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Persistence of fluazinam in soil under boreal conditions

Kati P. Hakala^{1*}, Markku J. Yli-Halla¹, Päivi M. Tuomainen², and Helinä Hartikainen¹

¹ *Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland*

² *Department of Food and Nutrition, University of Helsinki, Helsinki, Finland*

Abstract

Fluazinam, a widely used pesticide in conventional potato cultivation, is effective against epidemics of the fungal disease late blight. To assess fluazinam persistence in soil, laboratory experiments were conducted with fluazinam added to soil as a pure chemical or contained in the commercial product Shirlan[®]. In a follow-up experiment, the persistence was monitored under constant temperature and water content conditions during a maximum period of 1 year. In an annual climatic rotation experiment, fluazinam added to soil was exposed to the year-round temperature and water content conditions occurring in the boreal zone. A third experiment was undertaken to clarify the effect of soil organic matter (SOM) on the recovery of fluazinam. In the follow-up and annual climatic rotation experiments, more than half of the added fluazinam was recovered after 1 year of incubation. The estimated half-life of fluazinam ranged between 355 and 833 days. The degradation of fluazinam was enhanced by an abundance of SOM, a warm temperature, and wetness. Additionally, in over half of soil

* Address correspondence to Kati P. Hakala, Department of Agricultural Sciences, University of Helsinki, Viikinkaari 9, FI-00790 Helsinki, Finland; E-mail: kati.p.hakala@helsinki.fi

samples collected from fields where potato had been intensively cultivated for many years, varying concentrations of fluazinam were detected. Fluazinam can carry over to the next growing season in professional potato production.

KEYWORDS: Pesticide; Shirlan®; degradation; potato late blight; high-performance liquid chromatography

Introduction

Potato (*Solanum tuberosum*) is a widely used daily staple food, particularly in Europe, North America, and parts of Asia. In Finland, its yearly consumption is about 285 million kg, corresponding to about 52 kg per person, ^[1] and in 2016, the arable land area used for potato cultivation totaled 21,900 ha. ^[2] One of the most damaging potato diseases worldwide is potato late blight (*Phytophthora infestans* (Mont.) de Bary). ^[3] Fluazinam (3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)- α,α,α -trifluoro-2,6-dinitro-p-toluidine, CAS No. 79622-59-6) (Fig. 1), belonging to the chemical group of phenylpyridinamines, is a fungicide widely used against late blight epidemics of potato.

Fluazinam is an effective contact fungicide that inhibits fungal respiration and the production of energy within the fungus. ^[4] It also adversely affects luminescent soil bacteria, the vegetative vigor of terrestrial vascular plants, freshwater and marine organisms, and the dietary and reproductive capacity of wild mammals. ^[5, 6] Arguments for the severe toxicity of fluazinam to soil organisms have been presented. In terms of ecotoxicity, fluazinam is considered to be a hazardous pesticide. ^[7] In a Finnish study, fluazinam was demonstrated to be highly toxic in a microcosm test but less toxic in mesocosm and field tests. ^[8]

In Finland, the commercial product Shirlan® (containing 50% w v⁻¹ fluazinam) is normally sprayed onto potato foliage several times at the end of the spraying schedule. Its maximum individual dose is 0.4 L ha⁻¹ (corresponding to 200 g ha⁻¹ fluazinam) mixed with water, the maximum number of applications is 10 per crop, and the latest spraying time is 7 days before the harvest. An unsprayed 5-meter buffer zone adjacent to surface water bodies is recommended. In Finland, it is not recommended to use fluazinam on the same field plot in consecutive growing seasons in potato cultivation. ^[9]

Fluazinam ends up in soil during spraying and at the end of the growing season, when potato foliage is incorporated into the soil. It is generally classified as non-persistent or moderately persistent in soil. Fluazinam is not readily biodegradable, its mineralization is almost negligible, and photolysis may contribute moderately to its environmental dissipation in soil. ^[10] Its degradation is enhanced by anaerobic conditions and a high soil organic matter (SOM) content. ^[10-12] Fluazinam degradation has been found to be most effective at the beginning of experiments. ^[9] The reported degradation half-life (DT50) values obtained in field experiments carried out in China, Germany, and Finland have varied significantly, from 1–4 days ^[13] to 8–41 days ^[10] and even 45–165 days. ^[14] The European Food Safety Authority ^[10] (EFSA) demonstrated that the DT50 of fluazinam in soil is 17–226 days under aerobic and 4–26 days under anaerobic conditions.

The log K_{ow} (octanol/water partition coefficient) of 4.03 indicates that fluazinam is weakly polar and has low water solubility. ^[11] Thus, its retention most probably occurs via physical sorption onto SOM. Chloro groups, in particular, increase the molecular volume of the compound, which assists its sorption onto SOM by hydrophobic attraction. ^[15] Thus, a high

SOM content can notably increase the sorption tendency and favor its accumulation. The strong binding onto soil particles as well as the chloro and fluoro substituents in the fluazinam molecule retard microbiological degradation and photodegradation on the soil surface. ^[16] The gradual accumulation of pesticides with slow degradation tendencies is possible in professional potato production, which is often based on long-term monoculture. In fact, fluazinam is reported to accumulate in soil ^[9] and to be preserved to the next growing season. ^[8, 11] According to the Ministry of Water and Environment, ^[17] the proportion of fluazinam bound to soil particles was striking in a Finnish soil, being 47% of the added amount after 180 days under aerobic and 60% under anaerobic conditions.

Fluazinam displays little movement downwards in soil in the dissolved form, but in soils subjected to erosion, it can be transported with detached soil particles to watercourses. In a study undertaken in Canada, fluazinam was not found to leach through soil layers, ^[11] while a study in Denmark was unable to detect fluazinam in leachates from landfills. ^[18] In a field experiment conducted on a silty soil in Finland, 0.01% of the fluazinam applied was transported with surface runoff, whereas the fungicide was not detected in subsurface drainage water. ^[14]

In Finland, the cold temperatures retard the degradation of fluazinam in soil. ^[9] During winter, the topsoil is commonly frozen for several months each year, and even in Southern Finland, the mean temperature in surface soil (0–5 cm) exceeds 10°C only during 4 months per year and the mean soil temperature in July is 15°C at the depth of 20 cm. ^[19] Consequently, the degradation of fluazinam slows down in winter, ^[20] which contributes to the retention of its potential bioavailability over winter. ^[8]

Few studies have been conducted on the behavior of fluazinam in soil, especially in the boreal zone, where the characteristic low pH and low temperatures delay microbiological decomposition. Furthermore, in boreal conditions, the annual variation in temperature is large, with cold winters and a short growing season of about 170 days in Southern Finland and 110 days in Northern Finland. ^[21] In the humid boreal climate, the soils are saturated by water for long periods in autumn, winter, and spring, but the low temperature may not be conducive to the development of anaerobic conditions. On the other hand, soil in the boreal zone is typically high in SOM, which promotes the degradation of fluazinam through high microbiological activity. ^[14] Therefore, information on the net effect of environmental conditions on the persistence of fluazinam in boreal conditions is needed to predict the potential environmental risks of this pesticide. This information is needed because more aggressive strains of *Phytophthora infestans* have spread to northern latitudes, which will further increase the need to use fungicides. ^[22]

The aim of this study was to assess the persistence of fluazinam under various temperature and water content conditions in the boreal zone soils differing in SOM content. Three laboratory experiments were conducted with fluazinam added to soil as a pure chemical (fluazinam standard) or in the commercial product Shirlan[®] (containing 50% w v⁻¹ fluazinam), which is directly sprayed onto potato foliage as an aqueous solution in potato cultivation. First, in a follow-up experiment on plow layer soil and subsoil, the recovery of fluazinam was monitored under constant temperature and water contents during a maximum period of 1 year. Second, in an annual climatic rotation experiment, the recovery of added fluazinam from topsoil and subsoil was monitored under conditions simulating the temperature and water content conditions of the boreal zone for a maximum period of 1 year. Third, the effect of SOM on the degradation of fluazinam was examined using soil samples before and after the

removal of SOM. In addition to these laboratory experiments, soil samples were collected from fields under long-term potato monoculture. They were analyzed for fluazinam residues to assess the degradation and accumulation of this fungicide.

Materials and methods

Soil samples

Soil samples were collected from potato fields located in Helsinki (Province of Uusimaa) and in Ylistaro and Kurikka (Province of Southern Ostrobothnia) (Fig. 2). The soil samples for the follow-up, annual climatic rotation, and SOM experiments were collected from two depths of a sandy field at the Viikki research farm of the University of Helsinki. These fields have never been treated with fluazinam. To obtain paired soil samples with a similar texture but differing in SOM content, they were collected from the plow layer (topsoil, 0–20 cm) and from the subsoil underneath (20–35 cm). According to the World Reference Base for Soil Resources (WRB) classification system, ^[23] the soils were Endogleyic Arenosols. Their properties are presented in Table 1.

The Viikki soil samples were homogenized, crushed to pass through a 2-mm sieve, and stored at 5°C. Air-dried subsamples were used to determine the chemical soil properties. SOM was analyzed as the loss-on-ignition, assuming that the weight loss is equal to the SOM content. The potential cation-exchange capacity (CEC_{pot}) was determined as the sum of cations replaced by four subsequent extractions with 1 M ammonium acetate (pH 7). Soil pH was measured in a 0.01 M calcium chloride suspension (1:2.5).

In Southern Ostrobothnia, soil samples were collected in the regions of Ylistaro and Kurikka from commercial potato fields where long-term intensive potato cultivation had been practiced, in some fields even for decades. In October 2013, topsoil (0–10 cm) samples were taken after the growing season. On the basis of a preliminary study, fluazinam was not found in the subsoil, and only topsoil samples were therefore collected. Most of the fields had been sprayed with Shirlan[®] during the same growing season and many fields for a number of years. A total of 30 topsoil samples were collected from 15 different fields of five farms and one research institute. All samples were stored at 5°C, homogenized, crushed to pass through a 2-mm sieve, and air-dried before fluazinam analysis. The air-dried subsamples were analyzed for SOM using the loss-on-ignition method and their pH was measured in a 0.01 M CaCl₂ suspension (1:2.5). The farmers provided information on the soil types and the number of sprayings applied prior to soil sampling.

Experimental designs

A follow-up experiment was conducted to monitor the persistence of fluazinam in topsoil and subsoil under different temperature and water content conditions during a maximum period of 1 year. Initially, the water content of the homogenized soil samples was adjusted to about 25% for topsoil and 20% for subsoil (lower in SOM) or the samples were waterlogged. Moisturizing was performed because Finnish soils are moist for the majority of the year. Then, 25-g portions of soil samples were treated in triplicate with the fluazinam standard (dissolved in acetone) or Shirlan[®] (dissolved in water) by spraying with a syringe. The volume of solution sprayed was only 1 mL to minimize any side effects on the indigenous microbial population and degradation patterns. ^[24] The fluazinam standard (purity 98.5%) was purchased from Sigma-Aldrich Corporation (St. Louis, MO) and the commercial product

Shirlan[®] (containing 50% w v⁻¹ fluazinam) from Syngenta (ISK Biosciences Europe SA, Diegem, Belgium). In both cases, the fluazinam addition was 2.5 mg (corresponding to about 2 kg ha⁻¹), i.e. 10 times higher than the normal application rate (0.4 L Shirlan[®] ha⁻¹), assuming a homogeneous distribution of the chemical on the surface of the plow layer. The tenfold excess was incorporated into the soil to obtain clearly observable fluazinam residues in the analyses. Finally, the samples were incubated in constant-temperature rooms in darkness at 5°C or 22°C for 90, 180, 270, or 360 days. The maximum of 1 year as a reference period was chosen to obtain a reliable estimate of degradation [25] and because in potato monoculture it is possible to respray Shirlan[®] in the next growing season. During the incubation, the water content was checked every month, but it was not necessary to add any water. The samples were not stirred during the experiment.

An annual climatic rotation experiment was conducted to monitor the persistence of fluazinam in topsoil and subsoil. It simulated the year-round temperature and water content conditions typical of soil in the boreal zone. The moisturizing and the spraying of the soil samples were performed in triplicate, similarly as in the follow-up experiment described above. During the first 90-day incubation step at 22°C, the constant water content was about 25% in topsoil and 20% in subsoil. This combination of temperature, time, and water content was taken to simulate summer conditions. Thereafter, to mimic cool and wet autumn conditions, the samples were flooded and incubated at 5°C for 90 days. Then, the samples were exposed to winter conditions of -7°C for 90 days and finally to 5°C for 90 days, still in a waterlogged state, simulating snow melting (spring). During this experiment, the samples were kept in darkness and they were not stirred to avoid unintended oxidation reactions. In all sample sets the fluazinam concentration was determined after each 90-day incubation step by using all the sample from the incubation bottles.

A third experiment was undertaken to directly assess the effect of SOM on the behavior of fluazinam. In this experiment, SOM was removed from air-dried topsoil and subsoil samples by using 30% hydrogen peroxide (H_2O_2) ^[26] purchased from Merck & Co., Inc. (Whitehouse Station, NJ). Then, the soil samples were air-dried, sprayed with fluazinam standard or Shirlan[®] in triplicate as in the follow-up experiment, and incubated for 90 days at room temperature in darkness. Corresponding air-dried samples without SOM removal were sprayed and incubated in the same manner.

Soil analyses

The fluazinam concentration in soil was analyzed immediately after each incubation period. The method used is described in detail by Hakala et al. ^[27] Briefly, fluazinam was extracted from 25 g of sample soils with acetonitrile in triplicate. Then, after filtration and solvent change to heptane, the extract was purified using solid-phase extraction (SPE) with a mixture of diethyl ether and heptane (20:80). Finally, after nitrogen (N_2) evaporation, the fluazinam concentration was analyzed by HPLC-DAD (high-performance liquid chromatography equipped with a diode-array detector) in acetone. Strata[®] SI-1 silica cartridges (500 mg) were purchased from Phenomenex (Torrance, CA), HPLC-grade acetonitrile, heptane, and acetone from Rathburn Chemicals (Walkerburn, UK), and diethyl ether from Merck & Co., Inc. (Whitehouse Station, NJ).

Analyses of fluazinam were performed with an Agilent 1200 Series liquid chromatographic system equipped with a G1315B diode-array detector (DAD, 240 nm) (Agilent Technologies, Santa Clara, CA). A C18 column (Zorbax[®] SB-C18, 4.6 * 150 mm, 5 μm) (Agilent

Technologies) combined with a guard column (SecurityGuard™ cartridge C18, 4.0 * 3.0 mm) (Phenomenex) was used. The mobile phase consisted of 70% acetonitrile and 30% water (v v⁻¹), including 0.02% acetic acid (Merck). The water was purified with a Milli-Q® Plus system (Millipore Corporation, Burlington, MA). ChemStation® software was used for instrument control and data handling (Agilent Technologies).

To assure the quality of analyses, control soil samples were included in each extraction set. They had been treated with the fluazinam standard in the same manner as the samples used in the experiments but without incubation. To assess the repeatability of the results of the HPLC measurements, fluazinam standard solution (10 mg L⁻¹) was measured several times in each HPLC run. The limit of detection (LOD) was 0.03 mg kg⁻¹ and the limit of quantification (LOQ) 0.1 mg kg⁻¹.

Calculations and statistical analyses

DT50 is the time required for the concentration to decline to half of its initial value. Estimated DT50s for the follow-up and annual climatic rotation experiments were calculated from the results after 1 year of incubation by using Equation 1:

$$DT50 = t * \ln(2) / \ln(N_0/N_t) \quad (1)$$

where t = incubation time, i.e. 360 days,

N₀ = concentration of fluazinam at the beginning of the experiment (mg kg⁻¹) and

N_t = concentration of fluazinam after 360 days (mg kg⁻¹).

The results of the Shirlan[®]-sprayed samples of the follow-up experiment were used in a 4-factor design. First, the mean concentration was calculated from triplicates and then the dependent variable was constructed by calculating the percentage reduction in fluazinam for each incubation period (0–90, 90–180, 180–270, and 270–360 days). The factors were sampling depth (0–20 cm and 20–35 cm), soil water content (moist and waterlogged), temperature (5°C and 22°C), and duration of incubation (90, 180, 270, and 360 days). The normality of the results was graphically checked with quantile-quantile (Q-Q) plots, as well as using the Shapiro-Wilk test. The level of statistical significance (p) was taken to be 0.05. Data management and statistical analyses were performed with SPSS Statistics 21 (IBM Corporation, Armonk, NY).

Results

Follow-up experiment

In the follow-up experiment (Table 2), after 1 year, a prominent amount of added fluazinam was recovered in topsoil and in subsoil, ranging from 54–70% and 67–74%, respectively. After 1 year of incubation for fluazinam in Shirlan[®], the average recovery was higher in subsoil (73%) than in topsoil (64%), the recovery in moist soil (69%) being similar to that in waterlogged soil (68%), while the recovery at 5°C (71%) was higher than that at 22°C (66%). Accordingly, the average recovery of the fluazinam standard was higher in subsoil (69%) than in topsoil (58%). In moist soil (64%) it was similar to that in waterlogged soil (63%), but slightly higher at 5°C (65%) than at 22°C (62%). Somewhat more fluazinam in Shirlan[®] was recovered than fluazinam in the pure chemical form, the respective recoveries being 68% and 63%. After 1 year, the highest recovery was recorded in the moist subsoil at 5°C in the

Shirlan[®] treatment and the lowest one in the fluazinam standard treatment in waterlogged topsoil at 22°C. The loss of the fluazinam standard in topsoil is illustrated in Figure 3 and that of Shirlan[®] in Figure 4. The degradation of fluazinam (0 days, 180 days, and 360 days) in the Shirlan[®] treated topsoil sample (moist, 22°C) in the follow-up experiment is illustrated in the chromatograms in Figure 5.

For Shirlan[®], the percentage reduction in the fluazinam concentration in consecutive 90-day periods was generally greater during the first 90 incubation days, ranging from 15–26%, whereafter it drastically declined (Table 3). However, in the soils sprayed with the fluazinam standard, the situation was much more variable. In general, the percentage reduction was greater during the incubation period from 270 to 360 days, being 13–23%, than in the previous periods (0–90, 90–180, and 180–270 days), being 1–19%. For Shirlan[®], the concentration reduction during the incubation period from 270 to 360 days was only 0–6%.

The estimated DT50s for the fluazinam standard varied from 418–548 days for topsoil and 629–713 days for subsoil, while the corresponding figures for Shirlan[®] were 472–704 days and 719–833 days, respectively (Table 4). The estimated DT50 in subsoil was on average 201 days longer than in topsoil. At 5°C, the estimated DT50 was on average 79 days longer than at 22°C, while in the Shirlan[®] treatment it was 112 days longer than in the fluazinam standard treatment.

Annual climatic rotation experiment

In the annual climatic rotation experiment, the residual concentrations were also high after 1 year of incubation (Table 5). The estimated DT50 was 436 days in topsoil and 575 days in

subsoil for the soils treated with fluazinam standard, and 355 and 583 days, respectively, for the soils treated with Shirlan[®]. Generally, the fluazinam concentration diminished most rapidly under moist summer conditions, i.e. during the first part of the experiment. Under spring conditions (the last part of the experiment), which were similar to autumn conditions, the degradation was very slow, in some cases even negligible. It is noteworthy, however, that the degradation of fluazinam was recorded to continue under winter conditions.

The loss of fluazinam was faster in topsoil than in subsoil, its recovery being 56% in the standard-treated and 50% in Shirlan[®]-treated samples. In subsoil, the recovery of fluazinam was similar, being 65% in the standard-treated samples and 66% in Shirlan[®]-treated ones. Generally, the loss of fluazinam was most rapid during the first 90 days of the experiment, slowing down considerably during each subsequent 90-day period. After 1 year of incubation, the highest recovery was recorded in Shirlan[®]-treated subsoil and the lowest in Shirlan[®]-treated topsoil, the respective figures being 66% and 50%.

SOM experiment

The presence of SOM clearly enhanced the loss of fluazinam (Table 6). In the samples containing SOM, the reduction was 14–34% greater than in those from which SOM had been removed. When fluazinam was added in the standard solution, its recovery was similar in both the topsoil and subsoil samples. However, when it was added in Shirlan[®], the soil samples differed markedly in their responses, the recovery in topsoil being lower than in subsoil.

Field samples

From the total of 30 samples collected from fields that had been under intensive potato cultivation, fluazinam was detected in 17, the concentrations being 0.14–1.15 mg kg⁻¹ (Table 7). Among the samples containing fluazinam, 14 had been sprayed with Shirlan[®] during the same growing season. Fluazinam was also found in three fields that had not been sprayed in the same growing season. Out of the 13 samples in which fluazinam was not detected, six were collected from fields sprayed with Shirlan[®] and seven from those not sprayed with Shirlan[®] during the same growing season.

In the majority of the samples in which fluazinam was detected, its concentration was 0.14–0.37 mg kg⁻¹. The highest concentration (1.15 mg kg⁻¹) was recorded in sample A2, which was sprayed three times during the same growing season. Samples H1–H6 were collected from fields that had been under intensive cultivation for about 50 years and frequently sprayed with Shirlan[®]. From these fields, fluazinam was detected in four samples (H3–H6) at concentrations of 0.14–0.26 mg kg⁻¹.

In samples Y1 and Y3, fluazinam concentrations of 0.15 and 0.20 mg kg⁻¹, respectively, were detected, even though the fields had not been sprayed during the same growing season.

Interestingly, fluazinam was not detected in the corresponding sample Y2. Samples E1 and E2 were from potato fields with a history of Shirlan[®] spraying until 2011. However, fluazinam was not detected from these fields, while a field treated similarly (P1) had a fluazinam concentration of 0.14 mg kg⁻¹.

In two fields sprayed six times during the same growing season (P2 and P6), no fluazinam was detected from the soil samples. In four other similar samples (P3, P4, P5, and P7), the

concentrations were 0.33–0.53 mg kg⁻¹. Fluazinam was not detected in the organic soils T3 and T4, despite field T4 having been sprayed during the same growing season.

The control soil samples and HPLC repeatability samples confirmed the uniform quality of the analyses throughout the experiments. Figure 6 presents the HPLC chromatogram of a repeatability sample (fluazinam standard 10 mg L⁻¹).

Factorial design

The factorial design demonstrated that the sampling depth and the duration of incubation had the main effects, i.e. they significantly affected the fluazinam concentration, excluding the effects of all other factors. Interactions were found between the sampling depth and duration of incubation, the soil water content and duration of incubation, and the temperature and duration of incubation. In other words, for the samples sprayed with Shirlan[®] in the follow-up experiment, the factorial design demonstrated that the percentage reduction in fluazinam over time significantly differed between topsoil and subsoil, moist and waterlogged soil, and temperatures of 5°C and 22°C. In our experiment this meant that fluazinam degradation was enhanced by the abundance of SOM, waterlogging, and a warmer temperature.

Discussion

In the follow-up treatment, the decline in the fluazinam concentration during each 90-day period was taken to indicate the rate of degradation. In Shirlan[®] treatment, the loss of fluazinam was most rapid at the very beginning of the experiment. For the fluazinam standard, the concentration decreased in a less consistent manner. During the final incubation

period (270–360 days), its reduction rate in both topsoil and subsoil was quite high compared with the previous periods. Recently, Ren et al. ^[28] reported for some recalcitrant pesticides that microbial adaptation can enhance their dissipation over time, but it is unknown whether this is valid for fluazinam. The unknown inert ingredients in the Shirlan[®]-treated samples probably promoted the water solubility of fluazinam and thus enhanced its degradation at the beginning of the experiment.

Fluazinam is known to degrade more rapidly under anaerobic than aerobic conditions. ^[10] Our follow-up experiment demonstrated that within a time scale of 1 year, waterlogging tended to enhance the degradation only slightly as compared with that under moist conditions. Although the formation of anaerobic conditions will be retarded at a low temperature such as 5°C, such conditions can form at 22°C, at least in the topsoil, where a high SOM content favors microbiological activity. Because the samples were not stirred during the experiments, a significant supply of oxygen did not occur.

In comparison with the values reported in the literature, the estimated DT50s in our follow-up experiment were consistently much longer. This is attributable to factors such as differences in the soil types, the temperature, and the light conditions (the samples in this study were incubated in darkness), differences in the added amount of fluazinam, and the analytical methods. Under waterlogged conditions, the estimated DT50s were generally shorter than under moist conditions, as concluded by EFSA. ^[10]

In the annual climatic rotation experiment, a prominent amount of fluazinam was still present in soil after 1 year. The loss of fluazinam was most rapid at the beginning of the experiment (mimicking moist summer conditions). It clearly slowed down as the experiment proceeded,

being almost negligible in the last 90-day period (spring). In Finland, soil microbial activity is low during winter, but in our experiment, some loss of fluazinam still occurred. Degradation was more prominent in topsoil high in SOM than in subsoil, because SOM favors microbial degradation.^[14] The estimated DT50s (355–583 days) were generally shorter than those in the follow-up experiment (418–833 days), in which the samples were kept under constant conditions for 1 year. This outcome could have been attributed to the freezing and thawing processes, which altered the soil structure. In this experiment, the estimated DT50s were also considerably longer than those reported in the literature.

The experiment on the impact of SOM removal clearly demonstrated that the SOM content considerably contributes to the degradation rate of fluazinam. In the samples without SOM removal, the degradation of fluazinam was more rapid, obviously due to the enhanced microbial activity. The degradation-promoting effect of SOM was especially marked in topsoil. Although the subsoil was innately low in SOM, the treatment with H₂O₂ nevertheless reduced the loss of fluazinam. The abundance of SOM appeared to particularly enhance the loss of fluazinam in Shirlan[®], probably because its water solubility was higher than that in the fluazinam standard.

In the fluazinam-containing soil samples collected from the potato fields, its concentrations were rather variable. Fluazinam was found in three samples from fields that had not been sprayed with Shirlan[®] in the same growing season. This indicates that fluazinam in soil can be carried over from one growing season to the next. Even though some fields had been sprayed with Shirlan[®] six times in the same growing season, the highest fluazinam concentration was recorded in a sample from a field that was sprayed three times during the same growing

season. Three of the intensively cultivated potato fields had been sprayed frequently with Shirlan® for years until 2011, but in 2013, fluazinam was only detected in one of them.

On the other hand, fluazinam was not found in six samples from fields on which Shirlan® had been sprayed in the same growing season. Some of the samples were collected from fields that had been under intensive potato cultivation for decades and frequently sprayed with Shirlan®. Despite this, not all of these samples contained fluazinam. In three samples, fluazinam was not found even though the fields had been sprayed with Shirlan® three times per growing season during the current and the two previous growing seasons. Our previous finding ^[27] that fluazinam molecules were immobile and remained where they ended up with the incorporated potato tops is also valid for the potato field samples. Fluazinam was not found in the organic soil samples, probably because microbes had degraded it.

The sorption of pesticides onto soil particles can increase with a prolonged residence time. This phenomenon (aging) is due to pesticide diffusion to less accessible or stronger sorption sites, or both. ^[25] Sharer et al. ^[29] observed that as for pesticides with strong retention, little or no increase in sorption occurred with prolonged equilibration times. Therefore, most probably, the reduction in the fluazinam concentration in our experiments was mainly due to its degradation.

Conclusions

Despite some inconsistencies in the degradation rate, the main outcome was that a substantial amount of fluazinam remained in soil after 1 year of incubation. Our experiment

demonstrated that this chemical can be quite persistent in soil, raising a concern about its accumulation in soil upon repeated applications.

In the follow-up and the annual climatic rotation experiment, more than half of the added fluazinam was still present in the samples after 1 year. The estimated DT50s were much longer than the corresponding values reported in the literature, and this trend was seen throughout the experiments. Fluazinam degradation in topsoil appeared to be more rapid than in subsoil due to the higher SOM content favoring microbial activity.

The results of the potato field samples were quite variable when considering the spraying history of the fields. There seemed to be no relationship between how long and how many times the fields had been sprayed with Shirlan® and the amount of fluazinam detected in the soil. Because of the uneven distribution of the pesticide, a large number of samples should be collected from the fields and the analysis should be carried out with several replicates to obtain a comprehensive picture of fluazinam degradation.

Based on the results obtained in all the experiments and from field soil samples, in professional potato production based on monoculture, fluazinam can be carried over to the next growing season in the field, especially under the cold conditions typical in Finland. Despite this, field soil samples indicated no substantial accumulation of the pesticide in soil.

Our experiments call for local soil and climate conditions to be taken into account in estimating the behavior of chemicals. The results highlight the risk of directly transferring information from different climates when formulating regulations for pesticide use. Further

studies on the effect of photodegradation on the dissipation of fluazinam are needed to provide information on its fate in agricultural soils.

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FIGURE CAPTIONS

Figure 1. Fluazinam molecule.

Figure 2. Soil sampling locations.

Figure 3. Degradation of fluazinam in topsoil (mg kg^{-1}) following treatment with fluazinam standard in the follow-up experiment (with standard deviation bars in one direction, being 0–10 mg kg^{-1}): **a** moist soil at 22°C, **b** waterlogged soil at 22°C, **c** moist soil at 5°C, and **d** waterlogged soil at 5°C.

Figure 4. Degradation of fluazinam in topsoil (mg kg^{-1}) following Shirlan[®] treatment in the follow-up experiment (with standard deviation bars in one direction, being 1–7 mg kg^{-1}): **a** moist soil at 22°C, **b** waterlogged soil at 22°C, **c** moist soil at 5°C, and **d** waterlogged soil at 5°C.

Figure 5. HPLC chromatograms illustrating the degradation of fluazinam in the Shirlan[®] treated topsoil sample (moist, 22°C) in the follow-up experiment: **a** 0 days, **b** 180 days, and **c** 360 days.

Figure 6. HPLC chromatogram of a repeatability sample (fluazinam standard 10 mg L^{-1}).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Table 1. Soil properties and their standard deviations at the Viikki research farm of the University of Helsinki.

Soil	pH ¹		CEC _{pot} ²		SOM		Sand ³	
	SD		(cmol(+)kg ⁻¹)	SD	(%)	SD	(%)	SD
Topsoil	5.6	0.0	9.4	0.5	2.4	0.0	92	0
Subsoil	5.4	0.0	3.7	0.3	0.3	0.0	98	0

¹pH in 0.01 M CaCl₂ (1:2.5).

²CEC_{pot}, potential cation-exchange capacity, determined with 1 M CH₃COONH₄, pH 7.0.

³Sand (0.06–2 mm) content of the soil, dominated by medium sand (0.2–0.6 mm).

Table 2. Percentage recovery and standard deviation (%) of added fluazinam after 90, 180, 270, and 360 days of incubation ($n = 3$) in the follow-up experiment.

			Recovery (%)				SD (%)			
			90 d	180 d	270 d	360 d	90 d	180 d	270 d	360 d
Topsoil										
Fluazinam standard	Moist	22	81	80	71	59	3	3	1	1
		5	90	83	68	57	5	1	1	2
	Waterlogged	22	86	83	68	54	2	2	2	3
		5	96	92	80	63	2	3	2	1

Shirlan®	Moist	22	74	67	63	60	2	3	2	1
		5	80	76	71	70	1	2	4	4
	Waterlogged	22	74	64	59	59	4	2	1	1
		5	81	76	66	66	4	1	2	2
Subsoil										
Fluazinam standard	Moist	22	94	92	87	67	5	4	6	1
		5	96	95	90	71	2	5	5	2
	Waterlogged	22	94	93	87	67	8	1	1	3
		5	97	96	79	69	9	6	2	2
Shirlan®	Moist	22	80	77	76	71	3	2	1	4
		5	81	80	77	74	1	1	1	3
	Waterlogged	22	82	77	72	72	2	3	2	2
		5	85	79	75	73	3	1	1	1

Table 3. Percentage reduction in the fluazinam concentration in 90 days for successive incubation periods in the follow-up experiment.

			Reduction (%)			
	Water content	Temperature (°C)	0–90 d	90–180 d	180–270 d	270–360 d
Topsoil						
Fluazinam standard	Moist	22	19	2	11	17
		5	10	8	18	16
	Waterlogged	22	14	3	18	21
		5	4	4	13	21
Shirlan®	Moist	22	26	10	5	4
		5	20	4	7	2
	Waterlogged	22	26	14	7	1
		5	19	6	13	1
Subsoil						
Fluazinam standard	Moist	22	6	2	5	23
		5	4	1	5	21
	Waterlogged	22	6	1	6	23
		5	3	1	18	13
Shirlan®	Moist	22	20	4	1	6
		5	19	1	4	4
	Waterlogged	22	18	6	6	0
		5	15	8	5	2

Table 4. Estimated half-life (DT50) and standard deviation (days) ($n = 3$) in the follow-up experiment.

	Water content	Temperature (°C)	DT50 (days)	SD (days)
Topsoil				
Fluazinam standard	Moist	22	476	6
		5	443	30
	Waterlogged	22	418	31
		5	548	49
Shirlan®	Moist	22	485	21
		5	704	54
	Waterlogged	22	472	11
		5	588	63
Subsoil				
Fluazinam standard	Moist	22	633	69
		5	713	64
	Waterlogged	22	634	17
		5	629	23
Shirlan®	Moist	22	719	61
		5	833	48
	Waterlogged	22	785	68
		5	800	44

Table 5. Concentration (mg kg^{-1}), standard deviation (mg kg^{-1}), recovery (%), and percentage reduction of added fluazinam at the end of successive 90-day periods simulating the soil conditions during 1 year and estimated half-life (DT50) (days) ($n = 3$).

	Cumulative duration (d)	Water content	T (°C)	c (mg kg^{-1})	SD (mg kg^{-1})	Recovery (%)	Reduction (%)	DT50 (d)
Fluazinam standard								
Topsoil	90	moist	22	100	7	81	19	436
	180	wet	5	85	1	68	16	
	270	wet	-7	74	4	60	12	
	360	wet	5	70	2	56	7	
Subsoil	90	moist	22	105	7	88	12	
	180	wet	5	91	1	76	14	
	270	wet	-7	80	3	67	12	

	360	wet	5	77	2	65	3	575
Shirlan [®]								
Topsoil	90	moist	22	92	3	74	26	
	180	wet	5	75	1	60	19	
	270	wet	-7	64	3	52	13	
	360	wet	5	61	2	50	4	355
Subsoil	90	moist	22	95	3	80	20	
	180	wet	5	83	3	70	13	
	270	wet	-7	81	4	67	4	
	360	wet	5	77	3	66	1	583

Table 6. Percentage recovery of fluazinam and standard deviation (%) after 90 days of incubation with and without the removal of SOM ($n = 3$).

	Recovery (%)		SD (%)		SOM efficiency ¹
	SOM removed	SOM present	SOM removed	SOM present	
Topsoil					
Fluazinam standard	83	69	3	4	16
Shirlan [®]	80	53	4	2	34
Subsoil					
Fluazinam standard	84	73	3	0	14
Shirlan [®]	88	70	7	6	21

¹SOM-enhanced degradation of fluazinam (%).

Table 7. Properties, concentration of fluazinam (mg kg^{-1}), standard deviation (mg kg^{-1}) ($n = 3$), and number of sprayings of field soil samples.

Soil code	Soil type	pH ¹	SOM (%)	Concentration ² (mg kg^{-1})	SD (mg kg^{-1})	Number of sprayings ³		
						2011	2012	2013

P1	silt	6.3	2.7	0.14	0.01	3	0	0
P2	silt	5.0	5.5	nd		0	0	6
P3	silt	5.0	5.5	0.53	0.02	0	0	6
P4	silt	5.0	5.5	0.37	0.01	0	0	6
P5	silt	5.0	5.5	0.34	0.01	0	0	6
P6	silt	5.0	5.5	nd		0	0	6
P7	silt	5.0	5.5	0.33	0.05	0	0	6
P8	silt	5.0	5.5	nd		0	0	0
P9	silt	5.0	5.5	nd		0	0	0
T1	silt	4.9	5.8	0.22	0.03	0	1	1
T2	silt	4.9	5.8	nd		0	1	0
T3	organic	4.7	14.6	nd		2	0	0
T4	organic	5.1	12.8	nd		2	1	1
Y1	silt	5.1	3.4	0.15	0.02	1	1	0
Y2	silt	5.1	3.4	nd		1	1	0
Y3	silt	5.1	3.4	0.20	0.01	1	1	0
Y4	silt	4.7	1.7	0.23	0.04	2	1	1
A1	silt	4.9	5.0	0.22	0.05	n/a	n/a	3
A2	silt	4.9	5.0	1.15	0.06	n/a	n/a	3
A3	silt	5.2	2.1	0.26	0.03	n/a	n/a	3
H1	sand	5.0	1.7	nd		3	3	3
H2	sand	5.0	1.7	nd		3	3	3
H3	sand	5.0	1.7	0.26	0.02	3	3	3
H4	sand	5.0	1.7	0.14	0.00	3	3	3
H5	sand	5.0	1.7	0.20	0.03	3	3	3
H6	sand	5.0	1.7	0.18	0.02	3	3	3
H7	sand	5.0	1.7	nd		3	3	3
H8	sand	5.0	1.7	0.31	0.07	0	0	3
E1	silt	5.0	1.3	nd		3	0	0
E2	silt	5.0	1.3	nd		3	0	0

¹pH in CaCl₂ (1:2.5).

²nd, not detected.

³Spraying times, dosage per 1 spraying ca. 0.4 L ha⁻¹; n/a, not available.