KATI P. HAKALA

Fluazinam in Soil: Analytical Method and Persistence in Boreal Conditions

ENVIRONMENTAL SOIL SCIENCE
FACULTY OF AGRICULTURE AND FORESTRY
UNIVERSITY OF HELSINKI

Department of Agricultural Sciences
Faculty of Agriculture and Forestry
University of Helsinki, Finland

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Supervisors	**Professor Markku Yli-Halla** Department of Agricultural Sciences University of Helsinki, Finland
	Professor Helinä Hartikainen Department of Agricultural Sciences University of Helsinki, Finland
	Docent Päivi Tuomainen Department of Food and Nutrition University of Helsinki, Finland
Examiners	**Sari Autio** Research Manager Natural Resources Institute Finland (Luke)
	Suvi Ojanperä Head of Section Finnish Customs
Cover art	**Kati P. Hakala**

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
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Tiivistelmä – Referat – Abstract

Fungal disease late blight (*Phytophthora infestans*) causes considerable damage to potato crops worldwide. Fluazinam is a widely used pesticide employed against the late blight in potato cultivation. It ends up into soil during spraying and at the end of the growing season when potato foliage is incorporated into the soil. Nevertheless, there is very little literature about behaviour of fluazinam in soil, especially in the conditions that exist in Finland. Therefore, in the preparation of user guidelines, studies made elsewhere are used. From the environmental risks point of view, behaviour of fluazinam in Finnish conditions should be known better.

Soils in the boreal zone are characterised by low pH and low temperatures that delay microbiological decomposition and they are typically high in organic matter and saturated by water for long periods in autumn, winter and spring. A prerequisite for assessing the environmental risk of fluazinam is knowledge of its sorption and desorption tendency as well as its degradation rate in boreal conditions. This information is needed, because more aggressive strains of *Phytophthora infestans* have spread to northern latitudes, increasing the need to use fungicides.

In this study, a specific and repeatable high-performance liquid chromatography method utilizing a diode array detector was developed to determine the presence of fluazinam in soil. This method differs from most of the methods found in the literature, which used gas chromatography or gas chromatography-mass spectrometry as an instrument for analysing fluazinam. The method consists of acetonitrile extraction, clean-up with solid-phase extraction and separation using a mobile phase consisting of 70% acetonitrile and 30% water (v v^{-1}), including 0.02% acetic acid. The method was successfully applied to various laboratory experiments and to soil samples collected from potato fields in which fluazinam had been used.

In the systematic experiments carried out in controlled conditions, performed with both the fluazinam standard and the commercial product Shirlan®, the effect of soil organic matter on the fluazinam degradation was tested, as well as the persistence of fluazinam in the boreal zone soils for a maximum of one year. The major outcomes of the laboratory experiments were that fluazinam degradation was enhanced by the presence of soil organic matter and even after one year of incubation, more than half of the added fluazinam was recovered. Additionally, soil samples were collected from intensively cultivated potato fields. Over half of these field samples contained varying concentrations of fluazinam, but no substantial accumulation of fluazinam was detected.

Avainsanat – Nyckelord – Keywords

Soil, pesticide, degradation, fluazinam, Shirlan®, potato late blight, liquid chromatography, HPLC, boreal

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

Foreword

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Contents

List of original publications and participation

This study is based on the following publications, which are referred to by their Roman numerals:

I Hakala, K. P., Tuomainen, P. M., Yli-Halla, M. J. & Hartikainen, H. 2014. High-performance liquid chromatography (HPLC) as a tool for monitoring the fate of fluazinam in soil. *Journal of Environmental Science and Health, Part B* 49, 491–497.

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II Hakala, K. P., Yli-Halla, M., Tuomainen, P. & Hartikainen, H. Persistence of fluazinam in soil under boreal conditions. Submitted to *Journal of Environmental Science and Health, Part B*.

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In addition, some unpublished data are presented.

The author's contribution:

I The first author participated in planning the study together with the other authors. The first author was responsible for sampling and experimental work, with the exception of some basic analysis of soil. The author also had the main responsibility of writing the article in collaboration with the co-authors.

II The first author participated in planning the study together with Markku Yli-Halla and Helinä Hartikainen. The first author was responsible for sampling and experimental work. The author also had the main responsibility of writing the article in collaboration with the co-authors.

Abbreviations

%RSD	percentage relative standard deviation
a.i.	active ingredient
AMPA-fluazinam	4-chloro-6-(3-chloro-5-trifluoromethyl-2-pyridylamino)-*α*,*α*,*α*-trifluoro-5-nitro-m-toluidine
CEC_{ef}	effective cation-exchange capacity
CEC_{pot}	potential cation-exchange capacity
DAD	diode-array detector
DAPA	4-chloro-2-(3-chloro-5-trifluoromethyl-2-pyridylamino)-5-trifluoromethyl-m-phenylenediamine
DT50	degradation half-life, the time required for the concentration to decline to half of its initial value
DDT	dichlorodiphenyltrichloroethane
GC	gas chromatography
GC-ECD	gas chromatography equipped with electron capture detector
GC-MS	gas chromatography equipped with mass spectrometry
GC-MS-SIM	gas chromatography equipped with mass spectrometry and selective ion monitoring
HCH	hexachlorocyclohexane
HPLC	high-performance liquid chromatography
HPLC-DAD	high-performance liquid chromatography equipped with diode-array detector
HPLC-MS/MS	high-performance liquid chromatography equipped with tandem mass spectrometry
HYPA	5-(3-chloro-5-trifluoromethyl-2-pyridylamino)-*α*,*α*,*α*-trifluoro-4,6-dinitro-o-cresol
K_{ow}	octanol-water partition coefficient
LC	liquid chromatography
LC-MS/MS-ESI	liquid chromatography equipped with tandem mass spectrometry and electrospray ionization
LOD	limit of detection
LOQ	limit of quantification
MAPA	2-chloro-6-(3-chloro-5-trifluoromethyl-2-pyridylamino)-α,*α*,*α*-trifluoro-5-nitro-m-toluidine
R^2	coefficient of determination
SD	standard deviation
SOM	soil organic matter
SPE	solid-phase extraction
UPLC-MS/MS	ultra-performance liquid chromatography equipped with tandem mass spectrometry

1. Introduction

1.1 Survey of pesticides

A pesticide is any substance or mixture of substances, natural or synthetic, formulated to prevent, mitigate, repel or destroy weeds, diseases and pests of cultivated plants all over the world. Classes of pesticides are, but not limited to, herbicides, insecticides, fungicides, rodenticides, molluscicides, ovicides and pheromones.

The worldwide consumption of pesticides is about two million tonnes per year, of which about 47.5% is of herbicides, 29.5% of insecticides and 17.5% of fungicides (De et al., 2014). It is estimated that nearly one-third of the agricultural products are produced by using pesticides (Zhang et al., 2011). Pesticide use has increased 50-fold since 1950 (Tadeo et al., 2008) but declined since 2007 (Zhang, 2018). Most of the pesticides are consumed in Europe (45%) and in the USA (25%) (De et al., 2014). Pesticide application depends on the climatic conditions and on the outbreak of pests and diseases of a particular year. Crop loss from pest injury declined by 35–42% when pesticides were used (Pimentel, 1997). Globally, in total of approximately 50,000 species of plant pathogens injure crops (Zhang et al., 2011) and cause an estimated 13% loss in crop production (Pimentel, 2009).

At first, pesticides had a good reputation mainly due to the control of diseases like malaria transmitted by mosquitoes, killing millions of people over time (Tadeo et al., 2008). But since the 1960s, as the toxic effects of dichlorodiphenyltrichloroethane (DDT) on birds became evident, society awareness of chemical contaminants in the environment and consumer concerns on food safety have increased (Tadeo et al., 2008). At present, due to the possible toxic effects of pesticides on human health and on the environment, there are strict regulations for their registration and use all over the world. The registration of a pesticide for its application on a particular crop requires normally data on physicochemical properties, analytical methods, efficacy, toxicology, ecotoxicology and fate and behaviour in the environment (Tadeo et al., 2008).

The rate of pesticide degradation is estimated using a chemical property known as half-life (DT50). DT50 is the time required for the chemical concentration decline to 50% under defined conditions and is not constant for pesticides but dependent on climate and soil properties (Ruuttunen et al., 2008b). According to DT50, pesticides can be classified as nonpersistent (DT50 < 30 days), moderately persistent (DT50 30–100 days) and persistent (DT50 > 100 days) (Kerle et al., 1996).

1.2 History of pesticides

Elemental sulphur has been used against plant diseases as early as 3,500 years ago and 2,000–3,000 years ago oils of a different kind were used against insects, flower powder against flies and salt against plants. Professional chemical protection started in the 1900th century, when plant diseases were prevented first with copper salts and later with other inorganic chemicals, copper sulphate was used against wild radish and lead arsenate against Colorado beetle (Paasivirta and Rytsä, 1980).

A naturally occurring organic pesticide, rotenone, has been used as an insecticide since 1848. The first synthetic organic pesticides were organochlorine pesticides, the very first being hexachlorocyclohexane (HCH), which was invented in 1825, but used as pesticide not until 1941 (Paasivirta and Rytsä, 1980). The triumph of synthetic organic pesticides begun in 1939, when the insecticidal effect of DDT was first discovered and soon after organophosphorus pesticides and carbamates were developed. Nowadays the trend is to develop synthetic organic chemicals having a specific effect on one or few species (Paasivirta and Rytsä, 1980). In Finland, pesticides against

potato late blight have been used since 1953 (Ruuttunen et al., 2008b) and the active ingredients include fluazinam, metalaxyl, dimethomorph, cyazofamid, mandipropamid, fenamidone, propamocarb, maneb and mancozeb.

1.3 Fluazinam

Fluazinam is a fungicide with activity against *Phytophthora infestans* of the group of Oomycetes, Kingdom Stramenopila. Fluazinam belongs to the group of phenylpyridinamines and its IUPAC name is 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). Fluazinam has a molar mass of 465.1 g mol^{-1}, a vapour pressure of 2.3 * 10^{-5} Pa, Henry's law constant of 0.082 Pa m^3 mol^{-1}, log K_{ow} (octanol-water partition coefficient) of 4.03 and its solubility in water is 0.13 mg L^{-1} (pH 5) (Health Canada, 2003). These physical properties indicate that fluazinam has low volatility, low potential to volatilize from moist surfaces and water, a potential to accumulate in soil and low water solubility in acidic conditions. The trade names of fluazinam include Shirlan, Frownside, Ohayo, Winby, Allegro 500F, Omega 500F, Zignal 500 SC, Epok 600EC (contains also metalaxyl-M) and Banjo Forte (contains also dimethomorph).

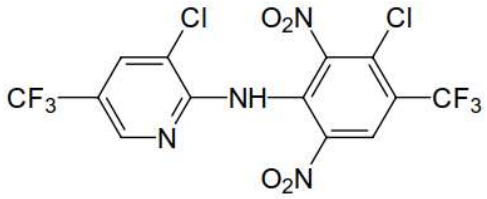

Figure 1. Fluazinam molecule.

Fluazinam is a wide-spectrum contact fungicide with good persistence and rain fastness. Fluazinam was developed by the company Ishihara Sangyo Kaisha, Ltd. (ISK), in Japan and it entered the market at the very beginning of the 1990s. In Finland, fluazinam gained a remarkable market share as soon as it became available in 1995 (Ruuttunen et al., 2008b). Fluazinam filled the gap at the end of the spraying program because its harvest interval is only seven days, it protected potato from late blight better than the former pesticides and it was reasonably priced (Ruuttunen et al., 2008b). In Finland, the use of fluazinam totalled 3.23 t in 2013 (Natural Resources Institute Finland (Luke), Statistical Services, email message to author, 4 August 2017).

Fluazinam is usually added as foliar spraying on potato foliage before potato is exposed to spores. As a contact fungicide, it coats the leaves to prevent infection but is not capable to stop infection once it occurs (Schumann and D'Arcy, 2000). Fluazinam has a multi-site mode of action that disrupts energy production in oomycetes, more exactly, it uncouples mitochondrial oxidative phosphorylation, inhibiting spore germination, hyphal penetration, growth and sporulation (EFSA, 2008).

Fluazinam is considered to be highly toxic to very highly toxic to fish with the potential to bioaccumulate (EPA, 2001). It is expected to adversely affect luminescent soil bacteria, the vegetative vigour of terrestrial vascular plants, the reproductive capacity of wild mammals, freshwater invertebrates, freshwater algae, aquatic vascular plants and marine invertebrates (Health Canada, 2008; Niemi et al., 2008). In a study undertaken in Finland, the effect of fluazinam on soil microbiota was studied (Niemi et al., 2009), where fluazinam was detected to be highly toxic to luminescent bacteria in microcosm test and less toxic in mesocosm and field tests, but changes in soil microbiota were mainly temporary. Arguments for the severe toxicity of fluazinam to soil organisms have been presented (Räsänen et al., 2014).

1.4 Shirlan®

The commercial product Shirlan® contains 50% w v^{-1} of the active ingredient (a.i.) fluazinam. Inert ingredients, which are combined with an active ingredient to make a pesticide product, are usually confidential business information. Inert ingredients play key roles in pesticide efficiency and product performance, e.g. they act as a binder and a solvent to help the active ingredient to penetrate leaf surface, improve water solubility of the active ingredient, improve the ease of application by preventing caking or foaming and extend the shelf-life of the product. Two generally known inert ingredients in Shirlan® are a toluidine because it causes adverse effects on mammals and an ammonium salt, for its chronic effects on aquatic organisms (EFSA, 2008).

Shirlan® is used e.g. against late blight, tuber blight, scab and powdery scab on potatoes, downy mildew on grapevines and onions, scab on apples, grey mould and mites on citrus, southern blight on peanuts, clubroot on cabbage, white mould on soybean and root rot on fruit trees. Against potato late blight, Shirlan® is recommended to be used up to a maximum of eight applications (Finnish Safety and Chemicals Agency, 2012) at an individual application rate of 0.4 L ha^{-1}, corresponding 0.2 kg ha^{-1} fluazinam per spraying (mixed with 300–400 L of water), with a spraying interval of 7–14 days during one growing season in Finland. Normally, Shirlan® is sprayed to the potato foliage several times at the end of the spraying schedule in Finland. While spraying, an appropriate unsprayed buffer zone adjacent to surface water bodies have to be maintained to mitigate the risk to aquatic organisms. To prevent resistance, the use of Shirlan® should alternate with fungicides with a different mode of action (Finnish Safety and Chemicals Agency, 2012).

1.5 Potato late blight

Phytophthora infestans was named by Anton de Bary, father of plant pathology. It was the first plant disease for which a microorganism was proved to be the causal agent, leading to the birth of plant pathology as a science (Schumann and D'Arcy, 2000). Potato late blight originated probably in Mexico, spread to North America in 1843 and from there to Europe in 1845 (Schumann and D'Arcy, 2000). The greatest catastrophe caused by potato late blight happened in Ireland 1845–1848, causing massive emigration and death of hundreds of thousands of people in famine.

Temperature and moisture are the most important environmental factors affecting late blight development. Sporangia are formed on the lower leaf surfaces and infected stems when relative humidity is < 90% and temperatures from 3 to 26 °C (the optimum range being 18–22 °C) (Schumann and D'Arcy, 2000). All parts of potatoes are susceptible to potato late blight and the disease may result in total plant loss or death and severe reduction of the yield (Health Canada, 2003). In addition to blighting foliage, *Phytophthora infestans* can infect potato tubers (tuber blight), as they become infected when sporangia are washed from the foliage into the soil (Schumann and D'Arcy, 2000). The disease continues to develop after the crop is harvested, causing the potatoes to rot in storage (Health Canada, 2003). Oospores may survive in soil over winter and for many years (Schumann and D'Arcy, 2000).

1.6 Potato cultivation and potato late blight in Finland

In Finland, the arable land area used for cultivation of potato totalled 22 100 ha in 2017 (Statistics Finland, 2018). The total consumption of potato was about 255 million kg per year, corresponding to about 46 kg per person per year in 2016 (Statistics Finland, 2018). Potato cultivation is intensive and practised mostly in coarse-textured mineral soils. The nutrient balance of these soils is disturbed easily and their water permeability is good, increasing the risk of leaching of pesticides to groundwater (Ruuttunen et al., 2008b). Intensive potato production is concentrated in the

specialised potato farms in relatively small areas in which rotation of crop is often hard to arrange (Lemola et al., 2000), therefore, potato is often cultivated in the same fields for many consecutive years, even for decades (Ruuttunen et al., 2008b). In monoculture of potato, e.g. the occurrence of plant diseases increases, leading to deterioration in crop (Lemola et al., 2000).

Potato late blight occurs in all areas of potato production and causes considerable economic losses. In Finland, the potato late blight populations have become more aggressive and outbreak of potato late blight takes place about two to four weeks earlier than in the middle of the 1990s due to the increased professional potato cultivation, prolongation of growing season and lack of rotation, resulting in increased pesticide use (Hannukkala et al., 2007; Ruuttunen et al., 2008b). Recently, in the Netherlands, the reduced efficacy of fluazinam against *Phytophthora infestans* was found, indicating that resistance has been developed by the fungus (Schepers et al., 2018).

1.7 Environmental fate of fluazinam in soil

To achieve peak efficacy, a pesticide must retain its biospecificity for a certain time, but after adequate pest or disease control, it is desirable that it degrades to minimize possible environmental hazard. Once introduced into the environment, the processes related to environmental behaviour of pesticides include sorption, degradation, leaching, runoff, volatilization and accumulation (Leistra and Green, 1990). By spraying most of the fluazinam ends up in potato leaves and stays there in the place in which it was hit. Some fluazinam enters soil surface during spraying, but most of it ends up into soil at the end of the growing season when potato foliage is incorporated into the soil (Laitinen, 2008).

1.7.1 Metabolism of fluazinam in soil

Fluazinam does not alter substantially its backbone structure in the environment. Instead, it goes through slight transformations of the functional groups. In soil, substitution of phenyl ring chlorine by hydroxyl yields the major soil metabolite HYPA (5-(3-chloro-5-trifluoromethyl-2-pyridylamino)-*α*,*α*,*α*-trifluoro-4,6-dinitro-*o*-cresol) (Fig. 2), which is considered to be moderately persistent to persistent in soil (DT50 54–148 d) (EFSA, 2008). Minor metabolites result from the reduction of the -NO_2 groups of the phenyl ring to form the corresponding anilines: MAPA (2-chloro-6-(3-chloro-5-trifluoromethyl-2-pyridylamino)-α,*α*,*α*-trifluoro-5-nitro-*m*-toluidine) (Fig. 2), DAPA (4-chloro-2-(3-chloro-5-trifluoromethyl-2-pyridylamino)-5-trifluoromethyl-*m*-phenylenediamine) (Fig. 2) and AMPA-fluazinam (4-chloro-6-(3-chloro-5-trifluoromethyl-2-pyridylamino)-*α*,*α*,*α*-trifluoro-5-nitro-*m*-toluidine) (Fig. 2) (EFSA, 2008). In anaerobic conditions, MAPA, DAPA and AMPA-fluazinam are formed relatively rapid in addition to HYPA, but MAPA and DAPA are not formed or are formed very sparingly in aerobic conditions (EFSA, 2008). The metabolism products of fluazinam appear to be relatively persistent under most conditions (EPA, 2001). Metabolites resulting from the cleavage of the bridging amino group have not been identified, resulting in negligible mineralization of fluazinam in soil (EFSA, 2008).

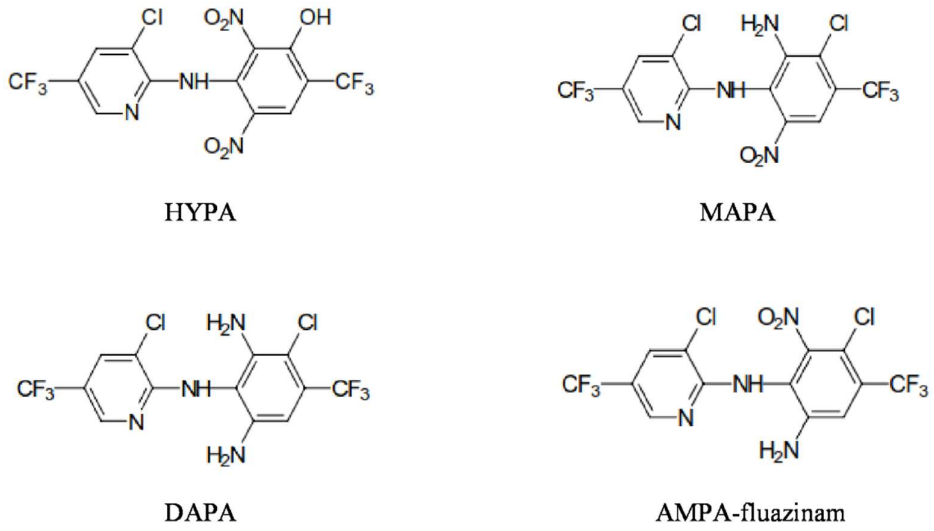

Figure 2. HYPA, MAPA, DAPA and AMPA-fluazinam molecules.

1.7.2 Sorption and retention

The retention of pesticides in soil is affected by the quality of soil (e.g. texture, soil organic matter content, cation exchange capacity), the chemical structure of the pesticide and environmental conditions (e.g. soil pH, soil water content, temperature, light) (Ruuttunen et al., 2008b). The chemical characteristics of pesticides are largely responsible for their behaviour in soil because the type, number and placement of the functional groups on an organic molecule influence the strength and mechanism of its chemical retention in soil. Substitution in the phenyl ring with a halogen (F^- and Cl^-) enhances the sorption of pesticides in soil (Koskinen and Harper, 1990) and chloro groups, in particular, increase the molecular volume of the compound, assisting its sorption onto soil organic matter by hydrophobic attraction (La Poe, 1985). Many times, the heterogeneous nature of soil preclude systematic determination of retention mechanisms.

The retention of very weakly polar fluazinam occurs most probably via physical sorption onto soil organic matter, particularly in the cationic parts (Luo and Liu, 2012; Xu et al., 2013). Hydrophobic fluazinam does not find the polar environment of water to be favourable, instead, it tends to collect at nonpolar interfaces. This physical sorption might be described as hydrophobic partitioning of fluazinam between the aqueous phase and the hydrophobic organic matter (Koskinen and Harper, 1990). The mechanism may not be that simple, e.g. some clays have hydrophobic sites. There is likely a continuum of mechanisms responsible for sorption in soil: an organic molecule may be sorbed initially by sites that provide the strongest mechanism, followed then by progressively weaker sites as the stronger sorption sites become filled (Koskinen and Harper, 1990). According to the Ministry of Water and Environment (1994), the proportion of fluazinam bound to soil particles 180 days after the application was substantial, being 47% under aerobic and 60% under anaerobic conditions.

1.7.3 Degradation

Besides the physicochemical properties of pesticides, their degradation rate depends on many factors, such as soil organic matter and water content, temperature, photolysis, soil pH and microbial degradation (Laitinen et al., 2000). Pesticides are subjected to biological and non-biological degradation processes happening simultaneously (Bollag and Liu, 1990). Fluazinam degradation is enhanced by anaerobic conditions and a high soil organic matter content (Hu et al.,

1997; Health Canada, 2003; EFSA, 2008). Degradation is more rapid in topsoil than in subsoil because both chemical and microbiological reactions are usually more prevailing in topsoil attributable to higher soil organic matter content (Laitinen et al., 2000). On the other hand, the chloro and fluoro substituents in fluazinam molecule and strong binding to soil particles lower its biological feasibility and retard microbiological degradation, while also photodegradation on the soil surface decelerates (Ruuttunen et al., 2008b). Fluazinam degradation slows down considerably as time passes (Autio and Mecke, 2008).

Degradation of fluazinam is strongly temperature-dependent, being slowest during winter and the warmer the temperature, the faster the degradation. In Finland, the cold temperatures have a retarding effect on fluazinam degradation (Autio and Mecke, 2008). Topsoil is commonly frozen for several months each year and even in Southern Finland, the monthly mean surface soil (0–5 cm) temperature exceeds 10 °C for only during four months per year (Heikinheimo and Fougstedt, 1992). As temperature decreases, the microbial function decelerates, and soil being frozen, microbial function is practically stopped. Freezing and thawing exert effects on physical, chemical and biological processes in soil (Andersson and Hartikainen, 2008). The formation of ice breaks soil structure (Hinman, 1970) and therefore might add reactive area and reveal new sorption sites, leading to stronger sorption of compounds. On the other hand, alternating freezing and thawing processes could release tightly sorbed molecules (Hinman, 1970). In a laboratory trial simulating winter conditions frozen soil decelerated degradation, but degradation still happened to some extent (Andersson and Hartikainen, 2008). Fluazinam has demonstrated to retain its bioavailability in the field after winter (Niemi et al., 2009).

Photolysis contributes somewhat to fluazinam degradation in soil (EFSA, 2008), not yielding major metabolites (Health Canada, 2003), but HYPA and AMPA-fluazinam as minor metabolites (EFSA, 2008). The DT50 of fluazinam in soil photolysis studies was 22 days (EPA, 2009).

Fluazinam may be considered stable at pH 4 and pH 5 but is hydrolysed at pH 7 and pH 9 (EFSA, 2008). Hydrolysis is expected to be an important route of degradation in soil under alkaline conditions (Health Canada, 2003).

Among biological processes, microbial metabolism is the primary force in pesticide degradation or transformation in soil. In many cases, microbes are more important in the degradation of a pesticide than are physical or chemical mechanisms (Bollag and Liu, 1990). However, some pesticides are resistant to microbial degradation and persist longer in the environment (Bollag and Liu, 1990). Fluazinam is not considered to be readily biodegradable (EFSA, 2008).

1.7.4 Leaching and runoff

In general, fluazinam is considered to be very slightly mobile in soil attributable to its low water solubility and strong sorption, therefore, it has a very low potential to leach through soil (EPA, 2001; Health Canada, 2003; EFSA, 2008). Potential groundwater contamination by fluazinam is considered negligible but it may be transported to watercourses by runoff with detached soil particles, especially in soils with low organic matter content (EPA, 2001). There was no data about amounts of fluazinam eroded with soil particles in the literature. In any case, the movement of fluazinam to lower soil layers is possible during soil tillage (Autio and Mecke, 2008).

1.7.5 Volatilization

Based on its low vapour pressure, low Henry's law constant and the fact that fluazinam binds strongly to soil and leaves, fluazinam is considered to be practically non-volatile in the environment (Health Canada, 2008; EPA, 2009). Therefore, fluazinam residues are not expected in the air.

1.7.6 Accumulation

The magnitude of the log K_{ow} of fluazinam indicates that there is a potential for accumulation in soil (Health Canada, 2003). Especially, a high soil organic matter content can notably enhance the sorption tendency and favour the accumulation of fluazinam in soil, although the degradation is fastest in soils high in soil organic matter, the soil being biologically active (Laitinen et al., 2000). The gradual accumulation of pesticides with slow degradation tendencies is possible in professional potato production, often carried out at monoculture fields year after year. In fact, fluazinam has been discovered to accumulate in soil (Autio and Mecke, 2008) and it may carry over to the next growing season (Health Canada, 2003; Niemi et al., 2009). In Finland, fluazinam can accumulate in soil already after three years of consecutive use (Ruuttunen et al., 2008b).

1.8 Methods of analysing fluazinam in soil

Degradation reactions of pesticides can be predicted from data obtained under controlled conditions in the laboratory or greenhouse because they are sensitive to environmental factors such as temperature, light intensity or humidity (Taylor and Spencer, 1990). Important points to consider when planning a pesticide study are e.g. choosing the extraction solvent, the degradation rate of the active ingredient in the extract (i.e. the effect of storage on pesticide concentration), the purification of the extract and choosing the analytical instrument. Regarding behaviour of fluazinam in soil a good correlation between laboratory experiments and field tests have been found in a comprehensive Finnish project (Niemi et al., 2008).

The methods reported for analysing fluazinam in soil generally include extraction, purification and quantification with gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). Individual studies have used e.g. GC-ECD (DEFRA, 1994; Dong et al., 2008), GC-MS-SIM (Laitinen et al., 2000; Nguyen et al., 2008; Ruuttunen et al., 2008a), LC-MS/MS-ESI (EPA, 2012; Zhang et al., 2012) and UPLC-MS/MS (Chen et al., 2018). The Austrian Agency for Health and Food Safety (2007) validated the analytical method for the detection of residues of fluazinam in soil with HPLC-MS/MS with the remark that GC methods are not robust enough for this purpose. Analytical methods for analysing fluazinam in soil found in the literature are presented in Table 1.

Table 1. Analytical methods for analysing fluazinam in soil found in the literature.

Analytical equipment	Substance	Extraction solvent	Extraction purification	Reference
GC-ECD, HPLC-MS/MS	fluazinam	acetonitrile	silica cartridge, elution with diethyl ether-hexane	Austrian Agency for Health and Food Safety, 2007
UPLC-MS/MS	fluazinam 17.5%-dimethomorph 17.5%	water-acetonitrile	primary secondary amine, C18, $MgSO_4$	Chen et al., 2018
GC-ECD	Shirlan®	acetonitrile	C18 SPE, elution with acetonitrile	DEFRA, 1994
GC-ECD	fluazinam 50% SC	acetone	Florisil cartridge, elution with hexane-dichloromethane	Dong et al., 2008
LC-MS/MS-ESI	fluazinam	methanol	-	EPA, 2012
GC-ECD	Shirlan®	ethyl acetate-acetone	-	Laitinen et al., 2000
GC-MS-SIM	Shirlan®	acetone-water-dichloromethane-diethyl ether	-	Laitinen et al., 2000
GC-MS-SIM	commercial product	water-acetonitrile	primary secondary amine, MgSO4, graphitized carbon black	Nguyen et al., 2008
GC-MS-SIM	Shirlan®	ethyl acetate-acetone	-	Ruuttunen et al., 2008a
LC-MS/MS-ESI			SPE	Zhang et al., 2012

1.9 Laboratory and field trials concerning fluazinam degradation and leaching in soil

A summary of the laboratory and field experiments found in the literature are presented in Table 2. Based on the studies referred in Table 2, the DT50s of fluazinam vary widely. Leaching of fluazinam is slight and fluazinam degradation in coarse-textured soils is mainly slower than in fine-textured soils.

According to the laboratory experiments of the United States Environmental Protection Agency (EPA, 2009), the DT50 for fluazinam in a sandy loam soil treated at 1 kg ha^{-1} was about 114–132 days and major metabolites were not observed. Another sandy loam soil treated at 5 kg ha^{-1} yielded a DT50 of 227 days and HYPA was observed at up to 11.4% at 30 days posttreatment. A loamy sand soil treated at 1 kg ha^{-1} yielded a DT50 of 165 days without major metabolites, but a major fraction (41%) was bound material at the end of the study (day 361).

In a laboratory study, fluazinam was applied at rates equivalent to 1 and 2 kg ha^{-1} into soil. The degradation was notably fast, DT50 4–26 days, under dark anaerobic conditions due to the relatively rapid reduction of the nitro groups to form MAPA, DAPA and AMPA-fluazinam metabolites (EFSA, 2008). Under dark aerobic conditions, fluazinam was applied at rates equivalent to 0.74, 1 and 5 kg ha^{-1} and according to this study the DT50 was 17–226 days and HYPA was formed (max 14% after 40 days) (EFSA, 2008).

DEFRA (1994) studied fluazinam degradation under laboratory and field conditions. Under aerobic laboratory conditions, sandy loam and loamy sand soil from southern England were used. The application rates were 1 kg ha^{-1} for both soils. The estimated DT50 for sandy loam was 37 days and for loamy sand 224 days. Field degradation studies were carried out in Germany, where

fluazinam was applied at 1.35 kg ha^{-1} in Shirlan®. The field trial soils were loamy sand, sandy loam, clay and clay loam and the DT50 values were 15, 6, 13 and 11 days, respectively. DEFRA (1994) also concluded that groundwater contamination following leaching of fluazinam through light-textured soils appeared unlikely, flooding the soil accelerated degradation and a high application rate (5 kg ha^{-1}) in a sandy loam soil produced deceleration of degradation.

Andersson and Hartikainen (2008) performed a 12-week laboratory study in 5 °C, -7 °C and alternating 5 °C and -7 °C (freezing-thawing simulation) for fluazinam in Shirlan® in sand soil (0–20 cm). In the beginning, the concentration of fluazinam was 0.325 mg kg^{-1}. Degradation was fastest at 5 °C, in which 0.14 mg kg^{-1} of fluazinam was detected at the end of the experiment. Freezing decelerated the degradation, fluazinam concentration being 0.29 mg kg^{-1} at the end of the study, and after freezing-thawing cycle, 0.22 mg kg^{-1} of fluazinam was detected.

Ruuttunen et al. (2008a) studied residues of fluazinam in three typical Finnish potato fields with a long history of potato monoculture during three years. Soil samples were taken from various soil depths from four to seven times per year. Two of the plough layer samples represented sand soil (soil organic matter (SOM) content 3.5% and 1.4%) and one silt soil (SOM content 11.2%). Sprayings were made with Shirlan® at normal doses (amount of fluazinam 0.2 kg ha^{-1}) 17–18 times in sand soils during three growing seasons and five times in silt soil during one growing season. The weather conditions varied widely in the three trial years. Fluazinam was detected in fields before the next spraying season, indicating that fluazinam had carryover over winter. In the last sampling before the plantings or sowings (in the next summer after the last sprayings), fluazinam was detected in sand samples in topsoil (0–6 cm) in the concentrations of 0.083 and 0.070 mg kg^{-1}. In silt soil, fluazinam was detected in topsoil (0–20 cm) in the concentration of 0.030 mg kg^{-1} after three years of the last spraying. The DT50 of fluazinam was 32–369 days. Also in the layer of 20–40 cm some fluazinam (0.2–2.1% of the added amount) was detected at the end of the experiment. Water samples were taken from puddles of the field and among these samples very little fluazinam was detected in comparison of the added amount and the time of the sprayings. It seemed that fluazinam was strictly bound to suspended soil material in the water samples.

Results of terrestrial field studies of dissipation and accumulation conducted in Canada indicated that fluazinam was moderately persistent in soil, with DT50 values of 82 and 95 days with a significant carryover (up to 52%) of residues to the next growing season and there was no evidence of leaching of fluazinam through soil layers (Health Canada, 2003). Field dissipation studies conducted in the USA yielded DT50 values of 19 and 33, indicating slight persistence (Health Canada, 2003). Unfortunately, there was no data about soil types and application rates in these studies.

Chen et al. (2018) studied the degradation rate of a mixture of fluazinam (17.5%) and dimethomorph (17.5%) in field soil. Fluazinam application rate was 0.42 and 0.63 kg ha^{-1} per spraying, repeated four times, with seven days between applications. The DT50 of fluazinam was 10 days and the concentrations in soil were < 0.05–0.183 mg kg^{-1} at the end of the experiment.

Laitinen et al. (2000) conducted a two-years leaching field experiment on fluazinam in silt soil in Finland. After eight months of the last application, fluazinam was detected in the plough layer (0–25 cm) in the concentration of 0.03 mg kg^{-1}, corresponding to about 7% of the added amount (0.2 kg ha^{-1} per application, six applications). Fluazinam was not detected in the subsoil beneath the plough layer. Fluazinam was detected in surface water samples in the concentrations of 0.4–0.6 μg L^{-1}, in the surface runoff the total emissions amounted to < 0.01% of the added amount and fluazinam was not detected in subsurface drainage water.

A soil column leaching study of fluazinam indicated low mobility of the residues of fluazinam: < 1% of the applied fluazinam was detected in the leachates and > 80% remained at the top of the soil columns (EPA, 2001). Unfortunately, there was no data about soil types or application rates in this study.

Table 2. Summary of the laboratory and field experiments found in the literature.

Experiment	Soil type	Application amount (kg ha^{-1})	Application times	Fluazinam concentration (mg kg^{-1})	DT50	Reference
field		0.420 and 0.630	4	< 0.05–0.183	10	Chen et al., 2018
laboratory	sandy loam	1	1		37	DEFRA, 1994
laboratory	loamy sand	1	1		224	DEFRA, 1994
laboratory	sandy loam	5	1		205	DEFRA, 1994
field	loamy sand	1.35	1		15	DEFRA, 1994
field	sandy loam	1.35	1		6	DEFRA, 1994
field	clay	1.35	1		13	DEFRA, 1994
field	clay loam	1.35	1		11	DEFRA, 1994
laboratory		0.74, 1 and 5	1		17–226	EFSA, 2008
laboratory (anaerobic)		1 and 2	1		4–26	EFSA, 2008
laboratory	sandy loam	1	1		114–132	EPA, 2009
laboratory	loam	5	1		227	EPA, 2009
laboratory	loamy sand	1	1		165	EPA, 2009
field					19, 33 and 82, 95	Health Canada, 2003
field	silt	0.2	6	0.03		Laitinen et al., 2000
field	sand	0.2	5–18	0.030–0.083	32–369	Ruuttunen et al., 2008a

2. Objectives of this study

The aim of this study was to gain a better understanding of fluazinam degradation in the boreal zone soils. In order to do that a method to analyse fluazinam in soil was developed first. Then, laboratory studies were performed and potato field samples from potato cultivated fields were analysed.

The specific objectives were to:

1. develop a reliable, straightforward and repeatable method to analyse fluazinam in soil;
2. test the applicability of the method in practice;
3. clarify the effect on soil organic matter (SOM) in fluazinam degradation in soil;
4. determine fluazinam degradation fastness in the boreal zone soils under various constant temperature and water content conditions;
5. determine fluazinam degradation fastness in conditions that mimic the annual climatic conditions of Finland;
6. analyse fluazinam levels in Finnish potato fields in which fluazinam had been sprayed.

3. Materials and methods

3.1 Soil samples

Soil samples used in the methodological development and laboratory experiments were collected in the Province of Uusimaa (I) from the fields of the Viikki research farm of the University of Helsinki in June 2010 and August 2012. These fields have never been treated with fluazinam. The samples were taken from the plough layer of sandy soil (0–20 cm, code: topsoil) and from the subsoil underneath (20–35 cm, code: subsoil) to obtain paired soil samples with a similar texture but differing in SOM content. In addition, samples from a clayey soil (0–28 cm (CS1) and 28–60 cm (CS2)) were taken in Viikki and an organic soil sample (OS), representing the uppermost layer of 0–28 cm, was received from a field located in Perho, Western Finland. According to the WRB classification system (IUSS Working Group WRB, 2014) the soils were classified as Endogleyic Arenosol (Viikki sand samples), Endogleyic Stagnosol (Viikki clay samples) and Umbric Gleysol, with the texture dominated by coarse silt and fine sand (Perho). The samples were homogenized, crushed to pass through a 2-mm sieve and stored at 5 ºC. Air-dried subsamples were used to determine soil pH, the potential cation-exchange capacity (CEC_{pot}), the effective cation-exchange capacity (CEC_{ef}) and soil organic matter (I).

For field soil analysis, samples of commercial potato fields were collected in the municipalities of Ylistaro and Kurikka, Province of Southern Ostrobothnia. Eight samples from three locations were collected in the depths of 0–20 cm (plough layer) and 20–40 cm (subsoil underneath) of packed furrow soil and 0–4 cm of loose ridge soil after the growing season in October 2011 (I). The samples represented silt soil and organic soil. In October 2013, after the growing season, a total of 30 topsoil (0–10 cm) samples were collected from 15 different potato fields of five farms and one research institute in Ylistaro and Kurikka (II), including the same fields as in 2011. All samples were homogenized, crushed to pass through a 2-mm sieve and stored at 5 ºC. The air-dried subsamples were analysed for pH and soil organic matter (II). The farmers provided information on the soil types and the number of sprayings applied prior to soil sampling (II).

3.2 Chemicals

Fluazinam standard (purity 98.5%) was provided by Sigma-Aldrich Corporation (St. Louis, USA) and the commercial product Shirlan® (contains 50% w v^{-1} fluazinam) by Syngenta (manufacturer ISK Biosciences Europe AS, Diegem, Belgium). The Strata® SI-1 silica cartridges (500 mg) were purchased from Phenomenex (Torrance, USA). The purification was accomplished in a Visiprep™ DL SPE vacuum manifold (Sigma-Aldrich Corporation, St. Louis, USA).

High-performance liquid chromatography (HPLC) grade reagents acetonitrile, heptane and acetone were purchased from Rathburn (Walkerburn, UK). Diethyl ether (for spectroscopy), acetic acid (glacial 100%, p.a.) and 30% hydrogen peroxide (H_2O_2) were obtained from Merck (Whitehouse Station, USA). Calcium chloride ($CaCl_2$) was purchased from Riedel-de Haën (Seelze, Germany). The water used was purified using a Milli-Q® -Plus system (Millipore Corp., Billerica, USA). All the glassware was either rinsed thoroughly with technical acetone (BDH Prolabo®, VWR International, Radnor, USA) or heated (two hours at 400 °C) in a muffle furnace (Nabertherm N 11, Lilienthal, Germany). The filter paper used was Whatman™ black ribbon and white ribbon (Maidstone, UK).

3.3 HPLC specifications

Analysis of fluazinam was performed with an Agilent 1200 Series liquid chromatographic system equipped with a G1315B diode-array detector (DAD), a G1312A quaternary pump, a G1367B autosampler and a G1379B degasser (Agilent Technologies, Santa Clara, CA). The column used was a C18 column (Zorbax® SB-C18, 4.6 × 150 mm, 5 µm) (Agilent Technologies) combined with a guard column (SecurityGuard™ cartridge C18, 4.0 x 3.0 mm) (Phenomenex). The mobile phase consisting of 70% acetonitrile and 30% water (v v^{-1}), including 0.02% acetic acid, was used in the isocratic elution with the flow rate of 1.0 ml min^{-1}. The run time was 10–12 min, the injection volume 10 µl and the column temperature 30 °C. The detection wavelength was 240 nm. ChemStation® software was used for instrument control and data handling (Agilent Technologies).

In the methodological development, the analyses of the soil samples in the 90-day incubation experiment were performed with an Alliance 2690 Separations Module with a Waters 996 photodiode array detector using Empower™ 2 software for instrument control and data handling (Waters Corporation, Milford, USA). Otherwise, the conditions were alike as mentioned above.

3.4 Method development

Fluazinam analysis method development (I) was started by familiarizing with the existing literature, which turned out to be scarce. In addition, there were some articles published in Asia, in which only abstracts were written in English. The report of the Austrian Agency for Health and Food Safety (2007) was chosen as the grounds for the methodological development for its rigour. The laboratory work was started by testing the solubility of fluazinam standard and Shirlan® in various solvents (water, heptane, diethyl ether, dichloromethane, acetone). This was necessary to carry out the spraying of fluazinam in a proper manner in the laboratory experiments.

The extraction efficiency was tested by adding 2.5 mg (corresponding to about 2 kg ha^{-1}) of fluazinam in the standard or in Shirlan® to 25-g portions of air-dried sandy topsoil and subsoil samples in triplicate. The added amount was ten times higher than the normal application rate of fluazinam (0.4 L Shirlan® ha^{-1}), assuming a homogeneous distribution of the chemical on the surface of the plough layer. The trial was started with acetonitrile because it is a solvent commonly used in pesticide extraction procedures and the report of Austrian Agency for Health and Food Safety (2007) used acetonitrile in the extraction of fluazinam from soil. The extraction efficiency was tested by refluxing (Büchi Rotavapor® RE-111, Flawil, Switzerland) in a round-bottom flask in a heated water bath and sonicating in an ultrasonic cleaner (Branson B5510-DTH, Branson Ultrasonics Corporation, Danbury, USA).

The purification of the filtered extracts was performed with SPE (solid-phase extraction) using silica cartridges. Diethyl ether and heptane in different combinations (20:80, 30:70, 40:60), as well as sequential elution (20:80, 50:50, 80:20), were tested with both fluazinam standard (1 mg L^{-1}) and soil extracts to obtain the optimal elution. Heptane used in the conditioning of silica cartridges which came out from the cartridge after introducing the extract was analysed for fluazinam.

Gentle nitrogen (N_2) flow was used twice for solvent evaporation when processing the samples. The effect of evaporation on the concentrations of the soil samples was tested.

HPLC-DAD was the first choice as the analytical equipment in trials. The detection wavelength of DAD was tested with 197, 240, 254 and 257 nm. Mobile phases consisting of 70% acetonitrile and 30% water (v v^{-1}), as well as 90% acetonitrile and 10% water (v v^{-1}), both including 0.02% acetic acid, were tested.

The effect of air-drying the soil samples on the recovery of added fluazinam was assessed. The field-moist and air-dried sandy topsoil and subsoil samples were treated in triplicate with fluazinam standard or Shirlan® (2.5 mg a.i. in 25 g soil) and extracted with acetonitrile and 0.01 M $CaCl_2$ immediately after sprayings to minimize fluazinam decomposition.

To assess the shelf-life of fluazinam, the stability of the standard solutions was analysed fresh and after 7-day storage at 5 °C. In this test, the standard solutions with six concentrations (2.5–

15.0 mg L^{-1}) and extracts from the sandy topsoil and subsoil samples were used. The soil samples were treated with both fluazinam and Shirlan® (2.5 mg a.i. in 25 g air-dried soil).

3.5 Performance of the HPLC and method confirmation

Several tests with HPLC were performed to verify its performance (I).

The linearity was determined by a calibration curve in the concentration range of 0–25 mg L^{-1} of fluazinam standard. The calibration curve was constructed by plotting the peak area of the analyte versus analyte concentration. The limit of detection (LOD) of soil samples was determined with sprayed sandy topsoil samples, using a signal-to-noise ratio of 3:1. In addition, the LOD for fluazinam standards was determined.

The specificity of the method for fluazinam standard- and Shirlan®-sprayed (2.5 mg a.i. in 25 g) sandy topsoil and subsoil samples was confirmed by analysing them through the procedure described in Chapter 4.2., to determine whether soil components, decomposition products or possible by-products of the procedure interfered with the analyte.

The precision (%RSD, percentage relative standard deviation) of the method was evaluated by analysing extracts of standard-treated (2.5 mg a.i. in 25 g air-dried soil) soil samples (sandy topsoil and subsoil, CS1, CS2) twice in one day. For the fluazinam standards, the precision was assessed by analysing six concentrations (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 mg L^{-1}) twice in one day (within-day) and over three consecutive days (between-days) in triplicate.

The repeatability was assessed by adding one concentration (10 mg L^{-1}) of fluazinam standard as control and by measuring it several times in every HPLC run during precision tests of fluazinam standards.

The quality of analysis was assured by adding a control soil sample in each extraction set during the laboratory experiments. Control soil samples 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 in the HPLC measurements, fluazinam standard solution (10 mg L^{-1}) was measured several times in each HPLC run in all the experiments and potato field analysis.

3.6 Testing the method with soil samples

A 90-day incubation experiment was carried out with air-dried subsamples (25 g) taken from sandy topsoil and subsoil, CS1, CS2 and OS (I). The samples were weighed into 100 ml glass bottles and 2.5 mg of fluazinam was added as the standard compound or with the commercial product Shirlan® in triplicate. The treatments were incubated for 90 days at room temperature in daylight with caps on and extracted with acetonitrile or $CaCl_2$. $CaCl_2$ was chosen as extractant because it mimics the soil solution.

The eight field soil samples collected from Ylistaro and Kurikka in 2011 were used to assess the functionality of the method for true potato cultivated field samples. All fields had been sprayed with Shirlan® two to three times during the same growing season and a maximum of one time in growing season in the two previous years (I).

3.7 Laboratory experiments

3.7.1 SOM experiment

An experiment was undertaken to directly assess the effect of soil organic matter on the behaviour of fluazinam (II). Therefore, SOM was removed from air-dried topsoil and subsoil samples by using 30% hydrogen peroxide (Kunze and Dixon, 1986). Then the soil samples were air-dried,

sprayed with fluazinam standard or Shirlan® (a.i. addition was 2.5 mg) and incubated for 90 days in darkness at room temperature in triplicate. Corresponding air-dried soil samples without SOM removal were sprayed and incubated in the same manner.

3.7.2 Follow-up experiment

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 one year (II). In addition to sandy topsoil and subsoil, this experiment was carried out also with clayey topsoil and subsoil (CS1 and CS2). Initially, the water content of the homogenized soil samples was adjusted to about 25% for topsoil and 20% for subsoil (lower in organic matter) or the samples were waterlogged. Water content modification was performed because Finnish soils are moist or wet for the majority of the year. Then, 25-g portions of the soil samples were treated with fluazinam standard (dissolved in acetone) or Shirlan® (dissolved in water) by spraying with a syringe in triplicate. In both cases, the fluazinam addition was 2.5 mg (corresponding to about 2 kg ha^{-1}), i.e. ten times higher than the normal application rate. The tenfold excess was incorporated into the soil to obtain clearly observable fluazinam residues in the analyses.

Finally, the samples (in a total of 384 sandy soil samples and 384 clayey 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 one year as a reference period was chosen to obtain a reliable estimate of degradation (Koskinen et al., 2001) and because in potato monoculture it is possible to respray Shirlan® in the next growing season. The water content was checked every month, but it was not necessary to add any water during the incubation. The samples were not stirred during the experiment.

3.7.3 Annual climatic rotation experiment

An annual climatic rotation experiment was conducted to monitor the persistence of fluazinam in sandy topsoil and subsoil by simulating year-round temperature and water content conditions typical to soil in the boreal zone (II). Moisturizing and spraying of the soil samples were performed similarly as in the follow-up experiment in triplicate (in total 48 samples). The first incubation step at 22 °C lasted 90 days at the constant water content of about 25% for topsoil and 20% for 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 -7 °C for 90 days (winter, freezing over) and finally to 5 °C for 90 days (spring) still in waterlogged state (simulating snow melting). In all sample sets fluazinam concentration was determined after each 90-day incubation step by using all the sample from the incubation bottles in the analysis. During this experiment, the samples were not stirred to avoid unintended oxidation reactions.

3.7.4 Potato field samples

The samples from the potato fields of Southern Ostrobothnia were air-dried and 25-g portions of the samples were analysed for fluazinam in triplicate by the method described in Chapter 4.2. (II)

3.8 Data and statistical analysis

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

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

where t = incubation time, i.e. 360 days,
N_0 = concentration of fluazinam at the beginning of the experiment (mg kg^{-1}) and
N_t = concentration of fluazinam after 360 days (mg kg^{-1}). (II)

For the follow-up experiment, a 4-factor design was made with the results of the samples sprayed with Shirlan®, resulting in 32 factor-level combinations (II). The factorial design was made only with Shirlan®-sprayed samples because spraying of the potato fields is made with Shirlan® in practice. 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 analysis were performed with PASW Statistics 18 and SPSS Statistics 21 (IBM Corporation, Armonk, USA) throughout the study.

4. Results and discussion

4.1 Method development

According to the solubility tests, visual perception and literature findings (the solubility of fluazinam in acetone is 853 g L^{-1} (EFSA, 2008)), fluazinam standard was decided to be dissolved in acetone and Shirlan® in water (as in the producer's instructions) to carry out the sprayings. It was important to dissolve fluazinam standard in acetone instead of water, because fluazinam standard had a poor water solubility, giving low and differing yields in the preliminary tests when analysing it in aqueous solution. Fluazinam in Shirlan®, on the basis of visual perception, is much more soluble in water, which is probably due to the inert ingredients in Shirlan®. Spraying of soil samples was performed with a syringe and the volume of solution sprayed was only 1 mL to minimize any side effects on the indigenous microbial population and degradation patterns (Brinch et al., 2002).

The results demonstrated that the extraction efficiency of acetonitrile was good in terms of extracting fluazinam from soil. As the results obtained by refluxing and sonication were nearly identical, sonication was selected for extraction for its simplicity. Heating was found to have a positive influence on the extraction efficiency in sonication, therefore, 60 °C was used. The extraction time of two hours was discovered to be appropriate.

For the silica purification, the most appropriate combination was verified to be 5 ml of diethyl ether-heptane mixture in the ratio of 20:80 for both fluazinam standard solutions and soil extracts. The recoveries of fluazinam standards (2.5–10.0 mg L^{-1}) in clean-up were 92–94%. In the sequential elution, fluazinam was not detected in the second and third fractions (diethyl ether-heptane mixtures 50:50 and 80:20). Heptane used in the conditioning of silica cartridges did not contain fluazinam.

The gentle nitrogen flow and the temperature of 50 °C in the stand had no effect on fluazinam concentration.

The detection wavelength of DAD was chosen to be 240 nm. The cutoff of acetonitrile (190 nm) was too close to 197 nm. The wavelengths of 254 and 257 nm gave slightly lower yields than 240 nm. For mobile phase, 70% acetonitrile and 30% water (v v^{-1}), including 0.02% acetic acid, was chosen for the convenient retention time of fluazinam (6.6–7.0 min in the methodological development).

In the field-moist soils sprayed with fluazinam standard, the acetonitrile extraction recovered 86% of fluazinam added to topsoil and 72% added to subsoil immediately after sprayings. The corresponding recoveries of fluazinam in topsoil and subsoil treated with Shirlan® were 64% and 61%, respectively. When fluazinam was added to air-dried soil, acetonitrile extracted 95% of it from topsoil and 86% from subsoil after application. For Shirlan®, the recoveries were again lower, 72% for topsoil and 73% for subsoil. Based on the difference between yields of fluazinam standard (95%) and fluazinam in Shirlan® (72%) in air-dried topsoil, the yield of fluazinam in Shirlan® is much lower. It was a true challenge to try to find out why this difference exists and in the literature this matter was not considered. One reason might have been the dissolving of Shirlan® in water and the inert ingredients. Shirlan® was mixed with water at the beginning of the experiments to make sprayings and in general, the presence of water seemed to hinder the analysis of fluazinam in Shirlan®. The possible effect of the inert ingredients was unknown. As air-drying evidently enhanced the extraction of fluazinam, it was decided to air-dry the soil samples before analysis. In the $CaCl_2$-extracted samples, fluazinam was not detected.

The standard solutions demonstrated an apparent increase of 5–7% in fluazinam concentrations after 7-day storage. For soil samples, the increase in the stability test was 3% in topsoil and 4% in subsoil for fluazinam standard and 3% and 5% for fluazinam in Shirlan®,

respectively. This increase in fluazinam concentration was possibly due to the evaporation of acetone from the samples and therefore, the extract became more concentrated. To avoid this source of error, the samples were prepared just before the HPLC run in the laboratory experiments and analyses of potato field soils. Furthermore, the same fluazinam standards were used for a maximum of two days.

4.2 The method

Fluazinam was extracted from 25 g of air-dried soil with 75 ml of acetonitrile by ultrasonication (2 h, 60 °C). The extract was filtered through filter paper and made up to a volume of 100 ml with acetonitrile. A 4-ml aliquot of the extract was evaporated under nitrogen flow (50 °C) and dissolved in 3 ml of heptane for purification. A volume of 1 ml of the above-mentioned solution was loaded onto heptane activated silica cartridge and eluted with 5 ml of a mixture of diethyl ether and heptane (20:80). The eluate was evaporated to dryness with nitrogen (50 °C), dissolved in 2 ml of acetone and analysed immediately or in the next morning with HPLC-DAD. The method had many steps but it was quite straightforward to perform (Fig. 3).

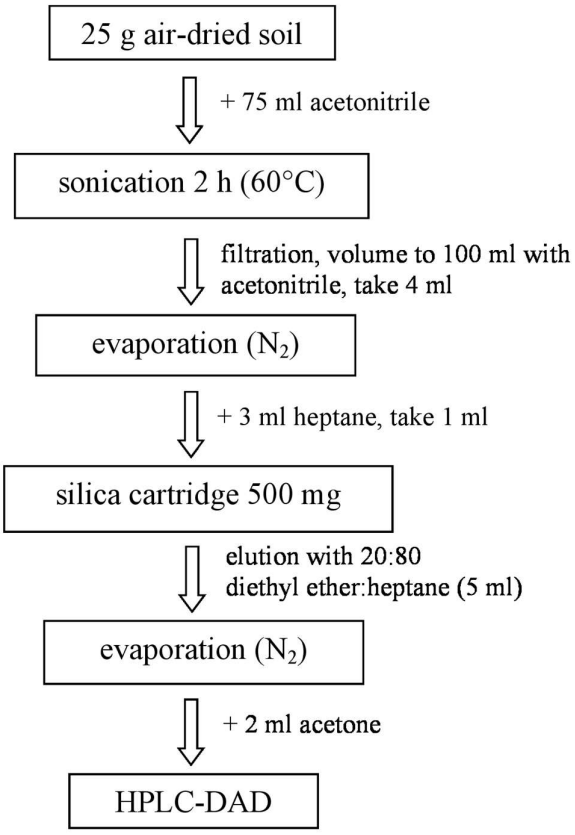

Figure 3. Method for analysing fluazinam in soil.

4.3 Performance of the HPLC and method confirmation

The calibration curve was linear at the concentration range of 0–25 mg L^{-1}, with the coefficient of determination (R^2) of 0.9999. The determined LOD was 0.03 mg kg^{-1} for soil samples and 0.006

mg L^{-1} for standards. The limit of quantification (LOQ) was defined as LOD, using a signal-to-noise ratio of 10:1. The LOQ was 0.1 mg kg^{-1} for soil samples and 0.02 mg L^{-1} for standards.

Interfering peaks were not observed at the retention time of fluazinam in the chromatograms, thereby confirming the specificity of the method.

The precision of the extracts of the fluazinam- and Shirlan®-treated soil samples was 0.0–0.3% and 0.0–0.7%, respectively. For fluazinam standards, the %RSD was 0.0–1.4% in the within-day evaluation and 0.7–1.3% in the between-days evaluation.

In the repeatability test, the %RSD for the within-day evaluation was 0.3–0.9% and 0.2% for the between-day evaluation.

4.4 Testing the method with soil samples

The recovered amount in acetonitrile extraction of fluazinam after 90 days was ranging from 49–73% for fluazinam treated samples and 40–70% for Shirlan®-treated samples, the recovery being lower the higher SOM was, being smallest in the organic soil sample (OS). In the blank samples, residues above LOD were not detected. Fluazinam was not detected in the $CaCl_2$ extracts.

From the eight collected samples, fluazinam was detected in two of the silt field topsoil samples at concentrations of 1.9 mg kg^{-1} and 2.1 mg kg^{-1}, standard deviations being 1.4 mg kg^{-1} and 1.6 mg kg^{-1}, respectively (I). The notable standard deviations might be attributable to the immobility of fluazinam in soil. In other words, fluazinam molecules remained in the place in which they ended up by spraying or with the incorporated potato foliage. Fluazinam was not detected in the subsoil samples or in the organic soil samples. It is likely that fluazinam had not reached the layer underneath the ploughing depth because the downward movement of fluazinam with water was slow or negligible. In the organic soil samples, SOM probably accelerated the fluazinam degradation.

4.5 SOM experiment

The presence of SOM clearly contributed to the degradation rate of fluazinam (II). In the samples containing SOM, the reduction was 14–34% greater than in those from which SOM had been removed, obviously due to 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 H_2O_2 nevertheless reduced the loss of fluazinam. When fluazinam was added in the standard solution, its recovery was similar in both topsoil and subsoil samples. However, when it was added in Shirlan®, the recovery in topsoil was lower than in subsoil. The results of the SOM experiment are presented in the Fig. 4, with standard deviations being 1–7 mg kg^{-1}.

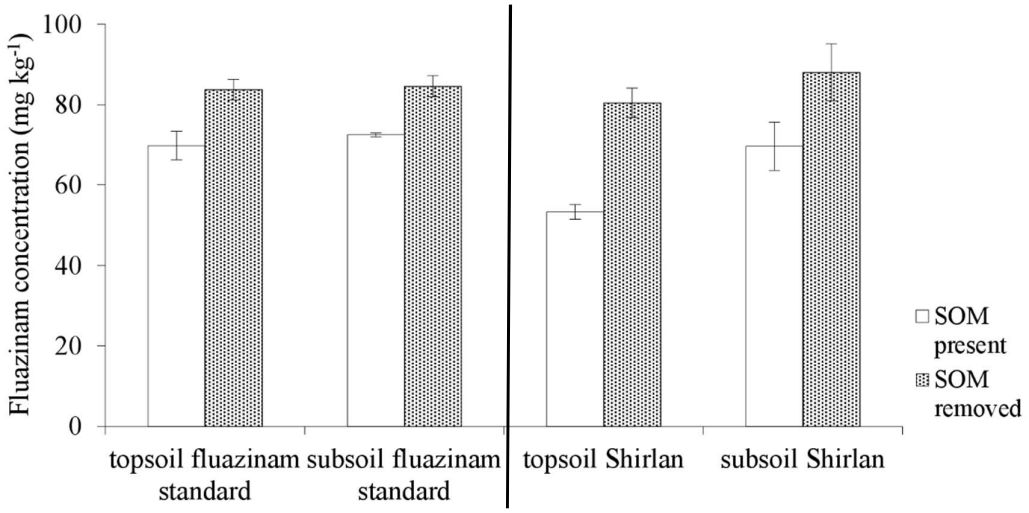

Figure 4. Fluazinam recovered (mg kg^{-1}) in SOM experiment in topsoil and subsoil in fluazinam standard and Shirlan® treatments with standard deviation bars.

4.6 Follow-up experiment

In the follow-up experiment, the results of the clay soil samples were rejected due to the high standard deviations, 0–38 mg kg^{-1}. One obvious reason for this is that during spraying the samples, it was challenging to mix the sticky soil in a manner that the spray would have been distributed evenly. Consequently, the spray did not reach the sorption sites of soil optimally.

The concentrations of fluazinam in sandy soil decreased systematically throughout the follow-up experiment but, nevertheless, the results (Tables 3 and 4) demonstrated that a prominent amount of fluazinam was recovered after one year, ranging from 54–74%. The recoveries were higher in subsoil than in topsoil and in moist soil being similar as in waterlogged soil. In continuation, the recoveries at 5 ºC were higher than that at 22 ºC and somewhat more fluazinam in Shirlan® was recovered than fluazinam in the pure chemical form. The estimated DT50s ranged from 418 to 833 days (Table 4). The standard deviations were 1–14 mg kg^{-1}.

Fluazinam is known to degrade more rapidly under anaerobic than aerobic conditions. Our experiment demonstrated that within a time scale of one year, waterlogging tended to only slightly enhance the degradation as compared with that under moist conditions. Although, the formation of anaerobic conditions will be retarded at a low temperature such as 5 ºC, they can be formed at 22 ºC, 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.

For Shirlan®, the percentage reduction in the fluazinam concentration in consecutive 90-day periods was generally greater during the first 90 incubation days, whereafter it declined, being only 0–6% in the last incubation period (II). However, in the soils sprayed with the fluazinam standard, the percentage reduction was quite high (13–23%) during the final incubation period compared with the previous periods (1–19%) (II). It has been reported for some recalcitrant pesticides that microbial adaptation can enhance their dissipation over time (Ren et al., 2018), but there was no such data in the literature for fluazinam degradation in soil.

Table 3. Fluazinam recovered (mg kg^{-1}) after 90, 180, 270 and 360 days of incubation (n = 3) in the follow-up experiment. The added amount of a.i. was 117–127 mg kg^{-1}.

	Soil water content	Temperature (°C)	90 d	180 d	270 d	360 d
Topsoil						
Fluazinam standard	Moist	22	100	100	88	73
		5	111	105	85	71
	Waterlogged	22	109	101	85	68
		5	120	113	99	78
Shirlan®	Moist	22	92	83	78	74
		5	98	96	88	88
	Waterlogged	22	91	80	73	73
		5	99	95	82	81
Subsoil						
Fluazinam standard	Moist	22	112	111	106	81
		5	115	110	108	86
	Waterlogged	22	112	112	104	81
		5	117	114	95	83
Shirlan®	Moist	22	95	92	92	85
		5	97	96	93	89
	Waterlogged	22	99	93	86	86
		5	103	95	89	87

Table 4. The percentage recovery of added fluazinam after 90, 180, 270 and 360 days of incubation and the estimated DT50s (n = 3) in the follow-up experiment.

	Soil water content	Temperature (°C)	90 d	180 d	270 d	360 d	DT50 (days)
Topsoil							
Fluazinam standard	Moist	22	81	80	71	59	476
		5	90	83	68	57	443
	Waterlogged	22	86	83	68	54	418
		5	96	92	80	63	548
Shirlan®	Moist	22	74	67	63	60	485
		5	80	76	71	70	704
	Waterlogged	22	74	64	59	59	472
		5	81	76	66	66	588
Subsoil							
Fluazinam standard	Moist	22	94	92	87	67	633
		5	96	95	90	71	713
	Waterlogged	22	94	93	87	67	634
		5	97	96	79	69	629
Shirlan®	Moist	22	80	77	76	71	719
		5	81	80	77	74	833
	Waterlogged	22	82	77	72	72	785
		5	85	79	75	73	800

In comparison with the values reported in the literature, 4–369 days (Table 2, p. 17), the estimated DT50s in the follow-up experiment were consistently much longer, 418–833 days. This may have been attributable to differences in the soil types, the temperature and the light conditions (the samples in this study were incubated in darkness) and the analytical methods. The results are most probably highly dependent on the extraction efficiency of solvents, in other words, fluazinam might stay adsorbed in soil rather than be extracted resulting in an incorrect conclusion that fluazinam has been degraded. The application amount in our experiment was 2 kg ha^{-1} and in the literature 0.74–5 kg ha^{-1} (Table 2, p. 17).

The factorial design (II) 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, e.g. the effect of sampling depth or soil water content or temperature on the fluazinam concentration changed depending on the 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 means that fluazinam degradation was enhanced by the abundance of SOM, waterlogging and a warmer temperature.

4.7 Annual climatic rotation experiment

After one year, a prominent amount of fluazinam was present in soil (Table 5) (II) and also in this experiment, the estimated DT50s were considerably longer than those reported in the literature. Due to the higher SOM content, the estimated DT50s were shorter in topsoil than those in subsoil, being 436 days in topsoil and 575 days in subsoil for fluazinam standard treated soils and 355 and 583 days for Shirlan®-treated soils, respectively. Generally, the estimated DT50s were shorter than those in the follow-up experiment (418–833 days), in which samples were kept under constant conditions for one year. This outcome could have been attributable to alternating freezing and thawing processes.

Table 5. Concentration (mg kg^{-1}) and recovery (%) of added fluazinam at the end of each subsequent 90-days periods simulating soil conditions during a year and estimated DT50s (days) (n = 3). The added amount of a.i. was 118–124 mg kg^{-1}.

	Simulated season	Cumulative duration (days)	Water content	Temperature (°C)	Concentration (mg kg^{-1})	Recovery (%)	DT50 (days)
Fluazinam standard							
Topsoil	summer	90	moist	22	100	81	
	autumn	180	wet	5	85	68	
	winter	270	wet	-7	74	60	
	spring	360	wet	5	70	56	436
Subsoil	summer	90	moist	22	105	88	
	autumn	180	wet	5	91	76	
	winter	270	wet	-7	80	67	
	spring	360	wet	5	77	65	575
Shirlan®							
Topsoil	summer	90	moist	22	92	74	
	autumn	180	wet	5	75	60	
	winter	270	wet	-7	64	52	
	spring	360	wet	5	61	50	355
Subsoil	summer	90	moist	22	95	80	
	autumn	180	wet	5	83	70	
	winter	270	wet	-7	81	67	
	spring	360	wet	5	77	66	583

The fluazinam concentration diminished most rapidly at the beginning of the experiment (moist summer conditions), generally slowing down as the experiment proceeded. In the last part of the experiment, under spring conditions that were like autumn conditions (wet, 5 ºC), the degradation was very slow, in some cases even negligible. Clearly, the fluazinam degradation decelerated over time. During winter soil microbial activity is slowed down in Finland, but in our experiment some loss of fluazinam still occurred. Fluazinam degradation following topsoil treated with Shirlan® in the annual rotation experiment is presented in Fig. 5. The standard deviations in this experiment were 1–9 mg kg^{-1}.

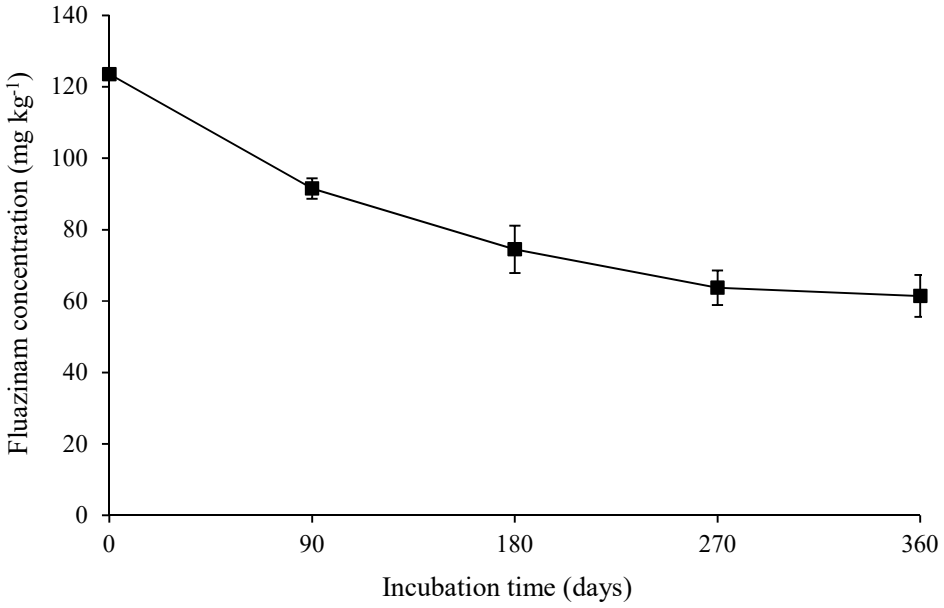

Figure 5. Fluazinam degradation in soil (mg kg^{-1}) following topsoil treated with Shirlan® in the annual rotation experiment with standard deviation bars.

It is worth considering whether the decreasing recovery of fluazinam in time is attributable to degradation or whether the sorption 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 (Koskinen et al., 2001). Unfortunately, there was no such data for fluazinam. Sharer et al. (2003) observed that for pesticides with strong retention, little or no increase in sorption occurred with prolonged equilibration times.

4.8 Potato field samples

Out of 30 soil samples, fluazinam was detected in 17 field samples at concentrations of 0.14–1.15 mg kg^{-1} (Table 6) (II). In 14 fields, Shirlan® had been sprayed during the same growing season when the samples were collected. Even though some fields (P2–P7) had been sprayed with Shirlan® six times during the same growing season, the highest concentration of fluazinam was detected in a sample from the field that had been sprayed three times during the same growing season (A2). In samples H3–H6, collected from fields that had been under intensive potato cultivation about 50 years and frequently sprayed with Shirlan®, fluazinam was detected in the concentrations of 0.14–0.26 mg kg^{-1}.

Fluazinam was detected in three samples collected from fields that had not been sprayed during the same growing season. In samples Y1 and Y3, fluazinam was detected in the concentrations of 0.15 and 0.20 mg kg^{-1}, respectively, and in P1 the concentration was 0.14 mg kg^{-1}. These findings indicated that fluazinam in soil can be carried over from one growing season to the next.

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 (II). Fluazinam was not detected in two of the fields that had been sprayed six times during the same growing season (P2 and P6). In three samples (H1, H2, H7) fluazinam was not detected even though these fields had been sprayed with Shirlan® during the same and the two previous growing seasons, three times per growing season. The field samples indicated variations at concentrations among the triplicates of the homogenized soil samples. A large number of samples

should be collected from fields and replicates should be used to overcome the spatial variation and to obtain a comprehensive picture of the fate of fluazinam in soil.

Comparing of the results of the three samples taken from the same field segments in 2011 and 2013, in two of them (KL1/T3 and KA1/T4) fluazinam was not detected in either year. In one sample (A3/P1), in turn, the concentration of fluazinam had decreased in two years, from 1.9 mg kg^{-1} to 0.14 mg kg^{-1}. Fluazinam was not detected in the subsoil samples collected in 2011. On the basis of this outcome and the literature findings that fluazinam moves hardly downwards in soil, subsoil samples were not collected in 2013.

Detected fluazinam concentrations in the field soil samples were higher than those in the literature (Laitinen et al. (2000); Ruuttunen et al. (2008a)) (Table 2, p. 17).

Table 6. Concentrations of fluazinam detected in the field soil samples (mg kg^{-1}) (n = 3) and standard deviations (mg kg^{-1}).

Soil code	Soil type	Fluazinam (mg kg^{-1})	SD (mg kg^{-1})
P1	silt	0.14	0.01
P3	silt	0.53	0.02
P4	silt	0.37	0.01
P5	silt	0.34	0.01
P7	silt	0.33	0.05
T1	silt	0.22	0.03
Y1	silt	0.15	0.02
Y3	silt	0.20	0.01
Y4	silt	0.23	0.04
A1	silt	0.22	0.05
A2	silt	1.15	0.06
A3	silt	0.26	0.03
H3	sand	0.26	0.02
H4	sand	0.14	0.00
H5	sand	0.20	0.03
H6	sand	0.18	0.02
H8	sand	0.31	0.07

The control soil samples and HPLC repeatability samples confirmed the uniform quality of the analyses throughout the experiments. The coefficients of determination for calibration curves were excellent ($R^2 \geq 0.9998$) in the analysis of the laboratory experiments and potato field soil samples.

4.9 An estimate of the environmental behaviour of fluazinam in Finland

Fluazinam causes environmental load in the potato field soils in which it is used. Fluazinam stays in the place in which it is hit during spraying and ends up into soil at the latest when potato foliage is incorporated into the field soil. Fluazinam binds tightly into soil and is hardly water soluble. Therefore, leaching of dissolved fluazinam to groundwater and other watercourses is expected only in very small amounts.

In Finland, potato is cultivated mainly in flat soils and, therefore, erosion might be considered negligible. Potato fields mostly consist of coarse-textured mineral soils, and they do not usually

crack. Therefore, only old root channels may be conducive to by-pass flow carrying fluazinam attached to soil particles downwards in the soil profile.

The expected warming of winters may accelerate fluazinam degradation in soil. On the other hand, in monoculture of potato the SOM content decreases in time producing deceleration of degradation. Fluazinam can accumulate in soil when using it regularly. However, the concentrations detected in the potato field soils were quite low and substantial accumulation was not observed even in the fields in which fluazinam had been sprayed regularly for many years.

Fluazinam is practically non-volatile, therefore, air pollution is not expected.

5. Conclusions

The main outcomes of this study were:

- The HPLC-DAD method developed and validated for analysing fluazinam in sandy soil proved to be selective, reproducible and rather easy to perform. Comparing the method with the majority of the methods found in literature, e.g. the extraction solvent and analytical equipment were different. The method is not valid for clay soil samples but works well with sandy soils, in which potato is normally cultivated. The method has good linearity and accuracy and it can be applied in laboratory studies on the fate of added fluazinam and to analyses of potato field soil samples for their residues.

- Fluazinam detected in soil was bound as residues extractable with acetonitrile, but not with $CaCl_2$, mimicking soil solution. Thus, it is unlikely that fluazinam will be leached from soil in dissolved form, but it can be transported to watercourses with eroded soil particles. This also explains the finding that air-drying clearly enhanced the recovery of fluazinam. In general, the presence of water seemed to interfere with the extractability of fluazinam to acetonitrile probably because the water molecules distracted the interactions between fluazinam molecules and acetonitrile. Consequently, as can be seen in the recoveries of fluazinam standard and Shirlan®, the dissolution of Shirlan® in water had an adverse effect on the extractability of the fluazinam added in this form.

- Despite some inconsistencies in the degradation rate, the major outcome is that there was a substantial amount (more than half of the added amount) of fluazinam remained in soil after one year incubation. Considering the results obtained in 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 that are common in Finland. Nevertheless, substantial accumulation according to field soil samples was not detected. The movement of fluazinam to other environmental compartments is slight from flat and coarse-textured mineral soils, in which potato is mainly cultivated in Finland.

- The SOM experiment demonstrated that the SOM content influenced the degradation rate of fluazinam in soil, the shortage of SOM decelerating the loss of fluazinam. In the follow-up and annual climatic rotation experiments, fluazinam degradation in topsoil was more rapid than in subsoil, due to the higher SOM content in topsoil.

- Fluazinam was detected reliably in many of the potato field samples. The field samples indicated variation at concentrations among the triplicates of homogenized soil samples, suggesting the immobility of fluazinam in soil. Furthermore, the results of the potato field samples were quite variable when observing the spraying history of the fields. It seemed that fluazinam molecules remained in the place in which they ended up with the incorporated potato foliage. Because of this uneven distribution, a large number of samples should be collected from fields and the analysis should be carried out with several replicates to overcome the spatial variation and to obtain a comprehensive picture of the fate of fluazinam in soil.

- Further studies regarding the fate of fluazinam in agricultural soils are needed, e.g. what is the effect of photodegradation on the fluazinam degradation, what is the effect of added concentration on DT50, what is the degradation rate of fluazinam in potato foliage before

introducing it into soil and are there great differences in the degradation rates between different types of field soils.

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