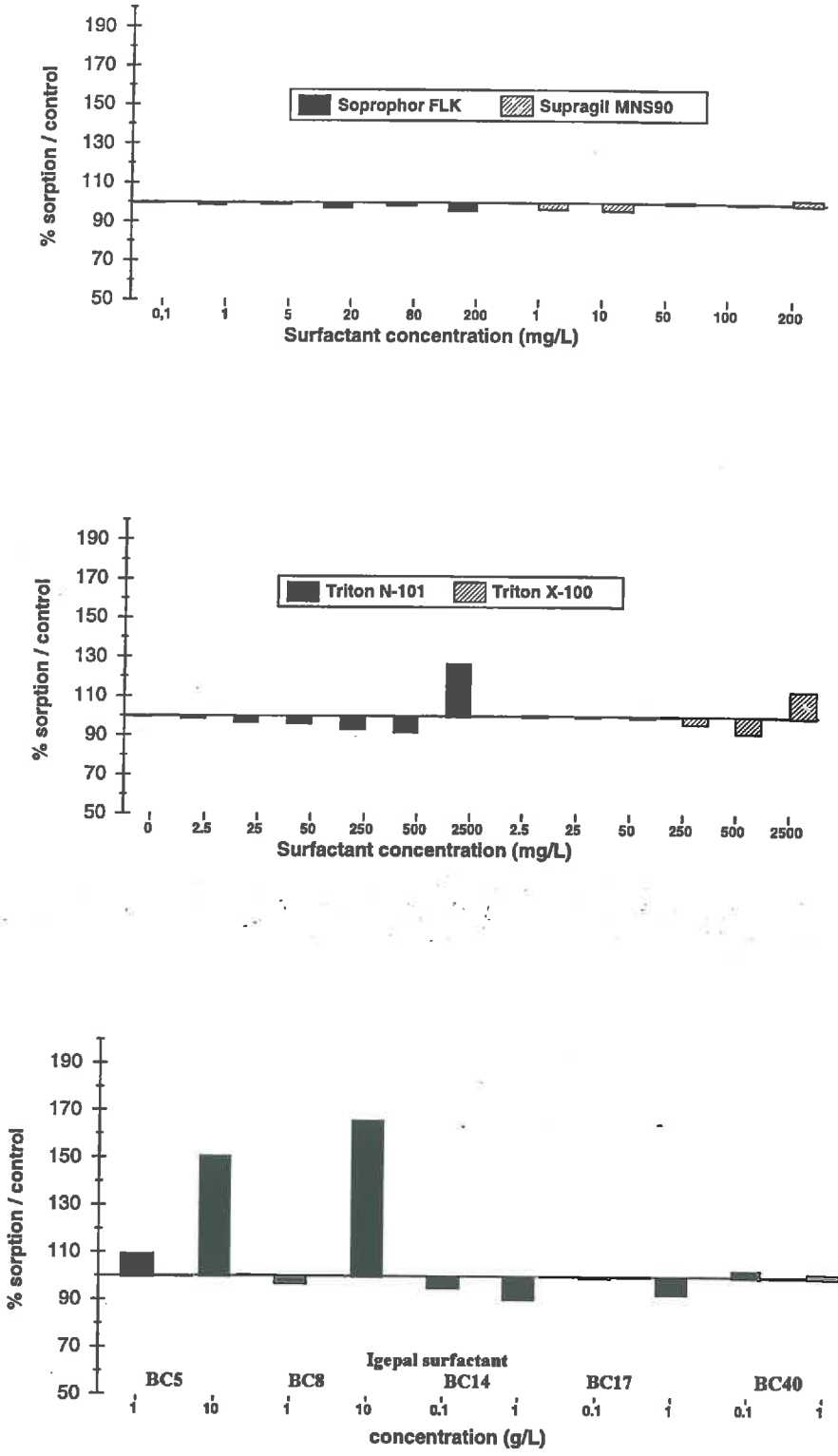

Fig. 5. Influence of different concentrations of Soprophor FLK and Supragil MNS90 anionic surfactants, and 7 alkylphenolethoxylates on the sorption of ^{14}C-triticonazole.

concentrations in the soil-water systems. Much higher amounts would be necessary to attain supra-CMC concentrations, but this would prove unrealistic in the pesticide formulations.

Similarily, strong sorption of the other surfactants on soil removed a major part of the surfactant molecules from the soil solution. Concentrations well below the C.M.C. would be expected in the soil solution for all combinations except for Triton X100, Triton N101, Soprophor BC5 and BC8 highest initial concentrations, where near CMC concentrations in soil solution would be attained. Triticonazole sorption on soil was little decreased or not affected in presence of low concentrations of the surfactants, whereas the presence of greater amounts of the most hydrophobic surfactants resulted in a near 50% increase in the sorption of triticonazole (Fig. 5). The most hydrophobic nonylphenol surfactants are expected to solubilize triticonazole in water systems. Indeed, enhanced solubility of the poorly soluble dichlobutrazol triazole fungicide in solutions of BC8 has been reported,[34] and thus there would be a potential for reduction of the sorption of other hydrophobic triazole compounds in soil. However, this author used high surfactant concentrations of 100 and 200 g L^{-1}, which are not representative of the agricultural application rates. Dilute nonionic surfactants may show no significant effect on pesticide sorption,[11] when most of the surfactant would be presumably sorbed, while not significantly increasing soil OM content. Other authors reported an increase in the sorption of moderatly water soluble and of hydrophobic compounds in soil-water systems at sub or near CMC concentrations of Triton X100.[26] This was attributed to an increase in the soil OM content due to surfactant sorption, which favored the partitioning of the hydrophobic molecules.

Sorption of the alkylethoxylate surfactants on soil is likely to modify the properties of the soil surfaces. At low concentrations, the nonionic surfactants would directly sorb on the soil surfaces through partitionning or hydrophobic interactions of the surfactant alkyl chain with soil organic matter. Under these conditions, the hydrophilic heads of the surfactants would extend in solution and thus lower the affinity of triticonazole for the soil surfaces. This may explain the slight decrease in triticonazole sorption to soil that was observed in the systems with low concentrations of the surfactants. However, at higher concentrations, the soil surfaces may be completely covered by a monolayer of surfactant, and the additionnal surfactant

monomers may sorb on the monolayer of hydrophilic heads. This would result in a bilayer of surfactant on the soil surfaces with the surfactant hydrophobic tails sticking out in aqueous phase, which would considerably enhance the sorption/partition of triticonazole on soil surfaces.

Estimates of the modifications of soil fractionnal OC content after addition of the more lipophilic surfactants (Triton X100, Triton N101, Igepal BC5 and BC8), and K_d and K_{OC} partition coefficients for triticonazole sorption in these soil/surfactant systems, are listed in Table 3. Addition of Igepal BC5 and BC8 surfactants at 10 g L^{-1} more than doubled the soil O.C. content from an initial 1.04 % to an approximate 2.3 %. Addition of these same surfactants at 1 g L^{-1}, and of Triton X100 and N101 surfactants at 2.5 g L^{-1} resulted in a more moderate increase, with OC content reaching approximately 1.15 % for the first two compounds, and 1.3 % for the two others. Addition of Triton X100 and N101 at 0.25 g L^{-1} did not alter significantly the soil OC content. These results are in accord with the observed increases in triticonazole sorption when applied with these surfactants, since triticonazole sorption was related to soil OM content, and would increase with soil fractionnal OC content. Compared to the K_d values, the calculated K_{OC} values for triticonazole sorption appeared much closer for all triticonazole/surfactant/soil combination systems. This confirms the hypothesis of an increase in triticonazole sorption on soil surfaces mediated by the sorption of the hydrophobic surfactants.

Surfactant	*No (blank)*	*Triton X100*		*Triton N101*		*Igepal BC5*		*Igepal BC8*	
Surfactant concentration (g L^{-1})		0.25	2.5	0.25	2.5	1	10	1	10
% OC	1.04	1.07	1.30	1.07	1.34	1.17	2.33	1.16	2.28
Triticonazole K_d (L kg^{-1})	4.35	4.17	5.15	4.02	6.24	5.05	11.77	4.19	19.74
Triticonazole K_{OC} (L kg^{-1})	418	389	396	376	466	432	505	361	866

Table 3. Triticonazole sorption K_d and K_{OC} in Grignon soil as influenced by the presence of different concentrations of Triton X100 octylphenolethoxylate, and Triton N101 (BC9), Igepal BC5 and BC8 nonylphenolethoxylates.

4 CONCLUSION

Addition of diluted sub- and supra-CMC concentrations of nonionic alkylphenolethoxylate surfactants and anionic formulation surfactant Soprophor FLK had little effect on triticonazole sorption. This was attributed to strong sorption of all the surfactants onto soil, which resulted in concentrations in soil solution well below the CMC. Sorption and CMC of the nonionic surfactants were strongly related to the length of their ethoxylate hydrophilic chain. The CMC was inversely related to the surfactant HLB, and sorption onto Grignon soil increased with the lipophilicity of the surfactant. Nonionic surfactant sorption is thus expected to occur predominantly on soil OM through hydrophobic interactions. As a consequence, higher doses of the most lipophilic surfactants, that resulted in soil solution concentrations approaching the CMC, significantly increased triticonazole sorption, by modifying the soil surface properties and increasing the soil fractionnal OM content.

As triticonazole sorption isotherm was not modified by the presence of diluted amounts of the two anionic commercial formulation surfactants, it is suggested that triticonazole equilibrium sorption may be characterized by Freundlich or linear partition coefficients of the active ingredient alone for incorporation in transport models. However, additional studies need to be carried out on the sorption on soil and influence on pesticide sorption of organic formulation adjuvants other than surfactants.

ACKNOWLEDGMENTS

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CHAPITRE 2

Effet des agents de formulation sur la solubilisation et la sorption du triticonazole dans le sol

Triticonazole Solubilization and Sorption in Soil-Water Systems: Influence of Formulation Adjuvants

C. Beigel, and E. Barriuso (1997)

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Triticonazole Solubilization and Sorption in Soil-Water systems: Influence of Formulation Adjuvants

C. Beigel, and E. Barriuso *

ABSTRACT

The influence of formulation adjuvants on sorption of systemic pesticides in soil needs to be known for a successfull utilization. We investigated the effect of low doses of 3 suspensed concentrate formulations, and 2 anionic surfactant adjuvants on the solubilization and sorption characteristics of triticonazole fungicide [(1RS)-(E)-5-(4-chlorophenyl-methylen)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol] in a loamy clay soil. Soil sorption of ^{14}C-triticonazole alone, in the formulations, and in the presence of increasing doses of blank formulations and surfactants was measured using the classical batch equilibration technique. Triticonazole solubilization in water-formulation systems was also evaluated using a batch procedure, and sorption of the formulation aqueous pseudophases on soil was performed. Solubilization of triticonazole in the formulation systems occured in excess of the water solubility (S_w). This was attributed to solubilization by association with surfactant monomers. Sorption of the formulation aqueous pseudophase onto soil resulted in an isotherm similar to that of triticonazole in water. Similarly, triticonazole sorption was not modified in the presence of the diluted blank formulations and surfactant additives. Sorption of the surfactants to soil probably removed a significant part of the surfactant available for solubilization. We concluded that in soil-formulation systems, triticonazole solubilization occured even at sub-critical micelle concentration (CMC) surfactant levels, and thus greater amounts may be available for plant absorption. However, triticonazole molecules preferentially associated with the soil surfaces, and diluted doses of formulation adjuvants would not modify triticonazole sorption.

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Triticonazole Solubilization and Sorption in Soil-Water systems: Influence of Formulation Adjuvants

INTRODUCTION

Sorption of pesticides on soil is a key process governing their fate and behavior in soils. It is particularly important for hydrophobic organic compounds (HOC) that are strongly retained to soil. Sorption of HOC significantly reduces their mobility in soils (Boesten and van der Linden, 1991; Guo et al., 1993), and their bioavailability to plants (Hance, 1989, Weber et al., 1993) and microorganisms (Ogram, 1985; Lehmann et al., 1992). Sorption of HOC on soil has been related to soil organic matter (SOM) through non specific interaction mecanisms like hydrophobic bounding or partitioning on SOM (Chiou et al., 1983). Soil sorption isotherms of HOC at diluted concentrations are usually linear (Calvet, 1989), and soil/solution partition coefficients (Kd) may be used to characterize HOC sorption on a particular soil. Strong correlation of Kd with SOM content has been frequently reported (Ainsworth et al., 1989; Barriuso and Calvet, 1992). However, sorption of organic chemicals may be modified by the presence of cosolutes. Unlike the conditions of most studies concerning pesticide sorption on soils, where the chemicals used are pure substances, pesticides are applied in formulations. Thus, it is essential to determine how the sorption of the pesticide is altered by the presence of formulation adjuvants when both coexist in soil.

Of particular interest are the surface active agents present in most of the formulations. Surfactants are used in the formulations as dispersants, spreaders or humectants, for solubilization, homogenization and stabilization of the active ingredients (Seaman, 1990). Their amphiphilic structure results in unique properties, reduction of interfacial tension and formation of aggregates or micelles above a certain concentration, the CMC. The influence of surfactants on the sorption of HOC on soil has been extensively studied for use for decontaminating soils

and aquifers polluted with HOC (Fountain et al., 1991; Haigh, 1996). Surfactants may affect HOC sorption indirectly by dispersing soil colloïds and thus altering soil physical properties (Kuhnt, 1993). Nevertheless, the most important property to be considered is their ability to enhance the water solubility of HOC (Nassetta et al, 1991; Jafvert et al, 1994), and to mobilize sorbed chemicals (Huggenberger, 1973; Amonette and O'Connor, 1980). If solubilization of HOC can occur at surfactant concentrations below the CMC, it is essentially a micellar process, usually attributed to partitioning of the pesticide in the surfactant micellar pseudophase (Dulfer et al., 1995).

Triticonazole is a new triazole systemic fungicide developed by Rhône-Poulenc Agro. REAL is triticonazole suspensed formulation for cereal seed treatment. It is used for cereal protection from seed to develop growth stages, as triticonazole controls major seed born, foliar and straw diseases. The incorporation of systemic fungicides and insecticides in seed treatments is a developing technology for high volume crops. A film of formulation containing the pesticide is coated on the seed. The extreme localization of the deposits on the target allows to use reduced dose rates of pesticides for an economical and less environmentally harmfull plant protection. The efficacy of systemic pesticides applied in seed treatments depends closely on their dissipation and localization in the soil profile in relation to the uptake by the plant roots system. The transport and fate of these molecules in soils is therefore of crucial interest for an optimal utilization. While the dissipation of pesticides in soil has been extensively studied, the influence of formulation additives like fillers, binders, adhesives/stickers, solvents, spreaders and humectants is of great concern and still needs to be determined.

Since only small amounts of formulation are applied for seed treatment, which will further dissolve and be diluted in the soil solution, the impact of low concentrations of surfactants and formulation adjuvants is to be considered more thoroughly. The present study was aimed at evaluating the influence on triticonazole solubilization and sorption on soil of low levels of REAL formulation adjuvants, with particular reference to the two anionic surfactants present in the formulation, Soprophor FLK, and Supragil MNS90.

MATERIALS AND METHODS

Soil

The soil (typic Eutrochrept) was sampled in the surface layer (0-20 cm) of a continuous wheat experimental plot located at Grignon (France). It had a pH in water of 8.2, with (g kg^{-1} of dry soil): 291 of clay, 540 of silt, 145 of sand, 24 of lime and 10.4 of organic C. Soil samples were air dried and passed through a 2-mm sieve. Soil residual gravimetric water content (g/g) was of 3.59%.

Chemical

^{14}C-U-benzyl-labeled triticonazole [(1RS)-(E)-5-(4-chlorophenyl-methylen)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol] (specific activity: 1184 MBq $mmol^{-1}$; radiopurity > 98 %) was provided by Rhône-Poulenc Agro (Lyon, France). Triticonazole water solubility is 8.4 mg L^{-1} at 20°C, vapour pressure is < 10^{-8} hPa at 50°C and distribution coefficient between octanol and water is 1950. Water solutions of ^{14}C-triticonazole were prepared at 5 different concentrations: 0.5, 1.0, 2.0, 3.0 and 5.0 mg L^{-1} by diluting 50 µL of the ^{14}C-triticonazole methanol stock solution in different volumes of a saturated water solution of triticonazole (analytical standard, purity > 92 %) and adjusting the concentration with MilliQ water (Millipore, Saint-Quentin, France). The solution concentrations were measured at 262.5 nm with a UV-visible spectrophotometer Lambda V (Perkin-Elmer, Überlingen, Germany).[1]

Formulations and surfactants

Formulated ^{14}C-triticonazole as three different concentrated aqueous suspensions was provided by Rhône-Poulenc Agro: REAL, [REAL+FLK], and [REAL+MNS90]. REAL is the commercial triticonazole formulation for wheat seed treatment. It comprises micronized triticonazole and anthraquinone active ingredients at 200 g L^{-1} and 85 g L^{-1} respectively, and

[1] Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the product listed by INRA.

formulation adjuvants: surfactant spreaders and dispersants, polymer, filler, cosolvant and dyes in various proportions not exceeding 100 g L^{-1}. Among those, Soprophor FLK is a phosphate tristyrylphenolethoxylate anionic micellar surfactant which is present at 30 g L^{-1}, and Supragil MNS90 is a sodium alkylnaphtalensulfate nonmicellar anionic surfactant present at 10 g L^{-1}. [REAL+FLK] is a formulation enriched with Soprophor FLK at four times its concentration in REAL (120 g L^{-1}), while [REAL+MNS90] does not contain Soprophor FLK and is enriched with Supragil MNS90 at 20 times its concentration in REAL (200 g L^{-1}. Specific activities of the ^{14}C-triticonazole in the formulations were 0.54, 0.38, and 0.61 kBq $mmol^{-1}$, for REAL, [REAL+FLK] and [REAL+MNS90]. Those stock suspensions were further diluted with water, and apparent concentration were expressed in function of triticonazole content (in mg L^{-1}).

Blank formulations, noted $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$ were also used. They comprise the same ingredients in the same proportions except triticonazole active ingredient. For convenience, we also expressed the doses of these blank formulations as equivalent of triticonazole in the normal formulations. Stock suspensions at 200 g L^{-1} were provided by Rhône-Poulenc Agro, which were diluted in water to obtain the required concentrations for the experiments.

Technical grade Soprophor FLK and Supragil MNS90 anionic surfactants were furnished by Rhône-Poulenc Agro. The surfactant solutions were prepared by diluting the proper amount of surfactant in water and solubilizing by ultrasound.

Triticonazole solubilization in the formulations

Diluted suspensions of the radiolabelled formulations REAL, [REAL+FLK] and [REAL+MNS90] were prepared at 10 different concentrations ranging from 1 to 200 mg L^{-1}. Fifteen milliliters aliquots were pipetted and put in glass centrifugation tubes, and shaken 24 h for equilibration. The suspensions were then centrifuged at 8000 g for 15 min to separate dissolved materials from the solid phase. Triticonazole concentration in the supernatants was calculated from the radioactivity content measured by liquid scintillation counting. Triticonazole concentrations in solution at equilibrium were then plotted in function of the

initial amount in the suspensions. Ten milliliters aliquots of the supernatants were also pipetted and stored for further sorption studies.

Triticonazole sorption isotherms

A classical batch equilibrium procedure was used to obtain triticonazole sorption isotherm at 22 ± 1°C. Ten milliliters aliquots of ^{14}C-triticonazole water solutions ranging from 0.5 to 5 mg L^{-1} were added to 5 g of air dried soil in 20-ml glass centrifuge tubes with Teflon caps. After shaking for 24 h, the samples were centrifuged at 8000 g for 15 min and triticonazole concentration in solution C_e was calculated from the supernatant radioactivity measurements by liquid scintillation counting. The amount of triticonazole sorbed was determined by difference between the initial and equilibrium concentrations in solution. The whole experiment was replicated twice.

The same batch slurry equilibrium procedure was used to evaluate the effects of the formulations on the sorption of triticonazole. Triticonazole sorption isotherms were measured in four different types of systems:

(1) In the radiolabelled formulations REAL, [REAL+FLK], and [REAL+MNS90], with initial apparent concentrations in the suspensions ranging from 1 to 200 mg L^{-1}.

(2) In the supernatants from the solubilization studies, with triticonazole initial concentrations corresponding to the dissolved concentrations measured at the solubilization equilibrium.

(3) In the presence of the blank formulations $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$, with initial solutions with ^{14}C-triticonazole concentration ranging from 0.5 to 5 mg L^{-1}, and blank formulations concentration at 100 mg L^{-1}.

(4) In the presence of Soprophor FLK and Supragil MNS90 surfactants, with initial solutions with ^{14}C-triticonazole concentration ranging from 0.5 to 5 mg L^{-1}, and surfactants concentration at 80 mg.L^{-1}.

The sorption isotherms were plotted as the amount of pesticide sorbed (x/m, mg per kg of dry soil) versus the equilibrium concentration Ce (mg L^{-1}). Freundlich empirical parameters

K_f and n_f were estimated using a least-square nonlinear regression method (S-Plus version 3.2, StatSci, Seattle, WA, USA), from the Freundlich equation:

$$x/m = Kf\, C_e^{nf} \qquad [1]$$

The averaged linear sorption partition coefficient K_d was calculated by linear curve fitting following the equation:

$$x/m = K_d\, C_e \qquad [2]$$

Finally, the K_{OC} was calculated from the relationship:

$$K_{OC} = K_d\ (\text{fraction of soil organic C})^{-1} \qquad [3]$$

Triticonazole sorption in presence of increasing amounts of formulation additives

In addition to the sorption isotherm studies in the presence of a single concentration of blank formulation and of surfactant additives, we investigated the influence of increasing amounts of the blank formulations $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$, and of the anionic surfactants Soprophor FLK and Supragil MNS90 on the sorption of triticonazole on soil. All these studies were performed using the batch equilibrium procedure as previously described, with triticonazole initial concentration in solution at 5 mg L^{-1}. Blank formulation and surfactants concentrations ranged from 0.1 to 200 mg L^{-1}. Samples at the same concentrations were also prepared without soil to evaluate triticonazole sorption due to formulation (on formulation additives). The results were expressed as the percentage of sorbed triticonazole in the presence of the additives relative to triticonazole sorption without additive.

RESULTS AND DISCUSSION

Surfactant influence on triticonazole sorption

Triticonazole sorption isotherms on Grignon soil, alone and in the presence of the anionic surfactants used in REAL formulation are presented in Fig. 1. Estimated values for K_f and n_f are listed in Table 1. The Freundlich equation allowed for a good description of the

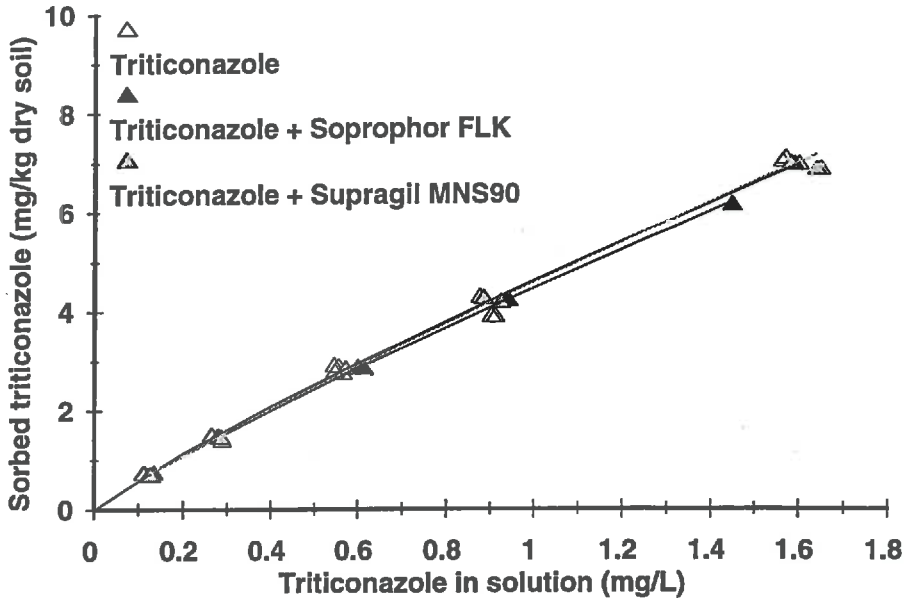

Fig. 1. Isotherms of ^{14}C-triticonazole sorption on Grignon soil, alone and in the presence of Soprophor FLK and Supragil MNS90 anionic surfactants at 80 mg L^{-1}. Initial triticonazole concentrations ranged from 0.5 to 5 mg L^{-1}. Freundlich fits are represented as solid lines.

experimental results for the range of concentrations tested. Triticonazole sorption isotherm is characterized by a high K_f of 4.61 ± 0.20 indicating strong sorption and a n_f of 0.87 ± 0.01 close to unity, indicating dominating non specific interaction mechanisms, which would be most likely hydrophobic bonding or solute partitioning onto soil organic matter. Consequently linearization of the isotherm and use of the linear partition coefficient K_d to characterize the equilibrium sorption of triticonazole is justified. A K_d value of 4.35 ± 0.08 L kg^{-1} was calculated, which corresponds to a K_{OC} value of 418 L kg^{-1}.

Triticonazole sorption in the presence of the anionic surfactants used in REAL formulation was not significantly modified as evidenced by the identical isotherms observed in the surfactant-free system and in systems containing a 80 mg L^{-1} dose of the surfactants K_f and n_f Freundlich values for Soprophor FLK and Supragil MNS90 addition were also extremely close to those for triticonazole alone.

	TREATMENT		
	^{14}C-Triticonazole	^{14}C-Triticonazole + Soprophor FLK	^{14}C-Triticonazole + Supragil MNS90
K_f	4.61 ± 0.20	4.48 ± 0.11	4.60 ± 0.13
n_f	0.87 ± 0.01	0.88 ± 0.01	0.89 ± 0.01

Table 1. Estimated values of the Freundlich parameters K_f and n_f for ^{14}C-triticonazole sorption in Grignon soil, alone and in the presence of Soprophor FLK and Supragil MNS90 anionic surfactants at 80 mg L^{-1}.

Modification of HOC sorption by surfactants usually occurs at supra-CMC concentrations, when a surfactant micellar pseudophase is considered (Sun et al., 1995). Hence, our results are not surprising for Supragil MNS90 surfactant since it does not form micelles.

In soil-water systems, surfactant-HOC interactions are very complex, depending mainly on soil type, type and concentration of the surfactant, and HOC physicochemical properties

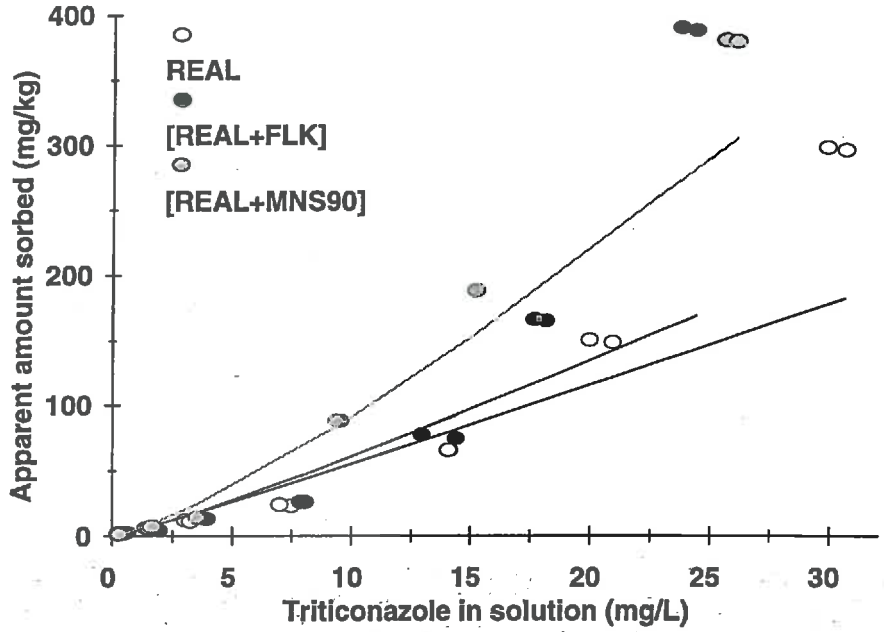

Fig. 2. Isotherms of apparent sorption on Grignon soil of ^{14}C-triticonazole in the suspensed concentrate formulations REAL, [REAL+FLK], and [REAL+MNS90]. Initial triticonazole amounts in the formulation suspensions ranged from 1 to 200 mg L^{-1}. Freundlich fits are represented as solid lines.

(Sanchez-Camazano et al., 1995; Haigh, 1996). In such systems, surfactant sorption as well as pesticide sorption need to be considered. Sorption of the surfactant onto soil surfaces decreases the surfactant concentration in solution and may result in much higher total surfactant doses being necessary to attain the CMC in soil solution. Also, sorbed surfactant molecules may increase HOC sorption on soil by increasing the soil fractionnal OM content (Edwards et al., 1994 a and b; Haigh, 1996).

In a previous paper (Beigel et al., 1997), we investigated the sorption characteristics of Soprophor FLK and other surfactants in Grignon soil. We evidenced that the CMC of Soprophor FLK was greatly affected by strong sorption of the surfactant onto soil. Soprophor FLK CMC in water was measured at 48 mg L^{-1} whereas its apparent CMC in the soil-water system was estimated at approximately 10000 mg L^{-1}. Anionic surfactant sorption on the negatively charged soil surface is usually expected to be minimal (Di Vincenzo and Dentel, 1996; Ou et al., 1996). Strong sorption of Soprophor FLK was attributed to sorption of hemimicelles (admicellization) on soil through hydrophobic interactions. In the present study, Soprophor FLK concentrations in the solutions added to the system ranged from sub-CMC concentrations to a supra-CMC concentration of 200 mg L^{-1}. Consequently, all Soprophor FLK amounts tested here would result in sub-CMC concentrations in the soil-water systems due to sorption of the surfactant on soil. Sorption of low levels of the surfactant would not significantly increase the soil fractionnal OC content. Thus, it is coherent that the low amounts of Soprophor FLK tested here showed no influence. Much higher amounts would be necessary to attain supra-CMC concentrations in the soil solution, but this would prove unrealistic in the formulations.

Formulation isotherms

Triticonazole sorption isotherms of the suspensed concentrate formulations REAL, [REAL+FLK] and [REAL+MNS90] (Fig. 2) were not correctly described by the Freundlich equation. This is not surprising since solid micronized cristals of triticonazole, that are artificially maintained in suspension in the formulations, would be separated from dissolved

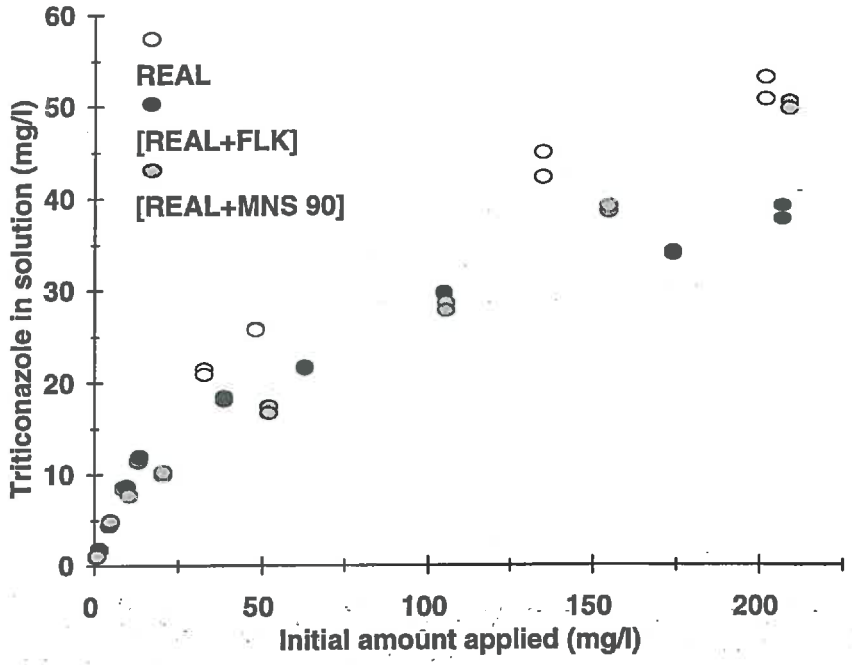

Fig. 3. Solubilization of triticonazole in the suspensed concentrate formulations REAL, [REAL+FLK], and [REAL+MNS90] in relation to the initial concentration of formulation.

material during centrifugation. The amount of triticonazole remaining in the centrifugation pellets would therefore account for solid phase triticonazole as well as sorbed material, and we will refer to apparent sorption. However, some interesting trends still appear.

First, equilibrium apparent concentrations measured in the supernatants continuously increased up to 23, 24, and 30 mg L^{-1}, for REAL, [REAL+FLK], and [REAL+MNS90] respectively, and largely surpassed triticonazole water solubility (S_w) of 8.4 mg L^{-1}. This means that the formulations have a great power to solubilize triticonazole molecules, and that at least part of the solubilized material is not sorbed.

Also, the three formulations showed a different behavior starting from equilibrium apparent concentration close to S_w. The apparent sorbed amounts of triticonazole were greater for the [REAL+FLK] and [REAL+MNS90] formulations than for REAL, whereas these formulations contained more surfactants. As previously evidenced, the presence of each of the two anionic surfactants at such diluted concentrations did not modify the sorption of triticonazole, and thus would not account for the increase in apparent sorption of triticonazole. However, mixtures of surfactants may behave differently.

Solubilization studies

Solubilization of triticonazole in the diluted formulations, expressed as the apparent concentration in solution in relation to the total amount of triticonazole initially in suspension (Fig. 3), show the different capacity of the three formulations to solubilize triticonazole in excess of the S_w. REAL and [REAL+FLK] curves were parabolic, with relatively greater efficiency for solubilization occuring at the lowest concentrations for both formulations. REAL exhibited a greater solubilizing capacity compared to [REAL+FLK] formulation, which contains more anionic micellar surfactant. Anionic surfactants have been reported to be less efficient in solubilizing hydrophobic compounds (Nassetta et al., 1991). Possible interactions between the added anionic surfactant Soprophor FLK with the other surfactants contained in REAL may lower the ability of the formulation to solubilize triticonazole. Apparent water concentrations of triticonazole as high as 50 mg L^{-1} for REAL and [REAL+MNS90], and 35

mg L^{-1} for [REAL+FLK] were observed at the 200 mg L^{-1} highest initial concentration, thus showing that triticonazole solubility in the water formulation systems was greatly enhanced. Since the formulations differ essentially in their surfactant contents, the variations in their solubilization capacity may be attributed mainly to surfactant effects.

Such substantial increase in triticonazole apparent solubility is quite surprising since the formulation amounts corresponded to surfactant concentrations above the CMC in all samples except [REAL+FLK] concentrations of 100, 150, and 200 mg L^{-1}, where the water CMC of Soprophor FLK surfactant is attained. However, solubilization enhancement of HOC by association with surfactant monomers at sub-CMC concentrations of surfactants has been reported (Kile & Chiou, 1989; Edwards et al. 1991; Dulfer et al., 1995). Also, in surfactant mixtures, CMC of the surfactants can be lowered by the presence of organic cosolutes that may stabilize the micelles (Rosen 1989). In [REAL+MNS90] formulation, Soprophor FLK surfactant is not included, triticonazole solubilization increased linearly with the formulation concentration, and seemed directly related to the increase in Supragil MNS90 nonmicellar anionic surfactant.

The differences in triticonazole solubilization would account at least for part of the variations in apparent sorption of triticonazole that were observed in the three suspensed concentrate formulations. A decreased dissolution in [REAL+FLK] and [REAL+MNS90] formulations would explain the increased apparent sorption of triticonazole.

Addition of blank formulations

The addition of blank formulation to triticonazole solutions allows to evaluate more accurately the impact of the formulation adjuvants on triticonazole sorption since, in these systems, triticonazole partitioning in soil/solution is not restricted by solubility.

In the control samples without soil, the total amount of triticonazole remained in solution, thus no sorption occured on the solid formulation compounds (fillers, polymers...) that were separated by centrifugation, and the sorption measured in the soil-blank formulation systems can be attributed to soil sorption.

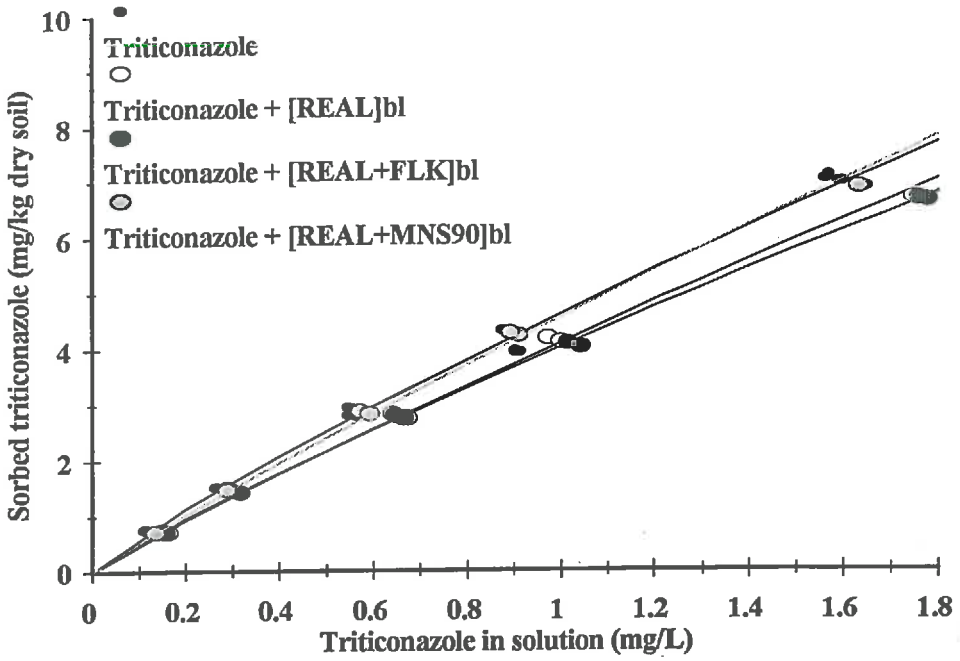

Fig. 4. Isotherms of ^{14}C-triticonazole sorption on Grignon soil, alone and in the presence of the blank formulations $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$ at 100 mg L^{-1}. Initial triticonazole concentrations ranged from 0.5 to 5 mg L^{-1}. Freundlich fits are represented as solid lines.

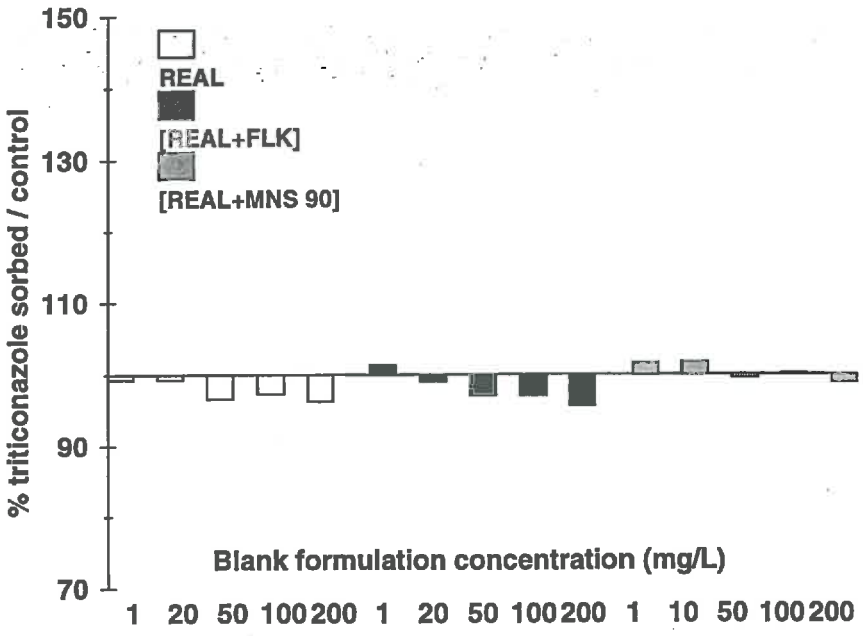

Fig. 5. Influence of different concentrations of the blank formulations $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$ on the sorption of ^{14}C-triticonazole on Grignon soil. Blank formulation concentrations are expressed as equivalent of triticonazole in the normal formulations. Control base line is for sorption of ^{14}C-triticonazole alone.

Triticonazole sorption isotherms in the presence of $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$ (Fig. 4) were not significantly different from the isotherm measured in pure water. K_f and n_f values were also very close to the Freundlich values calculated in water (Table 2.). Thus, the presence of formulation adjuvants at this concentration in the soil-water systems did not modify triticonazole sorption. Moreover, as the different blank formulations showed no difference in behavior, the influence of the additional amounts of the anionic surfactants Soprophor FLK and Supragil MNS90 on triticonazole sorption would be negligible.

	TREATMENT			
	^{14}C-Triticonazole	^{14}C-Triticonazole + $[REAL]_{bl}$	^{14}C-Triticonazole + $[REAL+FLK]_{bl}$	^{14}C-Triticonazole + $[REAL+MNS90]_{bl}$
K_f	4.61 ± 0.20	4.09 ± 0.20	4.02 ± 0.22	4.55 ± 0.13
n_f	0.87 ± 0.01	0.92 ± 0.02	0.89 ± 0.02	0.92 ± 0.01

Table 2. Estimated values of the Freundlich parameters K_f and n_f for ^{14}C-triticonazole sorption in Grignon soil, alone and in the presence of the blank formulations $[REAL]_{bl}$, $[REAL+FLK]_{bl}$, and $[REAL+MNS90]_{bl}$ at 100 mg L^{-1}.

Similarly, the addition of increasing concentrations of the blank formulations up to 200 mg L^{-1} did not affect significantly the sorption of triticonazole (Fig. 5). This is not surprising, since for such low amounts of blank formulations, the surfactants concentrations would be well below the CMC in the soil-water systems consequently to surfactant sorption on soil, as was evidenced in the surfactant experiments.

Supernatant isotherms

Triticonazole sorption isotherms obtained from the supernatants recovered by centrifugation of the formulations were not significantly different for the three formulations [REAL], [REAL+FLK], and [REAL+MNS90] (Fig. 6). The Freundlich equation fitted well to the experimental values for REAL and [REAL+MNS90], and K_f and n_f values were very close

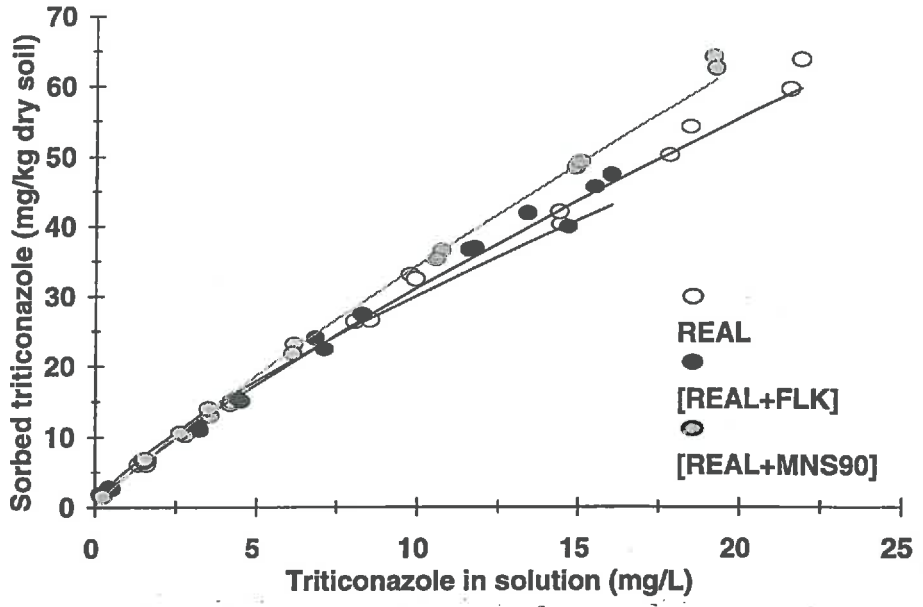

Fig. 6. Isotherm of sorption on Grignon soil of ^{14}C-triticonazole in the formulation aqueous pseudophases from REAL, [REAL+FLK], and [REAL+MNS90]. The pseudophases correspond to the supernatants recovered by centrifugation of the formulations in the solubilization experiments. Freundlich fits are represented as solid lines.

(Table 3). For [REAL+FLK], experimental results were more dispersed, and standard deviation of the K_f was greater. Therefore the small difference observed in K_f and n_f would not be significant. In this experiment, dissolved triticonazole in excess of the S_x would be associated with solubilizing formulation adjuvants, and the formulation aqueous pseudophase would include dissolved triticonazole molecules as well as triticonazole associated with surfactant monomers, or with other soluble adjuvants. The K_f and n_f values estimated from the supernatant isotherms were very close to the K_f and n_f values of respectively 4.61 ± 0.20 and 0.87 ± 0.02 estimated for the sorption of triticonazole in water. This suggests that the association of triticonazole molecules with solubilizing adjuvants at sub-CMC concentrations does not modify triticonazole sorption on soil. In the soil-triticonazole-soluble adjuvants systems, triticonazole partitioning on soil surfaces would be favored compared to water-adjuvants interactions. Thus, in the formulation systems, whereas some adjuvants or surfactants monomers have the capacity to associate and solubilize triticonazole molecules in the formulation aqueous pseudophase, these adjuvants can not compete with SOM as a sorbent phase.

	FORMULATION		
	REAL	[REAL+FLK]	[REAL+MNS90]
K_f	4.64 ± 0.28	5.45 ± 0.79	4.64 ± 0.17
n_f	0.83 ± 0.01	0.74 ± 0.02	0.87 ± 0.01

Table 3. Estimated values of the Freundlich parameters K_f and n_f for sorption in Grignon soil of ^{14}C-triticonazole in the formulation aqueous pseudophases from REAL, [REAL+FLK], and [REAL+MNS90]. The pseudophases correspond to the supernatants recovered by centrifugation of the formulations in the solubilization experiments.

CONCLUSION

Addition of anionic formulation surfactant Soprophor FLK had little effect on triticonazole sorption. This was attributed to sorption of the surfactants on soil, which resulted in concentrations in soil solution well below the CMC, while not significantly increasing the soil fractionnal organic carbon content.

In the suspensed concentrate formulations, triticonazole was greatly solubilized, leading to apparent concentrations in solution far above the water solubility. This is of importance concerning the efficacy of systemic compounds applied in seed treatments, as greater amounts of active ingredient may be available for transport and plant absorption. The extent of solubilization was related to the type and amount of surfactant used in the formulation. The decreased solubilization observed in the formulations enriched in the anionic surfactants was attributed to interactions between those molecules and other more solubilizing adjuvants present in the commercial formulation. However, the interactions of triticonazole with solubilizing formulation adjuvants monomers did not modify the sorption of the fungicide on soil, and in the soil-formulation aqueous pseudophases, triticonazole partitioned preferentially on soil organic matter.

As a result, sorption data of the pesticide as active ingredient alone may be used for modeling and prediciting their sorption on soil if low levels of formulation surfactants and other adjuvants are to be considered.

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CHAPITRE 3

Processus de dissipation du triticonazole dans le sol

Degradation of Formulated and Unformulated Triticonazole Fungicide in Soil: Effect of Application Rate

C. Beigel, M.-P. Charnay, and E. Barriuso (1997)

Soumis à *Soil Biology and Biochemistry*

DEGRADATION OF FORMULATED AND UNFORMULATED TRITICONAZOLE FUNGICIDE IN SOIL: EFFECT OF APPLICATION RATE

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SUMMARY

Laboratory incubation studies were conducted to evaluate the influence of commercial formulation adjuvants and initial dose of treatment over a range of 0.2 to 80 mg kg^{-1} on the dissipation of ^{14}C-radiolabeled triticonazole systemic fungicide in a loamy clay soil, at 22 and 28°C, and 80 % of water field capacity. Measurement of the balance of the ^{14}C-residues after incubation at 22 °C showed an increased resistance to desorption with time, as apparent desorption K_{app} increased from 2 to 10 l kg^{-1} in a 133 day period. Decreased extractability of the residues with incubation time, and formation of bound residues up to 20 % showed that the overall availability of triticonazole decreased with incubation time. The addition of diluted doses of formulation adjuvants did not significantly affect the degradation and binding of the active ingredient. Mineralization of triticonazole was slow, with a high activation energy of 130 kJ mol^{-1}. The persistence of triticonazole increased with initial dose rate applied, as the first-order mineralization rates at 28 °C decreased from 2 10^{-3} to 0.7 10^{-3} d^{-1} with amount applied increasing from 0.2 to 80 mg kg^{-1}. However, the absolute amount of triticonazole mineralized increased with increasing concentration in the soil solution, and the slow mineralization was attributed to limited availability in the soil solution due to high sorption.

INTRODUCTION

Biodegradation is one of the key processes controlling the fate of pesticides in soil, as microorganisms are the main agents responsible for the breakdown of most xenobiotics compounds in soil (Graham-Bryce, 1981). For an accurate prediction of the pesticide persistence, it is thus essential to have a quantitative knowledge of the environmental and nonenvironmental parameters that impact the microbial degradation of pesticides. This is of particular importance for systemic pesticides that are directly applied to soil, and which efficacy is closely related to their dissipation and bioavailability in soil.

The rate of degradation of pesticides in soil is largely governed by the importance and the activity of the soil microbial biomass, and by the availability of the organic substrate to the degrading organisms (Anderson, 1984; Torstensson, 1987). Temperature, moisture content, and organic carbon (OC) content of the soil are typically perceived to be the key environmental variables affecting the degradation of pesticides in soil. Pesticide degradation rates have been positively related to temperature and moisture content (Parker and Doxtader, 1983; Choi *et al.*, 1988; Walker *et al.*, 1992; Veeh *et al.*, 1996; Willems *et al.*, 1996). The influence of soil OC content is more complicated. An increase in OC may enhance the microbial activity by providing growth substrates for cometabolic degrading microrganisms (Nair and Schnoor, 1994). On the other hand, sorption of hydrophobic organic compounds (HOC) in soil, which is positively related to the OC content of the soil (Ainsworth *et al.*, 1989), strongly reduces the mobility and bioavailability of the pesticides (Ogram *et al.*, 1985; Boesten and van der Linden, 1991; Weber *et al.*, 1993). Higher soil OC content may thus considerably decrease the degradation rates of HOC (Lehmann *et al.*, 1992; Johnson *et al.*, 1995). Moreover, it has been evidenced that the strength of sorption of most HOCs in soil increases with incubation time (Lehmann *et al.*, 1990; Barriuso *et al.*, 1992; Beigel *et al.*, 1997). Slow desorption from restricted soil sites and formation of nonextractable (bound) residues after prolonged contact with the soil, which seems directly related to soil organic constituents, has been reported for most HOCs (Roberts, 1984; Miller *et al.*, 1997a, 1997b). This may further reduce the

availability of the pesticides to microbial attack, and thus further decrease their degradation rates (Smith *et al.*, 1992).

In addition to these environmental factors, parameters inherent to the pesticide application may also greatly affect the degradation of pesticides. Most studies deal with the degradation of the active ingredient as a solute, whereas pesticides are commonly applied as formulations where the active ingredient can be present at higher dose rates in both solute and cristalline phases. Formulation adjuvants may affect the degradation of pesticides by modifying the availability of the compound, as some surfactants have the ability to enhance the apparent solubility of poorly water soluble compounds and reduce their sorption to soil (Amonette and O'Connor, 1980; Jafvert *et al.*, 1994). The incorporation of formulation additives in soil may also directly affect the microflora, as some adjuvants may prove toxic or else may act as a C source for the soil microorganisms. Increasing the pesticide application rate could also influence their degradation, by increasing the concentration of the pesticide in the soil solution, or by providing a cristalline solid-phase reservoir of pesticide (Gan *et al.*, 1995, 1996).

The kinetics of degradation of pesticides in soil are often described with mineralization data. However, dissipation data would probably be more accurate for description of the disappearance of the pesticides, since they also account for the metabolization of the parent compound, and for the formation of bound residues. Degradation constants are usually estimated using pseudo first-order equations, which provide a useful simple parameter for incorporation in models describing the fate of pesticides in the environment. The first-order equation has been used satisfactorily to describe the mineralization and dissipation in soil of most pesticides at low levels (Parker and Doxtader, 1983; Patil *et al.*, 1988; Walker *et al.*, 1992; Yen *et al.*, 1994; Nair and Schnoor, 1994). However, its validity in more complex systems, when both solid-phase cristalline pesticide and formulation additives are present, is still questionable.

Triticonazole is a new triazole systemic fungicide developed by Rhône-Poulenc Agrochemicals Company, that is used in cereal seed treatment. The aims of the present study realized in controlled laboratory conditions were to evaluate the influence of some environmental and application factors, i.e. temperature, formulation and application rate, on

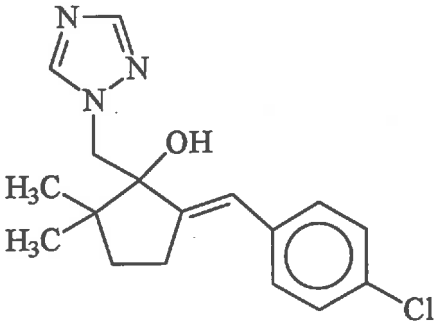

Fig. 1 Structure of triticonazole.

the degradation of triticonazole in soil, and to assess the validity of first-order dissipation constants for incorporation as input parameters in models.

MATERIALS AND METHODS

Soil

The soil (Typic Eutrochrept) was sampled in the surface layer (0-20 cm) of a continuous wheat experimental plot located at Grignon (France). It had a pH in water of 8.2 and field capacity of 24 % (w/w), with (g kg^{-1} of dry soil): 291 of clay, 540 of silt, 145 of sand, 24 of lime and 10.4 of organic C. Soil samples were air dried at room temperature and passed through a 4-mm sieve. Soil residual gravimetric water content (w/w) was of 4.9 %. Sterile soil for the mineralization experiment was obtained by gamma irradiation (30 kGy for 20 h).

Chemical and formulations

^{14}C-U-benzyl-ring-labeled triticonazole [(1RS)-(E)-5-(4-chlorophenyl-methylen)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol] (Fig. 1) (specific activity: 1184 MBq $mmol^{-1}$; radiopurity > 98 %) was provided by Rhône-Poulenc Agrochemicals Company (Lyon, France). Triticonazole water solubility is 8.4, 10.6, and 13.2 mg l^{-1} at respectively 20, 22, and 28°C, vapour pressure is < 10^{-8} hPa at 50°C, and distribution coefficient between octanol and water is 1950. Triticonazole methanol solutions were prepared by dissolving nonradiolabeled active ingredient (analytical standard, purity > 92%) in methanol to obtain the desired concentration, and adding the proper volume of ^{14}C-labeled triticonazole methanol stock solution to attain the desired radioactivity content.

Two water suspensions concentrate formulations, noted REAL and REAL + FLK were tested. REAL is the commercial triticonazole formulation for wheat seed treatment. It comprises micronized triticonazole and anthraquinone active materials at 200 g l^{-1} and 85 g l^{-1} respectively, and formulation additives, dispersants, polymer, filler, cosolvant and dyes in various proportions not exceeding 100 g l^{-1}. Among those, Soprophor FLK is a phosphate tristyrylphonolethoxylate anionic micellar surfactant, which is present at 30 g l^{-1}. [REAL +

FLK] is a formulation enriched with Soprophor FLK at 120 g l^{-1}. It was used to test the specific influence of the surfactant in the formulated triticonazole. ^{14}C-triticonazole-U-benzoil-labeled stock formulations at 200 g l^{-1} were provided by Rhône-Poulenc Agro. Specific activities were 540 and 380 Bq $mmol^{-1}$ respectively for ^{14}C-REAL and ^{14}C-[REAL + FLK]. Stock suspensions were further diluted with water for soil treatment, and the proper volume of ^{14}C-labeled triticonazole stock solution was added to adjust the radioactivity content. The apparent concentrations are expressed in function of triticonazole content (in mg l^{-1}).

A blank formulation, noted $(REAL)_{bl}$ was also used. It comprises the same ingredients in the same proportions as REAL except triticonazole active material. For convenience, we also expressed the doses of this blank formulation as equivalent to triticonazole content in the normal formulation. A suspension at 200 g equivalent triticonazole l^{-1} was provided by Rhône-Poulenc Agro, and was diluted in water to obtain the required doses for the experiments.

Before treatment, all solutions and suspensions were shaken 24 h on an end-over-end shaker for homogeneization.

Incubation setup

Triticonazole methanol solution were applied to 250 mg of microbially inactive soil (0.1 mm sieved and overnight oven dried at 110°C). The methanol was then evaporated overnight, and the treated soil was incorporated in 10 g biological active soil and thoroughly mixed, and placed in 0.5 l glass incubation flasks. The water content of the soil was then adjusted to 80 % of the soil water capacity (19.2 % w/w). Vials containing 2 ml of 1 M NaOH to trap CO_2 were placed in each incubation flask, which were then hermetically sealed and incubated in the dark at 22 ± 1°C or 28 ± 1°C in a thermostatic chamber. The NaOH traps were periodically removed for analysis and changed. The soil moisture content was adjusted every three weeks by weighing.

Triticonazole dissipation was studied during incubations at 22 ± 1 °C. 250 µl of ^{14}C-triticonazole methanol solutions at 10 Mbq ml^{-1}, and at concentrations of 8 and 3200 mg l^{-1} were applied, which, after incorporation to the 10 g of dry soil, corresponded to treatment rates of 0.2 and 80 mg kg^{-1}, and 208.3 kBq kg^{-1} dry soil. The radioactivity content of these

treatments was enhanced compared to the mineralization studies at 28°C to allow for accurate analysis, because degradation was expected to be slower at 22°C. The influence of the formulation additives of REAL was tested by incorporating 250 µL of the blank water suspension $(REAL)_{bl}$ at 8 and 3200 mg equivalent triticonazole l^{-1}. Dissipation studies at 22 °C were performed in duplicate for the two doses (0.2 and 80 mg kg^{-1}), two treatments (^{14}C-triticonazole and [^{14}C-triticonazole + $(REAL)_{bl}$]), and for ten incubation times during 133 days of incubation. At each incubation time, the corresponding soil samples were removed, weighed and stored at -20°C until analysis.

Complementary triticonazole mineralization studies were performed during incubations at 28°C. 250 µL of ^{14}C-triticonazole methanol solutions at 2667 Bq mL^{-1}, and at concentrations ranging from 8 to 3200 mg L^{-1} were applied to the soil aliquots, which corresponded to treatment doses of 6700 Bq kg^{-1}, and respectively 0.2, 1, 4, 20, 40 and 80 mg kg^{-1} dry soil. The same doses of triticonazole were applied for REAL and [REAL + FLK] treatments, but the water formulations were directly applied on the microbially active soil by pipeting 2 ml of suspensions ranging from 1 to 400 mg l^{-1} of triticonazole, and at 333 Bq ml^{-1}. Mineralization studies at 28°C were performed in triplicate for each dose and treatment (^{14}C-triticonazole, ^{14}C-REAL and ^{14}C-[REAL + FLK]) during 150 days. Triticonazole mineralization was also performed in sterile conditions with triticonazole at 1 mg kg^{-1} applied on γ-radiated sterile soil.

Sampling and chemical analysis

The evolved $^{14}CO_2$ trapped in NaOH was directly measured by liquid scintillation counting with Picofluor (Packard) as liquid scintillant using a Kontron Betamatic V scintillation counter (Kontron Ins, Montigny le Bretonneux, France).

Total ^{14}C-triticonazole residues remaining in the soil samples were measured by combustion of triplicate 300 mg aliquots of air dried and finely ground soil with a Sample Oxidizer 307 (Packard, Meriden, CT, USA). Extractable residues were analysed after extraction of 5 g of dry soil in a 20 ml-glass centrifuge tube with Teflon cap. This sample was first extracted with 10 ml of water. After 24 h shaking and centrifugation (15 min at 8000 g),

the radioactivity content in the extract was measured by liquid scintillation counting. The soil pellet was then extracted three successive times with 10 ml of methanol using the same procedure as for the water extraction, and the radioactivity content in the methanol extracts was measured. The addition of the ^{14}C-residues extracted in water and in methanol gave the total extractable residues. The soil containing the nonextractable ^{14}C-residues was air dried and finely ground, and its radioactivity content measured after combustion as previously described.

HPLC analysis of the residues contained in the water and methanol extracts was performed for each incubation time. The extracts from the 2 replicates were pooled. The water extracts were concentrated by solid/liquid extraction with Alltech C18 cartridge (200 mg), and eluted with 20 ml of methanol. The methanol extracts were concentrated by evaporation near dryness with a TurboVap II Concentrator (Zymark, Hopkinton, MA, USA) at 45°C on a helical flow of air with an operating pressure of 800 kPa. The residues were dissolved in 2 ml of the solvent used for the HPLC analysis, and filtered through a Cameo 13N syringe nylon filter (0.45 µm; MSI, Westboro, MA, USA). HPLC was performed using a Waters appliance (600E Multisolvent Delivery System, 717 Autosampler and a Novapak C18 column of 5 µm and 4.6 × 150 mm; Waters-Millipore, Milford, MA, USA) equipped with a photo diode array detector Waters 996 coupled on line with a radioactivity continuous flow detector Packard-Radiomatic Flo-one A550. Mobile phase was methanol/water (67/33) at 1 ml min^{-1}, and the injected volume was 300 µl.

Data analysis

Both triticonazole dissipation and mineralization were described using pseudo first-order kinetics:

$$S = S_0 e^{-kt} \quad (1)$$

where S is the amount of substrate at time t (mg kg^{-1} dry soil), S_0 is the initial amount of substrate, k is the first-order constant (d^{-1}), and t is time (d). The first-order constants were estimated by linear regression from the transformed first-order equation:

$$\ln S = \ln S_0 - kt \quad (2)$$

The first-order mineralization constants were estimated from the cumulative $^{14}CO_2$, assuming

that the amount of triticonazole remaining at time t was S_0 minus the cumulative $^{14}CO_2$ evolved at time t. The dissipation constants were estimated from the pooled amounts of triticonazole in the water and methanol extracts.

Zero-order mineralization rates (k_{zero} in mg kg^{-1} dry soil d^{-1}) were also by linear regression from the zero-order kinetics equation:

$$S = S_0 - k_{zero}\, t \qquad (3)$$

Arrhenius equation was used to evaluate the activation energies required for the mineralization of triticonazole:

$$k_T = A e^{-\frac{E_a}{RT}} \qquad (4)$$

where k_T is the first-order mineralization constant at temperature T (K), A is a constant related to soil chemical and other nonthermal factors, E_a is the activation energy (kJ mol^{-1}), and R is the gas constant (8.314 J K^{-1} mol^{-1}). The activation energy was calculated from the transformed equation:

$$E_a = R \frac{T_{22} T_{28}}{T_{28} - T_{22}} \ln \frac{k_{28}}{k_{22}} \qquad (5)$$

using the estimated mineralization constants at 22 and 28 °C.

The procedure for water extraction of the ^{14}C-residues was similar to that used to measure chemical sorption and desorption onto soils by a classical batch technique. Therefore we calculated an apparent desorption coefficient (K_{app}) for each incubation time as described in a previous paper (Beigel *et al.*, 1997):

$$K_{app} = x/m\, C_{des}^{-1} \qquad (6)$$

where x/m (mg kg^{-1} dry soil) is the amount of ^{14}C-triticonazole in the methanol extracts plus the ^{14}C-nonextractable residues, and C_{des} (mg l^{-1}) is the concentration of ^{14}C-triticonazole in the water extracts.

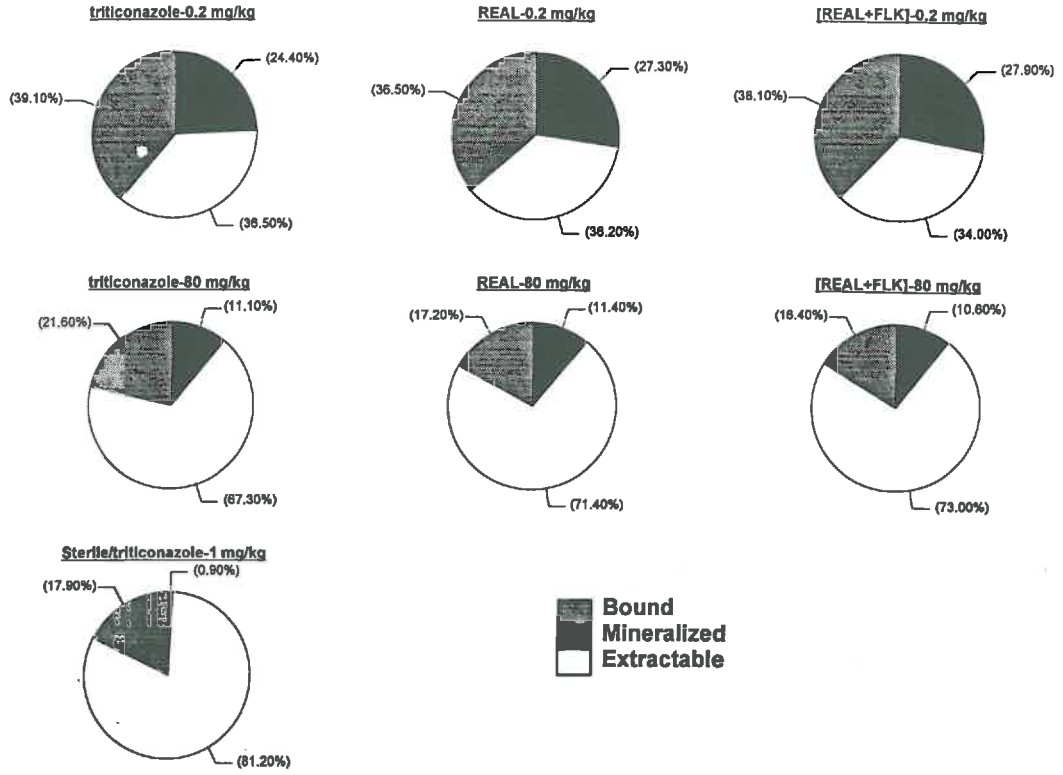

Fig. 2. Effect of treatment and application rate of triticonazole on the distribution of the ^{14}C-residues, after 150 days of incubation at 28°C. Extractable, bound and mineralized fractions are expressed as % of recovered radioactivity. Total recovery of the initial ^{14}C applied averaged 89.2 % in these samples.

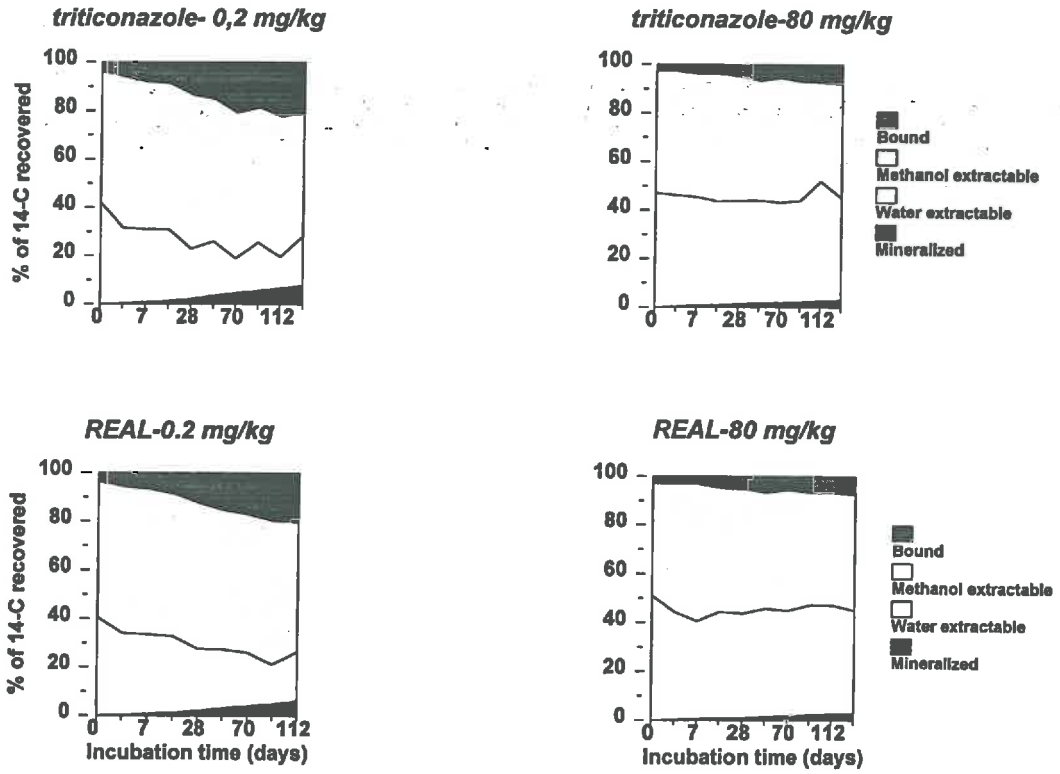

Fig. 3. Effect of treatment and application rate of triticonazole on the evolution of the distribution of the ^{14}C-residues during a 133 day incubation at 22 °C. Extractable, bound and mineralized fractions are expressed as % of recovered radioactivity. Total recovery of the initial ^{14}C applied averaged 95.7 % in these samples.

RESULTS

Triticonazole dissipation and evolution of ^{14}C-residues

Radioactivity balances between mineralized ^{14}C, extractable ^{14}C, and bound ^{14}C after 150 days of incubation at 28 °C (Fig. 2) showed different distribution patterns among the 0.2 and 80 mg kg^{-1} initial dose rates, while no differences were found between ^{14}C-triticonazole, ^{14}C-REAL, and ^{14}C-[REAL + FLK] treatments. For the low 0.2 application rate, ^{14}C-residues averaged 36, 26, and 38% in the extractable, mineralized, and bound fractions respectively. For the high application rate, the proportion of extractable residues was much more important, averaging 70%, whereas the mineralized and bound fractions were much lower, with respective averages of 18 and 12%. ^{14}C-residues from the sterile samples treated with ^{14}C-triticonazole at 1 mg kg^{-1} were mostly extractable (81%), while the bound residues attained 16%, and mineralization was negligible (< 1%). The evolution of the distribution of ^{14}C-residues at 22°C in function of incubation time (Fig. 3) was also dependent upon the initial dose rate whereas the addition of $(REAL)_{bl}$ blank formulation to the ^{14}C-triticonazole treatment did not change noticeably the results obtained. For both ^{14}C-triticonazole and [^{14}C-triticonazole + $(REAL)_{bl}$] treatments at the low dose, a bound fraction was rapidly formed, which increased continuously to attain almost 20% of the recovered radioactivity after 133 days of incubation. A continuous decrease in the extractable radioactivity was observed, from an initial 95% to 75% at the end of the incubation. This occured mainly in the water extractable fraction, which decreased from 40 to 10%. Mineralization also increased continuously, but remained low (< 5%). For the high dose rate, the bound and mineralized fractions also increased continuously, but to a lower extent, to attain respectively 8 and 3% of the applied ^{14}C. The extractable ^{14}C-residues decreased from 98 to 89%, but the proportion of water and methanol extractable residues remained constant, with approximately 50% of each.

Comparison of the extractable and bound ^{14}C-residues at the end of the incubations at 22°C (133 days) and 28°C (150 days, data not shown) indicated a strong effect of incubation temperature on the extractability of triticonazole residues. This was characterized in samples treated at 0.2 mg kg^{-1} by an important decrease in the extractable fraction (mean values among

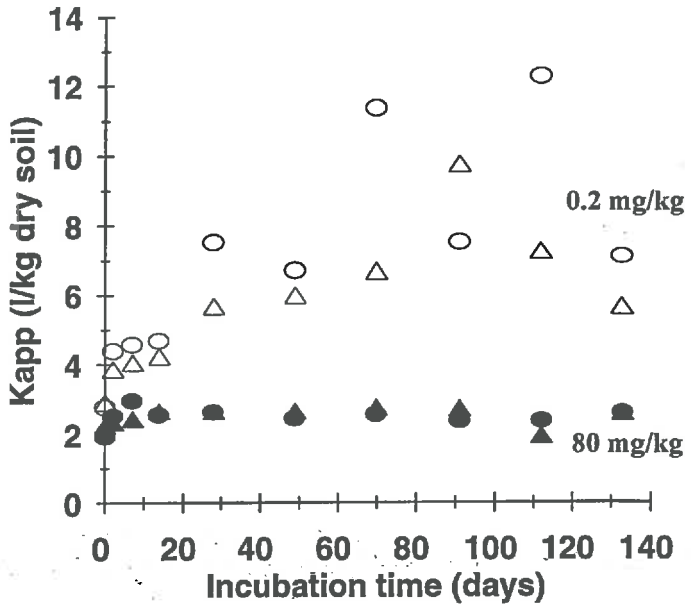

Fig. 4. Relationship between measured apparent desorption coefficients K_{app} and incubation time at 22°C, for treatments as ^{14}C-triticonazole (O) and ^{14}C-REAL (Δ), at 0.2 (empty symbols) and 80 mg kg^{-1} (plain symbols) application rates.

the treatments decreased from 72 to 36% of the recovered radioactivity) and an increase (from 21 to 36%) of the bound fraction with temperature increasing from 22 to 28°C. The same trends were observed for the high dose, to a lesser extent for the extractable residues, with a decrease from 89 to 71%, while bound residues increased from 8 to 17%.

The evolution of the proportion of water extractable residues with incubation time resulted in modifications of the apparent desorption coefficients K_{app} (Fig. 4). A time-dependence of the K_{app} for the low application rate treatments is revealed, while the K_{app} remained constant for the high dose treatment, with an average value of 2.2 l kg^{-1}. Results of K_{app} at 0.2 mg kg^{-1} increased with incubation time from an initial value of 2.2 l kg^{-1}, to values above 8 l kg^{-1}. Values close to the previously measured batch equilibrium K_d of 4.35 L kg^{-1} were rapidly attained, that were followed by a slower increase with time. No significant difference between ^{14}C-triticonazole and [^{14}C-triticonazole + $(REAL)_{bl}$] treatments was constated.

HPLC analysis (data not shown) revealed that the water and methanol extractable radioactivity of all the samples was mainly constituted of the parent compound (> 80%) with the presence of two unidentified metabolites in the same proportion. No significant effect of different application rate, temperature or formulation treatment was observed.

Modifications of the mineralization kinetics of triticonazole

Mineralization of triticonazole was strongly dependent on the application rate (Fig. 5). $^{14}CO_2$ evolved decreased from 9.5% to almost 2% when applied amount increased from 0.2 to 80 mg kg^{-1}. Triticonazole first-order mineralization constants k decreased for all treatments, with applied amount increasing from 0.2 to 80 mg kg^{-1} (Table 1). However, the absolute mass of triticonazole mineralized during the 49 days incubation period increased from approximately 0.2 µg at 0.2 mg kg^{-1} to 25 µg at 80 mg kg^{-1} for the three treatments. The zero order mineralization rates k_{zero}, which are expressed in absolute concentration per day, increased from 0.4 mg kg^{-1} d^{-1} to almost 50 mg kg^{-1} d^{-1} when applied dose increased from 0.2 to 80 mg kg^{-1}. All the measured parameters were similar at each dose for ^{14}C-REAL and ^{14}C-[REAL +

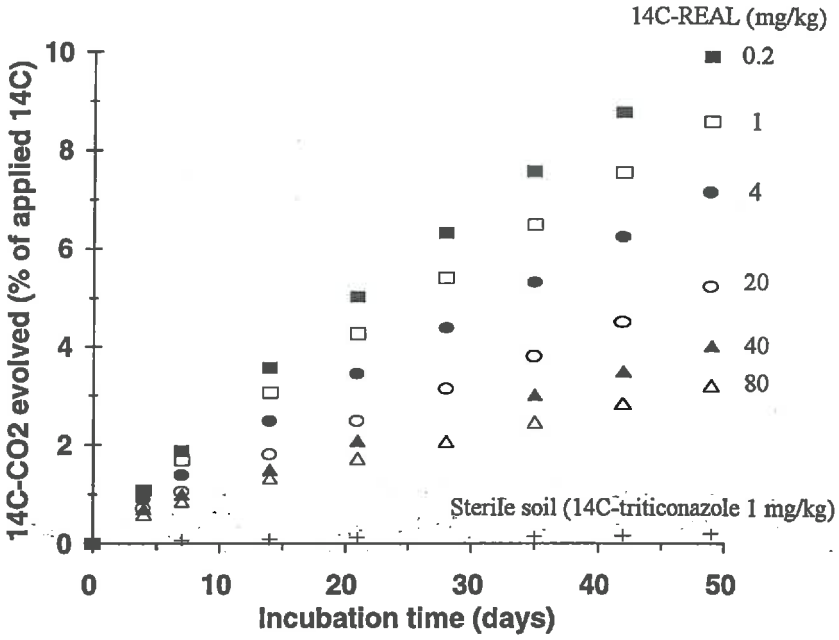

Fig. 5. Kinetics of mineralization at 28 °C of ^{14}C-REAL in relation to the application rate. The mineralization of ^{14}C-triticonazole at 1 mg kg^{-1} in the sterile soil samples is presented for comparison. Data points are average results of the triplicate incubations. Standard deviations are smaller than symbol size.

FLK] aqueous formulation treatments, while they were a little lower for triticonazole applied as methanol solution.

Applied triticonazole rate (mg kg^{-1} dry soil)	Mineralization constant k . 10^3 (d^{-1})*	Mineralization rate k_{zero} . 10^3 (mg kg^{-1} day^{-1}) *	Total % mineralized after 49 days	Mass mineralized after 49 days (µg)
^{14}C-triticonazole				
0.2	1.78 ± 0.02	0.33 ± 0.01	8.17	0.16
4	1.32 ± 0.02	4.95 ± 0.07	6.14	2.46
20	1.06 ± 0.02	19.74 ± 0.39	4.96	9.92
40	0.80 ± 0.01	29.73 ± 0.32	3.79	15.16
80	0.69 ± 0.03	48.73 ± 2.22	3.13	25.04
^{14}C-REAL				
0.2	2.23 ± 0.05	0.40 ± 0.01	9.89	0.20
1	1.85 ± 0.03	1.65 ± 0.04	8.60	0.86
4	1.56 ± 0.03	5.69 ± 0.13	7.15	2.86
20	1.12 ± 0.02	20.47 ± 0.47	5.22	10.44
40	0.65 ± 0.03	29.99 ± 0.43	4.01	16.08
80	0.71 ± 0.03	49.08 ± 2.46	3.22	25.76
^{14}C-[REAL + FLK]				
0.2	2.22 ± 0.04	0.40 ± 0.01	9.95	0.20
1	1.82 ± 0.03	1.62 ± 0.04	8.44	0.84
4	1.58 ± 0.03	5.82 ± 0.11	7.30	2.92
20	1.11 ± 0.02	20.17 ± 0.46	5.13	10.26
40	0.59 ± 0.05	29.83 ± 0.39	3.95	15.80
80	0.64 ± 0.03	44.77 ± 2.01	2.94	23.54

Tab. 1. Effect of treatment formulation and application rate on the first-order mineralization constants k, the zero-order mineralization rates k_{zero}, and on the amount of triticonazole mineralized after 49 days of incubation at 28° C.
*** Coefficient of determination r^2 were greater than 0.93 for all treatments**

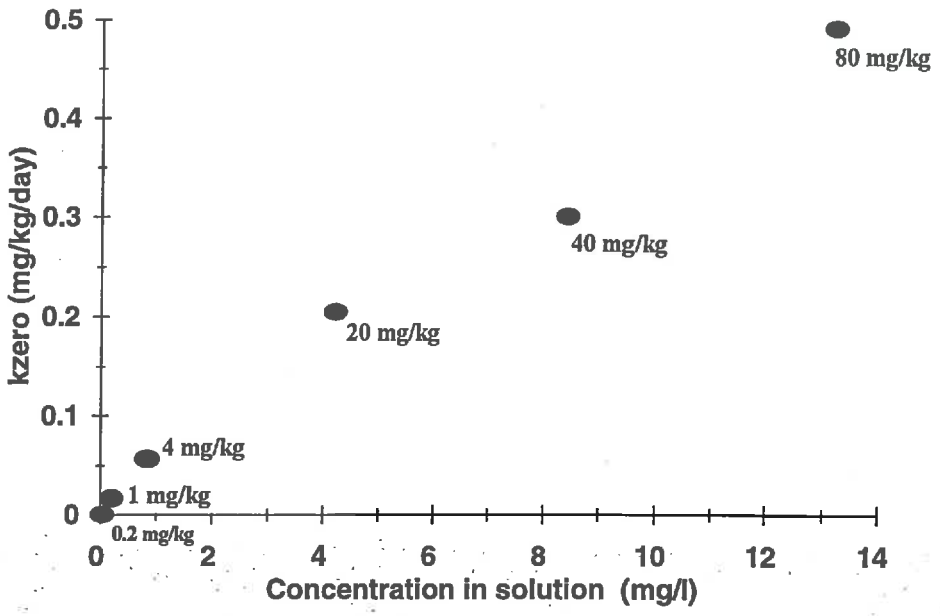

Fig. 6. Relationship between k_{zero} mineralization rates of REAL at 28°C and the concentration of triticonazole in soil solution [S] for the different application rates.

Triticonazole concentration in the soil solution [S] (in mg L^{-1}) can be estimated in function of the initial dose rate (D_{ini} in mg kg^{-1}), the water solubility of triticonazole (S_w = 13.2 mg l^{-1} at 28°C), and the soil/water equilibrium partition coefficient of triticonazole in Grignon soil (K_d = 4.35 l kg^{-1} Beigel *et al.*, 1997), using the following relations:

$$C_e = \frac{(x/m)}{K_d}, \text{ and } x/m = \frac{D_{ini} - C_e V}{m} \quad (7)$$

where C_e is triticonazole concentration in solution at equilibrium (mg l^{-1}), x/m is the amount sorbed (mg kg^{-1} dry soil), and V is the volume of soil solution (l), and

$$[S] = Ce \text{ if } Ce < K_s, \text{ and } [S] = S_w \text{ if } Ce \geq S_w \quad (8)$$

The estimated concentrations of triticonazole in soil solution would range from 0.04 mg l^{-1} for an application rate of 0.2 mg kg^{-1} to the water solubility of 13.2 mg l^{-1} at 80 mg l^{-1}. The absolute concentration mineralization rates k_{zero} were positively related to the concentration of triticonazole in soil solution (Fig. 6).

Temperature had a strong effect on the mineralization of triticonazole (Table 2). An increase from 22 to 28°C increased k mineralization constants by a mean of 2.95 folds. The difference between the k values among ^{14}C-triticonazole and ^{14}C-REAL treatments was only significative at the lowest dose and higher temperature. Activation energies (E_A) ranged from 115 to 157 kJ mol^{-1} (27.6 to 37.7 kcal mol^{-1}). The differences between the E_A values for the four treatments were not significant.

Treatment	Mineralization constant . 10^3 (d^{-1})	Regression coefficient (r^2)	Activation energy E_A (kJ mol^{-1})
^{14}C-triticonazole (0.2 mg kg^{-1})			
28 °C	1.78 ± 0.02	0.997	
			128 ± 28
22 °C	0.64 ± 0.02	0.963	
^{14}C-REAL (0.2 mg kg^{-1})			
28 °C	2.23 ± 0.05	0.988	
			158 ± 35
22 °C	0.62 ± 0.02	0.959	
^{14}C-triticonazole (80 mg kg^{-1})			
28 °C	0.69 ± 0.03	0.948	
			116 ± 35
22 °C	0.27 ± 0.02	0.720	
^{14}C-REAL (0.2 mg kg^{-1})			
28 °C	0.71 ± 0.03	0.927	
			129 ± 38
22 °C	0.25 ± 0.02	0.718	

Tab. 2. Effect of temperature on triticonazole first-order mineralization constants k for ^{14}C-triticonazole and ^{14}C-REAL treatments at 0.2 and 80 mg kg^{-1} application rates, and corresponding activation energies E_A calculated from the relation of Arrhenius.

Comparison of dissipation and mineralization kinetics

Mineralization kinetics appeared very slow whereas dissipation kinetics were much quicker (Fig. 7). The first-order degradation model allowed for a good description of the mineralization experimental data at 0.2 mg kg^{-1} ($k = 0.64 \pm 0.02\ 10^{-3}\ d^{-1}$, $r^2 = 0.96$) whereas it exhibited a poor fit to the mineralization data at 80 mg kg^{-1} ($k = 0.27 \pm 0.02\ 10^{-3}\ d^{-1}$, $r^2 =$

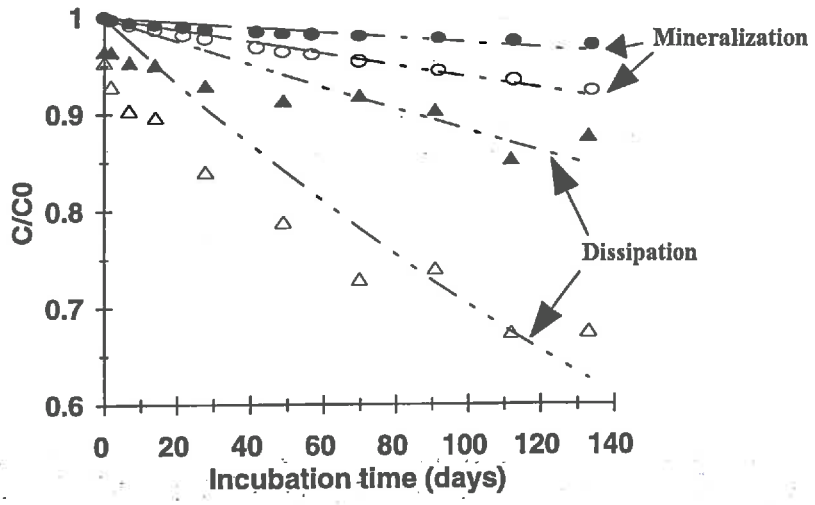

Fig. 7. Description of triticonazole mineralization (O) and dissipation (Δ) at 22°C by the first-order model, for ^{14}C-triticonazole treatments at 0.2 (empty symbols) and 80 mg kg^{-1} (plain symbols) application rates. Model fits are represented as dotted lines for the different treatments.

0.72), and to the dissipation results at both doses ($k = 3.52 \pm 0.30\ 10^{-3}\ d^{-1}$, $r^2 = 0.77$ at 0.2 mg kg^{-1}, and $k = 1.25 \pm 0.15\ 10^{-3}\ d^{-1}$, $r^2 = 0.43$ at 80 mg kg^{-1}), with large discrepancies appearing in the early stages of the incubation. From these results, no clear correlation could be determined among the mineralization and dissipation kinetics.

DISCUSSION

General trends of triticonazole degradation

Under optimal conditions of soil microbial activity (28 °C and 80 % field capacity), triticonazole mineralization in Grignon soil, appears very slow, with first-order mineralization constants ranging from 0.7 to 2.2 $10^{-3}\ d^{-1}$ in the same range as the mineralization constants of other persistent pesticides reported in the literature (Parker and Doxtader, 1983; Simon *et al.*, 1992; Ostrofsky *et al.*, 1997), indicating high stability of the molecule and little cleavage of the benzyl radiolabeled ring. This is in accord with previous results of Patil *et al.* (1988), showing triazole fungicides are very stable in soil. The negligible mineralization and metabolization of triticonazole in the sterile soil samples indicates that triticonazole degradation in soil resulted mainly from microbial transformation. The slow and linear kinetics of mineralization indicate a cometabolic degradation pathway. This is confirmed by the results of Charnay *et al.* (unpub. 1997), who showed that the addition of glucose and other easily metabolizable compounds enhanced the mineralization of triticonazole.

The extend of the positive influence of temperature is consistent with other published results, reporting an increase about 1.5-3 folds with each 5°C increase (Walker *et al.*, 1992; Helweg, 1993). Triticonazole E_A values for mineralization (mean: 130 kJ mol^{-1}) are relatively high compared to the degradation E_A in soils reported in the literature, which ranged from 50 to 80 kJ mol^{-1} for most pesticides (Helweg, 1987, 1993; Jolley and Johnstone, 1994; Veeh *et al.*, 1996). However, Vink and van der Zee (1996) also reported an E_A of 127 kJ mol^{-1} for the degradation of metamitron, which they related to high persistency in soil. High E_A are usually thought to reflect chemical mechanisms while low values are usually found for biological,

enzyme-catalysed reactions. High E_A may thus reflect an abiotic rate-limiting step in the mineralization of triticonazole, while the other steps would occur through biological means.

The availability of triticonazole was greatly affected by residence time in soil, as the distribution of ^{14}C-residues between water and methanol extracts gradually changed. Water-extractable residues represent the more readily available residues, while methanol extractable residues account for the potentially available residues, and bound residues are nonavailable. The stabilization of pesticides in soil as bound residues has often been related to biological activity (Benoit and Barriuso, 1997; Hundal *et al.*, 1997). The proportion of bound residues of triticonazole is also positively related to the extent of its mineralization, with more bound residues formed at the higher temperature and the lower application rates. However, the formation of a significant fraction of bound residues in the sterilized soil samples suggests that this is not only a biological process. Decreased extractability, and formation of bound residues resulted in a time-dependent, nonequilibrium sorption of triticonazole, as evidenced by the increasing desorption coefficient K_{app}. We observed similar changes in the sorption characteristics of triticonazole during diffusion studies in sterile soil, which we attributed to intra-organic matter diffusion, and trapping into restricted soil sites (Beigel *et al.*, 1997). Measurement of apparent desorption K_{app} would be a good indicator of the overall bioavailability of a pesticide to plants and microorganisms, which is related to its presence in the soil solution. Bioavailability in soil is of particular importance concerning the persistence, the bioactivity and the efficacy of soil-applied systemic pesticides. Triticonazole bioavailability apparently increases with increasing application rate, and decreases with prolonged soil contact time.

Modifications of triticonazole behaviour in relation to the conditions of application

The similarity of the results obtained for the different treatments, ^{14}C-triticonazole, ^{14}C-REAL, [^{14}C-triticonazole + $(REAL)_{bl}$], and [^{14}C-REAL + FLK] suggests that, in the range of application rates studied, the addition of formulation adjuvants, and of Soprophor FLK anionic surfactant in particular, had no significant effect on triticonazole bioavailability and on the growth and activity of the soil microorganisms degrading triticonazole. This is in accord with

the results of Jolley and Johnstone (1994), who compared the degradation of formulated and unformulated trifluralin, and found only small differences depending on the soil characteristics. On the other hand, Charnay *et al.* (unpub. 1997) observed significant modifications of the mineralization of triticonazole with addition of the commercial formulation adjuvants at high doses. Also, surfactants were reported to affect the degradation of pesticides (Rouse *et al.*, 1994), as some surfactants can enhance the availability of poorly water soluble compounds, while others, and in particular anionic surfactants, may prove toxic to soil microflora (Laha and Luthy, 1992). However, these effects mainly result from micellar interactions in the soil solution, and were reported for high doses of surfactants. When small amounts of formulation adjuvants are applied to the soil, as it is mostly the case for normal pesticide agricultural use, their effect on the dissipation of the active ingredient may be negligible.

The main action of formulation adjuvants is to allow the application of pesticides in a water solution at rates largely higher than the pesticide water solubility. The initial dose rate of treatment appeared to significantly affect the overall fate of triticonazole in Grignon. Persistence of triticonazole increased with increasing dose, while the proportion of triticonazole available also increased as the water extractability of the residues increased, and less bound residues were formed. Gan *et al.* (1995, 1996) reported similar dependence upon initial concentration for alachlor and atrazine dissipation in soils. The similar activation energies for triticonazole mineralization we observed at 0.2 and 80 mg kg^{-1} application rates suggest that the increased persistence at elevated levels would not arise from differences in degradation pathways. Limited water solubility of triticonazole and high sorption are more likely to control here the dissipation processes with regard to the applied amount. The linear positive relation observed between the absolute mineralization rates and the estimated concentration in soil solution suggests that the availability of triticonazole in the soil solution is the main limiting factor to triticonazole mineralization. As previously observed, triticonazole is strongly sorbed by soil constituents, which would significantly reduce its concentration in soil solution, and thus limit its bioavailability to the degrading microorganisms. On the other hand, the excellent fit to zero-order kinetics ($r^2 > 0.98$) suggests that the amount of triticonazole available for biodegradation is constant during the time course of the incubations, as pointed

out by Schmidt *et al.* (1985). Thus, rate-limiting desorption and formation of bound residues, which resulted in an increase in K_{app} with residence time that was observed at the lower application rate, seems not to significantly alter the mineralization rate of triticonazole. For the higher application rate, only a fraction of triticonazole would be directly available in solution, while a continued supply of triticonazole would be provided by dissolution of solid-phase cristalline triticonazole. Indeed, the constant K_{app} of 2.2 l kg^{-1} observed at 80 mg kg^{-1} indicates a continuous supply from solid-phase triticonazole allowed for a constant concentration close to the S_w of triticonazole.

Validity of the first-order constants for modeling triticonazole degradation

Based on the Monod approach, which implies that the rate of bacterial growth and therefore the rate of substrate utilization are determined by the concentration of the substrate, first-order kinetics would reflect the degradation of low doses of substrate by non growing microorganisms (Schmidt *et al.*, 1985). However, in complex systems such as our experimental design, where complete mineralization by soil microflora communities is to be considered, and where rate-limiting desorption, solid-phase substrate dissolution, and presence of formulation additives may interact, the measured kinetics would not be significant in terms of simple enzymatic reactions. Indeed, the estimated mineralization constants k of triticonazole varied with the applied dose rate, which is contradictory to enzymatic first-order kinetics, and thus k need to be considered as empirical. The first-order and zero-order models showed comparable fits to the mineralization data at 28°C, since the mineralization kinetics appeared essentially linear at the time-scale of the experimental incubations, with only little variations in relative concentrations. Nevertheless, the first-order model is usually preferred and widely used for modeling purposes, and first-order constants are thus needed to describe the degradation of triticonazole.

First-order kinetics are widely used to describe the dissipation of pesticides in soils, and the literature abounds with dissipation first-order constants and first-order half-lives of pesticides, that are to be used in transport models (Patil *et al.*, 1988; Walker *et al.*, 1992; Yen *et al.*, 1994; Jolley and Johnstone, 1994). However, the dissipation of triticonazole in Grignon

soil was not satisfactorily described by the first-order model. The significative discrepancies between fitted and experimental data mostly arised from the immediate formation of a fraction of triticonazole bound residues. Incorporation of triticonazole first-order dissipation constants in transport models would thus prove erroneous. Mineralization appears to be a good approximate of the biodegradation of triticonazole, as the proportion of metabolites in the extracts was always very low. Under these conditions, the stabilization of triticonazole residues in soil with prolonged residence time needs to be introduced in the transport models. Since the formation of triticonazole bound residue most likely arises from a rate-limiting diffusion process, it may be accounted by a time-dependent desorption coefficient K_{app}, as proposed by Walker (1987). Multicompartments transport models accounting for sorption nonequilibrium may also prove satisfactory to describe the transport and dissipation of triticonazole in soils.

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CHAPITRE 4

Dynamique de la rétention du triticonazole dans le sol et conséquence sur la diffusion

Time Dependency of Triticonazole Fungicide Sorption and Consequences for Diffusion in Soils

C. Beigel, E. Barriuso and L. Di Pietro (1997)

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Time Dependency of Triticonazole Fungicide Sorption and Consequences for Diffusion in Soil

C. Beigel, E. Barriuso and L. Di Pietro *

ABSTRACT

Solute transport by diffusion is a key process regarding the redistribution in soils of systemic pesticides applied in seed treatments. In this study we investigated the influence of nonequilibrium sorption on the diffusivity of triticonazole fungicide [(1RS)-(E)-5-(4-chlorophenyl-methylen)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol] in a loam clay soil from Grignon, France (typic Eutrochrept). Triticonazole diffusion coefficients were measured in saturated soil columns for time periods ranging from 1 to 25 days. Nonequilibrium sorption was evaluated from measurement of apparent desorption coefficients in the diffusion columns, and from estimation of apparent partition coefficients from a diffusivity model. Triticonazole availability during diffusion was also evaluated based on successive aqueous and methanol extractions, and measurement of the nonextractable residues fraction. Triticonazole diffusion in soil was characterized by a strong time-dependence, with apparent diffusion coefficient decreasing from 0.05 $cm^2 d^{-1}$ at 2 days to 0.02 $cm^2 d^{-1}$ at 25 days. Triticonazole apparent sorption coefficient increased with time of diffusion, from 5 L kg^{-1} at 1 day to 10.5 L kg^{-1} at 25 days, which was greater than the batch equilibrium value of 4.35 L kg^{-1}. The measured and calculated results were very close, showing that nonequilibrium sorption was the key process responsible of the decrease in triticonazole apparent diffusion rate. The availability of the diffusing residues diminished constantly with time. The water-extractable fraction corresponding to the more readily available residues decreased from 31 % at 1 day to 17 % at 25 days, while a resistant fraction of nonextractable residues was rapidly formed representing 9 % after 25 days. Triticonazole slow desorption behaviour was attributed to rate-limiting intrasorbent diffusion into restricted sorption sites.

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INTRODUCTION

Pesticide diffusion in soils is a slow process compared to mass flow, but it is the only means of pesticide transport under static water conditions, and it may prove significant when approaching equipotential soil matrix conditions. Also, the contribution of diffusion to movement may be relatively important for poorly water soluble compounds that are less available for transport by mass flow. The diffusion of pesticides in soils depends closely upon the physical conditions of the diffusing media. Diffusion coefficients increase with increasing temperature and soil water content, and decrease with increasing soil bulk density (Ehlers et al., 1969; Graham-Bryce, 1969; Ritter et al., 1973; Scott and Paetzold, 1978). However, the key process affecting pesticide diffusion in soils is sorption on the soil matrix (Walker and Crawford, 1970; Jacques and Harvey, 1979). Moreover, even if the diffusion coefficients in water among different pesticides vary little (Lavy ,1970; Scott and Phillips, 1973), differences in the sorption characteristics between the molecules may imply drastic variations among their diffusion coefficients in soil.

Sorption of non polar hydrophobic pesticides is directly related to soil organic matter (Ainsworth et al., 1989) mainly through non specific interactions like hydrophobic bonding or partitioning. Although usually regarded as instantaneous and reversible for modelling purposes, sorption may in fact require weeks to months to reach equilibrium (Pignatello and Xing, 1996). Moreover, sorption/desorption processes have been demonstrated to be partially nonreversible in many cases, with changes in sorption characteristics occurring when the residence time of the pesticide in soil increases (Lehman et al., 1990; Barriuso et al., 1992). This can be interpreted by intra-organic matter diffusion and trapping in soil colloidal structures (Brusseau and Rao, 1989). One consequence is the decrease in extractability of the pesticide residues with time, and the formation of nonextractable residues which seems directly related to soil organic matter, including non humified and humified constituents (Barriuso and Koskinen, 1996).

Pesticide sorption on soils are customarily described by equilibrium sorption coefficients obtained in the laboratory using batch equilibrium experiments. However, long term

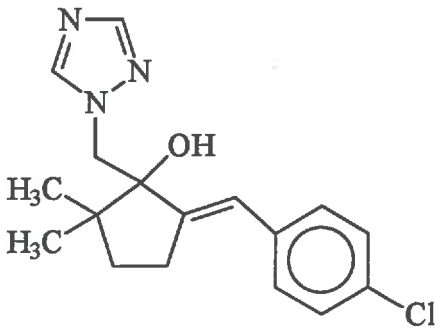

Fig. 1 Structure of triticonazole.

experiments have demonstrated that sorption coefficients are not constants and increase with time (Lehmann et al., 1990; Scribner et al, 1992). The use of sorption coefficients from batch experiments to assess pesticide transport and availability when nonequilibrium and/or nonreversible sorption occur could lead to wrong predictions. This may be particularly relevant when regarding a slow process like pesticide diffusion in soils.

Triticonazole is a new triazole systemic fungicide developed by Rhône-Poulenc Agrochemicals Company, that is used in cereal seed treatment. Seed treatment combines disinfection of the seeds with differed, long-term protection of the plants. The extreme localization of the deposits on the target allows for reduced rate doses, thus providing a cost effective and less environmentally harmful technique. The systemic mode of activity of the pesticides implies that the efficacy of the treatment depends strongly on the rate of supply to the plant roots relative to the requirements for optimum protection. Once introduced in the soil, the pesticide is progressively released from the coating into the soil matrix where it moves by diffusive-convective flow retarded by sorption. The amount of systemic active material that may be available to plant roots absorption is therefore closely related to its transport and retention characteristics in the soil. Detailed knowledge of the mobility of such pesticides in soil is of crucial interest for a successful utilization in seed treatments.

The aims of the present work were (1) to estimate the influence of sorption as a factor limiting triticonazole mobility in soil, (2) to evaluate realistic sorption parameters of triticonazole from different approaches, and (3) to estimate the changes in triticonazole sorption characteristics with time and their consequences on its mobility by diffusive transport.

MATERIALS AND METHODS

Chemical

Carbon 14-U-benzyl-labeled triticonazole (Fig. 1) (specific activity: 1184 MBq $mmol^{-1}$; radiopurity > 98 %) was provided by Rhône-Poulenc Agrochemicals Company, Lyon, France. Triticonazole water solubility is 8.4 mg L^{-1} at 20°C, vapor pressure is < 10^{-8} hPa at 50°C, and distribution coefficient between octanol and water is 1950. Water solutions of ^{14}C-triticonazole

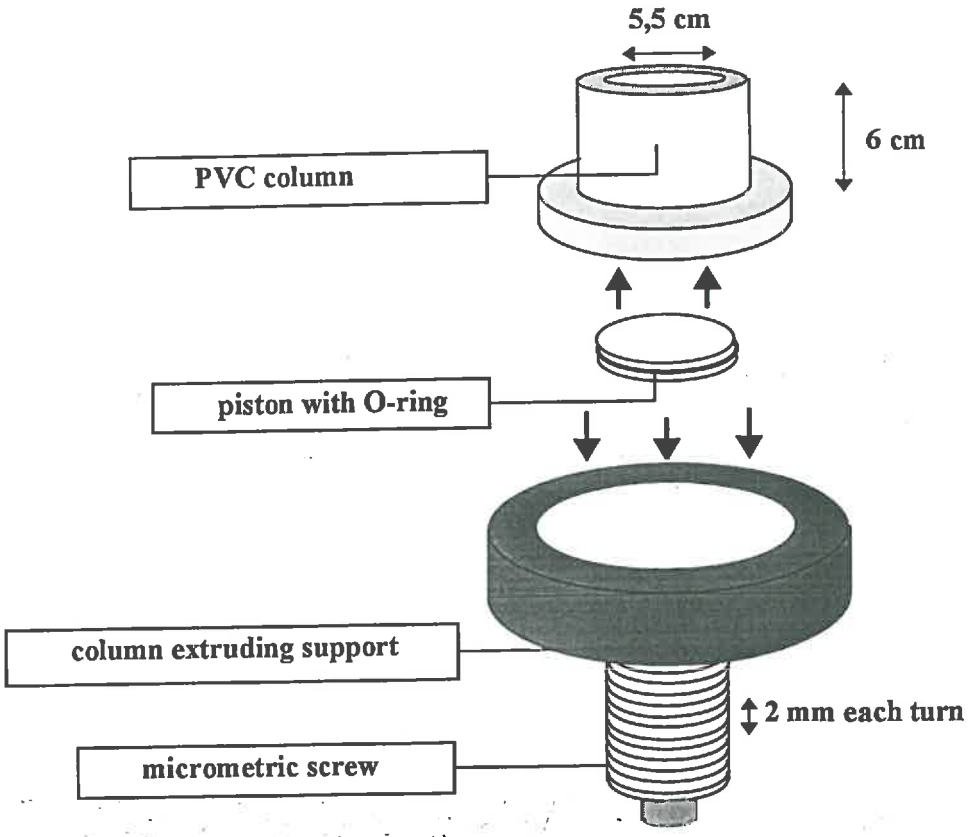

Fig. 2. Schematic layout of the columns soil extruding procedure.

were prepared at 5 different concentrations: 0.5, 1.0, 2.0, 3.0 and 5.0 mg L^{-1} by diluting 50 µL of the ^{14}C-triticonazole methanol stock solution in different volumes of a saturated water solution of triticonazole (analytical standard, purity > 92 %), and adjusting the concentration with water. The solution concentrations were measured at 262.5 nm with a U.V.-visible spectrophotometer Lambda V (Perkin-Elmer, Überlingen, Germany).[1]

Soil

The soil (typic Eutrochrept) was sampled in the surface layer (0-20 cm) of a continuous wheat experimental plot in Grignon (France). It had a pH in water of 8.2, with (g kg^{-1} of dry soil): 291 of clay, 540 of silt, 145 of sand, 24 of lime and 10.4 of organic C. Soil samples were air dried, passed through a 2-mm sieve and sterilized by gamma irradiation (30 kGy for 20 h).

On the other hand, Fontainebleau pure sand with a particle size of 150-210 µm (Prolabo, Fontenay-sous-bois, France) was used as a non reactive media for diffusion studies.

Incubations and experimental set-up for diffusion experiments

Incubation experiments allowing one-dimensional triticonazole diffusion studies were performed in columns packed with soil and with sand in water saturated, isotropic conditions. A piston at the base of the 60 mm long by 55 mm diam. PVC columns was used to extrude incremental sections of the soil for sequential sampling at the end of the experiment (Fig. 2; Calvet, personal communication). To obtain a homogeneous saturated media, 30 mL of water were initially put into the columns, and then successive layers of soil or sand were added while continuously gently shaking to allow mixing of the layers, degasification and homogenization. For the top layers the excess water was removed while little amounts of soil were added carefully in order to remain at saturation. The columns were then covered with plastic sheets to avoid evaporation, and allowed to equilibrate for 4 days at 22 ± 2 °C before treating with triticonazole.

[1] Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the product listed by INRA.

The homogeneity of the packed columns was tested for water content and bulk density by measuring them in each of 20 successive sections of 2-mm length. Tests were done with 8 replicates from sand and soil columns. The gravimetric water content (w/w) was 0.21 ± 0.01 and 0.55 ± 0.01, the volumetric water content (v/v) was 0.36 ± 0.01 and 0.56 ± 0.01, and the dry bulk density (Mg m^{-3}) 1.70 ± 0.02 and 1.02 ± 0.01, respectively for sand and soil columns. Columns were prepared for diffusion measurements after 1, 2, 3, 4, 6, 9, 12, 16, 20 and 25 days of incubation with two replicates per date. Treatment was performed by spraying 0.5 mL of the water solution of ^{14}C-triticonazole at 5 mg L^{-1} on the top of the columns, and the columns were once again covered with plastic sheets. At each date, the top 3 cm of the soil or sand of the columns were sectioned into 15 discs of 2-mm thickness using the micrometric screw extrusion procedure. The last 3 cm of the soil or sand remaining in the column were also collected for ^{14}C-balance. The samples were then weighed and stored at -20°C until analysis. For the sand experiment, triticonazole in each section of the columns was extracted with 20 mL of methanol. After 24 h shaking, the radioactivity in the extracts was measured by liquid scintillation with a Kontron Betamatic V counter (Kontron Ins., Montigny le Bretonneux, France). For the soil experiment, 2 g were taken from the soil sections and air dried, total radioactivity was then measured by scintillation counting of the $^{14}CO_2$ evolved (Sample Oxidiser 307, Packard, Meriden, CT, USA).

Estimation of diffusion coefficients

To estimate triticonazole diffusion coefficients in the columns, we used an analytical solution of Fick's second law to the particular case of one-dimensional solute diffusion in a semi-infinite cylinder, homogeneous isotropic media and instantaneous plane source (Crank, 1975). The residual concentration in the media (C, µg g^{-1}) is expressed as a function of depth (x, cm) and time (t, days) by:

$$C = \frac{M}{\sqrt{\pi D t}} e^{-x^2/4Dt} \qquad [1]$$

where D is the diffusion coefficient (cm^2 d^{-1}) assumed to be constant in the concentration range of the experiment, and M the amount of applied chemical by unit of cross section (µg cm^{-2}).

We calculated triticonazole diffusion coefficients in sand and in soil at each date by fitting the experimental results to the equation [1] using a nonlinear regression least-square method.

The diffusion coefficients of solutes in soils are commonly expressed as a function of the diffusion coefficients in free solution, D_0. Scott and Phillips (1973) defined the effective diffusion rate (D_{eff}) and the apparent diffusion rate (D_{app}) for chemical diffusion in a nonreactive and an adsorbing homogeneous media respectively:

$$D_{eff} = D_0 \lambda \theta_v \qquad [2]$$

$$D_{app} = \frac{D_0 \lambda \theta_v}{(K_d \rho_d + \theta_v)} \qquad [3]$$

where λ is the tortuosity factor, K_d is the linear partition coefficient (L kg^{-1}) for reversible instantaneous equilibrium sorption, θ_V is the volumetric water content (L L^{-1}), and ρ_d is the dry bulk density (Mg m^{-3}).

Sorption/desorption studies by batch equilibration

A classical batch equilibrium procedure was used to obtain triticonazole sorption and desorption isotherms at 22°C. Ten milliliters of ^{14}C-triticonazole water solutions were added to 5 g of air dried soil in 20 mL glass centrifuge tubes with Teflon caps. After shaking for 24 h, the samples were centrifuged at 8000 g for 15 min, and triticonazole concentration in solution Ce_{ads} was calculated from the supernatant radioactivity measurements by scintillation counting. The amount of triticonazole sorbed was determined by difference between the initial and equilibrium concentrations in solution. The whole experiment was replicated twice.

To measure desorption, the supernatant after sorption corresponding to the initial solution of highest concentration (5 mg L^{-1}) was removed and replaced by the same volume of water. The tubes were then vibrated to disperse the soil pellet, and the suspensions were shaken for 24 h and centrifuged as previously described. The amount of triticonazole desorbed was measured in the supernatant, and triticonazole concentration in solution Ce_{des} was calculated . This desorption cycle was repeated 6 times to obtain the desorption isotherm.

The sorption and desorption isotherms were plotted as the amount of pesticide sorbed (x/m, mg per kg of dry soil) versus the equilibrium concentration Ce (mg L^{-1}).

Freundlich empirical parameters Kf and nf were estimated by fitting the Freundlich equation:

$$x/m = Kf\, Ce^{nf} \quad [4]$$

to the experimental results using a least-square nonlinear regression method.

The linear sorption partition coefficient Kd was calculated by linear curve fitting following the equation:

$$x/m = Kd\, Ce_{ads} \quad [5]$$

Finally, the K_{OC} was calculated from the relationship:

$$K_{OC} = Kd\ (\text{fraction of soil organic C})^{-1} \quad [6]$$

Residues extractability and measurement of apparent desorption coefficients

The distribution of the ^{14}C-triticonazole residues between water-extractable, methanol-extractable and nonextractable (bound) fractions was also measured in the soil columns. Soil samples (7.5 g) of each section from the first centimeter of columns (corresponding to 5 g dry soil) were first extracted with 7.5 mL of water. After 24 h shaking and centrifugation (15 min at 8000 g), the radioactivity content in the water extract was measured by liquid scintillation. The soil was then extracted three successive times with 10 mL of methanol using the same procedure as for the water extraction, and the radioactivity content in the methanol extracts was measured by liquid scintillation. The addition of the ^{14}C-residues extracted in water and in methanol gave the extractable residues fraction. The soil containing the nonextractable ^{14}C-residues was air dried, and its radioactivity content measured.

The procedure for water extraction of the ^{14}C-residues was similar to that used to measure sorption and desorption by the batch technique. We calculated an apparent desorption coefficient (Kapp) for each soil sample:

$$Kapp = x/m\, C_{des}^{-1} \quad [7]$$

where x/m is the ^{14}C-triticonazole in the methanol extracts plus the ^{14}C of the nonextractable residues, and C_{des} is the concentration of ^{14}C-triticonazole in the water extracts. Because the soil/solution ratio, equal to 1:2, used in the water extraction procedure is identical to the ratio

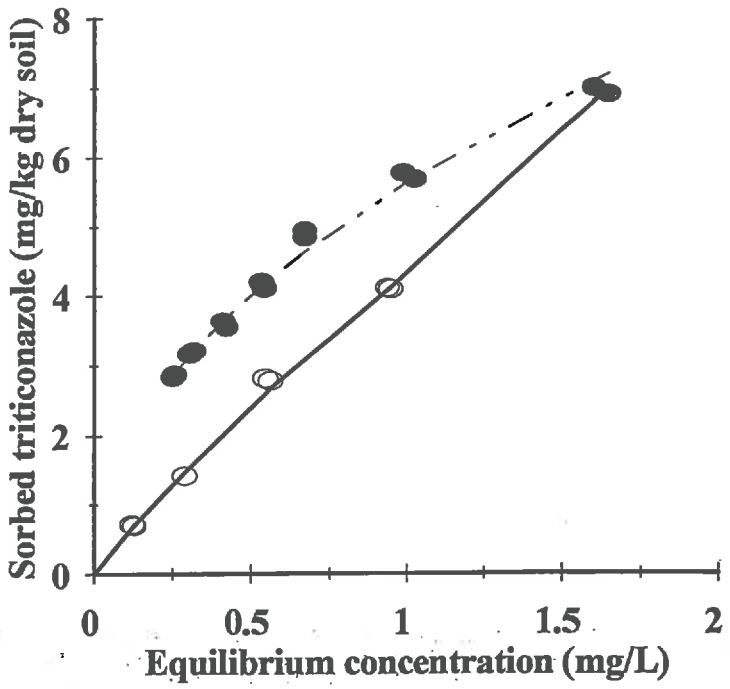

Fig. 3. Freundlich adsorption (open symbols) and desorption (closed symbols) isotherms for triticonazole in Grignon loamy clay soil.

used during sorption/desorption experiments with the batch technique, Kapp and Kd from equation [5] can be compared.

Results of Kapp and extractability measurements were expressed as mean values with respect to depth to allow comparison between columns of varying diffusion time.

RESULTS AND DISCUSSION

Batch equilibrium sorption

Triticonazole sorption/desorption isotherms on Grignon soil fitted to the Freundlich equation are presented in Fig. 3. The sorption isotherm was characterized by a high Kf of 4.45 ± 0.20 indicating strong sorption and a nf of 0.90 ± 0.02 close to unity, indicating mostly non specific interaction mechanisms. Since triticonazole is a nonpolar, hydrophobic organic molecule, those mechanisms would be most likely hydrophobic bonding / solute partitioning on soil organic matter. Consequently linearization of the isotherm and use of the linear partition coefficient Kd to characterize the equilibrium sorption of triticonazole was justified. A Kd value of 4.35 ± 0.08 L kg^{-1} was calculated, which corresponds to a K_{OC} value of 418 L kg^{-1}. Similar K_{OC} values were obtained for sorption of triticonazole on different English soils (Rhône-Poulenc, personal communication), thus supporting the hypothesis of organic matter-related sorption.

The triticonazole desorption isotherm was nonlinear and exhibited a significant positive hysteritic behavior, with fitted Freundlich desorption values Kf and nf of 5.62 ± 0.18 and 0.49 ± 0.01 respectively. Both the higher desorption Kf and the lower nf compared to sorption indicated a lower availability for the pesticide to desorb. Nonreversibility of the sorption/desorption process has been observed with numerous compounds (Singh et al., 1989; Bouchard and Lavy, 1985). It is usually attributed to possible differences in the rates of sorption and desorption, and nonattainment of equilibrium during desorption. Measured desorption Kf and nf may also depend on the amount of pesticide initially sorbed (Calvet, 1989; Barriuso et al., 1992), and on the residence time in soil of aged residues (Ma and Selim, 1994; Scribner et al., 1992).

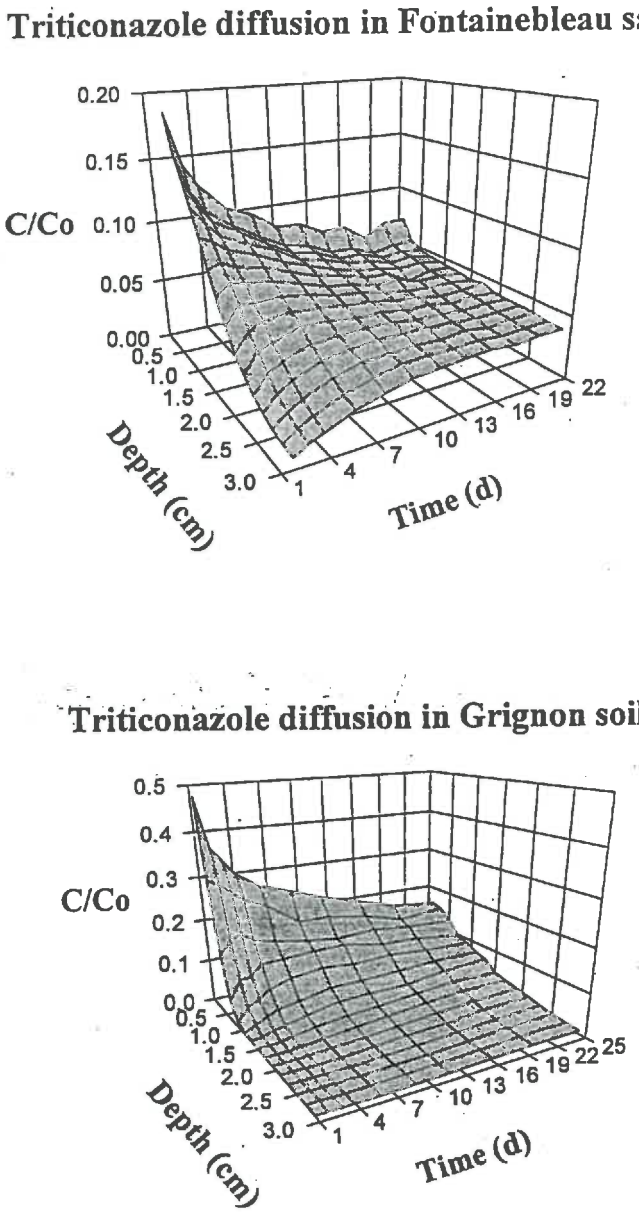

Fig. 4. Relative triticonazole concentration C/C_0 as a function of time and depth for diffusion in water-saturated sand (4a) and soil (4b) columns. The concentration scale for diffusion in soil is 2.5 times that for diffusion in sand.

Diffusion profiles

Plots of triticonazole normalized concentration relative to the initial amount applied in the media, C/C0, versus depth and incubation time show that triticonazole diffusion was rather quick in Fontainebleau sand compared to Grignon soil (Fig. 4). Diffusion in sand was characterized by a rapid homogenization of the relative concentration in the column, with a concentration gradient smoothing rapidly. After 2 days, triticonazole had already spread out to a 2.1 cm depth. At the end of the experiment (25 days), it was completely redistributed in the column, with a nearly homogeneous concentration throughout the column. In contrast, diffusion was much slower in soil with a diffusion profile presenting strong concentration gradients even after 25 days of incubation. Normalized concentration values were noticeably higher than in sand for the top soil section samples, indicating reduced mobility, with a major part of the residues remaining near the source. After 2 days, the amount of triticonazole was negligeable below a 1.1 cm depth. At the end of the experiment, the diffusion front had merely reached the 3 cm depth. The higher water content and lower bulk density in the soil compared to the sand would both tend to favor diffusion in the soil media rather than in sand. The strongly reduced diffusion observed in soil most probably illustrated the fundamental role of sorption as a retardation process decreasing the proportion of diffusible molecule in solution and thus limiting the transport of the solute. This would be in complete accordance with the high value of the measured triticonazole partition coefficient.

The plane source semi-infinite cylinder analytical model fitted well to the experimental results, and the model calculations provided accurate description of the diffusion profiles at each time (Fig. 5). The semi-infinite assumption was rapidly violated in the sand columns because of the rapid homogenization of triticonazole concentration relative to the diffusion times of the experiment, but it did not seem to perturbate the model predictions. A slight divergence was observed at the top of the columns corresponding to a 0 to 0.3 cm depth. This difference became more important as the incubation time increased. This was attributed to water evaporation occuring in spite of the plastic covers as verified by moisture content measurements. However, residuals of fitted versus experimental data were normally distributed, and thus we could assume that the fitted diffusion coefficients were relevant.

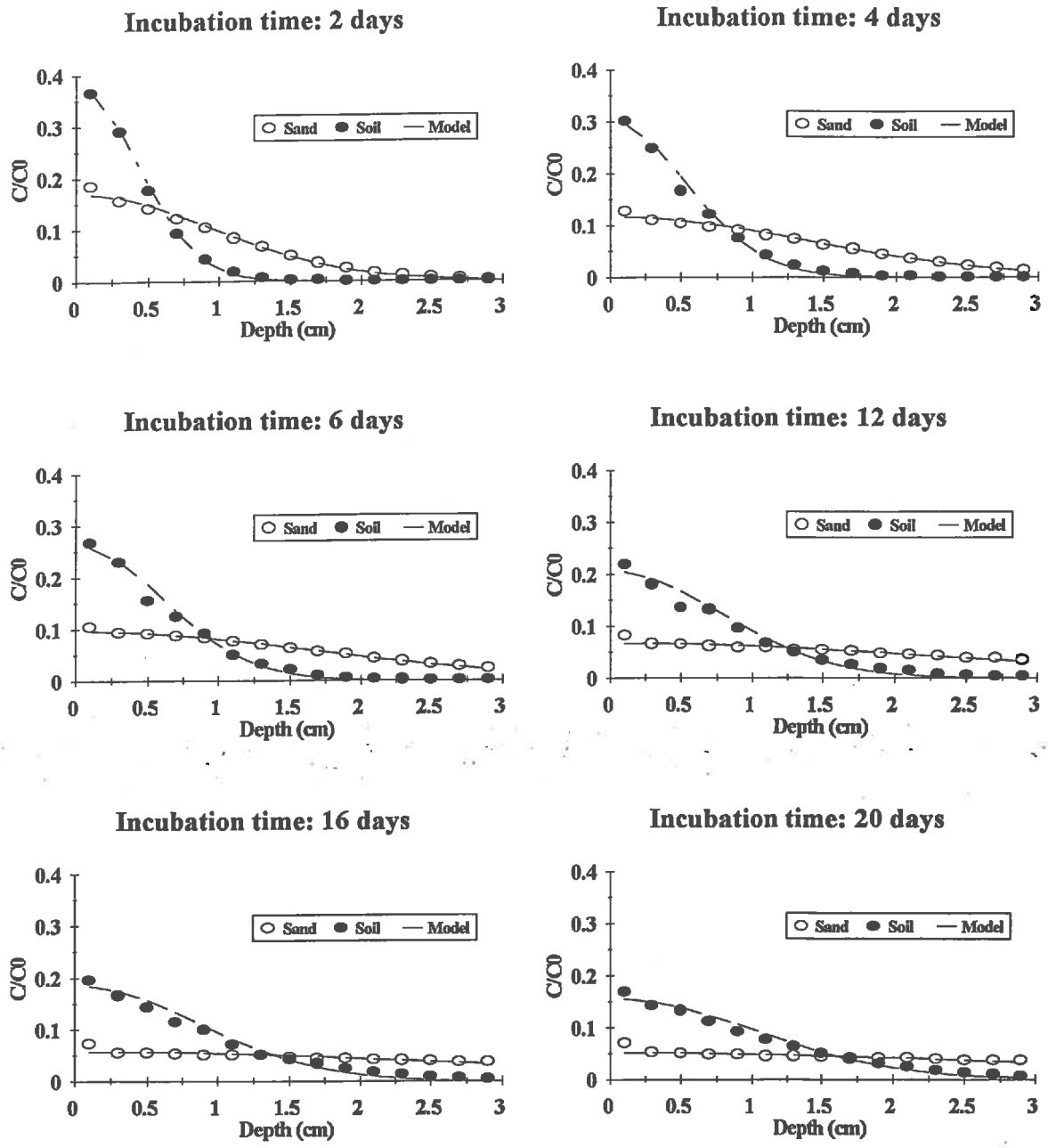

Fig. 5. Triticonazole diffusion profiles in the water-saturated sand and soil columns after 2, 4, 6, 12, 16 and 20 days of incubation. Open and closed symbols represent the experimental data in sand and soil respectively. Dotted lines represent the model fit.

Diffusion coefficients

The influence of the incubation time on triticonazole diffusion coefficients in sand and soil is shown in Fig. 6. Triticonazole effective diffusion coefficient in sand, Deff, was independent of time, with a mean value of 0.24 ± 0.01 $cm^2 d^{-1}$. This value is consistent with reported values of 0.39 $cm^2 d^{-1}$ for diffusion of salt in saturated sand (Mehta et al., 1995), and of 0.37 $cm^2 d^{-1}$ for 2,4-D diffusion in quartz sand (Lindstrom et al., 1968).

Apparent diffusion coefficients in soil, Dapp, were an order of magnitude lower (mean value 0.03 ± 0.01 $cm^2 d^{-1}$), and decreased significantly with incubation time, from 0.05 $cm^2 d^{-1}$ at 2 days to 0.02 $cm^2 d^{-1}$ at 25 days. The decrease was very rapid in the first 5-day period, followed by a slower decrease between 5 and 10 days, and attainment of a plateau after 10 days of incubation. As the diffusion coefficient in soil was not constant, the estimated value of Dapp for each diffusion time would not correspond to any specific time point value, but it would be a mean value over the duration of diffusion. Triticonazole diffusivities in soil were in the same range as reported apparent diffusion coefficients for various pesticides in water-saturated silt loam soils. Gerstl et al. (1979) reported a Dapp of 0.029 $cm^2 d^{-1}$ for parathion, with a partition coefficient of 3.59 close to our Kd of 4.35 for triticonazole sorption in Grignon soil. Shearer et al. (1973) also reported a solution phase Dapp of 0.016 $cm^2 d^{-1}$ for lindane diffusion, with a Kd of 2.88, while Graham-Bryce (1969) reported a Dapp of 0.14 for dimethoate, which was less subject to sorption (Kd of approximately 0.25).

Diffusivity in soils is usually assumed to be constant and few authors investigated the influence of time on the diffusion coefficients. Among them Farmer and Jensen (1970) found no influence of incubation time on the diffusion coefficient of dieldrin in soils when increasing the equilibration time from 1 up to 8 weeks, and they concluded that the diffusion coefficient was constant. However, dieldrin analysis conducted by toluene extraction and the formation of nonextractable bound residues would not be accounted in their calculations. Bode et al. (1973) reported decreasing diffusion coefficients with time for trifluralin, a volatile pesticide, in a silt loam soil. This decrease was attributed to the assumption of an infinite system while they worked in finite conditions. This would not be the case for triticonazole diffusion in soil since the spreading never reached the end of the column. Scott and Phillips (1972) found that the

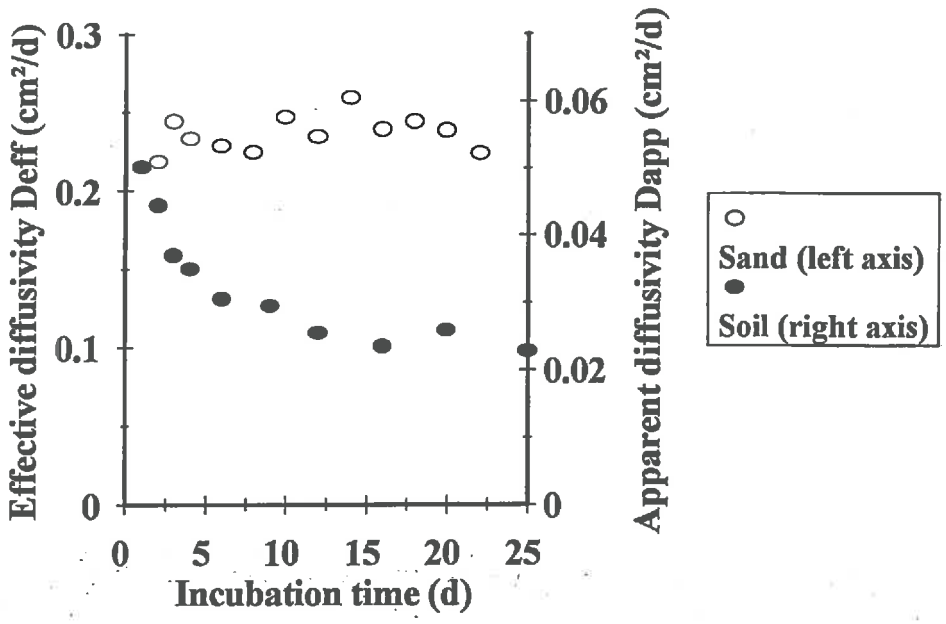

Fig. 6. Influence of time of incubation on the diffusivity of triticonazole in saturated sand and soil columns. The left ordinate axis corresponds to effective diffusion rates in sand (open symbols) and the right ordinate axis corresponds to apparent diffusion rates in soil (closed symbols).

diffusion coefficients of 2,4-D, chlorprophan, diphenamid and prometone decreased significantly during a 2 to 8 days diffusion study, and they attributed this time-dependence to microbial degradation and lower mobility of the degradation products. This interpretation can reasonably be excluded for our study in Grignon soil because we worked with initially sterile soil. Also, in a study concerning triticonazole biodegradation and mineralization kinetics in Grignon soil (unpublished data), we found that both the abiotic and microbial degradation of triticonazole in Grignon soil were extremely slow. Based on the relations (2) and (3), the effective bulk diffusion coefficient in soil should not depend upon time of incubation, as indicated the constant value of Deff we obtained for triticonazole diffusion in sand. We may thus attribute the time-dependence of Dapp in the soil to changes in the sorption characteristics of triticonazole with time resulting in a time-dependent, nonequilibrium apparent partition coefficient.

Evaluation of kinetic apparent sorption coefficients

Time-dependent sorption in soil during diffusion was evaluated from two approaches. First, the ability of triticonazole residues to desorb after prolonged incubation time was determined experimentally from the soil columns, providing apparent desorption coefficients Kapp. Also, we used the apparent diffusivity relation (3) to calculate apparent partition coefficients corresponding to each incubation time, assuming that a time-dependent Kd was the major factor responsible of Dapp decrease with time.

Volumetric water content and ρd were fixed to the mean values we measured in the blank columns, 0.56 and 1.02 respectively. Lavy (1970) and Scott and Phillips (1973) measured the diffusion coefficients in water D_0 for 8 pesticides and found that D_0 all fell in a narrow range around 0.53 $cm^2\ d^{-1}$, and were not particularly related to molecular weight or chemical structure. We used in equation (3) this mean value of 0.53 $cm^2\ d^{-1}$ for triticonazole D_0. Also, considering that Nye (1979) reported a good accordance between values of the tortuosity λ measured in saturated soils for small molecules and values of λ estimated with the equation of Cremers (1968), $\lambda=\theta_v^{0.5}$, we used this formula to calculate a tortuosity factor of 0.75 in the saturated soil columns. As for the Dapp values, these measured and calculated

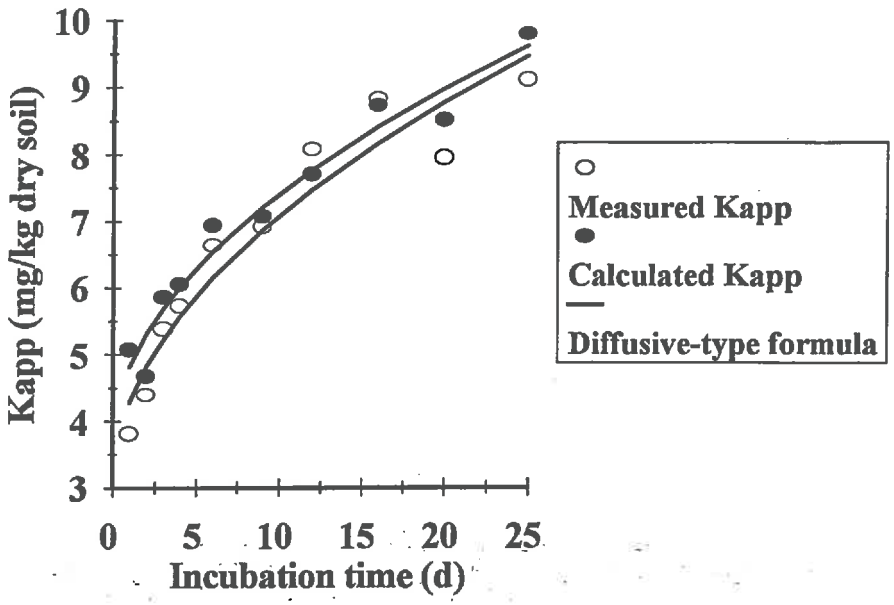

Fig. 7. Variation of the measured and calculated apparent sorption coefficients of triticonazole with incubation time. Measured Kapp correspond to the experimentally determined desorption coefficients. Calculated Kapp were determined using the diffusion rate equation: $D_{app} = \frac{D_0 \lambda \theta_v}{(K_d \rho_d + \theta_v)}$ **. Fitted values of a and b from the diffusive-type formula** $Kd = a + b\sqrt{t}$ **are respectively 3.62 and 1.20 for the measured Kapp and 2.33 and 1.29 for the calculated Kapp.**

apparent Kd would correspond to mean values over the range of diffusion time involved and the location of the residues in the column.

Results of Kapp obtained from desorption measurements were very similar to those calculated from the diffusion rate model, and were strongly time-dependent (Fig. 7). The assumption of nonequilibrium sorption responsible of the time-dependence of Dapp was thus verified. K_{app} increased rapidly from 5 L kg^{-1} at 1 day to approximately 7 L kg^{-1} at 6 days, and then increased continuously to reach nearly 10.5 L kg^{-1} after 25 days of incubation.
Whereas the apparent desorption coefficient was initially close to the equilibrium Kd, it rapidly became much larger, indicating that a significant fraction of the triticonazole was present in a slowly reversible sorbed state. Scribner et al. (1992), Pignatello and Huang (1991), and Lehmann et al. (1990) also reported similar results for aged herbicide residues in field soil samples, with increasing time-dependent apparent Kd and Koc values far above the batch equilibrium values. Thus as the equilibrium Kd significantly underestimated the true extent of sorption of triticonazole residues, it should prove irrelevant for describing triticonazole sorption during transport, and its incorporation in transport models would be erroneous when dealing with triticonazole long term behaviour. More complicated mechanisms including rate-limiting processes are apparently involved in triticonazole retention and retardation than what can be accounted by the classical batch equilibrium data, and kinetic rates of sorption/desorption would be needed to accurately predict triticonazole transport in soils.

Measured and calculated Kapp values could be fitted to the square root of time following a diffusive-type formula: $Kd = a + b\sqrt{t}$. The estimated values of a and b coefficients, respectively 3.62 and 1.20 for measured Kapp and 2.99 and 1.29 for calculated Kapp, allowed a good description of the nonequilibrium sorption (Fig. 7), which indicated that diffusion rate-limiting processes would be responsible of the time-dependence of sorption, as pointed out by Kookana et al. (1992). The mechanisms of nonequilibrium sorption of hydrophobic compounds in soils were discussed by Brusseau et al. (1989, 1991), Brusseau and Rao (1989) Pignatello and Xing (1996), who concluded that intraparticle (pore) diffusion and intra-organic matter (matrix) diffusion should be the most important processes accounting for slow desorption.

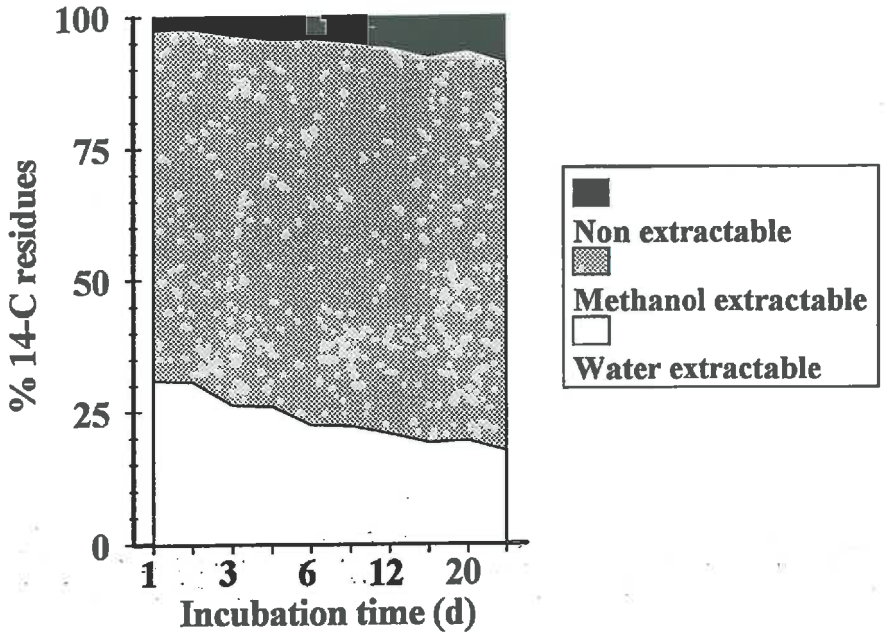

Fig. 8. Extractability of triticonazole ^{14}C-residues in saturated soil columns as a function of incubation time. Results are the average values of the 0 to 1 cm sections of the columns.

Indeed, models of intraparticle diffusion allowed good prediction of the sorption/desorption kinetics of hydrophobic compounds in soil (Wu and Gschwend, 1986; Ball and Roberts, 1991).

Residues extractability

As pointed out by Barriuso and Koskinen (1996), the desorptivity and consequently the availability of pesticide residues in soils can be evaluated in relation to their extractability. A water extractable fraction (here corresponding to a single and thus nonexhaustive aqueous extraction) would characterize the more easily desorbable residues and hence the more readily available fraction. An organic solvent extractable fraction (here corresponding to exhaustive methanol extraction) would characterize residues that are more difficult to desorb and therefore less readily available and potentially rate-limiting. Noteworthy, the residues extractable with an organic solvent could probably also be desorbed in water after exhaustive aqueous extraction, as demonstrated Barriuso et al. (1992) with atrazine. Hence, the solvent extractable fraction (water soluble residues plus organic solvent soluble residues) may indicate the potential availability of the compound, or the total amount susceptible to be desorbed in the soil aqueous solution. Finally, the nonextractable residues fraction can be defined as resistant or very slowly available and rate-limiting.

The changes in triticonazole ^{14}C-residues extractability during diffusion in soil, measured in the first cm of the soil columns are presented in Fig. 8. The water extractable fraction decreased as the residence time in soil increased, from 31 % of the recovered radioactivity after one day of incubation to 17 % after 25 days. Simultaneously, the methanol extractable fraction increased from 67 % to 74 %. As a result the solvent extractable fraction decreased from 98 % to 91 %. Hence, even if the total amount of triticonazole potentially available was always more than 90 % of the recovered residues, the significant decrease of the more easily desorbable residues with time would result in an important decrease in availability characterized by the increasing Kapp. A nonavailable nonextractable residues fraction was formed rapidly after triticonazole application, corresponding to 3 % of the recovered residues after 1 day, that increased continuously to attain 9 % at 25 days. The formation of such a resistant fraction of residues while ageing has been reported for various compounds (Barriuso

and Koskinen, 1996; Scribner et al., 1992). It was also evidenced during sorption/desorption kinetics studies of herbicides on much shorter time periods (Wauchope and Myers, 1985; Locke, 1992; Reddy et al., 1995). These authors showed that adsorption of the herbicides was characterized by a rapid initial phase followed by a slow approach to equilibrium and a concomitant increase in the nonextractable fraction. Nonextractable residues are highly stabilized in soil, and thus should be unavailable for transport. Koskinen and Harper (1990) pointed out that decreased availability of organic chemicals with incubation time may be due to physical trapping of the chemical in the soil matrix, implying slower desorption from restricted soil sites. Triticonazole gradual changes in extractability with time of diffusion could be interpretated as a rate-limiting diffusive transport of triticonazole molecules from an external part of the sorbent (easily accessible), to an inner part (exchanging slowly), where the extractability decreases as triticonazole penetrates deeper and becomes trapped.

CONCLUSION

This study demonstrated the major influence of sorption of triticonazole in limiting its mobility in soil, as indicated by diffusivities in sand and a loam clay soil. Moreover, nonequilibrium sorption and time-dependence of the apparent desorption coefficient, that was evidenced during triticonazole diffusion in the soil columns, resulted in a time-dependence of the diffusion coefficient. The decreased extractability of triticonazole residues after prolonged contact with the soil indicated that triticonazole nonequilibrium sorption was related to slow desorption and formation of nonextractable residues resulting from rate-limiting diffusion to restricted sites of the soil, probably involving soil organic constituents. Thus, as bulk diffusion of the fungicide was rate-limited by slow desorption, sorption itself was rate-limited by intrasorbent diffusion, and the retention and diffusion processes were highly associated.

In this context, the classical batch equilibrium sorption data collected over a 24 h equilibration period proved irrelevant in describing the true extent of sorption during diffusive transport in soil. Kinetic sorption data are thus needed, that can be provided either

experimentally during the diffusion studies, using an extraction procedure, or by calculations, from the apparent diffusion coefficient in soil.

Acknowledgments

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CHAPITRE 5

Modélisation du transport convectif-dispersif du triticonazole en sol homogène saturé

Transport of Triticonazole in Homogeneous Soil Columns: Influence of Nonequilibrium Sorption

C. Beigel, and L. Di Pietro (1997)

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Transport of Triticonazole in Homogeneous Soil Columns: Influence of Nonequilibrium Sorption

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ABSTRACT

Nonequilibrium sorption of pesticides is frequently reported to greatly affect their transport and dissipation in soil. This study was aimed at evaluating the performances of equilibrium convective-dispersive and 2 site/2 region nonequilibrium models for describing the sorption and decay characteristics during transport of triticonazole systemic fungicide in homogeneous soil. Chloride and ^{14}C-triticonazole column displacement experiments were carried out in a loamy clay soil under steady-state water flow at high pore-water velocities. The symmetrical breakthrough curves (BTC) obtained with chloride conservative tracer showed no significant physical nonequilibrium and were used to estimate a dispersivity of 0.06 cm. ^{14}C-triticonazole BTC were strongly asymmetrical and shifted to the right, with a broad extended tailing characteristic of sorption nonequilibrium. Chemical analysis of the soil after elution showed that a fraction of bound residues was rapidly formed during transport, that is accounted as decayed in the models. The two-site model correctly described the first part of the tailing, with an estimated partition coefficient K_d of 1.4 L kg^{-1} for instantaneous sorption, and predicted high values in the range of 60 d^{-1}, and 7 d^{-1} for respectively the sorption and decay first-order rates. However, the model failed to describe the slower, extended release of ^{14}C-triticonazole. Nonequilibrium sorption, and formation of bound residues of triticonazole were attributed to rate-limiting diffusive process. It was thus concluded that use of single first-order rate constants for description and prediction of both the nonequilibrium sorption and the stabilization of triticonazole in soil are not appropriate.

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INTRODUCTION

Triticonazole is a new triazole systemic fungicide developed by Rhône-Poulenc Agro, which is used in cereal seed treatments. Triticonazole controls major seed born, foliar and straw diseases, thus allowing for cereal protection from seed to developped growth stages. The efficacy of systemic pesticides applied in seed treatments depends closely on their dissipation, localization, and availability in the soil profile in relation to their uptake by the plant roots system. The transport and fate of these molecules in soils is therefore of crucial interest for an optimal utilization.

Solute transport of organic chemicals in soil depends on the soil structural and hydraulic properties, and it is controlled by sorption and degradation, which both limit the mobility of the pesticides in soil. These major dissipation processes have been extensively studied (Graham-Bryce, 1981; Weber and Miller, 1989; Weber, 1991). The difficulty of accurately accounting for these two interacting processes with simple input parameters is one of the main problems encountered for predicting and modeling solute transport in soil (Calvet, 1995).

Sorption of hydrophobic organic compounds (HOC) has been related to soil organic matter (OM) through non specific interaction mecanisms (Hamaker and Thompson, 1972; Ainsworth et al., 1989; Barriuso and Calvet, 1992). Soil sorption isotherms of HOC at diluted concentrations are usually linear (Calvet, 1989), and a soil-solution partition coefficient Kd (in L kg^{-1} for sorbed concentration S_e divided by solution concentration C_e at equilibrium) is frequently used to characterize HOC sorption on a particular soil. These coefficients are experimentally determined in batch systems, assuming reversible, quasi-instantaneous sorption. However, there is increasing evidence of nonreversible time-dependent sorption of most pesticides in soil (Lehmannn et al., 1990; Scribner et al., 1992, Barriuso et al., 1992). Sorption kinetics for HOC usually exhibit a two-stage approach to equilibrium, with a short initial phase of rapid uptake followed by an extended period of much slower uptake (Lee et al., 1988; Gaston and Locke, 1995).

The degradation of pesticides in soil is mainly due to biological transformations, and is thus controlled by the availability of the organic chemical, and by the activity of the soil microflora (Torstensson, 1987). It is usually characterized by pseudo first-order constants k calculated from mineralization or dissipation data, from the derived first-order relation: $C = C_0 e^{-kt}$, where C_0 and C are the substrate concentrations in soil at time 0 and t respectively. However, degradation may not always follow simple first-order kinetics if the pesticide is subject to significant abiotic degradation. Also, the degrading capacity of the soil microflora may vary with time because the growth and activity of the degrading microorganisms is extremely sensible to environmental conditions such as temperature and humidity (Walker et al. 1992, Veeh et al. 1996), and because adaptation phenomena may occur (Felsot and Shelton, 1993; Ou et al., 1993). Furthermore, changes in the pesticide availability with time due to nonequilibrium sorption may also affect the degradation rate.

The sorption and degradation characteristics of triticonazole systemic fungicide in a loamy soil of Grignon, France (Typic Eutrochrept) have been examined in previous studies (Beigel et al., 1997a and b). The degradation of triticonazole was essentially due to microbial, cometabolic transformations, that could be adequately characterized by first-order mineralization constants ranging from 0.3 10^{-3} to 0.6 10^{-3} d^{-1}, depending on the initial dose applied. Batch equilibrium studies showed that triticonazole equilibrium sorption was related to soil organic matter content and could be approximated by a linear isotherm, with a measured K_d of 4.35 L kg^{-1}. However, it appeared that triticonazole sorption onto Grignon soil during incubation and diffusion experiments was strongly time-dependent and increased as the time of contact with the soil increased. A time-dependent apparent partition coefficient was measured, which increased from 2.5 to 10 L kg^{-1} during a 130 day incubation. Rate-limiting desorption, and formation of bound residues were observed, which resulted in a decreasing apparent diffusion rate of triticonazole in soil. This was attributed to rate-limiting intra-sorbent (organic matter) diffusion to restricted sorption sites. Use of the batch equilibrium K_d to characterize triticonazole sorption and desorption during transport would thus prove erroneous, and nonequilibrium sorption conditions need to be accounted.

The objective of the present study was to test simple conceptual coupled-process models to describe the one-dimensional transport of triticonazole in homogeneous saturated soil columns. We evaluated the deterministic equilibrium CDE and the two-site / two-region deterministic nonequilibrium CDE models using the computer program CXTFIT 2.0 of Toride et al. (1995) which is an updated version of the CXTFIT code of Parker and van Genuchten (1984).

MATERIALS AND METHODS

Soil and chemicals

The soil used in this study was a loamy clay (typic Eutrochrept), sampled in the surface layer (0-20 cm) of a continuous wheat experimental plot located at Grignon (France). It had a pH in water of 8.2, with (g kg^{-1} of dry soil): 291 of clay, 540 of silt, 145 of sand, 24 of lime and 10.4 of organic C. Soil samples were air dried and passed through a 2-mm sieve. To avoid problems of clogging while packing of the columns and during elution, the finer particles were removed by further sieving at 0.5-mm. Soil residual gravimetric water content (g/g) was of 4.32 %.

^{14}C-U-benzyl-labeled triticonazole [(1RS)-(E)-5-(4-chlorophenyl-methylen)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol] (specific activity: 1184 MBq $mmol^{-1}$; radiopurity > 98 %) was provided by Rhône-Poulenc Agrochemicals Company, Lyon, France. Triticonazole water solubility is 8.4 mg L^{-1}, and 10.6 mg L^{-1} at 20 and 22 °C respectively, vapour pressure is < 10^{-8} hPa at 50°C and distribution coefficient between octanol and water is 1950. A solution of ^{14}C-triticonazole at 5.0 mg L^{-1} and 0.224 µCi mL^{-1} (8.332 Kbq mL^{-1}) was prepared for input tracer solution by adding ^{14}C-triticonazole methanol stock solution to a saturated water solution of triticonazole (analytical standard, purity > 92 %) and adjusting the concentration with MilliQ water (Millipore, Saint-Quentin, France). The solution

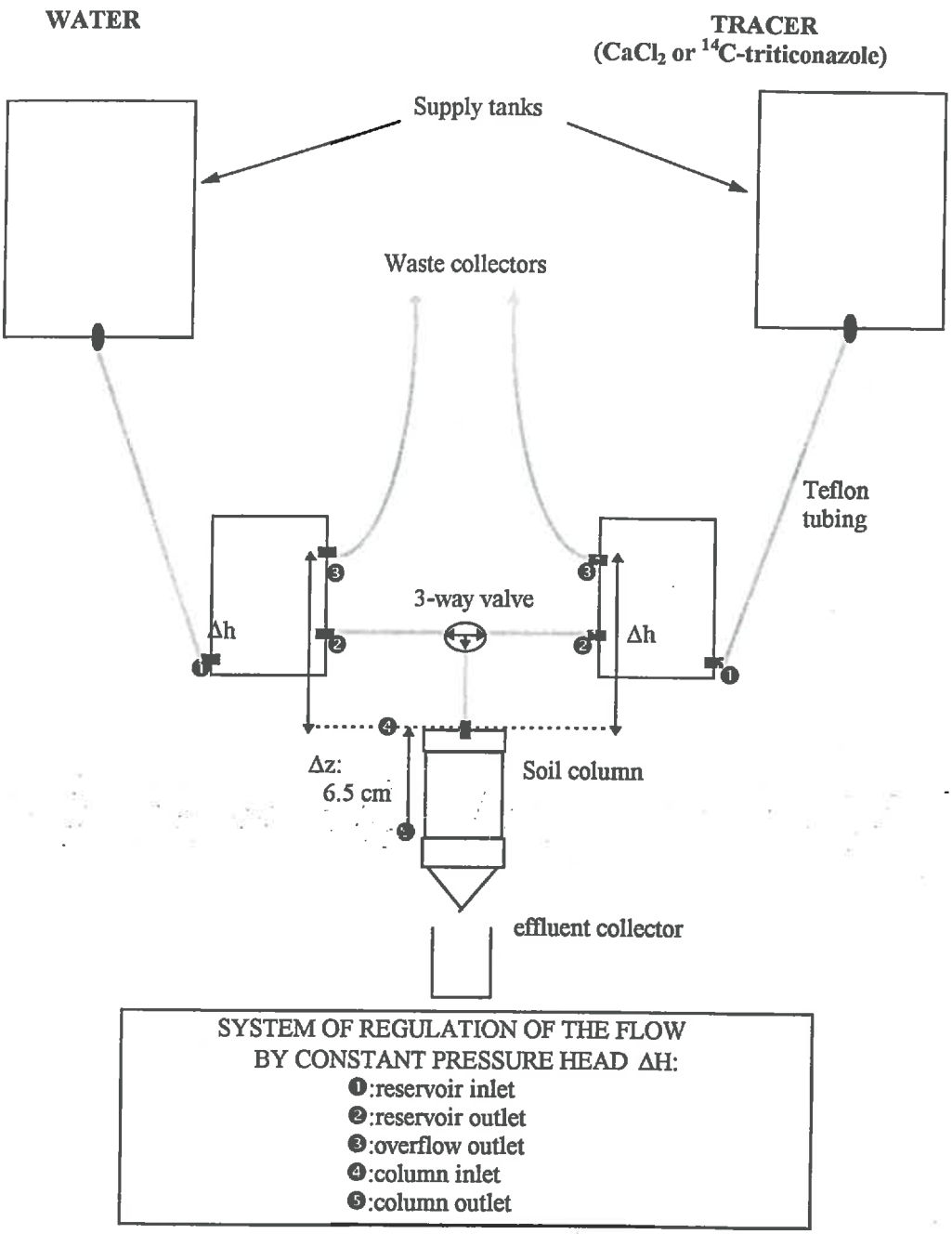

Fig. 1 Schematic layout of the experimental system for solute transport with constant pressure head.

concentrations were measured at 262.5 nm with a U.V.-visible spectrophotometer Lambda V (Perkin-Elmer, Überlingen, Germany).[1]

Chloride was used as a nonreactive conservative tracer to determine the soil hydrodynamic dispersion coefficient at different water velocities. A solution of $CaCl_2$ with Cl^- at 1 g L^{-1} was prepared for input tracer solution by diluting $CaCl_2,2H_2O$ analytical reagent > 98 % purity (R.P. Normapur, Prolabo, Paris, France) in the proper amount of MilliQ water.

Miscible displacement experiments

The breakthrough curves (BTC) for $CaCl_2$ and triticonazole were measured in water saturated, isotropic homogeneous soil columns. The system consisted of PVC columns of 60 mm long and 55 mm of diameter. A stainless steel porous filter of pore size 50 µm was used as bed support on the bottom of the column. At the top of the column, a void volume of 5 mm long was also provided to allow for the formation of a piston-like water front. Packing of the columns with soil was carried out under water saturation conditions in a water bath by adding successive layers of soil to establish uniform bulk density and water content. The columns were then covered to avoid evaporation, and allowed to equilibrate in the water bath for 24 h at 22 ± 2°C. Mean gravimetric water content, θ_g (w/w), volumetric water content, θ_v (v/v), and bulk density (Mg m^{-1}), measured in 10 test columns, were respectively of 0.65 ± 0.01, 0.60 ± 0.01, and 1.07 ± 0.01. The pore volume was calculated as the product of the column volume and θ_v at 86.5 cm^3. Soil saturated hydraulic conductivity (K_{sat}), measured in similar soil conditions, was of 97.2 cm d^{-1}.

A constant pressure head was applied on the soil columns for steady-state flow conditions, using the system described in Fig. 1. The desired pore water velocities were obtained by varying the position of the column on the vertical axe. The Darcy's velocities during the elution experiments were evaluated by measuring the elution fluxes Q (cm^3 d^{-1}) at the columns outlet by weighing the amount of solution eluted at regular time intervals, and

[1] Trade names and company names are included for the benefit of the reader and do not imply endorsement or preferential treatment of the product listed by INRA.

dividing by the soil column cross section (23.76 cm^2). We observed a good agreement between the measured and theoretical calculated Darcy's velocities q (cm d^{-1}) for different Δh.

All the elution experiments were performed in duplicate at 22 ± 2 °C. For the chloride miscible displacement studies, 3 different q of 125, 160, and 212.5 cm d^{-1}, corresponding to Δh of respectively 2, 4, and 8 cm were established. Assuming that piston flow conditions occured, the average pore water velocities v for the duplicates were estimated from $v = q / \theta_v$ at respectively 208, 263, and 354 cm d^{-1}. The elution of triticonazole was performed at the lowest chloride Darcian velocity of 125 cm d^{-1}. The columns were first supplied with MilliQ water until steady-state flow conditions were attained at the desired infiltration rate. A pulse of the tracer solution corresponding to 0.5 V_0 was then applied at the same rate, and the sytem was then switched back to the water reservoir for elution periods of 3 h (chloride BTC), and 11 h (triticonazole BTC). At regular time intervals, aliquot samples of the leachate were collected, weighed, and stored at -20 °C until chemical analysis. At the end of the elution time course, sequential sampling of the triticonazole soil columns was performed by extruding and slicing the soil in 10 incremental discs of 6 mm section using a soil extruding screw procedure described in a previous paper (Beigel et al. 1997a).

Chemical analysis

Chloride concentration in the effluent samples was measured by capillary analysis by direct UV detection with a sulfate/OFM/CHES electrolyte, using a CIA Analyser equipped with a AccuSep 75 µm × 60 cm capillary and Millenium 2.1 software (Waters, Milford, MA, USA).

Triticonazole concentration in the effluent samples was measured by liquid scintillation counting (LSC). 0.5 mL aliquots of the elution samples were pipetted and put in scintillation vials. 4 mL of Ultima Gold XR LSC Cocktail (Packard, Meriden, CT, USA) were added, and the amount of radioactivity was measured by LSC using a Kontron Betamatic V counter (Kontron Ins, Montigny le Bretonneux, France).

Total ^{14}C-triticonazole residues remaining in the soil samples were measured by LSC of the $^{14}CO_2$ evolved after combustion of triplicate 300 mg aliquots of air dried and finely ground

soil with a Sample Oxidizer 307 (Packard, Meriden, CT, USA). Combustion and trapping efficiencies averaged 74.9 %. Extractable residues were analysed after exhaustive extraction with methanol. The soil samples were extracted twice with 50 mL of methanol. After 24 h shaking, the samples were centrifuged for 15 min at 5000 tr min^{-1} (8000 g), and the radioactivity content in the supernatant was measured by LSC as previously described. After the second extraction, the soil pellets were air dried, and the remaining radioactivity in the soil was measured by combustion of triplicate 300 mg soil aliquots as previously described.

Models

CXTFIT 2.0 is a program presenting a number of analytical solution for one-dimensional transport models based on the convection-dispersion equation (CDE). Assuming steady-state flow in a homogeneous soil, and first-order transformation kinetics with uniformly distributed non growing biomass, this equation may be written as:

$$\frac{\partial C}{\partial t} + \frac{\rho}{\theta}\frac{\partial S}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \mu_{liq} C \qquad [1]$$

where t is time (d), x is depth (cm), ρ is soil bulk density (g cm^{-3}), θ is soil volumetric water content (cm^3 cm^{-3}), C is the concentration of the liquid phase (mg L^{-1}), S is the concentration of the adsorbed phase (mg kg^{-1}), v is the average pore water velocity (cm d^{-1}), D is the hydrodynamic dispersion coefficient D (cm^2 d^{-1}), and μ_{liq} is a first-order decay coefficient for degradation in the liquid phase (d^{-1}). We used the deterministic equilibrium CDE model, further noted as CDeq, and the deterministic 2 site / 2 region nonequilibrium model, further noted as CDnoneq.

The CDeq model assumes local equilibrium (LEA) for solute adsorption, and that sorption can be described by a single linear isotherm $S_e = K_d C_e$, where S_e and C_e are the concentrations in sorbed and liquid phases at equilibrium, and K_d is the equilibrium partition coefficient (L kg^{-1}).

The two-site / two-region bicontinuum model has been formulated to account for either sorption-related or transport-related nonequilibrium during solute transport. The two-site nonequilibrium concept assumes that sorption sites in soils can be classified into two fractions. In the first fraction, sorption is instantaneous and is described by an equilibrium sorption isotherm (Type 1, equilibrium). In the other one sorption is time-dependent and follows first-order kinetics (Type 2, kinetic). In this case, the kinetic rate limiting step for type-2 sites would be either chemical (chemisorption), or of diffusive type (intraparticle or intrasorbent diffusive mass transfer) as discussed by Brusseau et al. (1991). The two-region approach assumes that the liquid phase can be partitioned into mobile (flowing, macropore domain) and immobile (stagnant, matrix or micropore domain) regions. The exchange between the two liquid regions is modeled by a first-order kinetic rate. Flow occurs only in the mobile region. Sorption is assumed to be instantaneous on all sorption sites, and the sorption rate is limited here by the diffusion of the solutes to the exchange sites in the stagnant phase. If dimensionless parameters are employed, the two-site and two-region models reduce to the same dimensionless form:

$$\beta R_f \frac{\partial C_1}{\partial T} = \frac{1}{P}\frac{\partial^2 C_1}{\partial Z^2} - \frac{\partial C_1}{\partial Z} - \omega(C_1 - C_2) - \mu_1 C_1 \qquad [2]$$

$$(1-\beta)R_f \frac{\partial C_2}{\partial T} = \omega(C_1 - C_2) \qquad [3]$$

where $T = \frac{vt}{L}$, $Z = \frac{x}{L}$, P is the Peclet number $P = \frac{vL}{D}$, R_f is the retardation factor, defined as $R_f = 1 + \frac{\rho}{\theta} K_d$. The subscripts 1 and 2 refer to equilibrium and nonequilibrium sites respectively, β is a partitioning coefficient, and ω is a dimensionless mass transfer coefficient. The various dimensionless parameters have different significations for the two-site and two-region models, that are defined in the CXTFIT 2.0 code (Toride et al., 1995).

For the two-site model, β and ω are defined as:

$$\beta = \frac{\theta + f\rho K_d}{\theta + \rho K_d}, \qquad f = \frac{\beta(\theta + \rho K_d) - \theta}{\rho K_d} \qquad [4]$$

$$\omega = \frac{\alpha(1-\beta)RL}{v}, \qquad \alpha = \frac{v\omega}{RL(1-\beta)} \qquad [5]$$

where f is the fraction of type-1 sites, and α (d^{-1}) is the first-order rate for the kinetic, type-2 sites. The adimensional decay terms μ_{liq}, for degradation in the liquid phase only, reduces to:

$$\mu_l = \frac{L\mu_{liq}}{v}, \qquad [6]$$

For the two-region model, β and ω are defined as:

$$\beta = \frac{\theta_m + f\rho K_d}{\theta + \rho K_d} \qquad [7]$$

where θ_m is the volumetric water content of the mobile region, and f is the fraction of sorption sites in the mobile region

$$\omega = \frac{\alpha L}{\theta v} \qquad [8]$$

where α is a first-order mass coefficient governing the rate of solute exchange between the mobile and immobile liquid regions.

Parameters estimation

The hydrodynamic dispersion coefficient D of the soil was estimated from the chloride data with both models using the nonlinear least-squares parameter optimization method (inverse problem). The solute transport parameters v and D at the three infiltration rates were first estimated from the chloride BTC (pooled results from the duplicate columns) with the Cdeq model. The two-region approach from the CDnoneq was then used to estimate v, D, α, and β transport parameters, to evaluate the possible occurrence of physical nonequilibrium. For

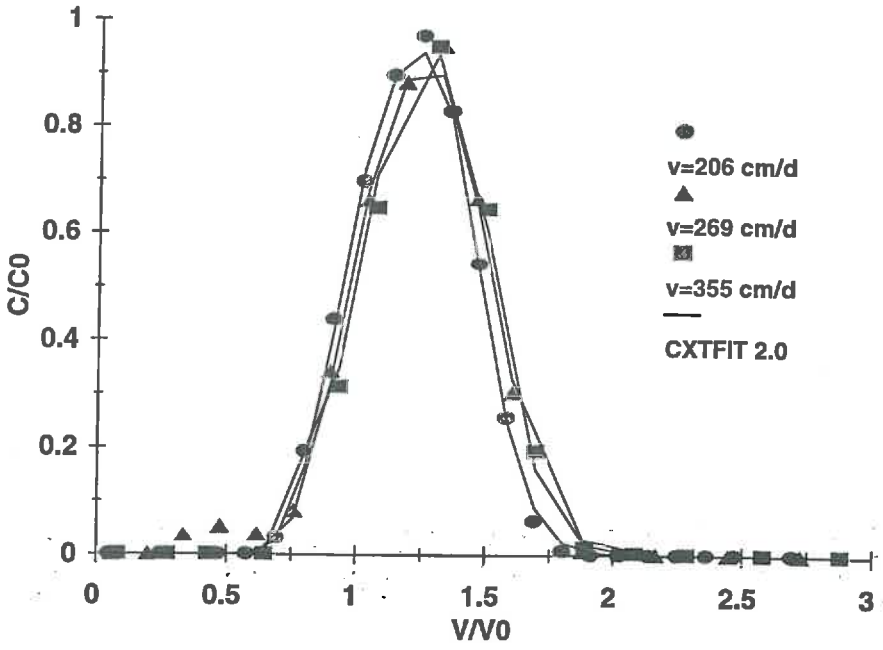

Fig. 2 Effect of pore water velocity on the measured (symbols) and simulated (lines) breakthrough curves in Grignon soil for displacement through Grignon soil of a pulse of 0.5 V_0 of chloride conservative tracer at 1 g L^{-1}.

the nonreactive tracer, R_f was set to 1. The estimated D was then introduced in CDeq to estimate the retardation factor R_f, adimensional parameter for linear sorption and the first-order degradation rate from the triticonazole BTC. Cdnoneq was then used to estimate the retardation factor, and the nonequilibrium adimensional parameters β, ω, and μ for triticonazole transport and decay.

Both models were used under the conditions of semi-infinite system, pulse input, linear sorption and first-order degradation in the liquid phase only. All these conditions were assumed to reasonably fit to our elution column experimental setup, and to triticonazole sorption and degradation characteristics. The corresponding initial and boundary conditions, and analytical solutions are detailed in the CXTFIT 2.0 code (Toride et al., 1995).

RESULTS

Chloride BTC

The breakthrough curves of the conservative tracer chloride measured at the three infiltration rates were almost identical (Fig. 2). The effluent curves appeared symmetrical and sigmoidal, with invariant frontal and distal portions. No significant retardation occured, as chloride was detected in the effluent after application of 0.8 V/V_0 of water, and relative concentration peaks were measured at 1.25 V/V_0, that is, after application of one pore volume and half of the pulse volume of water.

Results of the fits of CDeq and of the 2-region CDnoneq to the experimental chloride data are listed in Table 1. The CDE fitted well to the observed results, as indicates the high coefficient of determination, r, values obtained (> 0.97). Estimates of v for the duplicate columns at the three infiltration rates were close to the measured velocities, suggesting that the average velocity assumption was appropriate. However, the estimation of the dispersion coefficients D was not satisfactory, as the STD values were higher than the D values. Use of CDnoneq with the CDeq fitted values for v proved more efficient for the estimation of D, as STD were much lower. Estimated values for D were very low and increased from 16 to 25 cm^2 d^{-1} with increasing water velocity. As D appeared directly proportionnal to v, the adimensional

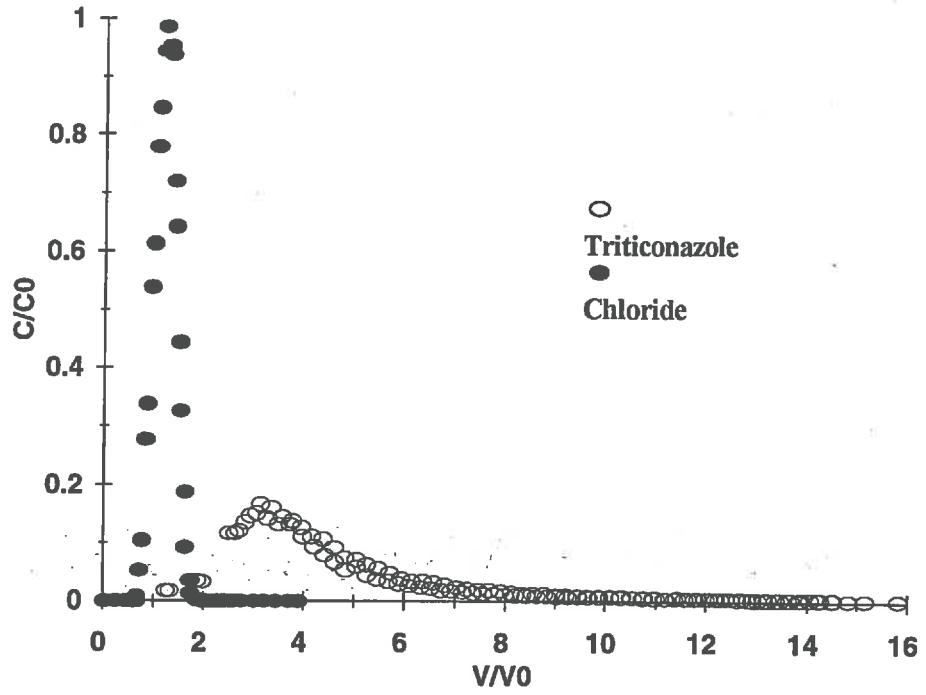

Fig 3. Comparison of chloride (plain symbols) and ^{14}C-triticonazole (empty symbols) measured breakthrough curves for displacement through Grignon soil of a pulse of 0.5 V_0 and a pore water velocity of 210 cm d^{-1}. Initial concentrations of the tracers input solutions were 1 g L^{-1} for chloride and 5 mg L^{-1} for triticonazole.

Peclet number P = Lv/D, and the dispersivity $\lambda = D/v$ were almost invariant, with mean values of respectively 86.7 ± 5.5 for P, and 0.06 ± 0.02 cm for λ. The estimated parameter β for physical nonequilibrium appeared dependent upon the water velocity, with β decreasing from 1 to 0.92 for v decreasing from 355 to 217 cm d^{-1}.

Experimental conditions			CDeq fit			CDnoneq Two-region fit			
Column	Measured v (cm d^{-1})	Pulse (V/V_0)	v (cm d^{-1})	D (cm^2 d^{-1})	r	D (cm^2 d^{-1})	β	ω	r
1	202	0.51	217±54.5	15.6±54.5	0.974	16.6±0.1	0.92±0.05	10^{-6}	0.977
2	215	0.54							
3	252	0.54	269±54.1	22.0±54.1	0.970	21.6±0.1	0.98±0.04	10^{-6}	0.986
4	275	0.58							
5	362	0.58	355±42.3	24.6±42.3	0.974	24.5±0.1	1.00±0.03	10^{-6}	0.988
6	346	0.52							

Table 1 Comparison of CDeq and CDnoneq parameter values estimated from the chloride data at three pore water velocities.

Triticonazole BTC

Triticonazole breakthrough measured at a water velocity of 202 cm d^{-1} is directly comparable to the breakthrough of chloride at the same velocity since a reduced concentration scale is used and similar pulse durations were applied. Triticonazole BTC appeared shifted to the right, and is strongly asymetrical in shape (Fig. 3). The distal part of the elution peak appeared skewed and biphasic, showing a rapid release in the early tailing followed by a much slower release in the extended tailing. The relative concentration of triticonazole in the effluent

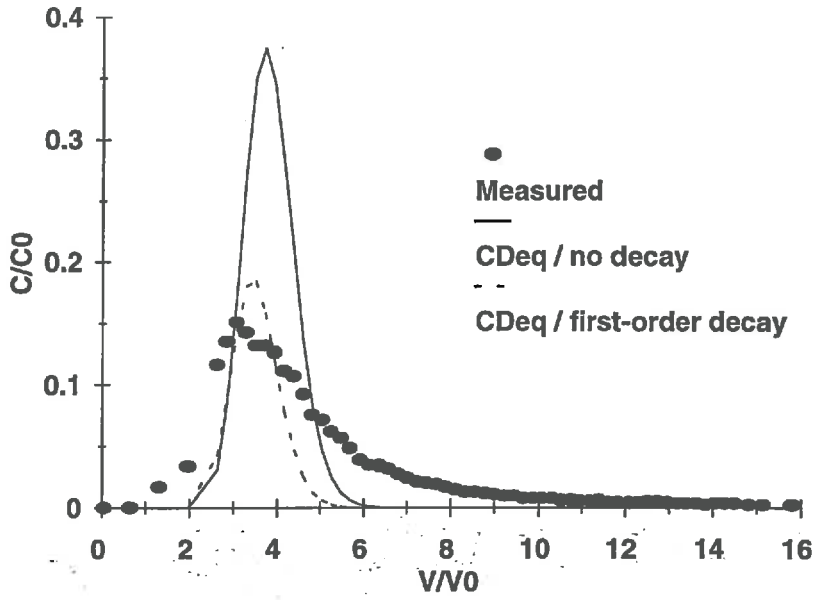

Fig. 4 Measured and CDeq simulated breakthrough curves for displacement through Grignon soil of a pulse of 0.5 V_0 of ^{14}C-triticonazole at 5 mg L^{-1}. Simulated curves were calculated with the model parameters v and D set to 208 cm d^{-1} and 16.34 cm^2 d^{-1}, without decay (solid line) or with first-order decay in the liquid phase (dotted line).

was comparatively much lower than the conservative tracer concentration, with delayed concentration peaks at a low C/C_0 of 0.16 that were attained after elution of about 3 pore volumes

	Model for parameter estimation			
	CDeq	CDeq	CDnoneq Two-Site	CDnoneq Two-Site
	No degradation	Degradation in solution phase	No degradation	Degradation in solution phase
R_f	3.57±0.06	3.33±0.03	3.64±0.01	3.77±0.01
K_d (L kg^{-1})	1.43±0.08	1.29±0.04	1.47±0.02	1.54±0.02
β			0.48±0.05	0.61±0.02
f (%)			0.52±0.18	0.85±0.12
ω			5.33±0.05	2.67±0.02
α (d^{-1})			88±15	57±6
μ	0	0.80±0.03	0	0.22±0.01
μ_{liq} (d^{-1})		25.6		7.0
r	-0.967	0.666	0.930	0.971

Table 2 Comparison of CDeq and CDnoneq parameter values estimated from the ^{14}C-triticonazole data, with v and D respectively set to 208 cm d^{-1} and 16.34 cm^2 d^{-1}.

Results of CDeq and CDnoneq fits with v set to 202 cm d^{-1}, and D set to 16.35 cm^2 d^{-1} are summarized in Table 2. The equilibrium model proved inefficient in describing the experimental data (Fig. 4). CDeq could not account for any of the asymmetry and tailing and could only describe the position of the delayed concentration peak, i.e. the retardation due to instantaneous equilibrium sorption. The CDeq fit without decay resulted in a peak twice as high as the observed data. The introduction of a first-order decay constant (sink term) ameliorated the description as it allowed to correctly describe the peak height. Use of the 2-site nonequilibrium model considerably improved the fit to the experimental BTC ($r > 0.93$).

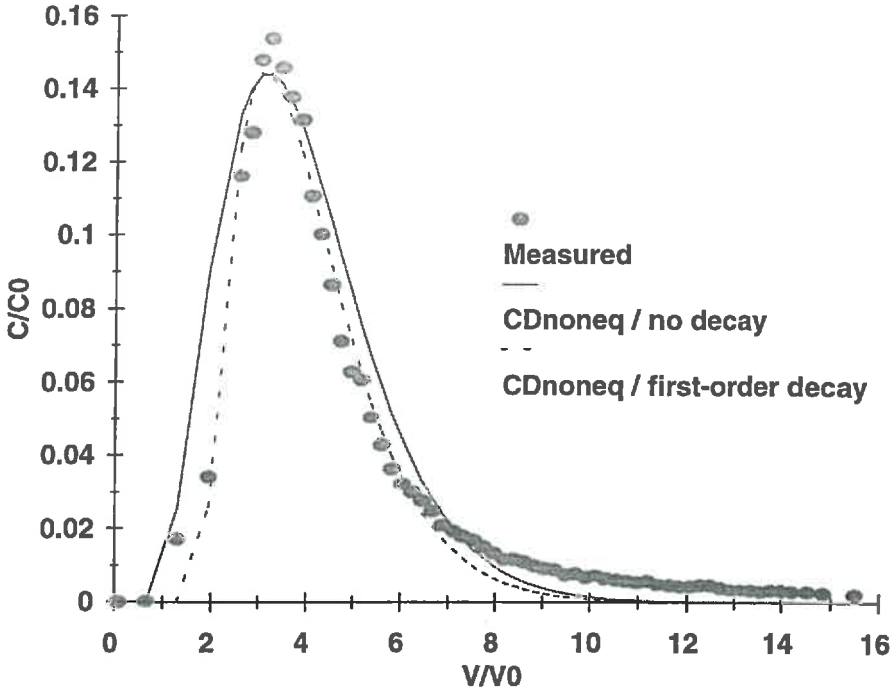

Fig. 5 Measured and CDnoneq simulated breakthrough curves for displacement through Grignon soil of a pulse of 0.5 V_0 of ^{14}C-triticonazole at 5 mg L^{-1}. Simulated curves were calculated with the model parameters v and D set to 208 cm d^{-1} and 16.34 cm^2 d^{-1}, without decay (solid line), or with first-order decay in the liquid phase (dotted line).

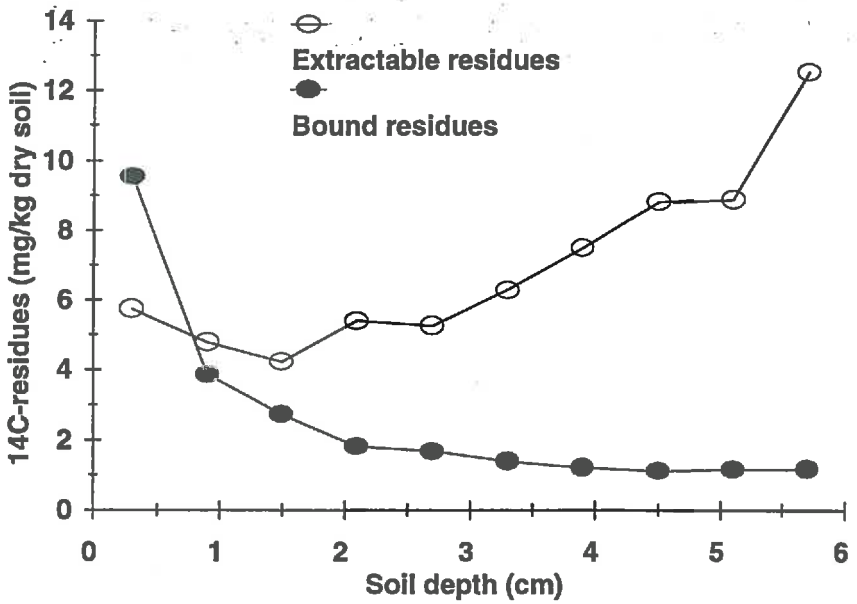

Fig. 6 Measured concentration profiles of the extractable (empty symbols) and bound (plain symbols) ^{14}C-residues remaining in the soil columns after elution of a pulse of 0.5 pore volume of ^{14}C-triticonazole at 5 mg L^{-1} with 16 pore volumes of water. Total recovered amounts of the extractable and bound fractions respectively averaged 2.7 and 2.5% of the initial amount applied.

CDnoneq allowed to correctly describe the asymmetry of the concentration peak, as it accounted for the early tailing, but it still failed to account for the extended tailing. The model predicted that all the applied radioactivity would be recovered in the effluent after elution of 12 pore volumes of water, whereas significant amounts of ^{14}C were still detected in the effluent after elution of the 16 pore volumes (Fig. 5). CDnoneq without decay slightly overpredicted the width of the concentration peak. Introduction of one or two decay terms improved the fit (r of 0.97), as it allowed to better describe the width of the elution peak. The adimensional parameters for decay μ_1 allow to calculate the first-order degradation rate in the soil liquid phase, μ_{liq}. Estimated value of μ_{liq} for the degradation of triticonazole, was of 7 d^{-1}.

In both models, instantaneous sorption is characterized by the adimensional retardation factor R_f, which allows to calculate the partition coefficient K_d. Estimated values of K_d from the CDeq (1.4 and 1.3 L kg^{-1}) and from CDnoneq (1.5 L kg^{-1}) were in the same range and were little affected by the introduction of decay in the fits.

In the 2-site model, the nonequilibrium adimensional parameters β and ω allow to calculate the fraction of type-1 sites, f, and the first-order rate α (d^{-1}) for the kinetic, type-2 sites. The estimated values for the parameters f and α were different for the fits with or without degradation term. If degradation is negliged, the fraction of sites at equilibrium f, at 0.52 indicates that half of the sites would be of kinetic type, whereas if a sink term is introduced, the proportion of kinetic sites would be much lower (< 25 %). The estimates of the first-order rates for the sorption kinetics are extremely high (> 50 d^{-1}), and increase if degradation is not accounted.

Results of the extraction and analysis of the soil sections at the end of the experiments showed that a significant fraction of 5.3 % of the total amount of radioactivity applied in the columns was recovered as methanol extractable (2.7 % of total applied), and bound ^{14}C residues (2.6 % of total applied). The distribution of the extractable and bound ^{14}C-residues in the soil profiles (Fig. 6) show that the amount of extractable residues increased with depth in the column whereas the bound residues were mainly located in the upper end of the column (at the inlet), and decreased with depth.

DISCUSSION

Physical characteristics of Grignon soil

Symmetrical, non retarded BTC of chloride are expected for the displacement of a nonreactive tracer in homogeneous water saturated repacked columns of sieved soil, as reported for instance by Gamerdinger et al. (1990), and Chen & Wagenet (1997). The invariant very low dispersivity α measured for Grignon soil is smaller than the aggregate size of the sieved soil which suggests that some destruction of the aggregates occured while packing of the saturated soil columns. The dispersivity will allow to directly estimate D from the water velocity, in the range of velocities studied. Low dispersivities and high Peclet numbers P indicating poor dispersion are characteristically reported in well packed homogeneous soil columns (Lee et al.,1988; Romero et al., 1997). In undisturbed soils, where preferential flow may occur, much higher dispersivities ranging from 4.5 to 65.8 cm were observed by Jaynes (1991), and low values of P of 0.7 were reported by O'Dell et al. (1992).

The adequate fit of CDeq to our experimental data, and the symmetrical shape of the BTC at the three measured water velocities in our study is representative of systems that are not influenced by transport nonequilibrium. Thus, physical nonequilibrium, which is suggested by the small decrease in β that was observed, can reasonably be assumed to be negligible in our columns, and nonequilibrium conditions for triticonazole reactive tracer would mostly arise from sorption nonequilibrium.

Nonequilibrium transport of triticonazole

The asymmetrical shape of triticonazole BTC and decreased solute peak height are indicative of nonequilibrium, and the considerable tailing suggests that it is primarily sorption related. Similar sorption-related nonequilibrium transport characteristics have been reported for various organic chemicals in repacked homogeneous soil columns (Lee et al., 1988; Angley et al., 1992; Gaber et al. 1995), and in field studies (Jaynes, 1991). Under these conditions, the failure of the CDeq model is expected, since the LEA would not be valid for triticonazole transport in soil.

Sorption nonequilibrium has been evidenced for a large number of pesticides, with a two-stage approach to equilibrium. A short initial fast phase of sorption is generally reported in the first few minutes (Kookana et al., 1993; Gaston and Locke, 1995), which is followed by an extended period of much slower uptake, occuring over periods of days or months. Brusseau and Rao (1989), and Brusseau (1991) reviewed the different rate-limiting processes of nonequilibrium, and they attributed the rate-limiting step in the sorption nonequilibrium of HOC in soil to intra-sorbent (intra-organic matter) mass transfer diffusion. Our previous results (Beigel et al., 1997a and b) support this hypothesis, as triticonazole sorption was related to nonspecific interaction mechanisms with soil OM, and rate-limiting slow desorption of triticonazole was evidenced during prolonged incubation, which was attributed to intrasorbent diffusion. Under these conditions, instantaneous type-1 sites would represent the sites that are directly accessible, while kinetic type-2 sites would be sites that are more remote in the soil organic constituents. The first-order rate α for sorption kinetics of the time-dependent sites would in fact reflect a rate-limiting diffusion process. If the water velocity is low enough for all the type 1, accessible sites to be reached, the 2-site assumption may be valid, and this approach has been successfully used to predict the nonequilibrium transport of pesticides in homogeneous soil columns under low velocities (Gamerdinger et al., 1990; Gaber et al. 1995).

The CDnoneq model correctly described the asymmetrical distal portion of triticonazole elution peak, i.e. the early tailing, but it failed to describe the extended tailing of triticonazole BTC. The failure of CDnoneq to describe the experimental BTC of triticonazole show that the two-site approach is not adequate for predicting the transport of triticonazole in soil at high velocity. Chen and Wagenet (1997) observed similar failure of a two-site first-order model to describe atrazine transport in homogeneous soil columns at high velocities. In such case, the rate of sorption-desorption from the kinetic sites cannot be described by a single first-order rate constant, as also reported by Connaughton et al. (1993). The two-stage tailing that we observed suggests that at least two types of kinetic sites need to be considered in addition to the instantaneous sorption sites.

The similar values for K_d obtained with both the CDeq and CDnoneq models with or without decay terms suggests that the estimated K_d adequately describes the instantaneous

sorption of triticonazole during transport. These estimates were much lower than the batch measured equilibrium K_d of triticonazole of 4.35 L kg^{-1}, indicating that the batch equilibrium K_d would considerably overpredict the retardation of triticonazole during transport at high flow rate. Such leftward shift of the experimental BTC compared to batch measured partition coefficients predictions has been frequently reported at high velocities, while better agreement is obtained at lower velocities (O'Dell et al., 1992; Gaber et al., 1995; Chen and Wagenet, 1997). This shows that the batch measured partition coefficient would not be appropriate for description of the transport of triticonazole, as the residence time in soil is not long enough for all the sorption sites to achieve equilibrium.

In the water-saturated soil columns packed with finely sieved soil, triticonazole would be directly in contact with the dispersed soil organic constituents, and could thus rapidly diffuse to accessible sorption sites in the internal voids of organic matter. A great part of the kinetic sorption sites would thus rapidly attain equilibrium, and contribute to a great extent to increase the overall sorption of triticonazole. This would be accounted by the very high value of the first-order rate α (>50 d^{-1}) estimated from the 2-site model.

Another part of the kinetic sites would be much more rate-limited, as evidenced by the extended slow and continuous release of sorbed residues and by the fraction of extractable residues remaining in the column. Methanol extractable residues may be subject to desorption in water, as observed by Barriuso et al. (1992), and thus are potentially available for transport. Indeed, their location at the effluent end of the soil indicates that they were subject to convective-dispersive transport, and release would continue after the 16 pore volumes of water were applied, resulting in a longer tailing than observed. These slow kinetics may be attributed to rate-limiting desorption controlled by mass transfer diffusion from remote soil sites. This was not apprehended in the two-site model, and needs to be determined independently for an adequate description of triticonazole transport in soil. Measurement of apparent desorption coefficients K_{app} at prolonged incubation times may be appropriate for evaluation of the time-dependent desorption, as showed by O'Dell et al. (1992).

Kinetics of bound residues formation

In our previous experiments (Beigel et al., 1997a and b)., the formation of a significant fraction of soil bound residues of triticonazole was evidenced immediatly after treatment. Bound residues may arise from physical trapping in the internal voids of soil organic matter (Khan, 1982; Calderbank, 1989). They are resistant to desorption and would thus be nonavailable for transport. This would greatly affect the elution of triticonazole in soil, and may also partly account for the two-site / two region incapacity to describe the experimental data. Indeed, significant amounts of bound residues were measured in the soil columns. Their localization at the inlet end of the soil profile show that the removal from soil solution and stabilization in the soil colloids occured immediatly after treating, and that those residues were not subject to convective transport. The nonreversible disparition from soil solution as bound residues cannot be accounted by sorption in the models, even in the two site approach, which explains why the fraction of kinetic sites is considerably higher when no sink term is provided. Bound residues would be partly accounted in the model in the degradation term, and the relatively high degradation rate μ_{liq} estimated from the two-site model, may be attributed to the rapid formation of triticonazole bound residues. However, the rapid formation of bound residues in soil is followed by a much slower, continuous stabilization (Beigel et al., 1997b), and a single first-order rate would not be suitable to describe the dissipation of triticonazole as stabilized bound residues. The kinetics of bound residues formation need to be further studied and correctly described to allow for an accurate modeling and prediction of transport in soil.

CONCLUSION

Our results clearly show that triticonazole convective-dispersive transport in homogeneous saturated soil columns is primarily influenced by its interactions with the soil matrix through nonequilibrium sorption and rate-limited desorption, and through the formation of bound residues. Both processes may be related to rate-limiting, intrasorbent diffusion into soil organic constituents, and thus would be strongly dependent upon the water flow rate. Failure of the two-site nonequilibrium CDE model to describe the extended tailing of

triticonazole experimental BTC at a high flow rate show that nonequilibrium sorption cannot be accounted by a single first-order rate constant. Similarily, whereas the immediate formation of bound residues may be accounted with a high first-order rate in the decay term, use of a single rate constant would prove erroneous for describing the much slower, continuous stabilization of triticonazole residues during prolonged incubation time which has been evidenced in previous studies.

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CONCLUSION GENERALE

I/ SYNTHESE DES RESULTATS

Rétention du triticonazole dans le sol

La sorption du triticonazole sur le sol de Grignon est caractérisée par une forte affinité du fongicide pour les constituants organiques du sol, résultant de mécanismes d'interactions non spécifiques de type hydrophobe. La sorption est donc essentiellement liée au caractère lipophile de la molécule (coefficient de partage octanol/eau élevé). L'isotherme de sorption mesurée en équilibres de suspensions de sol peut être considérée comme linéaire, avec un coefficient de partition K_d mesuré à 4.35 L kg^{-1} et un K_{oc} de 420 L kg^{-1} . L'hystérésis positive observée à la désorption montre que le processus n'est pas complètement réversible.

Le caractère dynamique complexe des interactions physico-chimiques du triticonazole avec la phase solide du sol a été mis en évidence lors des expériences de dissipation (sol non stérile à 80% de la capacité de rétention) et de transferts diffusif et convectif-dispersif (sol stérile saturé). L'évolution des processus de rétention avec le temps se traduit par une diminution de l'extractibilité et donc une stabilisation des résidus de triticonazole dans le sol. On observe simultanément une augmentation de la sorption apparente et la formation de résidus liés non extractibles. Ainsi, la proportion de résidus extractibles à l'eau directement

disponibles diminue au cours du temps et le coefficient de désorption apparent K_{app} augmente de 4 à 9 L kg^{-1}, dépassant rapidement la valeur du K_d mesuré en suspensions. Parallèlement, une fraction de résidus liés est formée quasi-immédiatement après application du triticonazole dans le sol. La proportion de résidus liés augmente avec le temps jusqu'à atteindre 8% après 25 jours d'incubation à 22°C en sol stérile saturé et 21% après 112 jours en sol non stérile non saturé.

Les deux processus sont caractérisés par des cinétiques biphasiques, avec une phase initiale rapide suivie par une évolution beaucoup plus lente dans le temps, qui peuvent être décrites par des équations de type diffusif (fonction de $\sqrt{t}$).

La stabilisation du triticonazole est donc attribuable à des transferts diffusifs limitants à l'intérieur de la matière organique du sol vers des sites de moins en moins accessibles, où les molécules de triticonazole peuvent être piégées physiquement.

Mobilité dans le sol

Les expériences de transferts unidimensionnels diffusif et convectif-dispersif en colonnes de sol homogène saturé ont montré une faible mobilité du triticonazole due à la sorption importante du fongicide. Ceci est particulièrement évident au vu des comparaisons avec des systèmes où les interactions avec la phase solide sont négligeables, dans le cas d'un traceur non réactif (chlorure), ou encore du transport dans un milieu poreux non adsorbant (sable de Fontainebleau).

La stabilisation du triticonazole dans le sol se traduit par un coefficient de diffusion apparent décroissant de 0.05 à 0.02 $cm^2\ j^{-1}$ pour une durée d'incubation de 1 à 25 jours, alors que le coefficient de diffusion effectif en solution, mesuré dans le sable, reste constant à 0.24 $cm^2\ j^{-1}$.

Le non équilibre de sorption se traduit aussi lors du transport dynamique par une courbe

d'élution asymétrique décalée sur la droite, avec une queue d'élution importante due à la désorption limitante.

Ainsi, les transferts de soluté verticaux rapides par entraînement du triticonazole (épisodes pluvieux) aussi bien que la migration plus lente et continue du fongicide par diffusion devraient être fortement réduits dans des sols très adsorbants.

Dégradation

La dégradation du triticonazole dans le sol est essentiellement d'ordre biologique et se traduit par une minéralisation lente et constante par co-métabolisme. Le principal facteur limitant est ici encore la sorption du fongicide qui réduit sa biodisponibilité pour les micro-organismes dégradants. Ainsi, la vitesse de minéralisation k_{zero}, mesurée entre 0.3 10^{-3} et 50 10^{-3} mg kg^{-1} j^{-1}, est directement proportionnelle à la concentration dans la solution du sol.

La description des courbes de minéralisation à l'aide de cinétiques empiriques d'ordre un s'est avérée satisfaisante. Les constantes de minéralisation de premier ordre k mesurées varient entre 0.65 10^{-3} et 2.2 10^{-3} j en fonction de la quantité de produit initialement apportée. Par contre, si la dissipation sous forme de résidus liés est aussi considérée, dans l'optique d'une caractérisation de la dissipation globale du triticonazole dans le sol, l'équation de premier ordre n'est plus valable. Les deux phénomènes, dégradation proprement dite et formation de résidus liés, devraient donc être dissociés et incorporés séparément dans les modèles de transport.

Influence des agents de formulation

La formulation du triticonazole en suspension aqueuse concentrée Réal permet de maintenir une concentration apparente en solution élevée. Des concentrations en solution jusqu'à 50 mg L^{-1}, largement supérieures à la limite de solubilité du triticonazole (8.4 mg L^{-1}), ont été observées dans la solution du sol lors des expériences de sorption. Cette solubilisation accrue, attribuée à une association aux monomères de tensioactifs dans la formulation, peut contribuer à augmenter ponctuellement le transport et la disponibilité du fongicide au voisinage de la semence.

Cependant, si l'on considère des concentrations faibles en agents de formulation, après dilution de la formulation dans la solution du sol, les adjuvants présents dans la formulation, notamment les tensioactifs anioniques Soprophor FLK et Supragil MNS90, n'affectent pas les différents processus étudiés, comme cela a été montré aussi bien au niveau de la sorption que de la biodégradation. Des résultats annexes ont par ailleurs montré que la présence d'adjuvants de formulation ne modifiait ni la diffusion dans le sol, ni les caractéristiques physiques du sol (courbe de rétention de l'eau).

L'étude plus spécifique de l'action des tensioactifs sur la sorption du triticonazole a montré que des concentrations en tensioactifs considérées comme réalistes dans une optique de formulation ne permettent pas de réduire l'adsorption du triticonazole. Au contraire, la sorption importante de certains adjuvants organiques hydrophobes tels que les tensioactifs alkylphénoléthoxylés Triton X100 et Triton N101 peut fortement augmenter la rétention du triticonazole en modifiant les propriétés de surface du sol et en augmentant significativement le K_{oc} du triticonazole.

Modélisation

L'utilisation de modèles déterministes de convection-dispersion a permis de déterminer correctement les paramètres physiques de transfert hydrique (vitesse moyenne d'écoulement poral v et coefficient de dispersion hydrodynamique D) par calage sur les courbes d'élution du traceur non réactif, le chlorure.

Les modèles testés diffèrent par leur prise en compte du processus de sorption, avec soit le concept d'équilibre local (CDEeq), soit celui de non équilibre chimique à deux types de sites (CDEnoneq). Dans ce modèle, nous supposons qu'une partie des sites de sorption est directement accessible (type 1: équilibre), alors que les autres sites sont plus éloignés et nécessitent un transfert physique par diffusion (type 2: cinétique de premier ordre).

La description par ces modèles du transport du triticonazole en conditions de sol homogène saturé à un v de 200 cm j^{-1} et un D de 16 cm^2 j^{-1} n'est pas satisfaisante.

Le modèle CDEeq n'est pas approprié pour décrire l'élution asymétrique du triticonazole due au non équilibre de sorption.

Le modèle à bicontinuum CDEnoneq procure une meilleure description des résultats expérimentaux, en particulier au niveau du pic d'élution correspondant à la sorption instantanée et à la phase initiale rapide du non équilibre. Le coefficient de partition de 1.5 L kg^{-1}, estimé par calage du modèle sur les résultats expérimentaux, est nettement inférieur au K_d mesuré en suspension. Cette méthode de mesure d'équilibre de sorption n'est donc pas appropriée pour caractériser la rétention du triticonazole lors du transport convectif-dispersif.

Le modèle CDEnoneq ne prend cependant pas en compte la queue d'élution, ce qui se traduit par une sous-estimation de la quantité de triticonazole réellement disponible et une surestimation de sa dissipation. Les paramètres caractérisant le non équilibre de sorption et la dissipation du triticonazole estimés à l'aide de ce modèle ne sont donc pas valides pour une

prédiction du transport en sol homogène saturé.

D'après l'ensemble des résultats obtenus concernant les processus physico-chimiques (sorption), biologiques (biodégradation), physiques (transfert diffusif et transfert convectif/dispersif) et leur couplage lors d'une étude de transport, il apparaît clairement que les interactions physico-chimiques entre le triticonazole et les constituants organiques du sol constituent la clé conditionnant le comportement du triticonazole dans le sol, en matière de persistance, de transport et de biodisponibilité. Deux phénomènes résultant de ces interactions sont à considérer en particulier: la désorption et la formation de résidus liés. Ces deux processus ont apparemment une même origine, un transfert de masse par diffusion, ce qui les rend difficilement dissociables et leur confère un caractère dynamique complexe. L'incapacité actuelle à décrire de manière simple cette dynamique à l'aide de paramètres facilement mesurables est le principal frein à une description correcte du transport et de la disponibilité du triticonazole dans les sols, et donc à la prédiction de son comportement.

En conclusion, la rétention forte et partiellement non réversible du triticonazole sur la matière organique du sol, évoluant vers une stabilisation croissante des résidus dans le sol, peut devenir limitante à long terme pour l'activité systémique de la molécule phytosanitaire et, par conséquent, pour l'efficacité des traitements. En effet, la mobilité et la biodisponibilité réduites du triticonazole dans la solution du sol résultant de la sorption pourraient rendre limitant l'approvisionnement au niveau racinaire, notamment dans le cas de sols riches en matière organique.

II/ PERSPECTIVES

L'étude ayant traité du comportement d'une molécule particulière, le triticonazole, dans un seul sol, la généralisation des résultats est à considérer avec prudence. Pour le triticonazole, la mise en place d'études similaires dans d'autres sols plus ou moins adsorbants apparaît nécessaire dans une optique d'optimisation des traitements. Néanmoins, en considérant que les processus majeurs impliqués dépendent des caractéristiques physico-chimiques des réactifs (lipophilie des molécules et des surfaces adsorbantes), les conclusions dégagées peuvent raisonnablement s'appliquer à d'autres sols comparables et à d'autres molécules organiques hydrophobes.

Tous les travaux ont par ailleurs été menés en conditions contrôlées et restrictives de laboratoire (sol homogène et saturé). La validité des résultats dans des conditions plus complexes de sol structuré (écoulements préférentiels), et de sol non saturé en eau demande à être étudiée. L'application des résultats en conditions naturelles de champs est donc encore une perspective à long terme.

A plus court terme, il convient d'améliorer la description de la dynamique des interactions physico-chimiques du triticonazole avec la matière organique du sol, en situant la problématique à deux niveaux : méthodologie et conception des modèles.

Au point de vue méthodologique, une meilleure caractérisation des cinétiques rapides d'échanges physico-chimiques apparaît nécessaire. Nous avons montré que les K_d mesurés en équilibres de suspensions n'étaient pas valables pour décrire la sorption instantanée pendant le transport convectif-dispersif en colonnes de sol saturé. Les conditions expérimentales de la technique de mesure en suspension, avec un rapport solution/sol très élevé et une agitation pendant 24 heures, augmentent la proportion de sites accessibles, ce qui se traduit par une

surestimation de la quantité directement sorbée. L'estimation des K_d dans les conditions mêmes du transport, par calage des modèles CDE sur les courbes d'élution, semble plus appropriée.

Nous avons par ailleurs mis en évidence la formation de résidus liés lors du transport. Il reste à déterminer précisément et à formaliser la phase initiale rapide (premières 24 heures) de la cinétique de ce processus de dissipation, ce que ne permet pas la méthode de mesures d'extractibilité actuellement employée.

Au niveau de la modélisation, nous avons montré que le modèle à bicontinuum deux sites ne permettrait pas de prédire le transport du triticonazole. Une approche à trois compartiments semble plus appropriée, avec une fraction de sites directement accessibles (type 1: équilibre) et deux types de sites cinétiques traduisant un transfert vers les sites plus éloignés dans la matière organique du sol (type 2: désorption initiale rapide, type 3: désorption lente), de manière à prendre en compte les deux phases du non équilibre de sorption du triticonazole. Enfin, il paraît intéressant d'introduire dans les modèles de transport un terme puits spécifique supplémentaire pour la formation de résidus liés afin de bien dissocier les processus biologiques et physico-chimiques impliqués dans la dissipation du triticonazole.