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EVALUATION OF A NOVEL NEMATICIDE FOR USE IN THE MANAGEMENT OF THE POTATO CYST NEMATODE, *GLOBODERA PALLIDA*

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DECLARATION

The information contained in this thesis is an original piece of work, and is a record of work carried out by the author on an original line of research.

Patrick M. Norshie

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ABSTRACT

The potato cyst nematodes (PCN) *Globodera rostochiensis* and *Globodera pallida* are two economically important potato pests constituting major limiting factors to production of the crop in the UK. Soil treatment with synthetic nematicides is widely practiced and is an essential part of an integrated management tactic for PCN. However, environmental concerns and human health issues have prompted the withdrawal of a number of active substances leaving a limited few, which are not only under European Union review (Hillocks, 2012; Anon, 2009), but are suggested to provide inadequate control of *G. pallida* (Trudgill *et al*., 2003), the predominant of the two species in England and Wales. Consequently, alternative nematicides and crop protection strategies are urgently required to ensure sustainable production of the potato. This thesis reports the findings of experiments conducted (i) to evaluate the efficacy of fluensulfone, a novel molecule belonging to the fluoroalkenyl group, for control of *G. pallida* and (ii) to determine factors likely to influence the efficacy of fluensulfone.

In all, five experiments (three as field and two as polytunnel pot experiments) determined fluensulfone soil treatments on the control of *G. pallida*. The field experiments were conducted in 2010 and 2011, and were undertaken in commercial potato fields that were predominantly infested by *G. pallida* at Woodcote and Howle near Newport in Shropshire, England. The field experiments 1 and 2 studied the control of *G. pallida* by fluensulfone application at five rates (1.95, 3.00, 4.05 (full rate), 5.05 and 6.00 kg a.s. ha^{-1}) as a 15% granular (G) formulation and a single rate (4.05 kg a.s. ha^{-1}) of a 480 g L^{-1} emulsifiable concentrates (EC) formulation. These treatments were compared with the currently commercially available fosthiazate (as Nemathorin 10G) and oxamyl (as Vydate 10G) applied at their respective recommended rates of 3.00 and 5.50 kg a.s. ha^{-1}. An assessment of the number of nematodes found within potato roots at ca. 4 and 6 weeks after planting

indicated that the treatments with fluensulfone had decreased the invasions of root by *G. pallida* and that fluensulfone had similar effects as the fosthiazate or oxamyl treatment. Besides the effects on the root invasions, the multiplication rate and final population density at harvest were reduced. Overall, the application of fluensulfone at the full rate gave more consistent controls than the two lower rates of 1.95 and 3.00 kg a.s. ha^{-1}, and the data showed no significantly greater effects for the applications higher than the full rate. Despite the clear reduction in PCN root invasion and population increase, no significant improvements in plant growth and tuber yields were recorded for the fluensulfone, as well as the two standard nematicide treatments. In 2012, a follow-up polytunnel pot experiment (Polytunnel experiment 1) determined the effects of granular fluensulfone treatments at 1.95, 4.05 and 5.05 kg a.s. ha^{-1} on *G. pallida* egg viability, juvenile hatching and root invasion. The data suggested that at the studied rates, fluensulfone may be more effective at suppressing activities of the infective juvenile *G. pallida* in the soil than in eggs within cysts. The field experiment 3, at Howle in 2011, determined the integration of granular fluensulfone application at the full rate and partially resistant potato (Santé or Vales Everest) treatment on *G. pallida*. The results showed significant interactive effects on the multiplication rate and the final population density, but not on the root invasions. Furthermore, there was evidence to suggest that fluensulfone had integrated effectively with Santé but not with Vales Everest in controlling *G. pallida* population increase.

In order to determine factors likely to influence efficacy, fluensulfone persistence and sorption on soil were investigated. The persistence studies involved measurements of the half-life (DT_{50}) as per the granular fluensulfone application at the full rate in the Field experiments 1 and 2, as well as in four arable soils in pot under polytunnel conditions (Polytunnel experiment 2). The data indicated persistence no longer than 24 days at an incorporation depth of 15-20 cm in the fields and in the soils in the polytunnel. Fluensulfone persisted similarly as fosthiazate in the field plots. Regression analyses, however, could not

establish a significant relationship between the DT_{50}, as well as the dissipation rates constant (*k*) and soil properties. It was, therefore, suggested that fluensulfone had dissipated independently of the prevailing conditions. Additionally, inferences from the reductions seen for the application of fluensulfone at the full rate on root invasion and population build-up, suggested that the determined dissipation rates did not affect the efficacy of fluensulfone. The sorption measurements were made both for the technical-grade and the granular product and were undertaken on a range of UK arable soil using the batch equilibrium technique. The results, as per both the Freundlich and equilibrium sorption coefficients K_F and K_D, respectively, showed variable, but generally, low sorption of fluensulfone. The formulation gave a four-fold decrease in the sorption, and the difference was due to limited availability of fluensulfone for uptakes. The pesticide mobility factor (K_{OC}), likewise, was low, ranging from 64 – 115 mL Kg^{-1} OC. In all instances, the soil organic carbon accounted for most of the variations in sorption, and both the K_F and K_D correlated positively with soil amendments with peat. The theory that soil organic matter would constitute a limiting factor to the efficacy of fluensulfone was, however, not supported by a follow-up polytunnel pot experiment (Polytunnel experiment 3) in 2012, which showed that the application of granular fluensulfone at the full rate had decreased root invasion by *G. pallida* in a peat-amended Shropshire sandy clay loam, irrespective of level of moss peat amendments. Fluensulfone analyses, as per water extraction in this experiment, showed that the solution concentration of fluensulfone, throughout the duration of the experiment, was higher than the minimum effective concentration proposed *in vitro* for control of *G. pallida* J2 hatching and mobility. The suggestion, therefore, was that sorption may not limit availability of fluensulfone in the soil for nematicidal activities.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 The potato crop

The cultivated potato (*Solanum tuberosum* L.) originates from South America (Hijmans and Spooner, 2001), where it has been grown for many years in the Andean highlands before was introduced to Europe in ca. 1562 (Hawkes and Francisco-Ortega, 1993). Ever since it has been accepted as a European food crop (Burton, 1989), the potato has spread as one of the world's principal food crops (Walker *et al*., 1999), and as of 2009, was grown in more than 125 countries (Lutaladio *et al*., 2009). In 2010, an estimated 18 million hectares were cultivated worldwide, with a production amounting to 314 million tonnes (FAO, 2012). Industrial production, however, is concentrated in a few countries in the northern hemisphere, and particularly to the temperate zone in Europe, which accounts for almost 51% of the total global area under cultivation. Great Britain is ranked 11th in terms of output (FAO, 2009), and the industry is currently made up of ca. 2300 growers (Anon, 2013a). Production remained relatively stable, though, with output in 2011 estimated at 6053 million tonnes, generating £947m at the farm gate and £3.8bn at the consumer level (Anon, 2012). However, recent challenging weather conditions are having effects on production levels, with output in 2012, for instance, fallen by 5.5% that of the previous year (NEPG, 2013).

1.2 Potato cyst nematodes

The potato cyst nematodes (PCN), *Globodera rostochiensis* (Wolleneweber 1923; Behrens, 1975) and *G. pallida* (Stone 1973; Behrens, 1975), are the most economically important and well-studied cyst-forming species within the genus *Globodera.* Both species are considered among the major biological constraints to potato cultivation (Zunke and Eisenback, 1998),

particularly in temperate agriculture, and are among the factors limiting productivity worldwide (Mehrdad *et al*., 2005). They are, primarily, root parasites, which cause a reduction in the marketable yield (both tuber quantity and quality) of the crop. Despite their pest status, however, empirical data on the extent of the damage caused to the potato are scarce, and this may be due, in part, to challenges associated with quantification of yield losses due to plant parasitic nematodes in general. Nonetheless, yield losses are suggested to range from 10 to 12% worldwide (Bates *et al*., 2002; Urwin *et al*., 2001) and about 9% in Europe (Tuner and Rowe, 2006). Besides causing direct damage, PCN predispose the potato to infections by other pathogens such as fungi (Back *et al*., 2006; Back *et al*., 2002; Evans and Haydock, 1993; Storey and Evans, 1987). To the potato grower, the need to manage PCN infestations requires significant financial input. Practice of crop rotation, for instance, may require allocation of resources to crops of lesser economic importance, and chemical control, on the other hand, is expensive, with granular nematicide application costing £400 to £650 per hectare (Anon, 2013b). Furthermore, *G. rostochiensis* and *G. pallida* are among the most regulated potato pests (Hockland *et al*., 2006). Consequently, the potato industry may incur costs as regulatory measures are implemented to control the spread of infestation. The economic loss due to PCN under the current UK production system is estimated at £26m annually (Clayton *et al*., 2008).

1.2.1 Origin and distribution

Globodera rostochiensis and *G. pallida* are widely accepted to have originated from South America (E.g. Evans and Rowe, 1998; Stone, 1985 and Inagaki *et al*., 1973), where they are thought to have evolved with the potato crop (Baldwin and Mundo-Ocampo, 1991). They were introduced to Europe in the 1850s (Turner and Evans 1998), probably, alongside potato germplasm imported for breeding for varietal resistance to the potato blight disease

(*Phytophthora infestans*) in Ireland in the 1840s (Evans *et al*., 1975). Nematodes do not have locomotive ability to traverse long distances (Den Nijs, 2007), and therefore, spread of the early introductions, most likely, occurred as a result of intra and inter-regional transport of plant and soil materials, possibly contaminated with PCN (Been and Schomaker, 2006; Whitehead, 1998).

Early populations were considered as a single species (*Heterodera rostochiensis)* until the 1970s, when differences in key morphological features led to the realization that the populations were not as homogenous as they were perceived (Stone *et al*., 1986; Stone *et al*., 1979). This prompted taxonomic examinations, which led to a re-description of the species (Golden and Ellington, 1972) and subsequent separation of *H. pallida* (Stone, 1973). Thereafter, Behren (1975) assigned both species to the genus *Globodera*. Further studies of interactions between PCN and resistant potatoes showed that populations varied in virulence on natural sources of genetic resistance. Based on these, pathotypes were recognised (Trudgill, 1985), with pathotypes being regarded as a group of individual nematodes capable of multiplying freely on an otherwise resistant potato (Fleming and Powers, 1998). Within Western Europe, the pathotype scheme (Kort *et al*., 1977) recognizes five pathotypes within *G. rostochiensis* (Ro1-Ro5) and three within *G. pallida* (Pa1-Pa3) based upon their abilities to reproduce on a range of potatoes carrying the qualitative resistance gene H_1 and quantitative resistance gene H_2, respectively.

1.2.2 Potato cyst nematodes in the UK

Both species of PCN are well established within the main potato-growing areas of the UK (Gratwick, 1992; Trudgill, 1986), but there has been an increasing incidence of *G. pallida* in England and Wales. By 1998, 67% of infested fields were found to comprise entirely of *G. pallida* (Minnis *et al*., 2002), and represented a significant shift in occurrence of this

species since an earlier survey (Brown, 1970) had shown that 50% of field infestations were purely *G. rostochiensis*. Many factors might have contributed to the epidemic of *G. pallida*, but the most widely accepted suggestion has been that of a widespread cropping of potato varieties such as Maris Piper carrying the major H_1 gene conferring complete resistance to *G. rostochiensis,* but is susceptible to *G. pallida*. Whereas these varieties have successfully controlled *G. rostochiensis* populations in the UK (Trudgill *et al*., 1996), repeated cropping has selected for *G. pallida* (Phillips and Block, 2008; Whitehead, 1991). Potato cyst nematodes are rather less common in Scotland and Northern Ireland, with *G. rostochiensis* being more frequently encountered (Pickup *et al*., 2012; Zaheer *et al*., 1993). In Scotland, for instance, only 1.8% of 14150 fields sampled in 2011 were found with PCN and the populations comprised 52% *G. rostochiensis*, 36% *G. pallida*, and 10% mixed species (Pickup *et al*., 2012). Even though there were no significant changes in the overall incidence as compared to the previous year, a marked increase in infestation foci was recorded in land already known to be infested (Pickup *et al*., 2012), suggesting that infestations may be spreading within individual fields. This increasing incidence of *G. pallida*, particularly in England and Wales, would suggest, most probably, that the current control strategies are not very efficacious against this species, thus warranting the search for alternative control options for effective PCN management for sustainable production of the potato crop.

1.2.3 General biology

Globodera rostochiensis and *G. pallida* exhibit a typical sedentary endoparasitic nematode life cycle (Plate 1.1), which comprises an egg, four juvenile stages, and sexually distinct male and female stages (Opperman and Bird, 1998). The active part of the life cycle commences when the second-stage juvenile (J2) hatches out of an encysted egg (Turner and Rowe, 2006). Soil factors such as moisture and temperature are known environmental

triggers for hatch in the cyst nematodes (Koenning and Sipes, 1998), but it is well established that significant hatch of J2 PCN occurs only in the presence of specific hatching factors present in exudate from developing host plant roots (E.g. Byrne *et al*., 2001; Devine *et al.*, 1996).

Once hatched, the J2 leaves the cyst in search of a host (Turner and Rowe, 2006), and relying on exudate such as carbon dioxide, it migrates towards the region behind the root tip (Noe, 2008). Penetration is direct and occurs in the zone of root elongation where tissues are in primary stage of growth (Sobsczak and Golinowski, 2008). The penetration of the root marks the onset of the parasitic life cycle (De Boer *et al*., 1999), and is achieved by combined action of cell wall modifying enzymes, secreted via the stylet (Vanholme *et al*., 2004) and throbbing thrusts of the stylet (Agrios, 2005); this creates a hole in the root, which the J2 enters. Once inside, the J2 penetrates further into the root, relying on the stylet thrusts and secretions to bore through the cortex, piercing, rupturing and destroying cells as it migrates to the vascular region (Mulder and Van Der Wal, 1997). Upon reaching the vascular cylinder, the J2 selects a single cell into which it injects stylet secretions originating from oesophageal glands (two sub-ventral and one dorsal) (Williamson and Gleason 2003), and are known for containing parasitism proteins, which transform this cell into an initial feeding site (Gheysen and Mitchum, 2009; Davies *et al*., 2008; Gheysen and Jones, 2006; Wyss and Grundler, 1992). The J2 soon becomes sedentary after feeding is initiated, and as it feeds, the stylet secretions trigger partial dissolution of walls of adjacent cells (Lee *et al*., 2011; Smant *et al*., 1997; De Boer *et al*., 1996), drawing their contents into the initial feeding cell, which then enlarges into a multicellular structure known as a syncytium (Tuner and Evans, 1998; Wyss and Grundler, 1992). The J2 continues to take nourishment from the syncytium as it undergoes a series of developmental stages (Tuner and Evans, 1998) into a sausage-shaped third-stage juvenile (J3) and then into a flask-shaped fourth-stage female or a filiform male juvenile (J4) at second and third moults. The female ruptures the root cortex at the fourth moult, with her

vulva exposed, whilst the male exits the root, still wrapped in its third-stage cuticle (Turner and Rowe, 2006). It remains free living and relies on pheromones to locate the female with which it mates (Riga *et al*., 1996) and dies soon afterwards. The female, containing developing eggs at this stage, continues to feed until completion of its life cycle, when it dies, her cuticle hardening into a leathery structure known as a cyst. The cyst, which may contain 400 – 600 eggs, each with a developing first-stage juvenile (J1), drops off the root into the soil, where it could remain viable for periods, which could extend over 20 years (Brodie *et al*., 1993). The J1 undergoes a first moult into a J2 whilst inside the egg and remains dormant until it is triggered to hatch (Seinhorst, 1985).

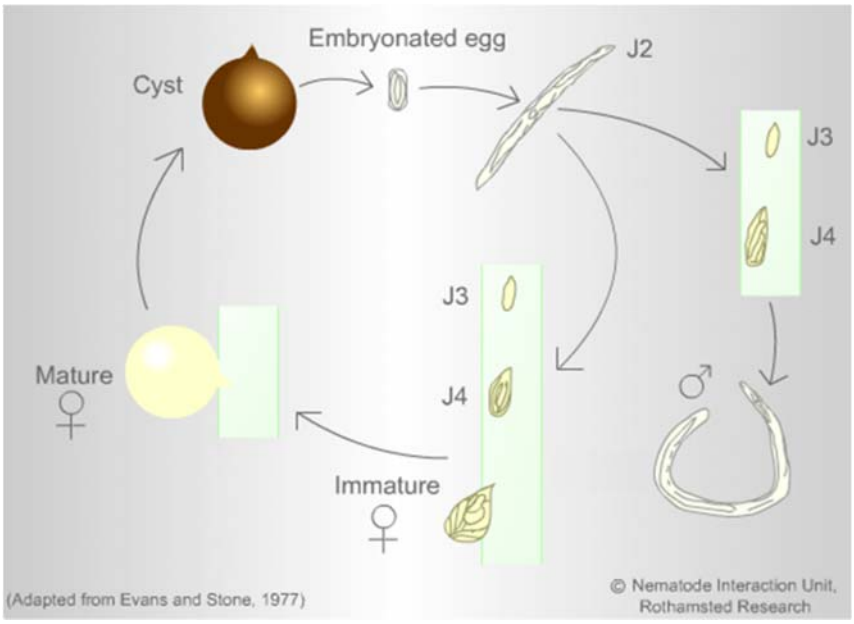


Plate 1.1. A typical life-cycle of *Globodera* species (according to Evans and Stone, 1977).

1.2.4 Damage and symptoms

The root invasion and feeding by the PCN are fundamental to the parasitic effects on the potato crop. During J2 invasion of the root cortex, tissues are irreparably damaged, and root development is impeded. Potatoes grown in PCN infested soils have been shown to have fewer vigorous roots (De Ruijter and Haverkort, 1999; Arntzen *et al*., 1994; Evans, 1982), implying that less soil volume would be explored (De Ruijter and Haverkort, 1999; Trudgill, 1980). This may limit availability of resources for physiological processes, for example, photosynthesis (Schans and Arntzen 1991) required to nourishing the plant. The feeding PCN, on the other hand, acts as a sink for photosynthates, and may deprive the plant of nutrients required for growth and development. The coupling effects of the damage to the root during invasion and the feeding by PCN, thus, reasonably, limits plant performance, and manifesting as reductions in growth and yield. Severely infected potatoes are stunted (Plates 1.2 and 1.3), with reduced foliage, retarded flowering and premature senescence (Mulder and Van Der Wal, 1997), all of which lead to considerable reductions in ground cover duration (Haydock, 1989).

Linear (Brown, 1969), log-linear (Oostenbrink, 1966), exponential (Seinhorst, 1971), and inversed linear (Elston *et al*., 1991) models that considered the relationship between PCN soil density and tuber yield had suggested inversed relationship between yield loss and the pre-cropping population density. However, it has become apparent that the severity of infection and/or the extents of yield loss are more of interplay of factors comprising, host status (being a susceptible, resistant or tolerant host), numbers of J2 that actually invade the root, weather and soil type (Phillip and Trudgill 1998; Evans and Haycock, 1990; Trudgill and Cotes, 1983).

Plate 1.2 PCN infested potato showing typical above-ground symptoms.

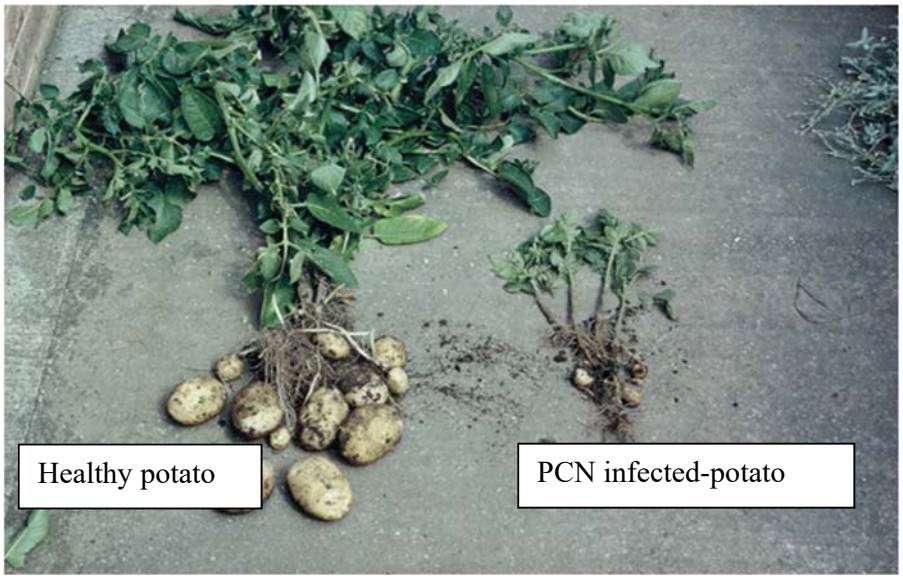


Plate 1.3 Comparison of healthy and PCN infected potato

1.2.5 Control options

Many tactics are suggested for control of plant parasitic nematodes, but just a few offers viable controls of PCN (Evans *et al*., 1993). For example, biological control with arbuscular mycorrhizal fungi (Deliopoulos *et al*., 2010; Ryan, *et al.*, 2000) or the pathogenic fungus, *Pochonia chlamydosporia* (Tobin *et al*., 2008), and trap cropping with *S. sisymbriifolium*

(Timmermans *et al*., 2006; Scholte, 2000; Scholte and Vos, 2000) have been shown to provide adequate reductions of PCN. In the UK, however, an integrated approach has been adopted (Haydock and Evans, 1998; Evans, 1993; Storey, 1984, Trudgill *et al*., 1987) and involves rotation of crops, soil treatment with nematicides and growing of resistant varieties.

1.2.5.1 Rotation of crops

Rotating potatoes with non-host crops is a well-known practice, and has been a reliable method for managing the population density of PCN between potato crops. Crop rotation is utilized to take advantage of the spontaneous hatch of *G. rostochiensis* and *G*. *pallida* in the absence of a host plant, coupled with the in-egg mortality of juvenile PCN causing a natural decline in population density. On average 30% annual decline of PCN is purported to occur in each year that potatoes are not planted, but decline rates may be low as 10% (Turner, 1996) and high as 40% (Whitehead, 1995). Even at these rates, long rotations are required to allow sufficient time for the population density to return to non-damaging levels by the next cropping of potato. Evans and Haydock, (2000), for example, suggested eight or more years between potatoes for effective population control. In practice, however, shorter rotations involving cropping of potato once in every five years is more commonly practiced in the UK (Minnis *et al*., 2002). The reason may be due to economics of the current production system, and probably the integration of nematicides and resistant varieties, hastening the population decline (Phillips and Trudgill, 1998). Even with this integrated approach, Trudgill *et al*., (2003) suggested shorter rotations had not been effective in preventing a build-up of *G. pallida*.

1.2.5.2 Cropping of resistant varieties

Growing resistant potatoes has been widely advocated as the simplest control tactic, the environmentally friendliest approach, the easiest to use method and the cheaper control option (Turner and Rowe, 2006) available for the management of PCN infestations. Indeed, it is the most extensively adopted control measure in major potato growing regions (Starr and Roberts, 2004), and is strongly recommended by the new EU Council Directive 2007/33/EC for control of PCN (http://eur-lex.europa.eu/).

Mechanisms of resistance to nematodes have been intensively investigated, and are reported to operate at any stage of the life cycle (E.g. Arntzen *et al*. 1993; Rice *et al*. 1987). However, resistance in potato to PCN is known to be triggered after the J2 had invaded the root, and operates by interrupting feeding (Lilley *et al*., 2004; Urwin *et al*., 2003) or limiting the development of the juvenile and/or reproduction in the female (Mullin and Brodie, 1988). In effect, resistant varieties may suppress population build-up of PCN, enabling shorter rotations (Starr and Roberts, 2004).

As mentioned previously under Section 1.2.2, existing resistance sources have narrow genetic basis, and are directed towards a few of the pathotypes within populations of *G. rostochiensis* and *G. pallida*, each of which exhibits a great range of virulence (Hockland *et al*., 2012). This makes these resistance sources easily overcome when challenged by populations containing virulent genes. The successful control of *G. rostochiensis* by varietal resistance in the UK is attributable to the fact that most populations are predominantly pathotype Ro1, which are avirulent on varieties carrying the H_1 gene (Stone *et al*., 1979). Control of *G. pallida* using varietal resistance has had little success since field populations exhibit a wider range of virulence and the lack of a single gene with complete resistance has made breeding very challenging (Starr and Roberts, 2004). A few partially resistant sources are available though, and some offer population control ranging from 10% to 90% (Trudgill

et al 2003). Nonetheless, they have been used only on a limited scale, probably, because they are not economically attractive.

1.2.5.3 Chemical control

Soil treatment with synthetic nematicides, with the aim of controlling damage caused by PCN, is a common practice in the UK (Whitehead, 1998; Whitehead, 1986) and has remained a key component of the integrated management of *G. rostochiensis* and *G. pallida* (Haydock *et al*., 2006). Currently, granular forms of the organophosphate, fosthiazate (as Nemathorin 10G; Syngenta, Crop Protection Ltd. Cambridge, UK) and the carbamate, oxamyl (as Vydate 10G; DuPont Crop Protection Ltd. Wedgwood Way, UK) are widely used. Available records on nematicide usage in Britain (https://secure.fera.defra.gov.uk, Table 1.1) showed that potato growing land receiving treatments of fosthiazate and oxamyl had increased significantly from 6.6% in 2000 to 24.6% by 2010; this would imply an increasing dependence on these products by growers for improving economic gross margin. To control PCN, granules of either nematicide are broadcasted onto the surface of potato beds and are incorporated by rotatory cultivation to 10 - 15 cm depth. Organophosphate and carbamate nematicides control nematodes by inhibiting the enzyme acetylcholinesterase (Gourd *et al*., 1993), which is crucial to the functioning of the nervous system. Inhibition of this enzyme results in paralysis and ultimately death due to the depletion of lipid reserves that occurs when the juveniles are unable to reach a feeding site (Opperman and Chang, 1990). This protects the growing crop from feeding damage, and perhaps, prevents a build-up of the population (Trudgill *et al*., 1996). No doubt, the effectiveness of these nematicides has impacted the current potato production system. However, changing EU legislation to safeguard the use of crop protection products (Hillocks, 2012; Anon, 2009) might restrict the availability of these nematicides. If these substances become unavailable, the Potato

Council estimated a doubling of the economic cost of PCN (£26m per annum) to the industry (Twinning *et al*., 2009; Clayton *et al*., 2008). In addition, the current increasing incidence of *G*. *pallida* in land treated with these nematicides provides evidence, which would support the suggestion of these substances not being effective in controlling this species (Whitehead *et al*., 1994; Evans, 1993; Whitehead, 1992), and that soil treatments might have selected *G*. *pallida* from mixed field populations (Evans and Haydock, 2000; Trudgill *et al*., 1996). Moreover, accelerated degradation has been widely suggested as a limiting factor to nematicide efficacy (Karpouzas and Walker, 2000; Karpouzas *et al*., 1999; Cox and Walker, 1996; Suett, 1986) and rotating nematicides has been proposed as a mean of managing nematicide efficacy (Osborn *et al.,* 2010). Therefore, more active substances are needed and testing of new products as they become available is justifiable to help fill some important gaps in designing integrated management of PCN (Haydock *et al*., 2006).

Table 1.1. Percentage of total potato area (ha) treated with nematicides fosthiazate and oxamyl from 2000 to 2010.

Year	Potato area (ha)	Area treated (ha)		% of total area treated		Mean % total area treated
		Fosthiazate	Oxamyl	Fosthiazate	Oxamyl	
2000	146200	2277	7302	1.55	4.99	6.55
2002	138700	7246	6581	5.22	4.74	9.96
2004	130900	9704	11546	7.41	8.82	16.23
2006	127200	9563	9693	7.51	7.62	15.13
2008	130200	13468	26769	10.34	20.55	30.9
2010	126900	15203	15978	11.98	12.59	24.57

Retrieved from https://secure.fera.defra.gov.uk on May 20, 2011.

1.2.6. Fluensulfone nematicide

Halogenation of pest controlling substances has played a major role in the quest for new pesticides, regarding improved efficacy, environmental safety, user friendliness, and economic viability (Jeschke, 2010). Fluensulfone (5-Chloro-2-(3, 4, 4-trifluoro-but-3-ene-1-sulfonyl) - thiazole), from ADAMA Agricultural Solutions Ltd, (formerly Makhteshim-Agan Industries Ltd) (Airport City, Israel), is a novel molecule of a new chemical class; fluoroalkenyl (Figure 1.1), and is intended for the reduction of nematode pests of crops (Karmon, 2010. Pers. Comm.).

1.2.6.1 Toxicology profile of fluensulfone

The acute and environmental toxicological data for fluensulfone are shown in Tables 1.2 and 1.3. Unlike the organophosphate and carbamate currently available, fluensulfone has a lesser acute toxicity for oral ingestion, dermal contact and inhalation, and it is a non-eye and skin irritant Tables 1.2. Furthermore, fluensulfone is deemed to have low toxicological activity on birds, fish, and bees, but is moderately toxic to Daphnia and earthworms Table 1.3.

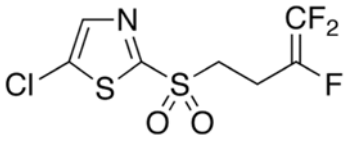


Figure 1.1. Chemical structure of fluensulfone. Retrieved from http://www.trc-canada.com on January 19, 2011.

Table 1.2. Toxicological summary for fluensulfone compared with organophosphate and carbamate nematicides.

Chemical class	Fluoroalkenyl	Organophosphate		Carbamate	
Active substance	Fluensulfone	Fosthiazate	Fenamiphos	Aldicarb	Oxamyl
Trade Name	MCW-2	Nemathorin[1]	Nemacur[2]	Temik[3]	Vydate[4, 5]
Acute oral toxicity (LD_{50}) Rat	671 mg/kg	73 mg/kg (Male) 51-64 mg/kg (Female)	2.7 mg/kg (Male) 3.0 mg/kg (Female)	0.8 mg/kg	5.4 mg/kg
Acute dermal (LD_{50}) Rat	> 2000 mg/kg	2396 mg/kg (Male) 861 mg/kg (Female)	225 mg/kg (Male) 178.8 mg/kg (Female)	20 mg/kg in water; 5 mg/kg in propylene glycol	2960 mg/kg
Acute Inhalation (LC_{50}) Rat	> 5.1 mg/L	0.83 mg/L (Male) 0.56 mg/L (Female)	> 0.1 mg/L	< 0.007 mg/L	0.12 - 0.17 mg/L
Irritation Rabbit	Non-irritant; eye and skin	Mildly eye irritating; non-skin irritating	Mildly eye irritating; non-skin irritating	Not relevant	Mildly eye irritating; non-skin irritating
Dermal sensitization Guinea pig	Positive	Positive	Negative	Negative	Negative

[1]Anon., 2004; [2]Anon., 2002; [3]Anon., 2007; [4]Anon., 2000; [5]Anon., 1996.

Table 1.3. Ecotoxicity of fluensulfone compared with organophosphate and carbamate nematicides

Chemical class	Fluoroalkenyl	Organophosphate		Carbamate	
Active substance	Fluensulfone	Fosthiazate	Fenamiphos	Aldicarb	Oxamyl
Trade name	MCW-2	Nemathorin	Nemacur	Temik	Vydate
Birds	Lowly toxic	Very toxic	Very toxic	Very toxic	Acutely toxic
most sensitive LC_{50}	>5620 mg/kg	10 mg/kg	0.8 mg/kg	3.4 mg/kg	3.16 mg/kg
Fish	Lowly toxic	Lowly toxic	Very toxic	Very toxic	Toxic
most sensitive LC_{50}	13 mg/L	114 mg/L	0.0093 mg/kg	0.56 mg/kg	3.13 mg/kg
Bioaccumulation	Log P_{ow}: 1.96	Low	Moderate	Low	Low
Daphnia	Moderately toxic	Very toxic	Very toxic	Very toxic	Very toxic
most sensitive LC_{50}	1-10 mg/L	0.282 mg/L	0.009 mg/L	0.42 mg/L	0.319 mg/L
Algae	Toxic	Lowly toxic	Lowly toxic	Lowly toxic	Lowly toxic
most sensitive LC_{50}	0.022 mg/L	> 4.51 mg/L	3.8 mg/L	50 mg/L	0.93 mg/L
Bees	Lowly toxic	Very toxic	Very toxic	Very toxic	Very toxic
most sensitive LC_{50}	170 µg/bee	0.256 µg/bee	0.28 µg/bee	0.09 µg/bee	0.38 µg/bee
Earthworms	Moderate	Moderate	Moderate	Moderate	Moderate
LC_{50}	153 mg/kg soil	209 mg/kg soil	444 mg /kg soil	65 mg/kg soil	112 mg/kg soil

1.2.6.2 Mode of action of fluensulfone

The exact biological processes affected by fluensulfone are not yet known. However, literature suggests that nematicidal effects may manifest at any stage of the life cycle of nematodes, and may involve disruption of hatching and infection processes (Woods *et al*., 1999; Steel, 1982; Greco and Thomason, 1980), suppression of feeding, development and reproduction of the nematode (Hague and Gowen, 1987; Evans and Wright, 1982).

1.2.6.2.1 Effects of fluensulfone on hatching

The effects of fluensulfone on hatching of *G. pallida* were determined *in vitro* (Deliopoulos *et al*., 2009) by measuring the emergence of J2 *G. pallida* from two-year-old cysts incubated in the technical-grade at concentrations ranging from 0.00425 to 0.608 mg L^{-1}. In comparison with potato root leachate and distilled water, all concentrations of fluensulfone tested reduced the hatch of *G. pallida,* and it was deduced from the cumulative hatch curve that there was complete inhibition of J2 emergence by the fifth week of incubation in fluensulfone. No further hatching was recorded within seven days of transferring the fluensulfone-treated cysts to either potato root leachate or water, suggesting irreversible effects on hatching. Subsequent assessments of the viability of the J2 from the fluensulfone-treated cysts indicated that the treatments might have caused in-egg mortality, with effects being significantly and positively dosage dependent. Fluensulfone was reported to have inhibited hatching of the root-knot nematode *Meloidogyne javanica* (Oka *et al*., 2009). The authors incubated approximately 150 *M. javanica* eggs in 10µL fluensulfone at 0.5, 1.0, 2.0, 4.0 and 8.0 mg L^{-1} and at an incubation temperature of 25 ± 2°C for 3 days. The data showed fewer J2 hatching from the fluensulfone-treated eggs at all concentrations in comparison with cadusafos at similar concentrations. However, hatching, in this study, resumed within three days of transfer of the eggs to water. Resumption of hatch when nematodes are

removed from a test compound is widely reported in the literature (E.g. Woods *et al*., 1999; Evans and Wright, 1982; Osborn, 1973). The apparent irreversible effects of the treatments in the study of Deliopoulos *et al*., (2009) could have been due to a higher dose effect from the longer exposure time of five weeks compared with three days in the case of *M. javanica* (Oka *et al*., 2009). A longer exposure to high concentrations is not likely to be achieved in field soils though. For example, percolating water flushes the nematicide from the site of the nematode, resulting in a shorter exposure and to low concentrations (Noling, 2003).

1.2.6.2.2 Effects of fluensulfone on mobility

Incubating J2 *M. javanica* for 24h in fluensulfone at 2.0, 4.0 and 8.0 mg L^{-1} inhibited mobility (Oka *et al*., 2009), and the immobilized juveniles became straight or rod shaped, which indicates death according to Wright *et al*., (1980). Similarly, the motility of J2 *G. pallida* was reduced following incubation in fluensulfone at 0.0078 – 32 mgL^{-1} for 24 – 72h, and the treatment effects correlated positively with the duration of incubation (Deliopoulos *et al*., 2009). Additionally, coiling or bent-body shape, indicating death, was observed. Swim frequency of *Caenorhabditis elegans* on solid and in liquid media was reduced following acute and chronic exposures to fluensulfone (Holden-Dye and O'Connor, 2010).

1.2.6.2.3 Effects of fluensulfone on root infection and population increase

Interference with the host seeking behaviour of J2 PCN is one mechanism of control widely reported for non-fumigant nematicides (E.g. Ibrahim and Haydock, 1999; Woods *et al*., 1999). Bhattarai and Haydock (2010) showed in a pot that soil treatments with granular fluensulfone (Fluensulfone 15G) at 6.75, 13.5, 20, 27 and 54 kg ha^{-1}, emulsifiable concentrate (Fluensulfone 480 g L^{-1} EC) and capsule suspensions (CS) forms, each at 4.22

and 8.44 l ha^{-1}, reduced the invasion of potato roots by *G. pallida* in a glasshouse experiment. All the rates and applications of fluensulfone reduced root invasions by the fourth week after planting. Besides reductions in the root invasion, the population increase of *G. pallida* was also decreased. Reduced infection of tomato by *M. javanica* in soil treated with EC fluensulfone has been reported as well (Oka *et al*., 2009). Under glasshouse and field conditions, soil treatment with EC fluensulfone reduced galling index and the number of eggs produced on tomato (*Lycopersicon esculentum (Mill.) cv. Daniela*). However, the control obtained from the treatment was variable. In a follow-up study, Oka *et al*., (2012) showed that a single foliar application to peppers (*Capsicum annum* cv. Hazera 1195) with an EC fluensulfone at 3.0 g L^{-1} gave ca. 80% reduction in galling index and reproduction of *Meloidogyne* species. Oka *et al*., (2009) and Oka *et al*., (2012) ascribed these reductions in infection to effects of fluensulfone on the root invasion.

Reduced invasion enables a certain fraction of the root, especially, during early stages of plant growth, to escape nematode attack (Schomaker and Been, 2006). Consequently, early reduction in plant nutrient uptake and associated harmful effects on plant growth are prevented (Grove *et al*., 1999a and 1999b). Trudgill *et al*., (2003) mentioned that nematicides, which reduce invasion, can affect the size of potential inoculum (*Pi*) and subsequent multiplication within the root tissues. Been and Schomaker (2006) however, suggested that contact nematicides mainly decreased *Pi* when they cause juveniles to become disorientated during the search for a host.

1.2.7 Factors affecting the efficacy of nematicides for PCN

1.2.7.1 Biology of PCN

Hatching of both species of PCN is triggered by potato root exudates, and therefore, it is reasoned that PCN will not be available in the soil until after root development has occurred. Moreover, the hatching process can have a rather slow start and has been reported to taking three weeks for *G. rostochiensis* and six weeks for *G. pallida* (Whitehead *et al*., 1984; Whitehead, 1992) to reach a maximum. Nematicides, however, may start to break down into non-toxic forms soon after application (Haydock *et al*., 2006). With the concentration beginning to fall with time or if the nematicide breaks down too quickly, it is suggested that the slow emergence of PCN may enable the hatching J2 to escape sufficiently high concentrations in the soil, thus rendering a nematicide ineffective (Evan, 1993). Indeed, the inadequate control of *G. pallida* by the currently available nematicides has been ascribed to the longer period of hatching (Whitehead *et al*., 1984). Haydock and Evans (1998), for instance, described the hatching behaviour of *G. pallida* in relation to the efficacy of oxamyl. Comparing oxamyl with two decay rates (Figure 1.2), it was postulated that extended hatching may render nematicides with a half-life less than three weeks ineffective. Furthermore, J2 G. *pallida* are reported to persist for a longer period in the soil in the absence of a host. Robinson *et al*. (1987b) found that at 20^{o} C of incubation, the lipid reserves of *G. rostochiensis* and *G. pallida* depleted in 15 and 22 days, respectively. Similar observations were made by Robinson *et al*. (1987a) who recorded the time for 50 % depletion in lipid reserve as 10 to 18 days for *G. pallid* and 7 to 11 days for *G. rostochiensis*. The nematicidal effects of fluensulfone on mobility reported for *M. javanica* (Oka *et al*., 2009), *C. elegance* (Holden-Dye and O'Connor, 2010) and *G. pallida* (Deliopoulos *et al*., 2009), suggest that fluensulfone may act by paralyzing J2 PCN. However, the longer persisting *G. pallida* may require a longer-lasting effect of fluensulfone to be effective against the free-living stages of this species.

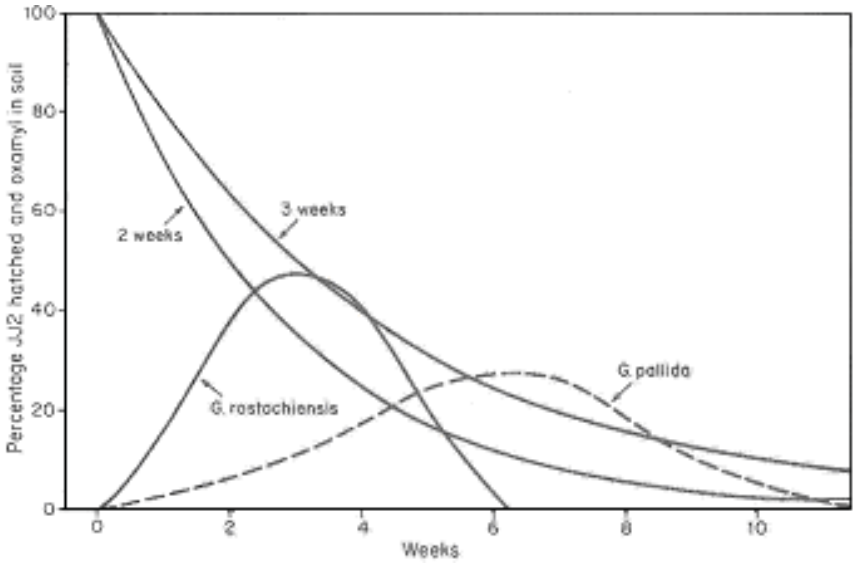


Figure 1.2. Hatching patterns for *G. rostochiensis* and *G. pallida* under a potato crop in relation to oxamyl with 2 or 3 week half-lives (Adapted from Haydock and Evans, 1998).

1.2.7.2 Water solubility

The water solubility of a nematicide determines the ease with which it partitions to the soil's aqueous phase, thus, its availability to be distributed by percolating soil water or to contact PCN, to be effective. Fluensulfone is known for having a water solubility of 622 mg L^{-1} (Karmon, 2010. Pers. Comm.), and is far lower than 9850 mg L^{-1} for fosthiazate and 28,000 mg L^{-1} for oxamyl. This lower solubility indicates that fluensulfone availability to soil water could be limited. In general, highly soluble nematicides such as oxamyl may also be susceptible to leaching, posing risks of contaminating underground water. They may perform poorly depending upon the amount and frequency of precipitation after application. Being soluble, however, does not provide a complete estimate of the potential to be available for transport since sorption couples with this property to determine the proportion available to the soil water.

1.2.7.3 Formulation

There is now evidence that soil treatments with the granular, the emulsifiable concentrate and capsule suspensions forms of fluensulfone could control PCN. However, granular nematicides are in widespread use in the UK, and it is reasonable to assume that most growers would preferably opt for the granular form of fluensulfone since they are already equipped for the application of dry granules. Besides, granular formulations may be more effective for reducing PCN, since granules release active substances slowly following application to the soil, and may help maintain a somewhat steady concentration in the soil (Leistra and Green, 1990). Granules are applied dry at planting, and must be wetted before the active substance is released to the soil water to be effective (Davies *et al*., 1996). The rate of release of fluensulfone from its carrier material may, thus, influence its effectiveness.

1.2.7.4 Depth of incorporation into soil

Investigations of techniques for applying non-fumigant nematicides to the soil for the reduction of PCN have shown that the depth of incorporation can have a substantial effect upon effectiveness (Woods and Haydock, 2000; Whitehead, *et al*., 1981). Control of PCN is obtained when granules are evenly spread on the surface of potato beds and immediately incorporated by rotatory cultivation to 10 – 15 cm deep at planting of potato. However, Woods and Haydock (2000) remarked that incorporation to the above depth may not work well for *G*. *pallida*, and demonstrated experimentally that superior control may be obtained when fosthiazate was incorporated to 20 cm deep. Their findings further showed that shallow incorporation (10 cm) failed to treat enough soil and deep incorporation (35 cm deep) merely dilutes the desired concentration for effective control.

1.2.7.5 Sorption

Sorption describes the interaction between a pesticide and the soil's solid phase, and determines the distribution of pesticides in the soil/water environment (Kah and Brown. 2007). A sorption phenomenon may involve both adsorption and absorption processes (Cornelissen *et al*., 2005; Accari - Dey and Gschwend, 2003), with adsorption representing the process by which pesticide molecules or ions attach to the surface of the soil particles (Calvet, 1989), whereas absorption involves the process by which the pesticide permeates the soil particle (Thompson and Goyne, 2012). Irrespective of the form in which it manifests, sorption entails the removal of a pesticide from the soil solution (Sposito, 2008), and therefore, may constitute a limiting factor to efficacy of non-volatile nematicides, which mostly exert their effects in the soil's aqueous phase. Furthermore, sorption is widely recognised as a key retention mechanism limiting pesticide mobility, as well as bioavailability in the soil (Spark and Swift, 2002; Kah and Brown, 2006; Boivin *et al.*, 2005; Wang and Keller, 2009). If sorption should influence fluensulfone mobility and bioavailability in the soil, it probably could affect the distribution and degradation, thus could impact efficacy against *G. pallida*. Sorption is the term used when adsorption and absorption cannot be distinguished; however, adsorption is widely used to represent both processes.

Both pesticide and soil properties influence the extents of sorption in a given system. The polarity of a molecule plays a major role in its sorption behaviour in the soil (Chen *et al*., 2005; Bromilow, 1980). Figure 1.1 shows the presence of a CF_3 group in the chemical structure of fluensulfone, and the influence of fluorine on a molecule's polarity is well documented in the literature (Biffinger *et al*., 2004; Smart, 2001). The introduction of F and CF_3 substituent often improves lipophilicity (Dinoiu, 2006; Ichino *et al*., 1990) and allows changes of the lipophilic parameters towards non-polarity (Smart, 2001). Park *et al*., (2001)

consider the CF_3 group as one of the most lipophilic of all substituents. Indeed, most fluorinated compounds disclosed for nematode control are non-polar (Phillion *et al*., 1999), and for non-polar organic molecules, adsorption is principally governed by the organic matter content of soils (Calvet, 1989). However, in soils with low organic matter content, adsorption is often related to the active components of the mineral fraction within the soil, which is predominantly the clay fraction (Spark and Swift 2002; Simon *et al*., 1992; Gerstl, 1984).

By convention, the soil adsorption coefficient is denoted by K_d, and is determined widely by the batch equilibrium method. With this method, an aqueous solution of the test compound (C_s) is added to soil slurry, and mixed gently for a period typically from 2 to 48h, with 24h being widely adopted (Boesten *et al*., 1988). The concentrations of the chemical in the soil solution (C_e) is then determined. The K_d value is defined by the equation in (1):

$$K_d = C_s / C_e \quad (1)$$

Since adsorption of non-polar molecules correlates mostly with organic matter, the K_d is often equalised for organic matter content of the soil (Walker, 2003), with organic matter usually expressed as organic carbon content (OC). The soil organic carbon adsorption coefficient (K_{oc}) of a chemical is then defined by the equation in (2):

$$K_{oc} = K_d / f_{oc} \quad (2)$$

where, f_{oc} is the organic carbon fraction of the soil. Once K_{oc} has been calculated from a K_d and a f_{oc} measurement for a pesticide in a soil, K_d for that pesticide is, in principle, calculable for any other soil whose f_{oc} is known (Wauchope *et al*., 2002). The sorption of fluensulfone on soil is yet to be determined, but the properties as per Table 1.3 would suggest that the fate and behaviour of fluensulfone in the soil may be influenced by the soil's organic fraction,

and a measure of fluensulfone sorption will be valuable in evaluating the efficacy against PCN.

1.2.7.6 Persistence

Ideally, a pesticide stays in the treated area long enough to produce the desired effect and then degrades via biological or chemical processes into non-toxic forms. The rate of degradation, however, has been the primary factor of concern regarding nematicide efficacy against PCN. In general, degradation rates of pesticides dependent on various properties of the soil, as well as the properties of the pesticide (Pantelelis *et al*., 2006; Ambrose *et al*., 2000; Jurado-Exposito and Walker, 1998; Wagenet and Hutson, 1990; Allen and Walker, 1987). Little is known regarding the fate of fluensulfone in soils, but most literature reveals that fluorinated chemicals generally environmentally persistent, owing to stability of the C-F bond towards microbial degradation (E.g. Kirk, 2006; Jeschke, 2004; Jeschke, 2010; Key *et al*., 1997). However, Key *et al*. (1998) reported microbial catalysed C-F bond cleavage reactions and showed that the stability of fluorinated chemicals depends upon the level of fluorination. Under laboratory conditions, a *Pseudomonas* species (Strain D2) completely defluorinated difluoromethane sulfonate ($CHF_2SO_3^-$), but could only partially degrade trifluoroethane sulfonate ($CF_3CH_2SO_3^-$) and perfluorooctane sulfonate ($C_6F_{13}C_2H_4SO_3^-$). Trifluoromethane sulfonate ($CF_3SO_3^-$) and perfluorooctane sulfonate ($C_8F_{17}SO_3$-) were not degraded at all. Key *et al*., (1998) then suggested that the transformation of fluorinated sulfonates may require the presence of hydrogen on the fluorinated alkyl chain.

The molecular structure of fluensulfone (Figure 1.1) shows that the molecule is partially fluorinated, and contains -CH_2CH_2- between a hydrophilic part and a fluorinated carbon chain. Natarajan *et al*., (2005) and Stenersen (2004) suggested that the presence of two hydrogen atoms between two functional groups, present sites, with high affinity for oxygen,

and therefore, may be reactive. Indeed, fluensulfone was suggesting for having an 11–22-day half-life (Manufacturer's information), and the hypothesis this molecule will degrade rapidly in soils is worthy of investigation.

1.3 Aims of the study

The study aimed at the following:

i. To establish the activity of the fluensulfone against *G. pallida*.

ii. To determine factors likely to influence the efficacy of fluensulfone in field conditions.

iii. To understand the persistence of the fluensulfone in field soil.

iv. To demonstrate how fluensulfone could be integrated into management program for *G. pallida*.

1.4 Hypotheses

This study tested the hypotheses that

i. Fluensulfone interferes with the host finding and root invasion activities of *G. pallida*, hence, its application to soil will protect the potato from infections and improves plant growth, development and tuber yields, as a consequent.

ii. Fluensulfone suppresses the development and reproduction of *G. pallida,* and therefore, its application to soil will control population increases.

iii. The efficacy of fluensulfone against *G. pallida* is limited by its persistence in soil.

iv. Fluensulfone will integrate into a management program for *G. pallida*.

CHAPTER 2

GENERAL METHODS

2.1 Soil sampling for PCN

Sampling of soil for PCN was informed by the field's history of PCN infestations provided by growers. Each field site was, preliminarily, sampled was to determine suitability for the study. Using a 30-meter measuring tape, a 10 metre square grid was laid out over the infested area, with the grid nodes serving as the sampling points. At each node, 10 soil cores were extracted (ca. 20 cm deep and at ca. 10 cm apart) using a 2.5 by 30 cm 'cheese corer' style auger. The cores were bulked (yielded ca. 500-g sample) in a secured cotton bag, labelled with the name of the field, the location of the node within the field and the date of sampling. The soils were kept in a drying cabinet at 25°C and processed for PCN whenever appropriate. During the field experiments, plots were sampled using the same equipment, prior to planting and after harvesting of potatoes, to determine, respectively, the initial (*Pi*) and final (*Pf*) population densities of PCN. For these, 50 cores were taken from each plot (followed a zig-zag sampling pattern) bulked in cotton bags (gave ca 2.5 kg sample). Soil for pot studies was collected from field sites with specific PCN population densities and species ratio. The soils were collected at 20 – 30 cm depth using a spade around the area where suitable population densities were, previously, recorded. The soils were either utilized immediately or stored in sacks outside for future use.

2.2 Quantification of PCN in soil

Cysts were extracted from soils using a Fenwick can (Fenwick, 1940) following standard methods (Shepherd, 1986). Dry soils were passed through a 5.6-mm aperture sieve (Endecotts Ltd. London, England) to separate soil from stones and other debris. The soil was

mixed thoroughly, by hand in a tray, before a 200 g sub-sample was weighed out and elutriated. The eluate was dried for at least 24h at 25°C, before being examined under a binocular microscope (magnification = x40). All PCN cysts were counted. The number of cysts used in the egg count depended on the total number of cysts recovered from each sample. Typically, 50 cysts were selected, but for samples that contained fewer cysts (i.e. less than 50), all the available cysts were included in the egg count. The population density of PCN was expressed as the number of viable eggs g^{-1} soil according to the equation in (3)

$$\text{Eggs } g^{-1} \text{ soil} = \left[\left[\frac{\text{Water (mL) in egg suspension}}{\text{No. Cysts used in egg count}} \right] \times \text{No. Egg ml}^{-1} \right] \times \left[\frac{\text{No. cysts in count}}{\text{Weight of soil}} \right] \quad (3)$$

The relative abundances of *G. rostochiensis* and *G. pallida*, at each site, were determined by polymerase chain reaction (Edwards, Pers. Comm.).

2.3 Assessments of plant growth and development

Percentage ground cover was determined by the grid method reported by Burstall and Harris (1983). At each time of sampling, plants were harvested, washed under slow running tap water, air-dried on filter paper for at least 30 minutes and divided into above and below-ground parts and weighed as per individual plants. The roots were cut into ca. 2 cm pieces, before a 2 g sub-sample was preserved in formal acetic alcohol (FAA) for PCN root invasion assessments (see Section 2.4 below).

2.4 Quantification of PCN root invasion

Root invasion was determined following the procedure outlined by Hooper (1986). Samples were stained by dipping in boiling 0.05% acid fuchsin solution (acid fuchsin dissolved in a 1:1:1 mixture of glycerol, lactic acid and distilled water) in a 1000 mL beaker for ca. 4 min. The stained roots were rinsed and transferred to a mixture of glycerol and water (1:1 v/v), which was then heated to boiling and allowed to cool. After rinsing with tap water, each sample was macerated in 100 mL of tap water using a waring blender, initially at the minimum speed for 30 seconds, and subsequently, at the maximum speed for 30 seconds. The suspension was transferred to a 300 mL beaker and made up to 200 mL with tap water. It was stirred thoroughly before 2 mL was taken with modified wide bore plastic 5 mL pipette and transferred to a De Grisse counting dish, where PCN was counted under a stereomicroscope at ×40 magnifications. The root invasion was expressed as the number of juveniles per gram root according to the equation (4).

$$\text{Juveniles g}^{-1}\text{ root} = \frac{\text{Total juveniles in 2 mL sub-sample} \times 100}{\text{Weight of root sample (i.e. 2 g)}} \quad (4).$$

2.5 Determination of the chemical and physical properties of soil

2.5.1 Sample processing

Soils were spread out in trays and kept at ca. 20 ºC for at least 7 days before passing through a 2-mm aperture sieve. If not analysed immediately, the samples were transferred to store dry in paper bags in the dark. All analyses were made in triplicate, and followed the standard methods of the Agricultural Development and Advisory Service (ADAS) (MAFF, 1986).

2.5.2 Soil texture

Soil texture was determined using the 'pipette method' following sedimentation procedures (MAFF, 1986). Twenty grams of processed soil were weighed out and transferred to a 500 mL beaker. Twenty millilitres of 30% hydrogen peroxide solution were added, and the suspension was stirred, whilst being gently heated on a hot plate, until it ceased giving off bubbles. The mixture was allowed to cool to room temperature overnight and transferred to a 250 mL shaking bottle. Ten millilitres of dispersing reagent (50 g sodium hexametaphosphate and 7 g anhydrous sodium carbonate in 1 L) and 150 mL of water was added. The suspension was agitated by placing on a HS 501 digital reciprocal shaker (IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 100 rpm for 5 min and poured through a 63 μm aperture sieves into a 500 mL graduated cylinder (Sigma-Aldrich Co. Ltd, UK). The content of the sieve was thoroughly rinsed with water from above to ensure that all particles had passed through. The particles remaining on the sieve, representing the sand fraction (> 63 μm), was washed off into an evaporating basin and dried in an oven at 105^{o}C overnight. Water was added to the graduated cylinder up to the 500 mL mark, and the suspension mixed for 30 seconds. Using a pipette, 25 mL sample was immediately taken from a depth of 100 - 150 mm below the surface and transferred to an evaporating basin for drying at 105^{o}C. This represented the silt + clay fraction (particle sizes less than 63 μm). The cylinder was then left to stand overnight before a 25 mL sample was taken from 90 mm below the surface and transferred to an evaporating basin for drying at 105^{o}C, to give the clay fractions (particles sizes less than 2 μm). The fractions were weighed, and their relative proportions in the original sample (i.e. 20 g) were expressed in percentages. A soil textural triangle was used to classify the soil.

2.5.3 Organic matter contents of soil

Both organic matter (OM) and total organic carbon (TOC) contents of soils were determined. The OM content of soils was determined by loss on ignition (LOI) using a Carbolite AAF 1100 furnace (Carbolite® Hope Valley, UK). Twenty grams of processed samples were transferred to ceramic crucibles and dried at 105°C overnight. The samples were cooled to room temperature in a desiccator and weighed (W_{105}) to determine the residual water content. The crucibles were then put in the furnaces and ignited at 550°C for 4 hours. They were re-weighed (W_{550}) after cooling in a desiccator and LOI determined as follows:

$$LOI = \left[\frac{(W_{105} - W_{550})}{W_{105}} \right] \times 100 \qquad (5)$$

The TOC was determined by dry combustion using a LECO sulphur-carbon analyser (LECO®Hazer Grove, Stockport, UK) equipped with a gas chromatograph. One gram samples were placed in ceramic crucibles and combusted at elevated temperature (> 1000°C).

2.5.4 Soil pH

Twenty grams of soil were transferred to a 100 mL beaker to which 50 mL of de-ionised water was added. The content was stirred thoroughly and transferred to a 100 mL shaking bottle. The suspension was mechanically shaken for 15 min., after which it was transferred back to the beaker and allowed to stand for 30 seconds before a pH reading taken with a Russell RL150 pH meter.

CHAPTER 3.0

EVALUATION OF THE EFFICACY OF FLUENSULFONE FOR CONTROL OF THE POTATO CYST NEMATODE, *GLOBODERA PALLIDA*

3.1 Introduction

Laboratory and glasshouse studies (Deliopoulos *et al*., 2009; Bhattarai and Haydock, 2009) have demonstrated nematicidal activities of fluensulfone against *G*. *pallida*. The experiments reported in this chapter were conducted to evaluate the efficacy of fluensulfone in controlling *G*. *pallida* in-field. In all, four experiments (three as field experiments and one as a polytunnel pot experiment) were conducted in soils infested predominantly by *G. pallida*. Two of the field experiments (established consecutively in 2010 and 2011) were dose-response studies, which evaluated the treatments shown in Table 3.1. The polytunnel experiment was also a dose-response study and was conducted in 2012 in the absence of a suitable field site with sufficiently high *G*. *pallida* population. The third field experiment (also established in 2011) determined the combined use of fluensulfone and partially resistant potatoes for control of *G*. *pallida*.

3.1.1 Aims

The aims of these experiments were to determine if fluensulfone soil treatments could protect the potato from PCN damages and could prevent the population from increasing.

3.1.2 Objectives

3.1.2.1 Field experiments 1 (Woodcote, 2010) and 2 (Howle, 2011)

The Field experiments 1 and 2 had the following objectives:

i. To determine the effects of fluensulfone soil treatments in the granular and emulsifiable concentrates forms on *G. pallida* root invasion, population increase, and associated plant growth and tuber yield losses.

ii. To compare the treatments of fluensulfone with the two commercially available nematicides fosthiazate and oxamyl at their recommended commercial rates of 3.0 and 5.5 kg a.s. ha^{-1}, respectively.

iii. To determine the minimum effective rate for fluensulfone treatment in the granular form for control of *G. pallida* in-field.

3.1.2.2 Field experiment 3

The Field experiment 3 at Howle in 2011 was conducted

i. To determine the combined treatment effects of granular fluensulfone soil treatment and partially resistant potatoes Santé and Vales Everest on the control of *G. pallida* root invasion and population increase.

ii. To compare this treatment of fluensulfone with fosthiazate and oxamyl treatments at their recommended commercial rates of 3.0 and 5.5 kg a.s. ha^{-1}.

3.1.2.3 Polytunnel experiment 1

The Polytunnel pot experiment 1 determined the effects of fluensulfone soil treatment on *G. pallida* viability, hatching and root invasion.

3.1.3 Hypothesis

The central hypothesis tested in these experiments was that fluensulfone possesses sufficient nematicidal activity to provide adequate control of *G. pallida* in-field, thus improving plant growth and tuber yields.

3.2 General methods applied to the field experiments

3.2.1 Field preparation and general agronomy

Each field was prepared by the grower and included sub-soiling, ploughing to a depth of 30 cm, bed-forming and de-stoning. The experimental plots were two beds wide (3.6 m) and each contained four rows; the two middle rows served as the harvest rows whereas the outer two served as guard rows. General agronomy was according to standard good practice.

3.2.2 Nematode population densities and soil properties

Before the application of nematicides and the planting of tubers, a 2.0 - 2.5 kg soil bulked sample (random 50 cores of 2.5 cm diameter × 20 cm deep) was taken from each plot and processed for the initial population densities (*Pi*) as per Section 2.2 and soil properties (pH, organic matter content and soil particle size distribution) as per Section 2.5. Whereas the soil pH and organic matter contents were determined for each plot, that of the soil texture was

estimated from a pooled sample for the entire experimental area. Within 24h of potato harvesting, soil sampling was repeated in the plots and the final population density (*Pf*) determined according to Section 2.2 (see General method). The rate of increase of the population during the experiment was determined as the ratio of *Pf*/*Pi*.

3.2.3 Experimental design

Each experiment was of a randomised block design with treatments replicated five times. Blocking of the treatments was informed by the *Pi*, and to form the blocks, plots with similar *Pi* were grouped and the treatments randomly assigned to the plots within the blocks using the Microsoft 2010 excel randomization function (Microsoft, Berkshire, UK). Differences between treatments within the blocks were checked by performing analyses of variance (ANOVA) where *Pi* was entered as treatment and blocks as replicates. The differences between the treatments were tested at $P < 0.05$; treatment arrangements were accepted only at $P > 0.05$. To justify blocking of the treatments, ANOVA was run on the blocks as treatments and *Pi* as replicates and the differences checked using similar statistics as for the treatments.

3.2.4 Application of nematicides

All granular nematicides were metered onto pre-formed beds using a Rickshaw type granule applicator (Plate 3.1), and incorporated into the topmost 15-20 cm depth (Woods and Haydock, 2000) by a tractor-mounted spike rotavator (Plate 3.3). Where applicable, emulsifiable concentrates (EC) fluensulfone was surface applied using a 2-Metre Oxford Precision Sprayer (Plate 3.2) and incorporated as the granules.

3.2.5 Seed potatoes, planting, harvesting and grading of tubers

Certified seed-potatoes (Super Elite grade II graded to 35 - 45 mm) were used throughout the study. The tubers were sprouted for at least three weeks (ca. 2 cm long sprout) by spreading in plastic trays under shed with natural lightening. Planting was done manually to 10 - 15 cm depth and at 25 cm within-row spacing using a hand-held potato planter. The tubers were harvested after the plants had senesced naturally, and involved mechanical lifting of tubers from 5 m length of each of the two middle rows. This was followed by hand forking to collect all potatoes present. The tubers were clean of soil and mechanically graded according to sizes less than 45 mm, 45 – 65 mm, 65 - 85, and greater than 85 mm. Each lot was counted and weighed, and yield was expressed in tonnes per hectare (t ha^{-1}).

3.2.6 Plant measurements

All measurements were made on plants in the two middle rows (harvest rows). Plant emergence was assessed visually during the first three weeks after planting (WAP). Ground cover was assessed by the grid method according to Burstall and Harris (1983). Plant weights, i.e. root and haulm weights, were determined from a pair of plants lifted from each plot at ca. 4 (3^{rd} positioned plant) and 6 (6^{th} positioned plant) WAP. After weighing, a 2-g sub-sample of the roots was examined for PCN root invasion as per section 2.4.

3.2.7 Soil temperature and precipitation

Soil temperature was recorded at 15 cm depth using a pair of Tinytag Plus 2 temperature data loggers (Gemini data loggers, West Sussex, UK). Rainfall records were taken at Harper

Adams University, Newport in Shropshire. The crops were irrigated as per standard practice in the area.

3.2.8 Data analysis

All plant growth and tuber yield data was checked for normality and transformed whenever appropriate before were subjected to ANOVA using GenStat for Windows® V.15 (VSN International Ltd. Hempstead, UK). All nematode counts were transformed to $Log_e(x +10)$ to establish normal distribution before analyses. Whenever appropriate, Fisher's protected LSD post hoc tests, at $P = 0.05$, were used to determine differences between individual treatments.

Plate 3.1. Surface applications of granular fluensulfone, fosthiazate and oxamyl to preformed beds using a Rickshaw type granule applicator.

Plate 3.2. Surface application of emulsifiable concentrates (EC) fluensulfone using a 2-metre Oxford Precision Sprayer.

Plate 3.3. Incorporation of nematicide into the top 15 – 20 cm layer of soil.

3.2.9 Field experiment 1 (Woodcote, 2010)

The experiment was undertaken in a commercial potato field at Woodcote (UK Ordnance Survey Grid Reference: SJ 76901 15708) near Newport in Shropshire. The soil type was a sandy clay loam (Table 3.2). The treatments that were evaluated are described in Table 3.1. The nematicides were applied on 19 May, 2010 to beds 6m long by 3.6 m wide. These were followed by tuber planting on 20 and 21 May, 2010. Tubers were harvested on October 17, 2010 (ca. 149 days after planting, DAP) by which time the plants had senesced naturally. Emergence of the plants was recorded at 10, 17 and 25 DAP. Ground cover measurement was started at 25 DAP, then at seven-day intervals until 53 DAP when the plant canopy was almost closed. The biomass of the shoots and roots, as well as and root infections by *G. pallida* were determined at 29 and 44 DAP.

3.2.10. Field experiment 2 (Howle, 2011)

The Field experiment 2 was, essentially, a repeat of that conducted in 2010 and as such, evaluated the same treatments shown in Table 3.1. The experiment was established in a commercial potato field at Howle (UK Ordnance Survey Grid Reference: SJ 69485 23830), near Newport in Shropshire. The soil type was a sandy clay loam (Table 3.10). The experiment was initiated on 20 April, 2011 when the nematicides were applied. Tubers were planted on 21 and 22 April, 2011 and harvested on 7 September 2011 (ca. 138 DAP). Plant emergence was recorded at 8, 17, 23, and 28 DAP. Percentage ground cover was measured at 21, 33, 40 and 56 DAP. The root invasion by *G. pallida* and the plant root and shoot weights were determined at 28 and 42 DAP.

Table 3.1. Descriptions of the treatments evaluated during the field experiment 1 and 2 at Woodcote and Howle in 2010 and 2011, respectively.

Treatment type	Formulation	Rate (kg a.s. ha^{-1})	Product rate ha^{-1}
Fluensulfone	Fluensulfone 15% w/w G	1.95	13.0 kg
Fluensulfone	Fluensulfone 15% w/w G	3.00	20.0 kg
Fluensulfone	Fluensulfone 15% w/w G	4.05	27.0 kg
Fluensulfone	Fluensulfone 15% w/w G	5.05	33.5 kg
Fluensulfone	Fluensulfone 15% w/w G	6.00	40.0 kg
Fluensulfone	Fluensulfone 480 g/l EC	4.05	8.44 l
Oxamyl	Vydate 10% w/w G	5.50	55.0 kg
Fosthiazate	Nemathorin 10%w/w G	3.00	30.0 kg
Untreated control		0.00	0.0 kg

G: granular formulation; EC: emulsifiable concentrates formulation; a.s. active substance.

3.2.11 Field experiment 3 (Howle, 2011)

This experiment was conducted alongside the field experiment 2 at the Howle site in 2011 (UK Ordnance Survey Grid Reference: SJ 69485 23830). It comprised a 4 × 3 factorial arrangement of treatments, where nematicide (fluensulfone at 4.05 kg a.s. ha^{-1}, fosthiazate at 3.0 kg a.s. ha^{-1}, oxamyl at 5.5 kg a.s. ha^{-1} and plot left untreated) was one factor and cultivar (*cv*) (Santé, Vales Everest and Estima) as the other factor. The treatments were replicated five times and arranged in randomised blocks, where the cultivars were allocated to plot using a separate randomization. The nematicides were applied to 9 m long and 3.6 m wide plots, on 20 April, 2011. Seed tubers were planted on a third, i.e. 3 m long (four rows each of 12 potatoes per cultivar), of the plots on 22 April, 2011. At harvest on 7 September, 2011, 2 m of the harvest rows was lifted (16 plants per cultivar) and assessed for tuber yield as per section 3.2.1.5. The *Pi*, *Pf* and *Pf*/*Pi* ratios were determined as per Section 2.2.

3.2.12 Polytunnel experiment 1 (Harper Adams University, 2012)

This experiment was conducted in the absence of a suitable field site with sufficiently high population. As mentioned above, it aimed at evaluating the nematicidal efficacy of fluensulfone in soil with high infestation of *G. pallida*.

3.2.13 Materials and methods

3.2.13.1 Soil and nematicide treatments

Soil for the experiment was collected from the untreated control plots at the end of the field experiments 2 and 3 at Howle (September 2011). Additional soils were collected from an area of the field planted with Maris piper in the same year. The soil was stored in sacks

outside until the start of the experiment on 16 May, 2012. Soil properties are given in Table 3.10. The experiment utilised 25-cm-diameter Optipot plastic pots (Congleton Plastic & Co. Ltd. Cheshire, UK) each filled with soil ca. 20 cm deep. The soil was infested by *G. pallida* at 50 eggs g^{-1} soil. Soil treatments with fluensulfone in the granular form at 1.95, 4.05 and 5.05 kg a.s. ha^{-1} were studied in comparison with fosthiazate at 3.0 kg a.s. ha^{-1} and soil left untreated. The above treatments were obtained by mixing the required quantity of soil and nematicide in a Belle mini 140 cement mixer (Belle Engineering [Sheen] Ltd, Sheen, Derbyshire) for approximately one minute during which the soil moisture was adjusted to ca. 50 % of the maximum water holding capacity (MWHC) as per Table 3.10, and slow-release N:P:K 14:13:13 Osmocote fertilizer (The Scotts Company Ltd, UK) added at 0.1%. After mixing, the soil was transferred to the pots, and a seed tuber (*cv*. Estima) planted at ca. 10 cm deep. The pots were arranged on a bench in the polytunnel in randomised blocks with 6 replicates. Afterwards, water was added to increase the soil moisture to ca. 90% of MWHC. The soil moisture during the experiment was monitored using a theta probe ML2x (Delta T, Cambridge) and adjusted accordingly to 80 – 90% of MWHC during the experiment. Soil and air temperatures were measured using Tinytalk temperature loggers (Gemini Data Loggers (UK) Ltd.) one buried at 10 cm depth in an untreated pot and the placed outside the pot. The experiment was terminated on 27 June, 2012 (ca. 43 DAP).

3.2.13.2 Assessments

Root and shoot weights, numbers of stem and stolon were determined at harvest (43 DAP). Root invasion by *G. pallida* were estimated in a 2-g sub-sample by standard methods (Section 2.4). Soil in each pot was thoroughly mixed and cysts were extracted from a 600 g sub-sample by the Fenwick Can (Fenwick, 1940). One hundred cysts were handpicked from each sample, and of these, a batch of 50 cysts was used to assess the viability of the remaining

encysted eggs, and the other batch used to assess J2 hatching. To assess the viability, the cysts were first placed in 1 mL of distilled water in a staining block for 24h after which the water was replaced with 1 mL of 0.05% Medola Blue stain (Sigma-Aldrich, Poole, UK) in which the cysts were left for 7 days at 20 ºC. Afterwards, the stain was replaced with 1 mL distilled water for a further 24h before the cysts were gently crushed by rolling on a glass slide. A 25 mL suspension was prepared of which 1 mL sub-sample was examined under a microscope and the numbers of non-stained (viable) egg/juvenile were counted. The hatching test was made by exposing the cysts to potato root leachate obtained from 5 weeks-old Estima. The cysts were placed in plastic tubes (1.0 cm wide × 2.0 cm high) the bottom ends of which were sealed with a 53 µm mesh sieve. The tubes were each placed into a well of a 24-well-plate containing 1 mL of the exudate. The plate was covered, sealed with parafilm and incubated at 16 ºC. Counts of emerged juveniles were made at weekly intervals until the number of juveniles observed was about zero.

3.3 Results

3.3.1 Field experiment 1 (Woodcote, 2010)

3.3.1.1 Initial population (*Pi*) densities and soil properties

Table 3.3 shows the initial population density (*Pi*), soil organic matter content (OM) and soil pH determined according to the treatments and blocks. The soil was classified as a sandy clay loam (MAFF, 1986); it was slightly acidic and organic matter constituted 1.8% (Table 3.2). The *Pi* ranged from 2 eggs g^{-1} soil to 34 egg g^{-1} soil, with a mean of 16.2 egg g^{-1} soil. The mean *Pi* and the soil properties did not differ significantly between the treatments. The block means, however, differed significantly according to the *Pi* ($P < 0.001$) and the soil pH ($P = 0.015$), but not according to the soil OM.

Table 3.2. Selected properties analysed for soil at the field site located at Woodcote (Shropshire, 2010).

% Clay	% Silt	% Sand	% OM	% MWHC at 5 KPa mbar	pH	Soil type
20.5	4.5	70.6	1.8	14.8	6.6	SCL

MWHC: maximum water-holding capacity; OM: organic matter content; SCL = sandy clay loam

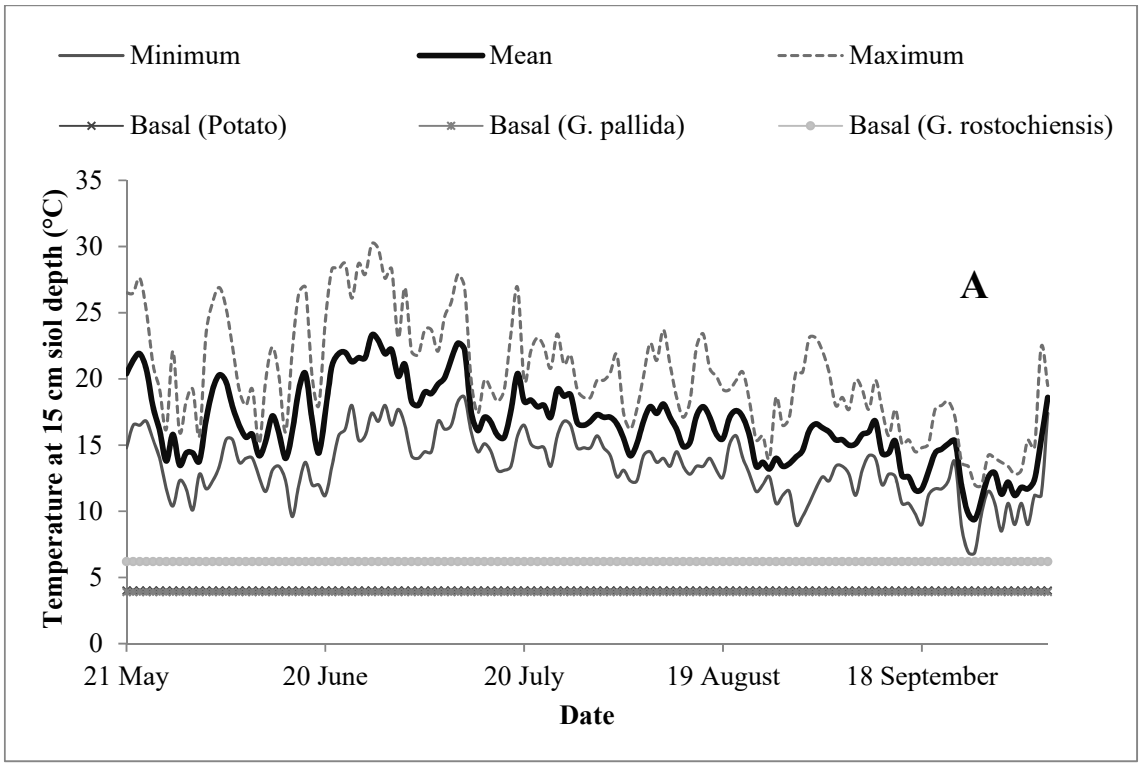
Table 3.3. Initial population densities (*Pi*) (eggs g^{-1} soil), soil pH and % soil organic matter content (OM) as per the treatments and blocks during the field experiment at Woodcote (Shropshire, 2010).

Treatment	Rate (kg a.s. ha^{-1})	Treatment means			Block means			
		Pi (eggs g^{-1} soil)	%OM	Soil pH	Block	*Pi* (eggs g^{-1} soil)	% OM	pH
Untreated	-	18.2 a	1.5 a	6.7 a				
Fluensulfone G	1.95	17.6 a	1.1 a	6.7 a	1	5.8 a	1.0 a	6.7 ab
Fluensulfone G	3.00	13.2 a	1.8 a	6.5 a	2	17.9 b	2.0 a	6.6 ab
Fluensulfone G	4.05	16.0 a	1.4 a	6.7 a	3	25.1 c	1.7 a	6.6 ab
Fluensulfone G	5.05	15.6 a	1.7 a	6.5 a	4	15.1 b	1.6 a	6.4 a
Fluensulfone G	6.00	18.2 a	2.0 a	6.5 a	5	17.3 bc	2.6 a	6.8 b
Fluensulfone EC	4.05	13.2 a	2.2 a	6.7 a				
Nemathorin G	3.00	18.0 a	2.9 a	6.6 a				
Vydate G	5.50	16.2 a	1.4 a	6.6 a				
Dosage mean		16.1	1.6	6.6		NA	NA	NA
$SED_{(DF = 16)}$		2.8	1.0	0.1		NA	NA	NA
CV%		27.7	101.4	2.6		NA	NA	NA
P-value $_{Dosage}$		0.457	0.680	0.057		NA	NA	NA
P-value $_{Linear}$		0.577	0.247	0.112		NA	NA	NA
P-value $_{Deviations}$		0.352	0.829	0.067		NA	NA	NA
Overall mean		16.2	1.8	6.6		16.2	1.8	6.6
$SED_{(DF = 32)}$		3.6	1.0	0.1		2.7	0.8	0.1
CV%		34.7	89.9	2.8		34.7	89.9	2.8
P-value		0.752	0.599	0.117		<.001	0.299	0.015

Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

3.3.1.2 Soil temperature and precipitation

The soil temperature at the 15 cm depth and the precipitation received during the experiment are shown in Figure 3.1. The mean soil temperature at 15 cm soil depth (see Figure 3.1a) ranged between 9.6 °C and 23.3 °C and was higher from planting time in May till late-July (18.3°C) than from August to tuber lifting in October (14.7 °C). The overall mean during the season was 16.6 °C, which was well above the basal requirements (4 ºC) for potato growth and development of the potato (O'Brien *et al*., 1983), and that required for the development of *G*. *pallida* (3.9 °C) and *G*. *rostochiensis* (6.2 °C) (Mugniery, 1978). It was, however, slightly above the range (15 – 20 °C) suggested for optimum activity of the two *Globodera* species (Robinson *et al*., 1987) later in May and mostly in June. Total precipitation received amounted to 379 mm, 29.8% of which was irrigated during May (8 mm), June (60 mm) and July (45 mm).



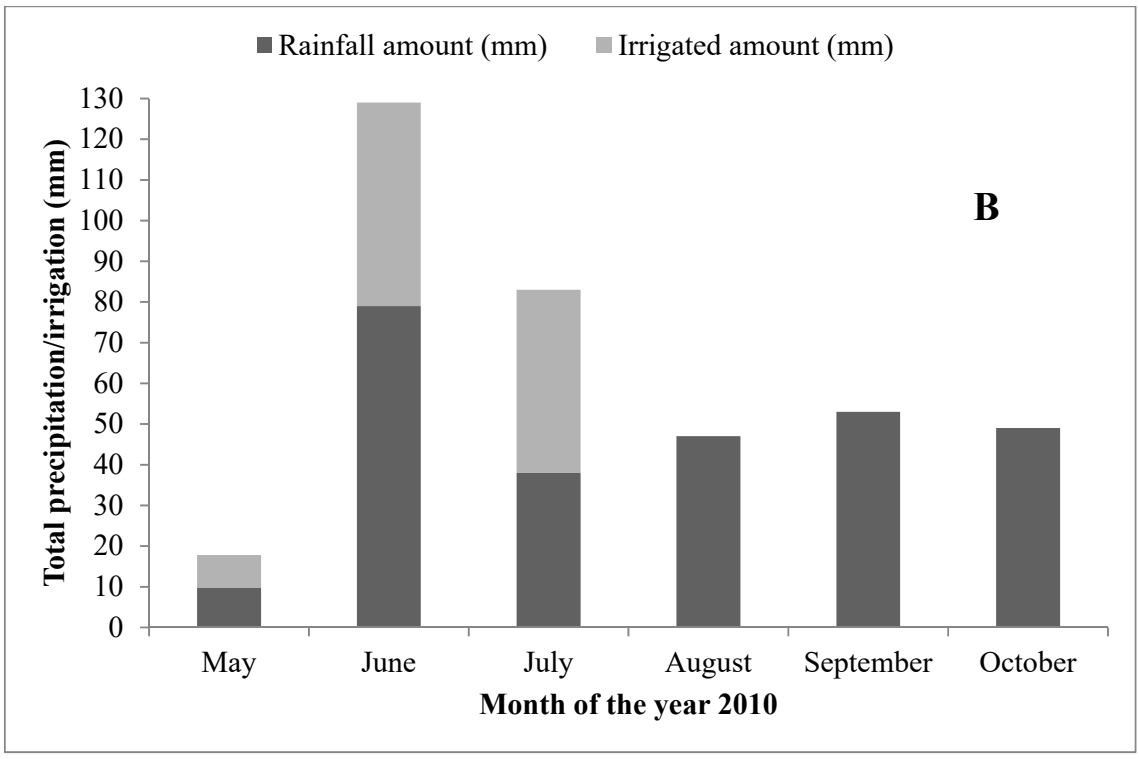


Figure 3.1. (A) Soil temperature recorded (°C) at 15 cm soil depth and (B) rainfall/irrigation (mm) received during Field experiment 1 at the site located at Woodcote (Shropshire, 2010).

3.3.1.3 Plant growth and tuber yield

The percentage emergence of plants at 10, 17 and 25 DAP (Table 3.4), percentage of ground covered by potatoes at 25, 32, 39, 46 and 53 DAP (Table 3.5), shoot and root weights at 29 and 44 DAP (Tables 3.6) and the yield of tubers at 149 DAP (Table 3.7) did not show significant differences between the treatments. Nearly all plants emerged by 25 DAP, and complete canopy cover was reached by 53 DAP.

Table 3.4. Percentage emergence of potato (*cv* Estima) from the middle two rows of plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plots left untreated at Woodcote (Shropshire, 2010).

Nematicide	Rate (kg a.s.ha^{-1})	Time in days after planting (DAP)		
		10 DAP	17 DAP	25 DAP
Untreated	-	73.5 a	85.2 a	100.0 a
Fluensulfone G	1.95	66.1 a	85.2 a	100.0 a
Fluensulfone G	3.00	75.2 a	91.3 a	100.0 a
Fluensulfone G	4.05	70.0 a	90.0 a	98.7 a
Fluensulfone G	5.05	66.5 a	84.8 a	99.1 a
Fluensulfone G	6.00	66.5 a	87.8 a	99.6 a
Fluensulfone EC	4.05	76.1 a	93.5 a	99.6 a
Nemathorin G	3.00	89.6 a	92.6 a	98.3 a
Vydate G	5.50	65.2 a	86.1 a	99.1 a
Dosage mean		68.9	87.8	99.5
$SED_{(DF = 16)}$		9.5	7.7	1.0
$LSD_{(P = 0.05)}$		20.2	16.2	2.1
CV%		21.9	13.8	1.6
P-value $_{Dosage}$		0.852	0.886	0.648
P-value $_{Linear}$		0.730	0.958	0.443
P-value $_{Deviations}$		0.754	0.773	0.604
Overall mean		72.1	88.5	99.4
$SED_{(DF = 32)}$		8.7	7.6	0.9
$LSD_{(P = 0.05)}$		17.8	15.5	1.8
CV%		19.1	13.6	1.4
P-value		0.168	0.910	0.479

Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

Table 3.5. Percentage of ground covered by potato (*cv Estima*) at 25, 32, 39, 46 and 53 days after planting in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Woodcote (Shropshire, 2010).

Nematicide	Rate (kg a.s. ha^{-1})	Time in days after planting (DAP)				
		25 DAP	32 DAP	39 DAP	46 DAP	53 DAP
Untreated	-	44.4 a	47.6 a	81.8 a	94.2 a	100.0
Fluensulfone G	1.95	28.4 a	53.0 a	77.4 a	88.4 a	100.0
Fluensulfone G	3.00	35.8 a	63.6 a	87.4 a	96.8 a	100.0
Fluensulfone G	4.05	39.0 a	57.8 a	82.8 a	93.2 a	100.0
Fluensulfone G	5.05	26.2 a	60.2 a	86.4 a	97.8 a	100.0
Fluensulfone G	6.00	34.6 a	58.4 a	84.4 a	95.6 a	100.0
Fluensulfone EC	4.05	41.6 a	65.4 a	87.0 a	99.2 a	100.0
Nemathorin G	3.00	32.0 a	57.8 a	79.4 a	95.2 a	100.0
Vydate G	5.50	38.6 a	57.0 a	79.6 a	97.8 a	100.0
Dosage mean		32.8	58.6	83.7	94.4	100.0
$SED_{(DF=16)}$		5.7	6.5	10.7	5.8	NA
$LSD_{(P = 0.05)}$		12.1	13.7	22.6	12.2	NA
CV (%)		27.5	17.5	20.2	9.7	NA
P-value $_{Dosage}$		0.188	0.597	0.892	0.517	NA
P-value $_{Linear}$		0.779	0.611	0.592	0.250	NA
P-value $_{Deviations}$		0.115	0.483	0.851	0.593	NA
Overall mean		35.6	57.9	82.9	95.4	100
$SED_{(DF=32)}$		5.7	6.9	9.3	4.8	NA
$LSD_{(P = 0.05)}$		11.6	14.0	18.9	9.8	NA
CV%		25.3	18.8	17.7	25.3	NA
P-value		0.052	0.333	0.957	0.528	NA

NA: not applicable. Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

Table 3.6. Weights of fresh root and shoot (g) of potato (*cv Estima*) at 29 and 44 days of planting (DAP) in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Woodcote (Shropshire, 2010).

Nematicide	Rate (kg a.s. ha^{-1})	Root weight (g)		Shoot weight (g)	
		29 DAP	44 DAP	29 DAP	44 DAP
Untreated	-	6.3 a	5.5 a	109.5 a	374.5 a
Fluensulfone G	1.95	6.3 a	5.5 a	110.2 a	368.8 a
Fluensulfone G	3.00	9.1 a	5.5 a	94.0 a	438.0 a
Fluensulfone G	4.05	6.7 a	4.9 a	97.4 a	446.3 a
Fluensulfone G	5.05	8.3 a	4.4 a	91.0 a	422.5 a
Fluensulfone G	6.00	6.4 a	4.0 a	94.1 a	379.1 a
Fluensulfone EC	4.05	5.0 a	5.1 a	92.7 a	380.3 a
Nemathorin G	3.00	6.5 a	6.3 a	93.1 a	430.8 a
Vydate G	5.50	4.4 a	4.7 a	81.1 a	379.5 a
Dosage mean		7.4	4.9	97.4	411
$SED_{(DF=16)}$		1.3	1.0	29.7	72.4
$LSD_{(P = 0.05)}$		2.7	2.1	53.5	153.4
CV%		27.0	31.4	48.2	27.8
P-value $_{Dosage}$		0.138	0.45	0.785	0.758
P-value $_{Linear}$		0.833	0.074	0.827	0.955
P-value $_{Deviations}$		0.081	0.969	0.651	0.61
Overall mean		6.4	5.1	104.1	402.2
$SED_{(DF=32)}$		1.8	0.9	31.0	68.2
$LSD_{(P = 0.05)}$		3.5	1.9	63.0	139.0
CV%		45.8	28.2	51.2	26.8
P-value		0.173	0.36	0.993	0.898

Means followed by same letter within a column are not significantly different according to Fisher's protected LSD post hoc test.

Table 3.7. The tuber yields (t ha^{-1}) of potato (*cv Estima*) after growing for 147 days in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Woodcote (Shropshire, 2010).

Nematicide	Rate (kg a.s. ha^{-1})	Yield (t ha^{-1})					
		< 45 mm	45 - 65 mm	65 - 85 mm	> 85 mm	Ware	Total
Untreated	-	3.3 a	30.6 a	16.2 a	0.2 a	46.7 a	50.2 a
Fluensulfone G	1.95	2.7 a	27.9 a	21.3 a	0.9 a	49.1 a	52.7 a
Fluensulfone G	3.00	2.5 a	22.1 a	18.9 a	0.8 a	41.0 a	44.3 a
Fluensulfone G	4.05	3.1 a	29.1 a	22.1 a	0.2 a	51.2 a	54.5 a
Fluensulfone G	5.05	2.8 a	26.3 a	24.6 a	0.9 a	50.9 a	54.6 a
Fluensulfone G	6.00	2.6 a	20.1 a	16.2 a	0.4 a	36.3 a	39.3 a
Fluensulfone EC	4.05	2.8 a	36.1 a	27.1 a	0.4 a	40.5 a	43.7 a
Nemathorin G	3.00	3.2 a	24.8 a	20.6 a	0.3 a	45.4 a	48.9 a
Vydate G	5.50	3.1 a	26.7 a	25.5 a	1.9 a	52.2 a	57.2 a
Dosage mean		2.8	26.1	21.5	0.7	47.8	49.1
$SED_{(DF=16)}$		0.7	4.9	4.7	0.7	8.1	11.4
$LSD_{(P = 0.05)}$		1.4	10.5	10	1.5	17.1	18.0
CV%		35.5	29.8	34.1	162.1	26.7	36.8
P-value $_{Dosage}$		0.886	0.422	0.445	0.706	0.319	0.322
P-value $_{Linear}$		0.718	0.154	0.412	0.485	0.18	0.175
P-value $_{Deviations}$		0.805	0.606	0.389	0.652	0.398	0.408
Overall mean		2.9	26.8	21.4	0.7	45.9	49.5
$SED_{(DF=32)}$		0.8	4.9	4.9	0.7	10.6	11.38
$LSD_{(P = 0.05)}$		1.6	10.0	10.0	1.5	15.9	17.0
CV%		45.2	28.7	36.2	181.7	36.6	36.4
P-value		0.967	0.640	0.511	0.332	0.590	0.573

Means followed by same letter within a column are not significantly different according to Fisher's protected LSD post hoc test.

3.3.1.4 Invasion of potato roots by *G. pallida*

Root invasion by *G. pallida* (Table 3.8) differed very significantly between the treatments at 29 DAP ($P < 0.001$) and significantly at 44 DAP ($P = 0.026$). The applications of oxamyl and fosthiazate, as well as those of fluensulfone significantly decreased root invasion at both sampling times, except the application of G fluensulfone at 1.95 kg a.s. ha^{-1}, compared with the untreated control at 42 DAP. Oxamyl decreased invasion, mostly but differed significantly just from the fluensulfone applications in the granular form at 5.05 and 6.00 kg a.s. ha^{-1} at 28 DAP ($P < 0.05$) and that at 1.95 kg a.s. ha^{-1} at 44 DAP ($P = 0.029$). The applications of fluensulfone decreased the root invasion by similar margins as the application of fosthiazate. The effects of fluensulfone applications in the granular form were not significantly affected by the dosage rates at both sampling times. However, a trend towards decreasing root invasion with increasing dosage was observed at 42 DAP.

Table 3.8. Log_e transformed numbers of *G. pallida* g^{-1} root of potato (*cv Estima*) at 29 and 44 days of growing in plots treated with fluensulfone in either granular (G) or emulsifiable concentrates (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Woodcote (Shropshire, 2010).

Nematicide	Rate (kg a.s.ha^{-1})	Number of *G. pallida* (g^{-1} root)	
		29 DAP	44 DAP
Untreated	-	5.6 a (258.5)	6.2 a (602.8)
Fluensulfone G	1.95	4.3 bc (68.8)	5.3 ab (277.5)
Fluensulfone G	3.00	4.4 bc (81.6)	4.3 bc (104.2)
Fluensulfone G	4.05	4.1 bc (61.6)	4.1 bc (95.6)
Fluensulfone G	5.05	4.6 b (91.4)	4.2 bc (72.2)
Fluensulfone G	6.00	4.6 b (97.4)	4.2 bc (89.2)
Fluensulfone EC	4.05	4.5 bc (79.7)	4.5 bc (157.5)
Nemathorin G	3.00	4.5 c (95.6)	4.3 bc (135.8)
Vydate G	5.50	4.1 c (50.4)	3.8 c (37.3)
Dosage mean		4.4	4.4
$SED_{(DF = 16)}$		0.2	0.5
$LSD_{(P = 0.05)}$		0.5	1.1
CV%		7.8	18.7
P-value $_{Dosage}$		0.145	0.190
P-value $_{Linear}$		0.113	0.068
P-value $_{Deviations}$		0.204	0.397
Overall mean		4.5	4.5
$SED_{(DF = 32)}$		0.2	0.7
$LSD_{(P = 0.05)}$		0.5	1.3
CV%		7.8	22.9
P-value		<.001	0.026

Back transformed means are shown in parentheses. Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

3.3.1.5 Rate of increase (*Pf*/*Pi*) and final population density (*Pf*) of *G. pallida*

The rate of the increase of *G. pallida* (*Pf*/*Pi* ratio) on Estima was significantly lower ($P < 0.001$) in the soils treated with oxamyl and with fluensulfone in the granular form at 5.05 kg a.s. ha^{-1} in comparison to the untreated control (Table 3.9). When compared with the treatments of fluensulfone, oxamyl and fosthiazate had significantly greater effects on the *Pf*/*Pi* ratio than fluensulfone in the granular form at 1.95 and 3.0 kg a.s. ha^{-1} ($P < 0.05$). These two rates of fluensulfone in turn gave significantly higher *Pf*/*Pi* ratio than the untreated ($P < 0.05$). Dose rate of fluensulfone in the granular form significantly affected the *Pf*/*Pi* ratio ($P < 0.001$); it decreased linearly ($r^2 = - 0.761$; $P < 0.001$) with increasing rate. The final population density (*Pf*) was significantly lowered ($P = 0.005$) by the treatment of oxamyl and that of fluensulfone in the granular form at 5.05 kg a.s. ha^{-1} in comparison with the untreated. The oxamyl treatment had significantly greater effects on the *Pf* ($P < 0.05$) than the fluensulfone treatments except that applied in the granular form at 5.05 kg a.s. ha^{-1}. No significant differences were found between the *Pf* after fosthiazate and the fluensulfone treatments. The *Pf* was not significantly affected by dose rate but there was a trend towards decreasing *Pf* at a higher dosage.

Table 3.9. Log_e transformed multiplication rate (*Pf*/*Pi* ratio) and final population density (*Pf*) attained by *G*. *pallida* in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plots left untreated at Woodcote (Shropshire, 2010).

Nematicide	Rate (kg a.s. ha^{-1})	Log_e(*Pf*+10)		log_e(*Pf*/*Pi*+10)	
Untreated	-	5.0 a	(149.1)	2.9 b	(9.2)
Fluensulfone G	1.95	5.0 a	(137.2)	3.6 a	(25.6)
Fluensulfone G	3.00	4.8 ab	(123.8)	3.3 a	(17.7)
Fluensulfone G	4.05	4.4 ab	(92.4)	2.7 bcd	(5.2)
Fluensulfone G	5.05	4.2 bc	(60.6)	2.6 cd	(3.5)
Fluensulfone G	6.00	4.7 ab	(128.2)	2.7 bcd	(5.7)
Fluensulfone EC	4.05	4.7 ab	(107.6)	2.7 bcd	(5.5)
Nemathorin G	3.00	4.4 ab	(94.8)	2.8 bc	(7.8)
Vydate G	5.50	3.7 c	(30.5)	2.5 d	(2.4)
Dosage mean		4.6		3.0	
$SED_{(DF=16)}$		0.3		0.2	
$LSD_{(P = 0.05)}$		0.6		0.4	
CV%		10.2		9.2	
P-value $_{Dosage}$		0.096		<.001	
P-value $_{Linear}$		0.123		<.001	
P-value $_{Deviations}$		0.119		0.031	
Overall mean		4.5		2.9	
$SED_{(DF=32)}$		0.32		0.2	
$LSD_{(P = 0.05)}$		0.3		0.3	
CV%		11.0		8.7	
P-value		0.005		<.001	

Back transformed means are shown in parentheses. Means followed by same letter within a column are not significantly different according to Fisher's protected LSD post hoc test.

3.3.2 Field experiment 2 (2011)

3.3.2.1 Initial population (*Pi*) densities and soil properties

The *Pi*, soil pH, and soil OM (Table 3.11) did not differ significantly between the treatments but between the block according to the *Pi* ($P < 0.001$) and the soil OM ($P = 0.008$). The field at Howle was infested by *G. pallida* at population density within range of 2 eggs g^{-1} soil to 28 eggs g^{-1} soil, with a mean of 9.6 egg g^{-1} soil. The soil properties are given in Table 3.10.

Table 3.10. Selected soil properties at site used for Field experiment 2 at Howle (Shropshire, 2011)

%Clay	%Silt	%Sand	%Soil OM.	%MWHC at 5 KPa	pH	Soil type
24.8	5.6	67.1	2.2	13.9	5.6	SCL

MWHC: maximum water-holding capacity; OM: organic matter content; SCL: sandy clay loam

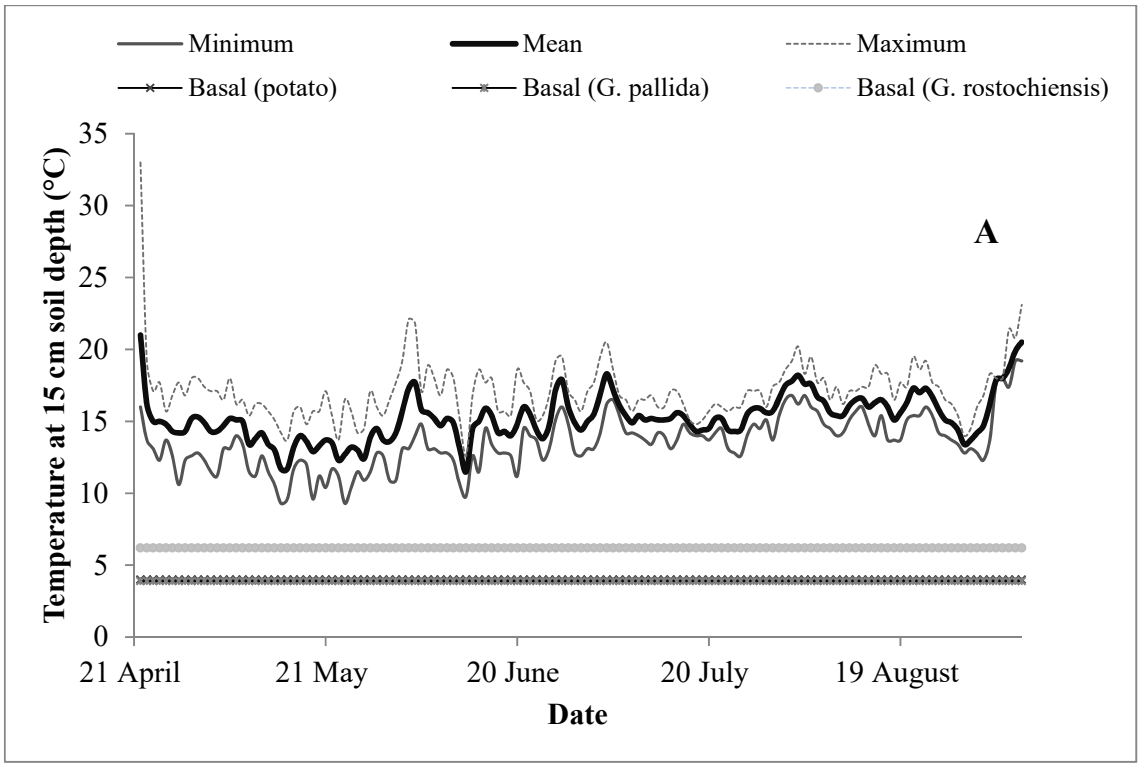
Table 3.11. Initial population densities (*Pi*) (eggs g^{-1} soil), soil pH and soil % organic matter content (OM) according to treatments and blocks at Howle (Shropshire, 2011).

Nematicide	Rate (kg a.s.ha^{-1})	Treatment mean			Block mean			
		Pi (eggs g^{-1} soil)	%OM	Soil pH	Block	*Pi* (eggs g^{-1} soil)	%OM	Soil pH
Untreated	-	10.9 a	2.2 a	5.9 a	1	7.9 b	2.3 b	5.4 a
Fluensulfone G	1.95	11.9 a	2.4 a	5.3 a	2	3.9 a	2.2 ab	5.7 a
Fluensulfone G	3.00	7.7 a	2.1 a	6.0 a	3	19.0 c	2.3 b	5.9 a
Fluensulfone G	4.05	9.1 a	2.3 a	5.3 a	4	8.4 b	2.0 a	5.6 a
Fluensulfone G	5.05	9.2 a	2.2 a	5.4 a	5	8.6 b	2.4 bc	5.7 a
Fluensulfone G	6.00	10.1 a	2.2 a	5.5 a				
Fluensulfone EC	4.05	8.2 a	2.3 a	6.0 a				
Nemathorin G	3.00	10.7 a	2.1 a	6.0 a				
Vydate G	5.50	8.2 a	2.1 a	5.5 a				
Dosage mean		9.6	2.2	5.5		NA	NA	NA
$SED_{(DF = 16)}$		1.9	0.1	0.3		NA	NA	NA
$LSD_{(P = 0.05)}$		4.1	0.2	0.7		NA	NA	NA
CV%		31.6	6.8	9.7		NA	NA	NA
P-value $_{Dosage}$		0.309	0.056	0.188		NA	NA	NA
P-value $_{Linear}$		0.598	0.084	0.88		NA	NA	NA
P-value $_{Deviations}$		0.218	0.079	0.113				
Overall mean		9.6	2.2	5.7		9.6	2.2	5.7
$SED_{(DF = 32)}$		1.7	0.2	0.4		1.7	0.1	0.4
$LSD_{(P = 0.05)}$		3.5	0.3	0.7		4.1	0.2	0.7
CV%		28.3	10.3	10		28.3	10.3	10
P-value		0.242	0.409	0.166		<.001	0.008	0.388

Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

3.3.2.2 Soil temperature and precipitation

The soil temperature at 15 cm soil depth and the precipitation received during the experiment at Howle are shown in Figure 3.2. The mean temperature ranged from 11.5°C to 21°C, with an overall mean of 15.3 °C. It was highest at planting (21°C) but dropped steeply soon afterwards and fluctuated mostly about 15°C during the experiment. It began to rise steeply in early September and was about 20°C by tuber lifting on 7 September, 2011. As illustrated in Figure 3.2a, the mean temperature was well above the basal temperature requirements for good performance of the potato and that required for activities of *G*. *pallida*. The amount of precipitation received (i.e. rainfall + irrigation) during the experiment summed up to 370. 1 mm, 46 % of which was irrigated during April (25 mm), May (80 mm) June (40 mm) and July (25 mm).



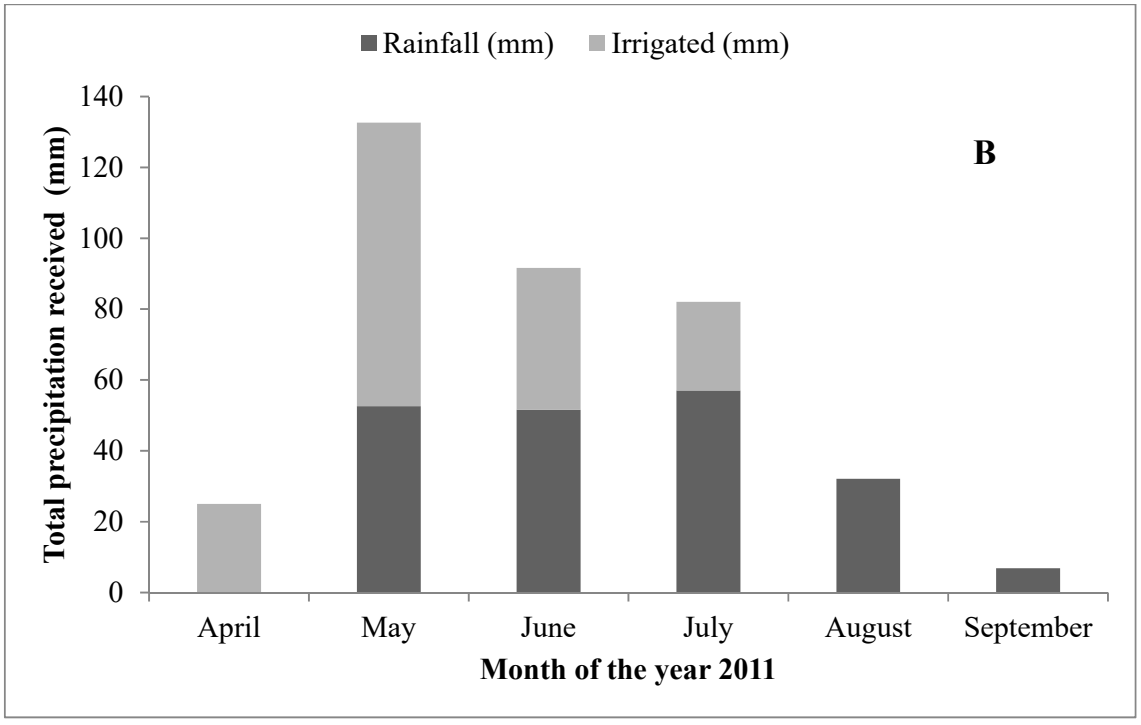


Figure 3.2. (A) Soil temperature recorded (°C) at 15 cm soil depth and (B) total precipitation (mm) received during the Field experiment 2 at Howle (Shropshire, 2011).

3.3.2.4 Plant growth and tuber yield

The percentage ground cover assessed at 21, 33, 40, and 56 DAP (Table 3.12) and the plant weights measured at 28 and 42 DAP (Tables 3.13) did not differ significantly between the treatments. Tuber yields at 138 DAP are given in Table 3.14. Except for tubers of size graded less than 45 mm, the yields were greater in the nematicide treated-plots than in the plots left untreated. However, significant difference was shown just for the 65 – 85 mm size grade (P = 0.023); the difference was seen between the treatment of oxamyl, fosthiazate and granular fluensulfone at 1.95 kg a.s. ha^{-1} compared with the untreated. When averaged over the fluensulfone treatments in the granular form, the ware and total yields were, correspondingly, 8.9 and 9.3 t ha^{-1} greater than was obtained from the untreated control. The application of the EC fluensulfone gave, respectively, 11.3 and 5.2 t ha^{-1} more of ware and total yields than the untreated control. The yields after the treatments of oxamyl or fosthiazate were about two-fold that seen for the untreated control. None of the yield categories were affected significantly by the dose rates of fluensulfone applied in the granular form.

Table 3.12. Percentage of ground covered by potato (*cv Estima*) at 21, 33, 40 and 56 days after planting in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Howle (Shropshire, 2011).

Nematicide	Rate (kg a.s. ha^{-1})	Time (days after planting, DAP)			
		21 DAP	33 DAP	40 DAP	56 DAP
Untreated	-	26.2 a	40.4 a	58.6 a	76.3 a
Fluensulfone G	1.95	20.6 a	39.8 a	53.4 a	73.0 a
Fluensulfone G	3.00	23.4 a	48.4 a	58.4 a	73.7 a
Fluensulfone G	4.05	25.4 a	42.2 a	64.2 a	74.4 a
Fluensulfone G	5.05	25.3 a	42.4 a	62.4 a	69.1 a
Fluensulfone G	6.00	19.6 a	41.7 a	67.5 a	74.1 a
Fluensulfone EC	4.05	25.4 a	41.5 a	58.7 a	79.9 a
Nemathorin G	3.00	27.3 a	43.6 a	61.5 a	76.9 a
Vydate G	5.50	24.3 a	39.3 a	55.4 a	78.4 a
Dosage mean		22.8	42.9	61.2	72.9
$SED_{(DF=16)}$		4.6	5.9	8.4	4.6
$LSD_{(P = 0.05)}$		9.8	12.5	17.8	10.0
CV%		31.9	21.8	21.7	9.9
P-value $_{Dosage}$		0.626	0.666	0.515	0.773
P-value $_{Linear}$		0.982	0.861	0.104	0.817
P-value $_{Deviations}$		0.470	0.515	0.933	0.638
Overall mean		24.0	42.0	60.0	75.0
$SED_{(DF=32)}$		4.7	6.3	6.8	5.9
$LSD_{(P = 0.05)}$		9.6	12.8	13.9	11.9
CV%		30.9	23.6	18.0	12.3
P-value		0.780	0.927	0.592	0.771

Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

Table 3.13. Weights of fresh root and shoot of potato (*cv* Estima) at 28 and 42 days after planting in soil treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available commercial nematicides or soils left untreated at Howle (Shropshire, 2011).

Nematicide	Rate (kg a.s. ha^{-1})	Root weight (g)		Shoot weight (g)	
		28 DAP	42 DAP	28 DAP	42 DAP
Untreated	-	3.1 a	7.4 a	27.0 a	128.9 a
Fluensulfone G	1.95	2.1 a	5.8 a	26.2 a	88.1 a
Fluensulfone G	3.00	2.1 a	8.7 a	21.1 a	105.6 a
Fluensulfone G	4.05	2.8 a	7.5 a	34.8 a	103.6 a
Fluensulfone G	5.05	3.5 a	9.7 a	32.8 a	98.0 a
Fluensulfone G	6.00	2.5 a	8.6 a	26.7 a	115.9 a
Fluensulfone EC	4.05	3.4 a	7.7 a	31.2 a	165.7 a
Nemathorin G	3.00	3.7 a	7.4 a	35.6 a	91.1 a
Vydate G	5.50	4.3 a	6.7 a	27.9 a	137.3 a
Dosage mean		2.6	8.1	28.3	102.2
$SED_{(DF = 16)}$		0.9	1.7	7.0	9.8
$LSD_{(P = 0.05)}$		2.2	3.9	16.1	22.7
CV%		44.0	25.5	30.2	11.8
P-value $_{Dosage}$		0.362	0.165	0.57	0.273
P-value $_{Linear}$		0.425	0.061	0.309	0.112
P-value $_{Deviations}$		0.302	0.343	0.609	0.431
Overall mean		3.1	7.7	29.3	114.9
$SED_{(DF = 32)}$		0.9	1.6	6.4	25.2
$LSD_{(P = 0.05)}$		1.8	1.6	13.5	53.5
CV%		35.3	24.8	26.7	26.9
P-value		0.260	0.415	0.414	0.115

Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

Table 3.14. The tuber yield for potato (*cv Estima*) after growing for 138 days in soil treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available commercial nematicides or soils left untreated at Howle (Shropshire, 2011).

Nematicide	Rate (kg a.s. ha^{-1})	Size grades (t ha^{-1})					
		<45 mm	45-65 mm	65-85 mm	> 85 mm	Ware	Total
Untreated	-	3.3 a	14.4 a	1.2 a	0.0 a	15.6 a	18.8 a
Fluensulfone G	1.95	3.4 a	19.8 a	3.9 bcd	0.0 a	23.7 a	27.1 a
Fluensulfone G	3.00	3.4 a	24.5 a	2.8 abc	0.1 a	27.2 a	30.7 a
Fluensulfone G	4.05	3.7 a	19.2 a	1.4 ab	0.0 a	20.7 a	24.4 a
Fluensulfone G	5.05	3.4 a	23.3 a	3.0 abc	0.1 a	26.3 a	29.7 a
Fluensulfone G	6.00	3.7 a	22.9 a	2.0 ab	0.0 a	24.8 a	28.5 a
Fluensulfone EC	4.05	2.9 a	21.8 a	2.2 ab	0.0 a	26.9 a	24.0 a
Nemathorin G	3.00	3.1 a	26.0 a	6.1 d	0.1 a	32.1 a	35.2 a
Vydate G	5.50	2.8 a	25.6 a	5.0 cd	0.0 a	30.6 a	33.4 a
Dosage mean		3.5	21.9	2.6	0.0	24.5	28.1
$SED_{(DF = 16)}$		0.8	4.4	1.1	0.1	5.2	5.6
$LSD_{(P = 0.05)}$		1.6	9.4	2.3	0.2	11.1	11.9
CV%		34.8	32	66.6	364.7	33.6	31.6
P-value $_{Dosage}$		0.982	0.715	0.257	0.599	0.75	0.815
P-value $_{Linear}$		0.781	0.635	0.165	0.982	0.918	0.894
P-value $_{Deviations}$		0.958	0.607	0.322	0.443	0.603	0.681
Overall mean		3.4	22	3.2	0.0	25.1	28.5
$SED_{(DF = 32)}$		0.6	3.9	1.3	0.1	4.8	5.0
$LSD_{(P = 0.05)}$		1.2	8.2	2.5	0.1	10	10.4
CV%		30.1	28.2	63.6	378.9	30.1	28.0
P-value		0.77	0.155	0.006	0.619	0.071	0.085

Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

3.3.2.7 Invasion of potato roots by *G. pallida*

Root invasions at both sampling times of 28 and 42 DAP were significantly decreased ($P < 0.05$) in the plots treated with oxamyl, fosthiazate and EC fluensulfone, compared to the plots left untreated (Table 3.15). The fluensulfone treatment in the granular form did not decrease the root invasion significantly, except the 6.0 kg a.s. ha^{-1} rate, which was significantly ($P < 0.05$) lower than the untreated control at 42 DAP. Oxamyl had significantly greater effects on the root invasion when compared with fluensulfone treatments in the granular form at 1.95, 5.05 and 6.00 kg a.s. ha^{-1} at 28 DAP ($P < 0.05$) and then again with 1.95, 3.00 and 4.05 kg a.s. ha^{-1} rates at 42 DAP ($P < 0.05$). The application of fosthiazate gave significantly greater control than fluensulfone at 1.95 kg a.s. kg^{-1} ($P = 0.038$) at 28 DAP and significantly greater effects ($P < 0.05$) than fluensulfone at 1.95, 3.00 and 4.05 kg a.s. ha^{-1} at 42 DAP. Figures 3.3 and 3.4, respectively, show the juvenile stages present within the root system at 28 and 42 DAP. The numbers did not differ between the treatments except for the treatment of oxamyl, which significantly decreased the numbers of J2 and J3 ($P < 0.05$), and the EC fluensulfone which had significantly reduced the J4 ($P < 0.05$) in comparison with the untreated control. The numbers of juvenile stages at 42 DAP, differed significantly ($P = 0.02$) just for the J4, and the numbers were lower after oxamyl, fosthiazate, compared with the untreated plot.

Table 3.15. Log_e transformed number of *G. pallida* g^{-1} root of potato (*cv Estima*) at 28 and 42 days after planting (DAP) in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Howle (Shropshire, 2011).

Nematicide	Rate (kg a.s. ha^{-1})	Numbers of *G. pallida* (g^{-1} root)			
		28 DAP		42 DAP	
Untreated	-	5.8 a	(358.3)	7.6 a	(1893.3)
Fluensulfone G	1.95	5.6 a	(283.3)	6.9 ab	(1200.0)
Fluensulfone G	3.00	5.1 abc	(156.7)	6.9 ab	(1106.7)
Fluensulfone G	4.05	5.0 abc	(140.0)	6.9 ab	(1120.0)
Fluensulfone G	5.05	5.3 ab	(183.3)	6.7 abc	(826.7)
Fluensulfone G	6.00	5.3 ab	(206.7)	6.0 bc	(600.0)
Fluensulfone EC	4.05	4.1 c	(86.7)	5.9 bc	(453.3)
Nemathorin G	3.00	4.3 c	(45.0)	5.6 c	(336.7)
Vydate G	5.50	4.0 c	(83.3)	5.6 c	(352.0)
Dosage mean		5.3		6.7	
$SED_{(DF = 16)}$		0.6		0.5	
$LSD_{(P = 0.05)}$		0.7		1.2	
CV%		13.9		9.1	
P-value $_{Dosage}$		0.508		0.338	
P-value $_{Linear}$		0.713		0.11	
P-value $_{Deviations}$		0.386		0.582	
Overall mean		4.9		6.5	
$SED_{(DF = 32)}$		0.6		0.6	
$LSD_{(P = 0.05)}$		1.2		1.2	
CV%		13.9		10.9	
P-value		0.038		0.028	

Back transformed means are in parentheses. Means followed by similar letters within a column are not significantly different according to Fisher's protected LSD post hoc test.

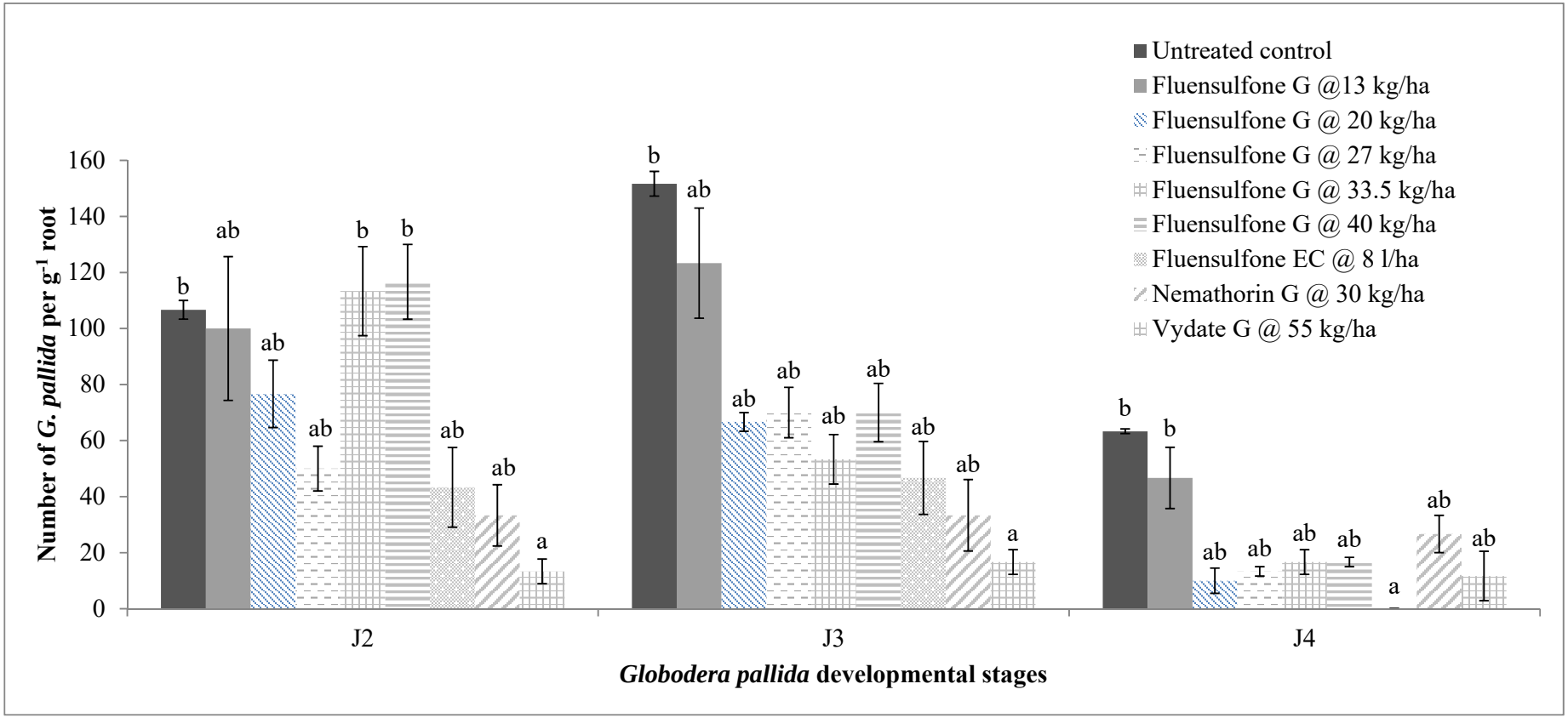


Figure 3.3. Numbers of *G. pallida* stages determined per g^{-1} root of Estima (Mean ±SE) at 28 days after planting in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Howle (Shropshire, 2011). Bars with the same letter are not different according to Fisher's protected LSD.

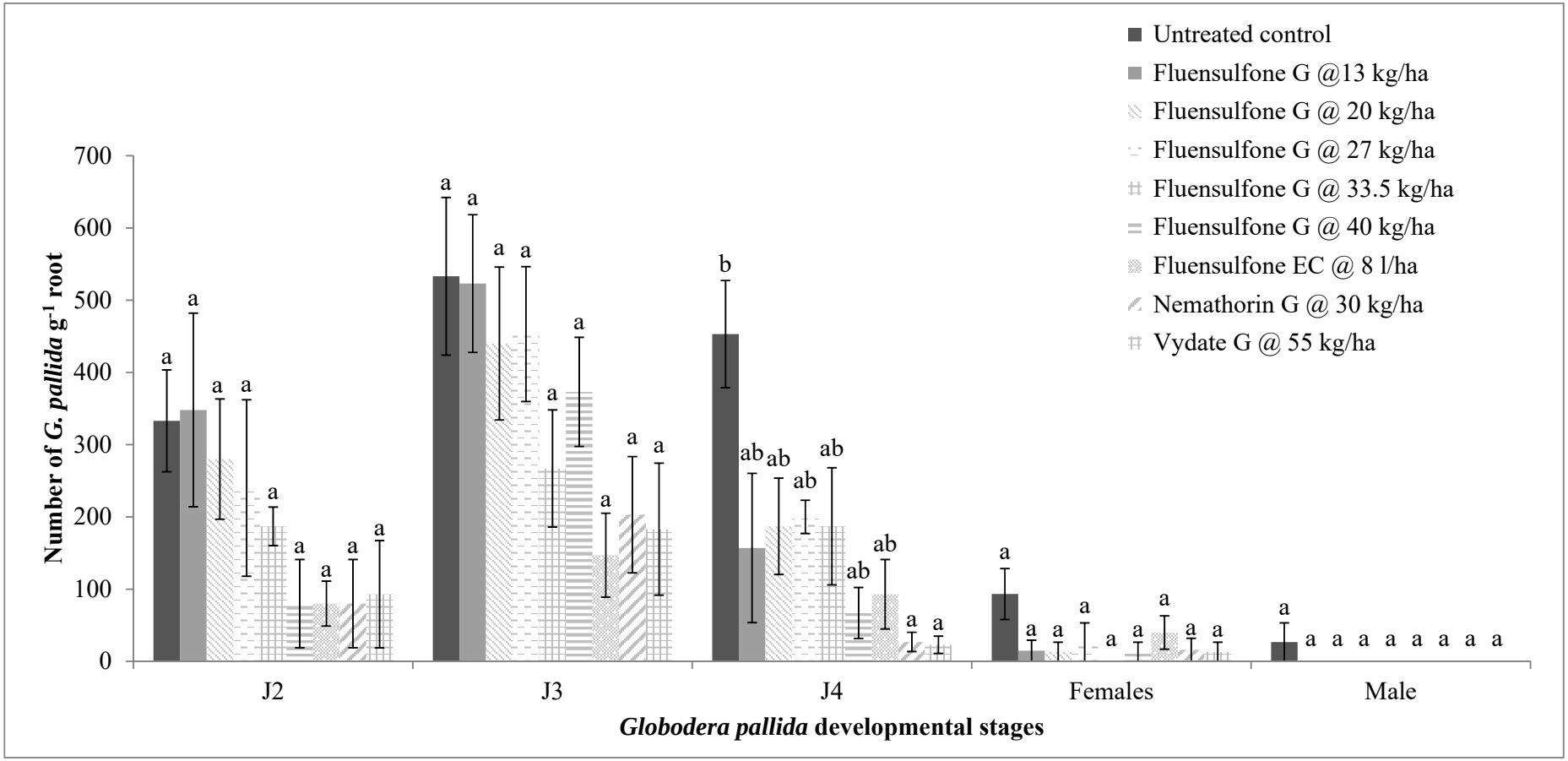


Figure 3.4. Numbers of *G. pallida* stages determined per g^{-1} root of Estima (Mean ±SE) at 42 days after planting in plots treated with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Howle (Shropshire, 2011). Bars with the same letter are not different according to Fisher's protected LSD.

3.3.2.8 Rate of increase (*Pf*/*Pi*) and final population density (*Pf*) of *G. pallida*

Table 3.16 shows the rates of the increase of *G. pallida* (*Pf*/*Pi* ratio) during the season and the final population densities (*Pf*) at the end of the experiment. The treatments of oxamyl and EC fluensulfone gave significantly lower *Pf*/*Pi* ratio ($P = 0.003$) and *Pf* ($P = 0.013$), compared with the untreated plot. No other significant differences were found between the treatments. Dose rate effects of fluensulfone treatments in the granular form were apparent but were not significant.

Table 3.16. Log_e transformed rate of increase (*Pf*/*Pi* ratio) and log_e transformed final population density (*Pf*) of *G*. *pallida* following soil treatments with fluensulfone in either granular (G) or emulsifiable concentrate (EC) form in comparisons with currently available nematicides (fosthiazate and oxamyl) or plot left untreated at Howle (Shropshire, 2011).

Nematicide	Rate (kg a.s. ha^{-1})	Loge(*Pf* +10)		Loge(*Pf*/*Pi* +10)	
Untreated	-	4.9 a	(147.5)	3.2 a	(14.2)
Fluensulfone G	1.95	4.3 b	(85.2)	2.9 bc	(8.8)
Fluensulfone G	3.00	4.3 b	(88.1)	3.0 ab	(10.6)
Fluensulfone G	4.05	4.2 b	(71.5)	3.0 ab	(9.9)
Fluensulfone G	5.05	4.1 bcd	(75.5)	2.9 bc	(8.9)
Fluensulfone G	6.00	4.1 bc	(66.5)	2.9 bc	(8.0)
Fluensulfone EC	4.05	3.6 cd	(40.5)	2.7 c	(5.5)
Nemathorin G	5.50	3.5 bcd	(66.6)	2.9 bc	(8.6)
Vydate G	3.00	4.1 d	(38.3)	2.7 c	(5.6)
Dosage mean		4.2		2.1	
$SED_{(DF = 16)}$		0.2		0.3	
$LSD_{(P = 0.05)}$		0.4		0.5	
CV%		9.0		5.7	
P-value $_{Dosage}$		0.818		0.765	
P-value $_{Linear}$		0.299		0.523	
P-value $_{Deviations}$		0.944		0.708	
Overall mean		4.1		2.0	
$SED_{(DF = 32)}$		0.2		0.3	
$LSD_{(P = 0.05)}$		0.5		0.1	
CV%		11.5		23.9	
P-value		0.003		0.013	

Back transformed means are in parentheses. Means followed by same letter within a column are not significantly different according to Fisher's protected LSD.

3.3.3 Field experiment 3 (Howle, 2011)

3.3.3.1 Shoot and root weights

Table 3.17 shows the weights of shoot and root at 28 and 42 DAP. The weights of shoot at 28 DAP were affected significantly by cultivar ($P = 0.006$), nematicide ($P = 0.019$), but not by cultivar x nematicide interaction. The shoots of Santé and Estima (71.1 and 58.9 g respectively) weighed significantly heavier than that of Vales Everest (39.9 g). When averaged over the nematicide applications, the shoot weights were greater after fluensulfone (50.7 g) and fosthiazate (61.5 g) but only that after oxamyl (73.9 g) was significantly greater than the untreated control (40.0 g). The weights of shoot at 42 DAP was affected significantly by cultivar ($P = 0.017$) and very significantly ($P < 0.001$) by nematicide but not by cultivar x nematicide interaction. The shoot of Santé (134.3 g) and not Estima (122.7 g) weighed significantly heavier than was seen for Vales Everest (87.2g). Only the oxamyl treatment gave significantly greater shoot weight (167.2 g) than the untreated control (81.9 g). The root weight was affected significantly by cultivar at 42 DAP, but not at 28 DAP ($P = 0.024$); the roots of Santé weighed significantly heavier (12.3 g) than those of Estima (8.9 g) and Vales Everest (7.3 g).

Table 3.17. Weights of fresh shoot and root of potato *cvs.* Estima, Santé and Vales Everest at 28 and 42 days after planting in soil (DAP) treated with granular fluensulfone, oxamyl, fosthiazate, respectively, at 4.05, 5.50 and 3.00 kg a.s. ha^{-1} or left untreated at Howle (Shropshire, 2011).

Treatment	28 DAP		42 DAP	
	Shoot weight	Root weight	Shoot weight	Root weight
CULTIVAR				
Estima	58.9 b	7.3 a	122.7 b	9.0 ab
Santé	71.1 b	9.1 a	134.3 b	12.3 b
Vales Everest	39.9 a	7.6 a	87.2 a	7.3 a
$SED_{DF=44}$	8.7	1.0	15.6	1.7
LSD ($P = 0.05$)	18.1	2.1	32.4	3.5
P-value	0.006	0.191	0.017	0.024
NEMATICIDE				
Untreated	40.4 a	7.0 a	81.9 a	7.8 a
Fluensulfone	50.7 ab	8.9 a	109.8 b	10.9 a
Fosthiazate	61.5 bc	7.0 a	100.0 b	11.6 a
Oxamyl	73.9 c	9.0 a	167.2 b	7.8 a
$SED_{DF=44}$	10.1	1.2	18.0	2.0
LSD ($P = 0.05$)	21.0	1.2	37.4	4.0
P-value	0.019	0.174	<.001	0.125
CULTIVAR X NEMATICIDE INTERACTION				
Estima untreated	38.4 a	7.7 a	82.0 a	6.3 a
Estima +fluensulfone	47.3 a	4.4 a	120.3 a	11.5 a
Estima +fosthiazate	73.8 a	7.1 a	101.9 a	9.3 a
Estima +oxamyl	76.2 a	9.9 a	186.5 a	8.7 a
Santé untreated	50.1 a	5.7 a	93.4 a	9.9 a
Santé +fluensulfone	60.5 a	8.3 a	120.8 a	13.3 a
Santé +fosthiazate	77.2 a	12.9 a	115.7 a	17.7 a
Santé +oxamyl	96.5 a	9.4 a	207.2 a	8.2 a
V.Everest untreated	32.6 a	7.7 a	70.2 a	7.2 a
V.Everest +fluensulfone	44.4 a	8.3 a	88.2 a	7.9 a
V.Everest +fosthiazate	33.4 a	6.8 a	82.4 a	7.7 a
V.Everest +oxamyl	49.1 a	7.5 a	108.0 a	6.5 a
$SED_{DF=44}$	17.5	2.0	31.2	3.4
LSD ($P = 0.05$)	36.2	2.0	64.7	7.0
CV%	37.7	31.2	33.3	43.5
P-value	0.647	0.05	0.616	0.498

Means followed by same letters within column as per cultivar, nematicide and cultivar x nematicide interaction, are not different according to Fisher's protected LSD post hoc

3.3.3.2 Invasion of potato roots by *G. pallida*

Table 3.18 shows the total numbers of juveniles per gram of root at 28 and 42 DAP. Significant treatment effects on root invasions were shown for nematicide treatments only at 28 DAP, when the root invasions were significantly ($P < 0.001$) and similarly decreased by fluensulfone (256 juveniles g^{-1} root), fosthiazate (227 juveniles g^{-1} root) and oxamyl (189 juveniles g^{-1} root) treatments, compared with the untreated (523 juveniles g^{-1} root). Invasion at 42 DAP was affected significantly by cultivar ($P = 0.044$) very significantly by nematicide ($P < 0.001$) and significantly by cultivar x nematicide interaction ($P = 0.03$). Among the cultivars, Santé was invaded significantly more (686 juveniles g^{-1} root) than Vales Everest (560 juveniles g^{-1} root) and Estima (628 juveniles g^{-1} root). When averaged over the nematicide treatments, the root invasions were decreased significantly in soils treated with fluensulfone (600 juveniles g^{-1} root), fosthiazate (493 juveniles g^{-1} root) and oxamyl (421 juveniles g^{-1} root), compared with the untreated control (984 juveniles g^{-1} root). The treatments of fosthiazate and oxamyl, however, had significantly greater effects on the root invasion ($P < 0.05$) than the fluensulfone treatment. No significant interactions were found for fluensulfone x Santé or Vales Everest interaction at 42 DAP. There were significant interactive treatment effects of Oxamyl x Santé, Oxamyl x Vales Everest and fosthiazate x Vales Everest on the root invasion at 42 DAP. Treating Estima with fluensulfone significantly ($P < 0.01$) reduced invasion from 1224 juveniles g^{-1} root (untreated control) down to 584 juveniles g^{-1} root, but significantly greater reductions ($P < 0.05$) were obtained by the treatments of fosthiazate (344 juveniles g^{-1} root) and oxamyl (360 juveniles g^{-1} root). Juvenile stages (J2, male and female stages of the J3, J4 and J5) within the root at both assessments (28 and 42 DAP) are given in Tables 3.22 and 3.23, respectively. Although each stage was reduced after treatment with fluensulfone, only 2 out of 12 comparisons showed significant treatment effects for the fluensulfone x Santé or Vales Everest interaction.

Table 3.18. Log_e transformed number of *G. pallida* g $root^{-1}$ of potato *cvs* Estima, Santé and Vales Everest at 28 and 42 days after planting (DAP) in soil treated with granular fluensulfone, oxamyl, fosthiazate, respectively, at 4.05, 5.50 and 3.00 kg a.s. ha^{-1} or left untreated at Howle (Shropshire, 2011).

Treatment	Numbers of *G. pallida* (g^{-1} root)			
	28 DAP		42 DAP	
CULTIVAR				
Estima	5.7 a	(376)	6.3 a	(628)
Santé	5.4 a	(268)	6.5 b	(686)
Vales Everest	5.4 a	(252)	6.3 a	(560)
$SED_{(DF = 44)}$	0.2		0.1	
$LSD_{(P = 0.05)}$	0.4		0.2	
P-value	0.099		0.044	
NEMATICIDE				
Untreated	6.2 a	(523)	6.9 c	(984)
Fluensulfone	5.5 b	(256)	6.4 b	(600)
Fosthiazate	5.3 b	(227)	6.1 a	(493)
Oxamyl	5.1 b	(189)	6.0 a	(421)
$SED_{(DF = 44)}$	0.2		0.1	
$LSD_{(P = 0.05)}$	0.4		0.2	
P-value	<.001		<.001	
CULTIVAR X NEMATICIDE INTERACTION				
Estima untreated	6.6 a	(712)	7.1 f	(1224)
Estima + fluensulfone	5.8 a	(320)	6.4 cd	(584)
Estima + fosthiazate	5.5 a	(288)	5.7 a	(344)
Estima + Oxamyl	5.1 a	(184)	5.9 ab	(360)
Santé untreated	6.2 a	(472)	6.8 ef	(912)
Santé + fluensulfone	5.3 a	(224)	6.5 cde	(680)
Santé + fosthiazate	5.1 a	(160)	6.4 cde	(624)
Santé + Oxamyl	5.2 a	(216)	6.3 bc	(528)
Vales Everest untreated	6.0 a	(384)	6.7 def	(816)
Vales Everest + fluensulfone	5.4 a	(224)	6.3 bcd	(536)
Vales Everest + fosthiazate	5.3 a	(232)	6.2 bc	(512)
Vales Everest + Oxamyl	4.9 a	(168)	5.9 ab	(376)
$SED_{(DF = 44)}$	0.4		0.2	
$LSD_{(P = 0.05)}$	7. 3		0.4	
CV%	10.5		5.3	
P-value	0.864		0.030	

Back-transformed means are in parentheses. Means followed by same letters within column as per cultivar, nematicide and cultivar x nematicide interaction, are not different according to Fisher's protected LSD post hoc test.

Table 3.19. Number of *G. pallida* stages per gram root (back-transformed data) of potato *cvs.* Estima, Santé and Vales Everest at 28 days after planting in soil treated with granular fluensulfone, oxamyl, fosthiazate, respectively, at 4.05, 5.50 and 3.00 kg a.s. ha^{-1} or left untreated at Howle (Shropshire, 2011).

Treatment	J2	J3		J4		J5	
		Male	Female	Male	Female	Male	Female
CULTIVAR							
Estima	104 a	2 a	54 b	6 a	66 b	0 a	16 b
Santé	150 ab	22 b	2 a	26 ab	8 a	0 a	2 a
Vales Everest	119 b	14 ab	0 a	40 b	10 a	2 a	4 a
SED($_{DF = 44}$)	16.4	6.8	7.9	10.9	9.2	1.6 a	5.2
LSD ($_{P = 0.05}$)	33.0	13.7	16.1	22.1	9.2	3.3	10.5
P-value	0.023	0.018	<.001	0.012	<.001	0.376	0.021
NEMATICIDE							
Untreated	203 a	13 a	40 b	53 b	45.3 a	0 a	16 b
Fluensulfone	117 b	11 a	13 a	13 a	26.7 a	0 a	11 ab
Fosthiazate	93 b	13 a	16 a	13 a	21.3 a	3 a	3 a
Oxamyl	83 b	13 a	5 a	16 a	18.7 a	0 a	0 a
SED($_{DF = 44}$)	18.9	7.8	9.2	12.6	10.7	1.9	6
LSD ($_{P = 0.05}$)	19.0	15.8	18.6	25.5	21.5	3.8	12.2
P-value	<.001	0.981	0.003	0.006	0.068	0.402	0.042
CULTIVAR X NEMATICIDE INTERACTION							
Estima untreated	152 a	0 a	120 c	0 a	136.0 d	0 a	48 c
Estima +fluensulfone	96 a	8 a	32 ab	0 a	64.0 c	0 a	16 ab
Estima +fosthiazate	80 a	0 a	48 b	16 a	40.0 bc	0 a	0 a
Estima +oxamyl	88 a	0 a	16 ab	8 a	24.0 ab	0 a	0 a
Santé untreated	264 a	16 a	8 a	88 c	0 a	0 a	0 a
Santé + fluensulfone	120 a	16 a	0 a	8 a	16 ab	0 a	8 a
Santé + fosthiazate	112 a	24 a	0 a	0 a	0 a	0 a	0 a
Santé + oxamyl	104 a	32 a	0 a	8 a	16 ab	0 a	0 a
V. Everest untreated	192 a	24 a	0 a	72 bc	0 a	0 a	0 a
V. Everest + fluensulfone	136 a	8 a	0 a	32 ab	0 a	0 a	8 a
V. Everest + fosthiazate	88 a	16 a	0 a	24 ab	24 ab	8 a	8 a
V. Everest + oxamyl	56 a	8 a	0 a	32 ab	16 ab	0 a	0 a
SED($_{DF = 44}$)	16.4	13.6 a	15.9	21.9	18.5	3.3 a	10.4
LSD ($_{P = 0.05}$)	66.2	27.3	32.1	44.1	37.2	6.6	21.0
CV%	38.8	169.2	135.1	144.2	104.3	774.6	225.2
P-value	0.234	0.654	<.001	0.05	<.001	0.437	0.006

Means followed by same letters within column as per cultivar, nematicide and cultivar x nematicide interaction, are not different according to Fisher's protected LSD post hoc test.

Table 3.20. Number of *G. pallida* stages per gram root of potato (back-transformed data) *cvs* Estima, Santé and Vales Everest at 42 days after planting in soil treated with granular fluensulfone, oxamyl, fosthiazate, respectively, at 4.05, 5.50 and 3.00 kg a.s. ha^{-1} or left untreated at Howle (Shropshire, 2011).

Treatment	J2	J3		J4		J5	
		Male	Female	Male	Female	Male	Female
CULTIVAR							
Estima	220 a	38 a	100 b	32 a	106 b	38 a	94 c
Santé	244 a	76 ab	66 a	118 b	46 a	90 b	46 b
Vales Everest	194 a	64 b	34 a	110 b	40 a	96 b	22 a
SED(DF = 44)	27.7	13.6	16.7	13	15.5	15.4	11.8
LSD (*P* = 0.05)	73.8	27.4	67.3	26.2	31.3	30.9	23.8
P-value	0.689	0.024	0.001	<.001	<.001	<.001	<.001
NEMATICIDE							
Untreated	405 b	85 a	133 b	85 a	96 c	80 ab	99 b
Fluensulfone	163 a	51 a	51 a	109 a	83 bc	67 a	77 b
Fosthiazate	152 a	56 a	35 a	85 a	48 ab	107 b	11 a
Oxamyl	157 a	45 a	48 a	67 a	29 a	45 a	29 a
SED(DF = 44)	32	15.7	19.3	15.1	17.9	17.7	13.6
LSD (*P* = 0.05)	85.2	31.6	38.9	30.3	36.1	35.7	27.4
P-value	<.001	0.064	<.001	0.056	0.002	0.011	<.001
CULTIVAR X NEMATICIDE INTERACTION							
Estima untreated	6 a	72 a	200 a	64 bc	208 c	80 a	192 f
Estima + fluensulfone	160 a	24 a	96 a	40 ab	104 b	32 a	128 e
Estima + fosthiazate	128 a	40 a	56 a	16 ab	64 ab	40 a	0 a
Estima + oxamyl	184 a	16 a	48 a	8 a	48 ab	0 a	56 bcd
Santé untreated	6 a	88 a	136 a	64 bc	32 a	80 a	80 d
Santé + fluensulfone	200 a	64 a	24 a	176 e	72 ab	80 a	64 cd
Santé + fosthiazate	192 a	56 a	40 a	136 de	56 ab	128 a	16 ab
Santé + oxamyl	152 a	96 a	64 a	96 cd	24 a	72 a	24 abc
V.Everest untreated	6 a	96 a	64 a	128 de	48 ab	80 a	24 abc
V.Everest + fluensulfone	128 a	64 a	32 a	112 cd	72 ab	88 a	40 abcd
V.Everest + fosthiazate	136 a	72 a	8 a	104 cd	24 a	152 a	16 ab
V.Everest + oxamyl	136 a	24 a	32 a	96 cd	16 a	64 a	8 a
SED(DF = 44)	55.4	27.2	33.4	26.0	31.1	30.7	23.6
LSD (*P* = 0.05)	147.5	54.7	33.7	52.4	62.7	61.9	47.6
CV%	46.7	72.4	79.2	47.4	76.8	65.0	69.2
Significance	0.974	0.306	0.169	0.008	0.005	0.263	<.001

Means followed by same letters within column as per cultivar, nematicide and cultivar x nematicide interaction, are not different according to Fisher's protected LSD post hoc test

3.3.3.3 Initial (*Pi*) and final (*Pf*) population densities, and multiplication rates (*Pf*/*Pi*) of *G. pallida*

3.3.3.4.1 *Pi* and *Pf*

The *Pi* did not differ significantly ($P > 0.05$) between the treatments and was at a mean of 12.1 eggs g^{-1} soil. The *Pf* on the other hand, varied significantly (Table 3.21) and was affected by cultivar ($P < 0.001$), nematicide ($P < 0.001$) and cultivar x nematicide interaction ($P < 0.001$). The *Pf* after cropping of Estima has 75.9 eggs g^{-1} soil and was significantly lower ($P < 0.001$) for Vales Everest (3.9 eggs g^{-1} soil) and Santé (6.3 egg g^{-1} soil), with Vales Everest having significantly greater effects ($P = 0.009$) than Santé. The mean *Pf* after soil treatments with fluensulfone and oxamyl, but not fosthiazate, were significantly lower ($P < 0.05$) than the untreated. When grown in plots left untreated, Santé and Vales Everest reduced the *Pf* very significantly ($P < 0.001$) from 109.9 eggs g^{-1} soil (after Estima) down to 6.7 and 4.1 eggs g^{-1} soil, respectively. The *Pf* after Santé but not Vales Everest was lowered much further and significantly when the crop was grown in soil treated with fluensulfone (*Pf* = 4.5; $P = 0.0034$) or oxamyl (*Pf* = 2.8; $P = 0.0006$). The fluensulfone x Santé and the oxamyl x Santé interactions had affected the *Pf* similarly. There were no further reductions in *Pf* following treatment of Vales Everest with any of the nematicides. The *Pf* after Estima (109.9 eggs g^{-1} soil) was lowered by all three nematicide but significantly ($P < 0.001$) only by the oxamyl treatment (16.5 eggs g^{-1} soil).

3.3.3.4.2 *Pf*/*Pi* ratio

The *Pf*/*Pi* ratio (Table 3.21) was affected significantly by cultivar ($P < 0.001$), nematicide ($P < 0.001$) and cultivar x nematicide interaction ($P < 0.001$). The *Pf*/*Pi* ratio was

significantly lessened ($P < 0.001$) on Santé (x0.53) or on Vales Everest (x0.35) than it was on Estima (x6.5). All three nematicide treatments significantly lessened the *Pf*/*Pi* ratio ($P < 0.05$) but not similarly; oxamyl but not fosthiazate lowered the *Pf*/*Pi* ratio by a significantly greater margin ($P < 0.001$) than fluensulfone. In the untreated plot, Santé and Vales Everest significantly lessened the *Pf/Pi ratio* ($P < 0.001$) compared with Estima (x10) down to x0.64 and x0.40 respectively. The *Pf/Pi* ratio after Santé was decreased significantly ($P < 0.001$) further down to x0.27 and x0.24 when Santé was combined, respectively, with fluensulfone and oxamyl. The fluensulfone x Santé interactive effect was not significantly different from the oxamyl x Santé interaction but gave significantly lower *Pf*/*Pi* ratio ($P < 0.001$) than the fosthiazate x Santé interaction (x0.98). The *Pf*/*Pi* ratio after Vales Everest was similar in plots treated with a nematicide and that left untreated. Growing Estima in soil treated with fluensulfone gave significantly lower *Pf*/*Pi* ratio ($P < 0.001$) and the effect was similar to that of fosthiazate but lesser than seen for oxamyl ($P < 0.001$).

Table 3.21. The initial (*Pi*) and final (*Pf*) populations densities (eggs g^{-1} soil) and multiplication rate (*Pf*/*Pi* ratio) of *G*. *pallida* on potato *cv*s Estima, Santé and Vales Everest grown in soil treated with granular fluensulfone, oxamyl, fosthiazate, respectively, at 4.05, 5.50 and 3.00 kg a.s. ha^{-1} or left untreated at Howle (Shropshire, 2011).

Treatment	*Pi*	Log_e (*Pf* +10)		Log_e (*Pf*/*Pi* + 10)	
CULTIVAR					
Estima	12.1 a	4.3 a	(75.9)	2.8 a	(6.5)
Santé	12.1 a	2.8 b	(6.3)	2.5 b	(0.5)
Vales Everest	12.1 a	2.6 c	(3.9)	2.3 b	(0.4)
$SED_{(DF = 44)}$	0.7	0.1		0.0	
$LSD_{(P = 0.05)}$	1.3	0.1		0.0	
P-value	1.0	<.001		<.001	
NEMATICIDE					
Untreated	11.2 a	3.4 a	(40.3)	2.5 a	(3.7)
Fluensulfone	11.9 a	2.8 b	(32.9)	2.4 b	(2.8)
Fosthiazate	12.9 a	3.4 a	(34.0)	2.6 a	(2.7)
Oxamyl	12.2 a	3.3 a	(7.7)	2.5 a	(0.7)
$SED_{(DF = 44)}$	0.8	0.1		0.0	
$LSD_{(P = 0.05)}$	1.6	0.1		0.1	
P-value	0.198	<.001		<.001	
CULTIVAR X NEMATICIDE INTERACTION					
Estima untreated	11.2 a	4.7 a	(109.9)	3.0 a	(10.1)
Estima + fluensulfone	11.9 a	4.6 a	(91.1)	2.9 b	(7.6)
Estima + fosthiazate	12.9 a	4.5 a	(86.3)	2.8 b	(6.8)
Estima + Oxamyl	12.2 a	3.3 b	(16.5)	2.4 c	(1.4)
Santé untreated	11.2 a	2.8 c	(6.7)	2.4 cd	(0.6)
Santé + fluensulfone	11.9 a	2.6 cd	(4.5)	2.3 d	(0.3)
Santé + fosthiazate	12.9 a	3.1 b	(3.3)	2.4 cd	(1.0)
Santé + Oxamyl	12.2 a	2.6 d	(2.8)	2.3 d	(0.2)
V.Everest untreated	11.2 a	2.6 cd	(4.1)	2.3 cd	(0.4)
V.Everest + fluensulfone	11.9 a	2.7 cd	(4.5)	2.3 cd	(0.4)
V.Everest + fosthiazate	12.9 a	2.6 cd	(3.3)	2.3 d	(0.3)
V.Everest + oxamyl	12.2 a	2.6 cd	(3.9)	2.3 d	(0.3)
$SED_{(DF = 44)}$	1.34	0.12		0.05	
$LSD_{(P = 0.05)}$	1.3	0.2		0.1	
CV%	2.6	5.9		3.0	
P-value	1.000	<.001		<.001	

Back-transformed data are in parentheses. Means followed by same letters within column as per cultivar, nematicide and cultivar x nematicide interaction, are not different according to Fisher's protected LSD post hoc test.

3.3.3.3 Tuber yield

Tubers of size graded less than 45 mm

Yields of tubers of size graded less than 45 mm (Table 3.22) was affected significantly by cultivar (P = 0.006) but not by nematicide. The cultivar x nematicide interaction was significant (P < 0.001). Vales Everest yielded similarly as Santé but significantly lower (P = 0.001) than Estima. The Santé x fluensulfone interaction significantly reduced (P = 0.0001) the yield from 1.32 t ha^{-1} (untreated Santé) down to 0.64 t ha^{-1}, and had similar effects as the Santé x oxamyl (0.76 t ha^{-1}) and Santé x fosthiazate (0.48 t ha^{-1}) interactions. The yield of Vales Everest was not affected significantly by the treatments of fluensulfone or oxamyl but the fluensulfone x Vales Everest interaction gave a significantly lower yield (0.63 t ha^{-1}) than seen for the fosthiazate x Vales Everest interaction (0.87 t ha^{-1}). Estima yielded significantly more (P < 0.01) when treated with any of the three nematicide than when grown untreated.

Tubers of size graded 45 – 65 mm

Table 3.22 shows no significant main treatment effects on yield of tubers graded 45 – 65 mm but the cultivar x nematicide interaction was significant (P = 0.002). The yield of Vales Everest but not Santé was significantly (P < 0.01) increased from 9.8 t ha^{-1} to 10.2 t ha^{-1} by the treatment of fosthiazate, which gave a significantly greater (P = 0.042) yield than treating Vales Everest with fluensulfone (7.03 t ha^{-1}) or oxamyl (7.59 t ha^{-1}). All three nematicide treatments significantly (P < 0.001) and similarly increased the yield of Estima.

Tubers of size graded 65 – 85 mm

This yield fraction was affected significantly by cultivar ($P < 0.001$), nematicides ($P < 0.001$) and cultivar x nematicide interaction ($P < 0.001$). Vales Everest yielded most (12.01 t ha^{-1}), followed by Santé (8.17 t ha^{-1}) and then Estima (5.43 t ha^{-1}). The treatment of fluensulfone increased the yield significantly from 4.78 t ha^{-1} (in the untreated soil) to 8.52 t ha^{-1}, and had affected the yield similarly as the treatments of fosthiazate and oxamyl. The Santé x fluensulfone interaction affected the yield very significantly ($P < 0.0001$), which was increased from 1.32 (untreated Santé) to 9.60 t ha^{-1}, and had similar effects as Santé x fosthiazate (15.5 t ha^{-1}) or Santé x oxamyl (6.5 t ha^{-1}) interactions. Although not significantly affected by any of the nematicide treatments, the yield of Vales Everest was greater in the soil treated with a nematicide than when grown in the soil left untreated. The yield of Estima was greatly and significantly ($P < 0.001$) increased by all three nematicides.

Tubers of size graded 45 – 85 mm (Ware yield)

The ware yield (Table 3.22) was affected very significantly by cultivar ($P < 0.001$), nematicide ($P < 0.001$) and by significantly by cultivar x nematicide interaction ($P = 0.003$). Vales Everest yielded significantly greater (20.70 t ha^{-1}) than Santé (15.90 t ha^{-1}) and Estima (13.2 t ha^{-1}). Santé, in turn, gave a greater yield than Estima ($P = 0.043$). The treatment of fluensulfone significantly increased the ware yield from 12.24 t ha^{-1} (untreated control) to 16.36 t ha^{-1}, and this increment was by a similar margin as seen for the oxamyl (18.25 t ha^{-1}) and fosthiazate (19.65 t ha^{-1}) treatments. The fluensulfone x Santé interaction gave significantly greater ($P < 0.001$) yield (18.37 t ha^{-1}) than the oxamyl x Santé interaction (13.56 t ha^{-1}), but gave significantly lower yield, compared with the fosthiazate x Santé interaction (23.03 t ha^{-1}). The nematicide x Vales Everest interactions did not affect the ware yield of the crop. All three nematicide significantly ($P < 0.05$) increased the ware yield of

Estima, which was increased by greater margin after fosthiazate (15.1 t ha^{-1}) and oxamyl (18.5 t ha^{-1}) than fluensulfone (11.9 t ha^{-1}).

Total yield

The total yields of tubers (Table 3.22) were affected very significantly by cultivar and by nematicides ($P < 0.001$) and the cultivar x nematicide interaction was significant at $P = 0.003$. Vales Everest yielded more (42.4 t ha^{-1}) than Santé (32.9 t ha^{-1}) and Estima (27.5 t ha^{-1}). All three nematicide treatments significantly ($P < 0.01$) and similarly increased the total yields. The fluensulfone x Santé interaction increased the total yield significantly ($P = 0.001$) and gave a greater yield (37.6 t ha^{-1}) than the oxamyl x Santé interaction (28.2 t ha^{-1}) but lesser yield than the fosthiazate x Santé interaction (46.8 t ha^{-1}). Treating Vales Everest with fluensulfone did not significantly affect the total yield and gave a significantly lower yield (39.0 t ha^{-1}) than where the crop was treated with fosthiazate (42.7 t ha^{-1}) or oxamyl (46.6 t ha^{-1}). The yield of Estima was significantly ($P = 0.01$) by all three nematicide treatments.

Table 3.22. Tuber yield (t ha^{-1}) obtained for potato *cv*s Estima, Santé and Vales Everest after g in soil treated with granular fluensulfone, oxamyl, fosthiazate, respectively, at 4.05, 5.50 and 3.00 kg a.s. ha^{-1} or left untreated at Howle (Shropshire, 2011).

Treatment	Yields (t ha^{-1})					
	< 45mm	45-65 mm	65-85 mm	> 85 mm	Ware	Total
CULTIVAR						
Estima	1.0 b	7.8 a	5.4 a	0.0 a	13.2 a	14.3 a
Santé	0.8 ab	7.8 a	8.2 b	0.2 a	16.0 a	17.0 a
Vales Everest	0.5 a	8.7 a	12.0 c	0.5 b	20.7 b	21.7 b
$SED_{DF=44}$	0.1	0.6 a	1.1	0.0	1.4	1.4
LSD ($P = 0.05$)	0.2	1.2	2.2	0.1	2.8	2.8
P-value	0.005	0.706	0.003	0.002	<.001	<.001
NEMATICIDE						
Untreated control	0.6 a	7.5 a	4.8 a	0.2 a	12.2 a	13.2 a
Fluensulfone	0.8 a	7.9 a	8.5 b	0.3 a	16.4 a	17.8 b
Fosthiazate	0.8 a	8.6 a	11.0 b	0.1 a	19.7 a	20.6 b
Oxamyl	0.7 a	8.4 a	9.8 b	0.4 a	18.3 a	19.4 b
$SED_{DF=44}$	0.1	0.7	1.3	0.1	1.6	1.6
LSD ($P = 0.05$)	0.3	1.4	2.5	0.1	3.2	3.2
P-value	0.686	0.796	0.023	0.30	0.183	<.001
CULTIVAR X NEMATICIDE INTERACTION						
Estima untreated	0.4 a	5.0 bcd	2.6 ab	0.1 a	7.5 cde	8.0 a
Estima +fluensulfone	1.4 e	7.7 abcd	4.1abc	0.0 a	11.9 abc	13.3 abc
Estima +fosthiazate	1.2 cde	8.2 ab	6.9 bcde	0.0 a	15.1 a	16.3 cde
Estima +oxamyl	1.1 bcde	10.4 d	8.1 cdef	0.0 a	18.5 def	19.5 def
Santé untreated	1.3 de	7.6 abc	1.3 a	0.0 a	9.0 ab	10.3 ab
Santé +fluensulfone	0.6 ab	8.8 cd	9.6 def	0.2 a	18.4 def	19.2 def
Santé +fosthiazate	0.5 a	7.5 abcd	15.5 g	0.2 a	23.0 f	23.7 f
Santé +oxamyl	0.8 abc	7.3 abc	6.3 bcd	0.4 a	13.6 bcd	14.7 bcd
V.Everest untreated	0.5 a	9.8 bcd	10.4 ef	0.5 a	20.2 ef	21.2 ef
V.Everest + fluensulfone	0.6 ab	7.0 a	11.8fg	0.8 a	18.8 def	19.9 def
V.Everest + fosthiazate	0.9 abcd	10.2 d	10.7 def	0.1 a	20.9 f	21.8 ef
V.Everest + oxamyl	0.4 a	7.5 abcd	15.1 g	0.7 a	22.7 f	23.9 f
$SED_{DF=44}$	0.3	1.2	2.2	0.2	2.8	2.7
LSD ($P = 0.05$)	0.5	2.4	4.5	0.2	5.6	5.6
CV%	48.1	23.6	40.5	3.1	26.5	24.8
P-value	<.001	0.004	<.001	0.260	<.001	0.003

Back-transformed means are in parentheses. Means followed by same letters within column as per cultivar, nematicide and cultivar x nematicide interaction, are not different according to Fisher's protected LSD post hoc test.

3.3.4 Polytunnel experiment 1 (2012)

3.3.4.1 Air and soil temperatures

Mean soil and air temperatures during the polytunnel experiment (Figure 3.5) ranged between 9 and 24 ºC and 10 and 24 ºC, respectively.

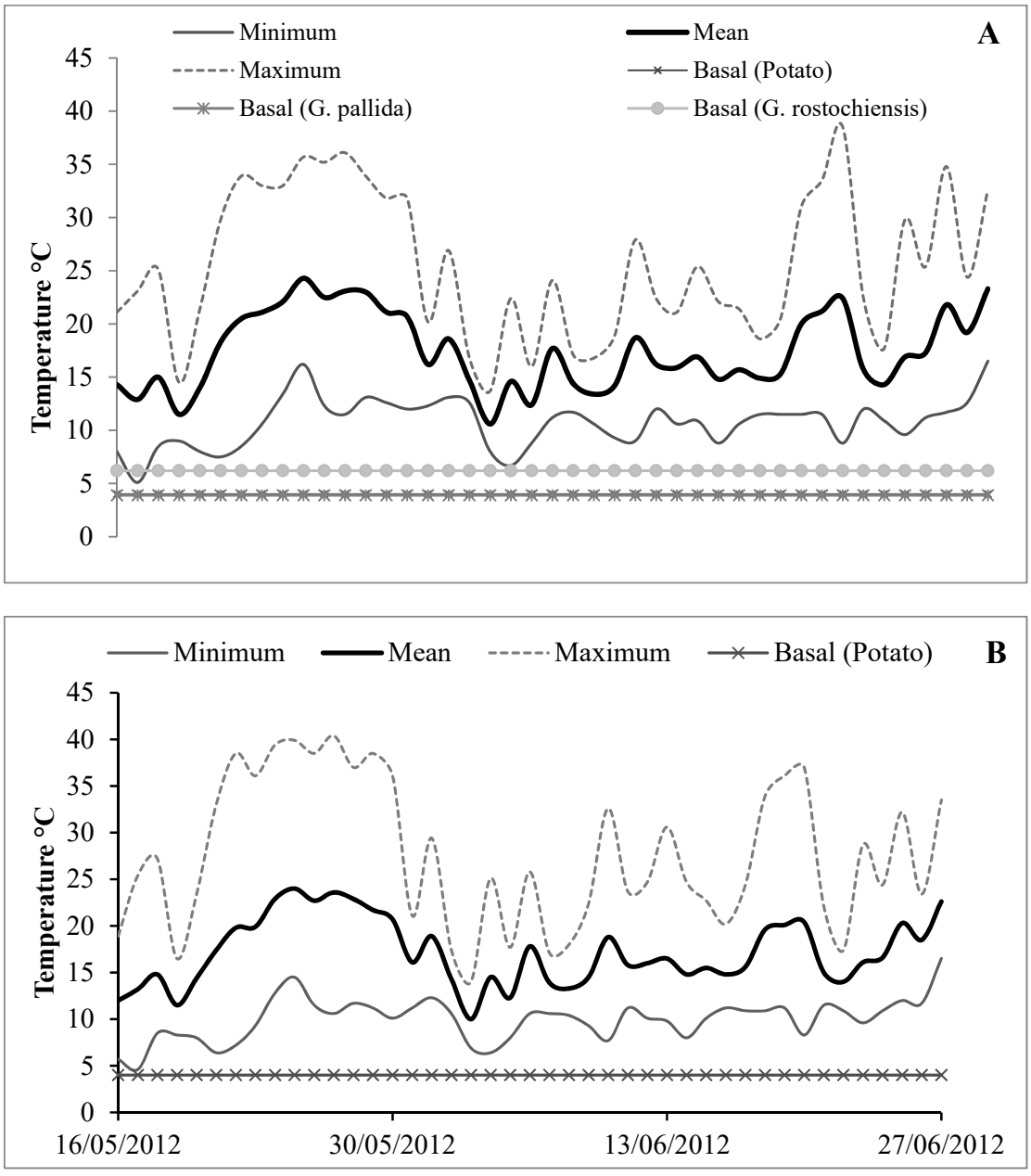


Figure 3.5. Air (A) and soil (B) temperature recorded (°C) during Polytunnel Pot Experiment 1 at Harper Adams University (Shropshire, 2012)

3.3.4.2 Viability and hatching of *G. pallida*

Figure 3.6 shows the number of viable eggs remaining in the cysts recovered from the soil at 43 DAP and the number of juveniles, which hatched from these cysts over six weeks of incubation in potato root leachate. No significant treatment effects were observed. The rates of emergence of juvenile (Figure 3.7) was similar across the treatment, with juvenile hatch occurring mostly within the first three weeks of incubation in potato root leachate.

3.3.4.3 Invasion of potato roots by *G. pallida*

The total numbers of juvenile per gram root at 43 DAP (Figures 3.8) were significantly (P = 0.002) and similarly lowered after the treatments of fluensulfone at 4.05 and 5.05 kg a.s. ha^{-1}, and fosthiazate than the untreated control. All juvenile stages found within the root were affected by the nematicide treatments but just the numbers of J3 and J4 were significantly decreased after fluensulfone treatments at 4.05 and 5.05 kg a.s. ha^{-1}, and fosthiazate, compared with the untreated control (Figure 3.9). The effects of fluensulfone on the root invasion increased significantly with increasing dose rate (P = 0.044), and the numbers found per gram root were reduced significantly (P < 0.01) when the dosage was increased from 1.95 to 4.05 kg a.s. ha^{-1} but was not decreased further with the increase of dosage to 5.05 kg a.s. ha^{-1}.

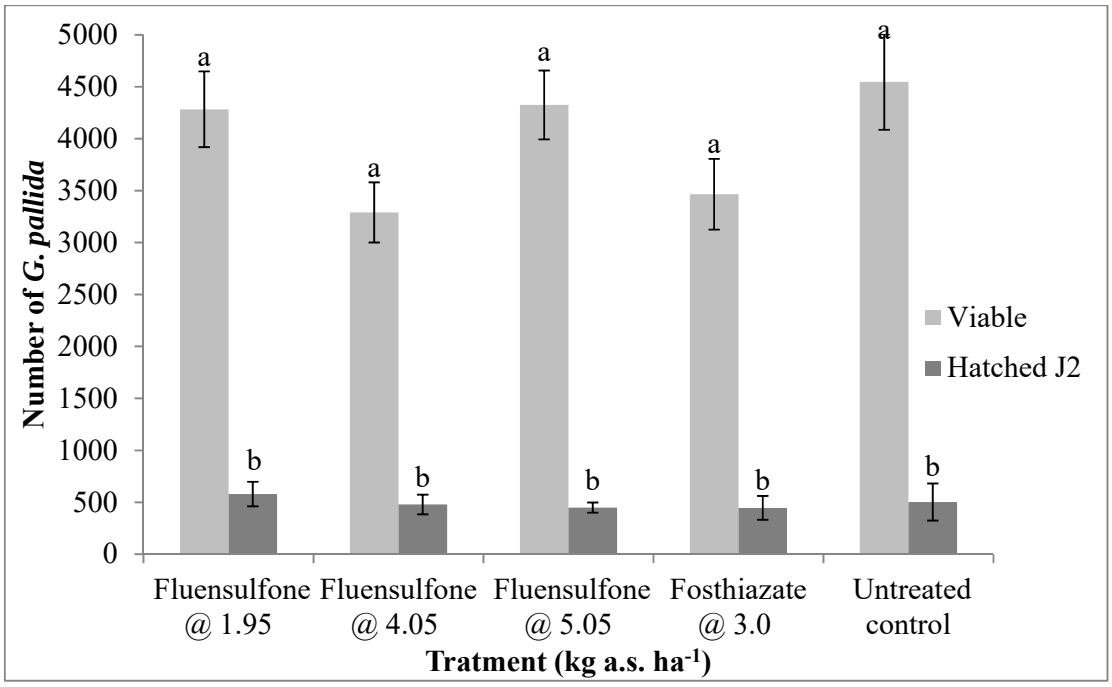


Figure 3.6. Numbers of viable *G. pallida* (Mean ± SE) remaining in cysts extracted from soil treated with fluensulfone, fosthiazate or soil left untreated and emergence of juvenile *G. pallida* after six weeks of incubation in potato root leachates at 16ºC.

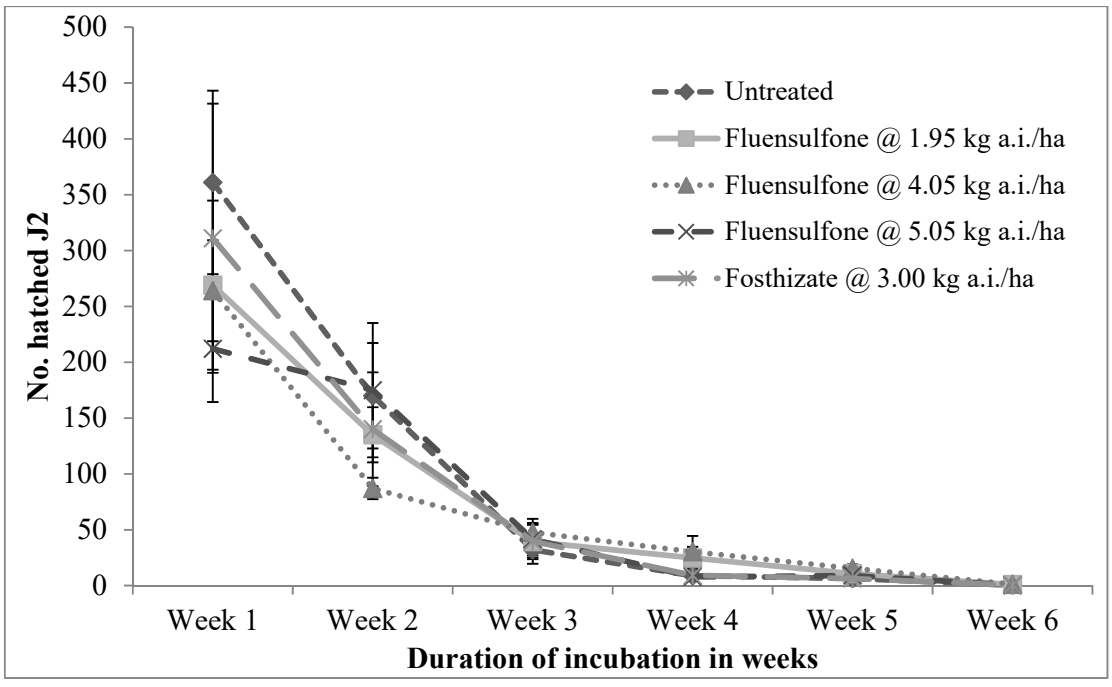


Figure 3.7. Time dependent hatching of *G. pallida* (Mean ± SE) from cysts extracted from soil treated with fluensulfone, fosthiazate and soil left untreated.

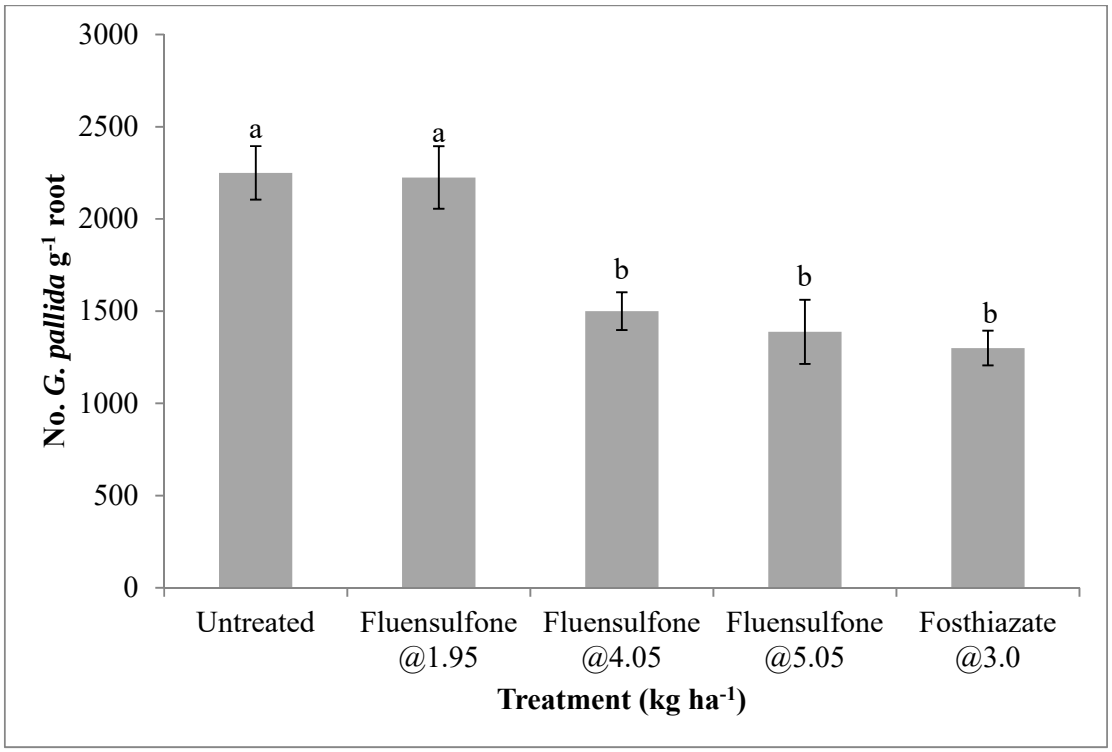


Figure 3.8. Number of *G. pallida* per gram root of potato (*cv* Estima) (Mean ± SE) at 43 days after planting in soil treated with fluensulfone in comparisons with fosthiazate or soil left untreated. Bars with the same letter are not different according to Fisher's protected LSD.

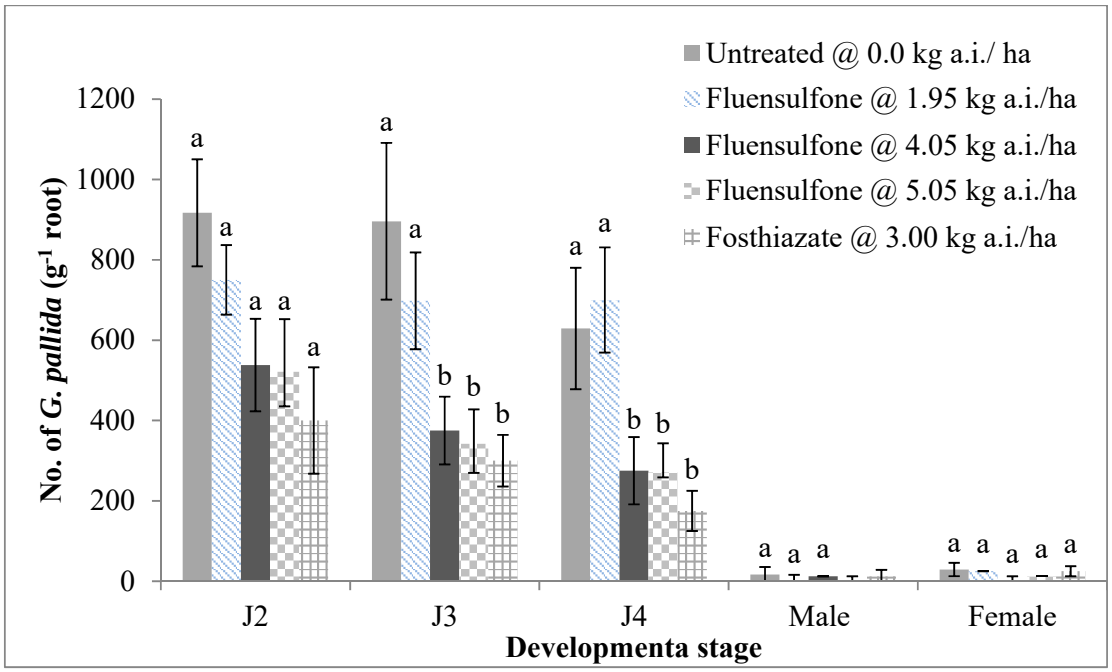


Figure 3.9. *Globodera pallida* stages per gram root of potato (*cv* Estima) at 43 days after planting in soil treated with fluensulfone in comparisons with fosthiazate or soil left untreated. Error bars indicate standard error of mean. Bars with the same letter are not different according to Fisher's protected LSD post hoc test.

3.3.4.4 Growth of plants

The shoot and root weights of Estima were greater, but not significantly, in the fluensulfone treated soil, compared with the soil left untreated (Table 3.23). However, there appeared to be an increase in these parameters with increasing dosage rate, and that seen for root weight was significantly linear ($P = 0.043$). Interestingly, increasing the dose rate of fluensulfone had a decreasing effect on the stolon and stem numbers, with the number of stems being significantly decreased ($P = 0.03$) by the application at the 5.05 kg a.s. ha^{-1} rate.

Table 3.23. Weights of fresh shoot and root, and number of stems and stolon of potato (*cv* Estima) at 43 days after planting in soil treated with fluensulfone in comparisons with fosthiazate or left untreated in the polytunnel.

Nematicide	Rate (kg a.s. ha^{-1})	Fresh shoot (g)	Fresh root (g)	No. stolon	No. stem
Untreated	-	3.9 a (57.1)	2.2 a (10.9)	1.6 a (5.7)	1.3 a (4.5)
Fluensulfone G	1.95	3.8 a (63.5)	2.1 a (11.4)	1.8 a (7.0)	1.8 a (6.7)
Fluensulfone G	4.05	4.1 a (65.6)	2.6 a (16.7)	2.1 a (7.8)	1.7 a (7.0)
Fluensulfone G	5.05	4.4 a (86.7)	2.8 a (16.2)	1.4 a (4.3)	0.9 a* (2.5)
Nemathorin G	3.00	4.0 a (69.9)	2.4 a (15.1)	2.1 a (10.0)	1.3 a (4.7)
Dosage mean		4.1	2.5	1.7	1.5
$SED_{(DF=10)}$		0.4	0.3	0.3	0.3
$LSD_{(P = 0.05)}$		0.9	0.7	0.6	0.7
CV%		16.8	22	26.5	35.8
P-value Dosage		0.33	0.112	0.082	0.026
P-value Linear		0.148	0.043	0.17	0.016
P-value Deviations		0.863	0.434	0.065	0.165
Overall mean		4	2.4	1.8	1.4
$SED_{(DF= 20)}$		0.4	0.5	0.3	0.4
$LSD_{(P = 0.05)}$		0.9	1	0.7	0.8
CV%		19	32.9	32.5	49
P-value		0.653	0.532	0.206	0.206

Back-transformed means are in parentheses. *: Significantly lower as per dose-response analysis of Fluensulfone G treatments. Means followed by same letter within column are not different according to Fisher's protected LSD post hoc test.

3.4 Discussion

3.4.1 Invasion of potato roots by *G. pallida*

The results from the field and polytunnel experiments show that fluensulfone soil treatment applied at planting reduced the invasion of Estima by *G. pallida*, at least, during the first 44 days of planting. Presumably, therefore, the current treatments could provide some protection to the potato from the damage associated with invasion, during the early stages of growth and development when the crop is most vulnerable and could very easily succumb to infections. At the Woodcote site (Field experiment 1), the granular and EC fluensulfone treatments decreased root invasion by margins ranging from 69 to 79 %. Even though all the granular treatments had affected invasion, 3.0 kg a.s. ha^{-1} application was required to sustaining an effective control beyond the first 28 days following treatment; a likely indication that the 1.95 kg a.s. ha^{-1} application could be below the minimum effective rate for control of root invasion. Except for the applications of 1.95, 5.05 and 6.00 kg a.s. ha^{-1}, which compared variably to oxamyl, the treatments of fluensulfone, generally, affected root invasion similarly as the two commercially available nematicides. The root invasions in the fluensulfone treated-plots at Howle were 21 – 68 % less than in the soil left untreated; however, just the EC and the granular application at 6.0 kg a.s. ha^{-1} showed significant effects. The EC treatment in this experiment, generally, had greater, but not significant, effects on root invasion than the granular treatments, and was as effective as fosthiazate and oxamyl. A plausible explanation to this superior performance of the EC may have to do with availability of fluensulfone in the soil following treatment. The release of active substances from granular formulations is generally known to occur at a slower rate than liquid formulation (Flury, 1996). Davies *et al*., (1996), for example, found that it took 8h and 24h, respectively, for complete release of fenamiphos from the EC and granular formulation. Therefore, if fluensulfone were released more quickly from the EC formulation, it could

become available sooner in the soil to act on *G. pallida*. The results from the polytunnel pot experiment agreed somewhat with those from the two field experiments and seemed to suggest that the activities of fluensulfone against *G. pallida* under the polytunnel conditions might have been no different from that under the field conditions. The application of fluensulfone at the 4.05 and 5.05 kg a.s. ha^{-1} rates proved to be as effective as the treatment of fosthiazate at reducing the invasion of Estima in the pots.

Judging from the current results, it will be logical to suggest that fluensulfone may control *G. pallida* by mechanisms similar to those reported for oxamyl and fosthiazate. The latter nematicides are known acetyl-cholinesterase inhibitors, and have been shown to control PCN by interfering with activities, such as juvenile hatching, mobility, and host seeking processes (Woods *et al*., 1999; Evans and Wright, 1982, Wright *et al*., 1980), all of which may reduce the chances of the PCN to locating and invading the potato. Oka *et al*., (2009) showed *in vitro* that fluensulfone (then MCW-2) temporarily inhibited the hatching and mobility of juvenile *M. javanica*. Furthermore, an *in vitro* evaluation of the nematicidal activities of fluensulfone against *G. pallida* (Deliopoulos *et al*., 2009) showed irreversible inhibitory effects on the hatching and mobility of the J2, as well as viability of encysted eggs. The reduced root invasions, therefore, may very likely be explained by suppressive activities of fluensulfone on juvenile hatching, and the J2 activities in locating and invading the root. The results from the polytunnel pot experiment, however, showed no significant fluensulfone treatment effects on the viability of encysted eggs, as well as on the hatching of the J2 upon exposure to root leachate. This may imply that, at the current treatments, fluensulfone may act effectively against *G. pallida* stages in the soil and not encysted stages.

3.4.2 Increase of *G. pallida* population

Besides reducing the invasion of Estima, the results from the Field Experiments 1 and 2 showed further that the treatments of fluensulfone lessened the rate of the increase of *G. pallida,* as well as the size of the final population density. In the field experiment 1 at the Woodcote site, however, only the treatment in the granular fluensulfone at 5.05 kg a.s. ha^{-1} effectively lowered the *Pf* and *Pf/Pi* ratio though (ca. 62% and 59 %, respectively, less that of the untreated control). At this rate, fluensulfone was a better treatment than fosthiazate treatment at 3.0 kg a.s.ha^{-1}, and was as effective as the oxamyl treatment at 5.5 kg a.s.ha^{-1}. The situation was different in the Field Experiment 2 at Howle, where both the *Pf/Pi* ratio and the *Pf* were controlled by the fluensulfone treatments but the granular treatments at 3.0 and 4.05 kg a.s. ha^{-1}; both treatments controlled the *Pf* but not the *Pf/Pi* ratio. The reasons for the lesser effects of fluensulfone at these two rates on the *Pf/Pi* ratio are not yet known. The lower populations seen after fluensulfone would suggest probable effects of the current treatment on the post-invasion activities of *G. pallida*, which presumably, could involve suppression of feeding, development and/or reproduction. The Field Experiment 2 at Howle and the polytunnel experiment have provided evidence, which would suggest likely suppression of the development of *G. pallida* inside the root by fluensulfone. Oka *et al*., (2009) suggested that suppression of reproduction in the females were plausible. Effects of nematicides on post infection stages of juveniles have been reported for other non-fumigant nematicide, but more often, for those with systemic properties (for example, Evans and Wright 1982; Wright and Roland, 1982). A recent study by Oka *et al*., (2012) has suggested systemic action of fluensulfone against *M. incognita* on pepper. In their study, both root infection and reproduction of *M. incognita* were reduced following soil treatments or foliar sprays with fluensulfone. If fluensulfone possesses systemic properties as suggested, then activities against *G. pallida* stages within the root are probable and could explain the reduced population development on Estima. Another plausible explanation to the reductions in

population may be that of delay root invasion in the fluensulfone treated plot, which could cause the development of late invading juveniles to lag behind those in the untreated soil.

3.4.3 Plant growth and tuber yield

The reduction of *G. pallida* seen for the fluensulfone, as well as fosthiazate and oxamyl treatments was not consistent with the growth and yield of Estima in the experiments, particularly at the Woodcote site. There is insufficient data as at this stage for conclusions on responses of potatoes to fluensulfone treatments, but other published experiments such as those of Evans *et al*., (2003), Whitehead *et al*., (1994), Whitehead *et al*., (1991), and Whitehead *et al*., (1984), for example, have shown that soil treatment with nematicides are not always accompanied by improved plant growth and yield parameters. The *Pi* (Trudgill, 1986), soil pH (Haverkort *et al*., 1993) and plant nutrition (Grove *et al*., 1999a and Grove *et at*., 1999b) are some of the factors suggested to influencing responses of potatoes to nematicide treatments. The field at Woodcote was infested by *G. pallida* at ca. 16 egg g^{-1} soil, which was well above the damage threshold suggested for this species in the UK (Haydock *et al*., 2006; Trudgill, 1986). Since damage was likely at this *Pi*, the crop was expected to benefit from the reductions in root invasion seen for the nematicide treatments. Apparently, this was not the case, and presumably, Estima may have tolerated the infestations of *G. pallida* at the above population density under the conditions at Woodcote in 2010. Since the field was moderately infested, it could plausibly be that the crop damage by *G. pallida* may have been too little to suppress the untreated crop to reflect the net benefits of the reductions seen in the root invasions by the nematicide treatments. The situation was somewhat different in the Experiment 2 at Howle, where there was a trend towards positive response of tuber yields to the nematicide treatments. The ware yield, for instance, was increased by 42 % after treatment with fluensulfone in the EC form, and those seen for the

treatment in the granular form were ca. 25 – 42 % greater than the untreated control. The responses to the oxamyl and fosthiazate treatments were even greater; the yield were about two-fold that of the untreated control. Even though this field had lower *Pi* (9.6 eggs g^{-1} soil), the root invasion was generally greater (1125.8 juveniles g^{-1} root) than was observed at Woodcote (444.2 eggs g^{-1} root). This could suggest that the crop was prone to suffer more damages at Howle, and probably, was more likely to reflect the benefits from the nematicide effects on root invasion. Perhaps, more than five replicates were needed to efficiently minimise variability within the experiment, and this should be considered in future experiments.

3.4.5 Integrated control with fluensulfone and partially resistant potato

When averaged over the nematicide treatment in the experiment 3 at Howle, fluensulfone treatment gave adequate control of invasion of the three cultivars and was as effective as the treatments of fosthiazate and oxamyl. However, there were no significant interactive effects of the fluensulfone treatment and partial resistance on the root invasion. As expected, the partially resistant potatoes Santé and Vales Everest gave good control of both *G. pallida* multiplication rate and final population density and were found to have provided far greater control when grown untreated than growing the susceptible Estima in soil treated with a nematicide. When each cultivar was treated with fluensulfone, the population control by Santé, but not by Vales Everest, was greatly enhanced, yielding a further 50% reduction in the population parameters. This interactive effect between fluensulfone and Santé is reflected in the tuber yield; the ware, as well as the total yield was improved significantly by fluensulfone. Unlike Santé, Vales Everest, generally, did not respond positively to fluensulfone, and reasons to this are not yet known.

3.5 Conclusions

The main findings of the current experiments are that control of root invasion and population increase of *G. pallida* are likely to be achieved following treatments of fluensulfone at planting of potatoes. Treatment using the granular form at a minimum rate of 3.0 kg a.s. ha^{-1} may be required to provide adequate control of root invasions by *G. pallida*; however, a higher rate may be required for a more robust effect. The experiments, also, highlighted the potential of controlling PCN with the EC formulation of fluensulfone, which may be as effective as the currently available nematicides. However, further studies are needed, particularly, those involving testing of efficacy of fluensulfone in soils other than a sandy clay loam and in fields with high infestation of PCN to providing further evidence to substantiate these early claims.

CHAPTER 4

PERSISTENCE OF FLUENSULFONE IN SOIL

4.1 Introduction

The prospects of controlling field infestations of *G. pallida* with fluensulfone have been highlighted by the experiments reported in Chapter 3. However, the activities of fluensulfone and the control achieved, in that regard, will depend on its persistence in the soil. Indeed, the challenge to effective control of *G. pallida* with the currently available nematicides is commonly ascribed to short persistence in the soil (Haydock *et al*., 2012; Halford *et al*., 1995; Whitehead *et al*., (1991) and Whitehead *et al*., (1984), in view of the prolonged hatching exhibited by this species of PCN (Ryan *et al*., 2003; Whitehead, 1992). A number of studies, for example, Whitehead 1992 and Whitehead *et al*. 1984 have shown that *G. pallida* required 6 weeks of incubation in potato root leachate to reach the maximum hatch as compared with 3 weeks for *G. rostochiensis*. This hatching behaviour is purported to allow for peak juvenile hatch to escape effective nematicide concentrations in the soil (Evans, 1993). In their description of the hatching behaviour of *G. pallida* in relation to efficacy of oxamyl, for instance, Haydock and Evans, (1998) proposed that effective *G. pallida* control will required a persistence extending over 3 weeks. The degradation of fluensulfone has not been researched, and therefore, its field dissipation rates, as well as persistence are not yet known. Being such an influential determining factor of nematicide efficacy, the knowledge of the persistence of fluensulfone in the soil will be needed to inform its efficient use in controlling PCN. Furthermore, human and environmental safety concerns will benefit from the knowledge of the persistence of fluensulfone in the soil.

The experiments reported in this chapter were in two following two parts. The first part studied the field dissipation rate of granular fluensulfone application at the full rate (4.05 kg

a.s. ha^{-1}) in comparison to that of fosthiazate application at 3.0 kg a.s. ha^{-1} during the experiments at Woodcote and Howle in 2010 and 2011, respectively (Chapter 3). The second part involved the same treatments of fluensulfone determined the dissipation rate of fluensulfone at the same treatment in four contrasting arable soils in a pot under polytunnel conditions at Harper Adams University.

4.2 Aims

The experiments sought to determine the persistence of fluensulfone when applied to soil in the granular form at the full rate of 4.05 kg a.s. ha^{-1} and to determine which soil and/or environmental factors could impact persistence in potato beds.

4.2 Objectives

The objectives were

i. To quantify the time dependent loss of parental fluensulfone from the 15 – 20 cm depth of potato beds, as per high pressure chromatography analyses (HPLC) and to determine the soil dissipation rate constant (k) and the half-life (DT_{50}).
ii. Determined correlations between the parameter k and DT_{50} and soil properties such as organic matter content, pH, as well as the clay, sand and silt fractions.

4.3 Hypothesis

The hypothesis tested was that fluensulfone does not possess the persistence required for control of *G. pallida*.

4.4 Materials and methods

4.4.1 Field experiments

4.4.1.1 Field sites and nematicide application to the plots

The locations of the site at Woodcote and Howle are given in Table 4.1, and the experimental procedure regarding nematicide application and the treatments layout has been detailed previously in sections 3.2.3 and 3.2.4 (see Chapter 3). In this chapter, the properties of the soils from the plots treated with fluensulfone at 4.05 kg a.s. ha^{-1} and fosthiazate at 3.0 kg a.s. ha^{-1} in the Woodcote and Howle experiments are given in Table 4.1.

4.4.1.2 Soil sampling in field plots

Ten soil cores, to the depth of 20 cm, were randomly taken from the two middle rows (harvest rows) in each plot using a cheese corer (2.5 cm diameter by 30 cm deep), Sampling started on the day of application (0 DAA) and then at 7 – 8 day intervals over the entire duration of the experiments. At each sampling time, the cores were bulked, thoroughly mixed and transferred in polypropylene bags, sealed and stored at -20ºC until analysis.

4.4.1.3 Precipitation and soil temperature

Soil temperature and precipitation (rainfall/irrigation) during the experiments at Woodcote and Howle were measured as per Section 3.2.7 (see Chapter 3).

4.4.2 Polytunnel experiment

4.4.2.1 Methodology

The polytunnel experiment was conducted between October 2009 and January 2010 and was carried out on soils collected from four agricultural fields in Shropshire (Table 4.1). The study utilized 3 kg soil in 25 cm diameter pots, and comprised treatments of fluensulfone at 4.05 kg a.s. ha^{-1} and fosthiazate at 3 kg a.s. ha^{-1}. These treatments were achieved by mixing the required quantity of fluensulfone (as Fluensulfone 15G) and fosthiazate (as Nemathorin 10G) soil in a Belle mini 140 cement mixer (Belle Engineering Ltd, Sheen, UK). Treatments were replicated five times and arranged in randomised blocks. No tubers were planted in the soils. Soil sampling started shortly after application and then at weekly intervals over 12 weeks. On each sampling occasion, five soil cores were randomly removed (ca.100 g soil) from each pot and transferred to polypropylene bags and stored at -20°C until analysis.

Table 4.1. Source and property of the soils used in the persistence studies

Source of soil	UK ordinance survey map reference	Sand (%)	Silt (%)	Clay (%)	pH	O.M. (%)	*Textural class
Field experiment							
Howle, Shropshire	SJ 69485 23830	69.12	5.55	24.76	5.3	1.20	SCL
Woodcote, Shropshire	SJ 76901 15708	70.57	4.46	20.51	6.6	1.80	SCL
Polytunnel pot experiment							
Cherrington, Shropshire	SJ 668186	35.3	0.16	64.85	6.2	45.40	LP
Cherrington uphill, Shropshire	SJ 668186	71.61	0.08	28.47	5.9	10.00	P
Woodcote, Shropshire	-	74.82	9.81	13.37	7.0	4.00	LS
Tibberton, Shropshire	SJ 664198	75.69	4.56	17.04	6.6	2.20	SL

O.M. = organic matter content; SCL= sandy clay loam; LP = loamy peat; P = peaty; LS= loamy sand; SL= sandy loam. Soil pH, soil texture and were determined according to standard methods (MAFF, 1986). *: classification based on a textural triangle according to MAFF (1985).

4.4.3 Analytical procedures

4.4.3.1 Nematicide standards and reagents

Technical-grade fluensulfone (> 95% purity, lot number 130291-PF-2) was supplied by the manufacturer, ADAMA Agricultural Solutions, Airport City, Israel. Analytical standard grade fosthiazate (98.6% purity) was purchased from Fluka Analytical UK Ltd. Acetonitrile (99.99% purity) and orthophosphoric acid (85% purity) were purchased from Fisher Scientific Ltd and BDH Laboratory supplies UK Ltd, respectively. Water for all analyses was prepared by Purite Stillplus HP Pack.

4.4.3.2 Analytical methods

Nematicides in the soil samples were quantified by high pressure liquid chromatography (HPLC) analysis on an Agilent Technologies 1100 series apparatus (Agilent Technologies Ltd, Stockport, UK). It was equipped with an auto-sampler, a binary pump system, a multiple wavelength UV detectors and operated by Agilent ChemStation B.03 software for windows. The chromatographic conditions for analysing fluensulfone were set according to a method provided by the manufacturer, ADAMA Agricultural Solutions, Airport City, Israel. The only modification to the method was an increase of the injection volume from 10 to 20 µL. The separation column was a reversible Hypersil Gold column (250 x 4.6 mm, and 5 µm particle size) and was used at 40°C oven temperature. The mobile phase consisted of acetonitrile (eluent A) and 0.1% orthophosphoric acid in water (eluent B) mixture, and was set to flow at the gradient shown in Table 4.2. The injected volume (20 µL) was monitored at 254 nm peak area. Fosthiazate was analysed using the same column but under conditions reported by Osborne *at al*., (2010). A 20 µL aliquot was injected and monitored at 230 nm peak area. The mobile phase was acetonitrile and water mixture (1/1 v/v) and flowed at 1

mL min^{-1}. Under the above conditions, fluensulfone and fosthiazate eluted at ca. 18.1 and 5.9 min respectively (see Appendixes 2 and 3).

Table 4.2. Time phase of the mobile-phase system for analyzing fluensulfone

Step	Time (minutes)	Interval	Acetonitrile (%)	0.1% Orthophosphoric acid (%)
0	0 - 1	1	30	70
1	10 - 19	9	45	55
2	24 - 29	5	95	5
3	31 - 33	2	30	70

4.4.3.3 Calibration of the HPLC equipment

A stock standard each of fluensulfone and fosthiazate was prepared by dissolving 10 mg chemical in 100 mL solvent (1/1 v/v mixture of acetonitrile and water) to give a concentration of 100 µg mL^{-1}. From these stocks, calibration solutions of 0.001, 0.01, 0.1, 0.25, 0.50, 1.00, 2.5, 5 and 10 µg mL were prepared. Each solution was injected four times, starting with the lowest concentration, and analysed with the appropriate methods. The stock standards were stored at 4°C and fresh working solutions were prepared whenever needed. The validity of the methods was determined by correlations between peak areas and concentrations. The correlations in each case were positively linear and occurred over the concentration range of 0.1, 0.25, 0.50 and 1.0 µg mL for fluensulfone (a = 11.175b + 0.21, r^2 = 0.996) and over 0.10, 1.0, 2.5 and 5.0 µg mL for fosthiazate (a = 14.56b + 0.25, r^2 = 0.999).

4.4.3.4. Validation of the extraction methods

Prior to extraction of the nematicides from the soils, the accuracy of methods for extracting fluensulfone was determined by comparing the recoveries from soils fortified with either the technical grade or treated with the granular formulation. The soils used were collected from the untreated plots at the Woodcote and Howle sites, and were air dried and sieved to < 2 mm. Triplicate 20 g sub-samples were weighed into 100 mL glass shaking bottles and was either spiked with 1 mL of 50 µg mL^{-1} of fluensulfone or mixed with 27 mg of granular fluensulfone. The samples were allowed to stand for ca. 30 min before fluensulfone was extracted by shaking in 20 mL of acetonitrile:water mixture (1:1, v/v) for 1h on a HS 501 Digital reciprocal shaker (IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 300 rpm. After allowing standing for ca. 1h, 1 mL of the supernatant was drawn with a 2 mL syringe (BD Plastics Ltd, UK) and sieved through a 0.2 µm pore size Polyvinylidene Difluoride (PVDF) syringe filters (GE Healthcare UK Ltd) into a screw cap 2 mL HPLC glass vial to analyse for fluensulfone. A similar set-up was made for fosthiazate but just with the analytical grade and was shaken for 3h according to the method of Osborn *et al*., (2010). The mean recovery seen for granular fluensulfone was 84% (82.8 – 88.7%) and was lower than 98.2% (92.4 – 103%) seen for the technical grade. For this reason, the soils treated with the granular formulation were agitated further, and after 2, 3, 4 and 12h, 1 mL of the supernatant was sampled and analysed for fluensulfone. These subsequent analyses showed that greater fluensulfone could be extracted after a shaking period lasting 3 – 4 h rather than 1h suggested in the available method. Based on these, three hours was then chosen as the shaking time for extracting fluensulfone from the soils. The recovery of fosthiazate averaged 92 % (86 – 102%).

4.4.3.5 Extracting and quantifying nematicides in the soils

Samples to be extracted were removed from -20ºC storage and left in plastic trays overnight to thaw. Each sample was then thoroughly mixed, and a 20 g sub-sample was transferred in a 100 mL glass shaking bottle and shaken in 20 mL of acetonitrile and water (1/1 v/v) at 300 rpm for 3h. The bottle was left to stand for at least 1h when 1mL of the clear supernatant was sampled, and either analysed immediately for fluensulfone or was stored at -20°C for future analyses.

4.4.4 Release of fluensulfone from granular formulation

In order to optimise the extraction of fluensulfone from the soil samples, two tests were conducted to examine the release rates of fluensulfone from the granular formulation. Test 1 was run three times and involved quantification of total fluensulfone in the formulation. Test 2 was made two times to determine a water-induced release of fluensulfone from the formulation. In Test 1, triplicate 27 mg of granules (4.05 mg fluensulfone) were transferred in 100 mL bottles and shaken at 300 rpm for 1, 2, 3, 4 and 12 h in 20 mL of acetonitrile without soil. At each time point, 1 mL of the solvent was sampled and analysed for fluensulfone. In Test 2, 27 mg of the granules were placed in each of ten plastic tubes (1.0 cm wide × 2.0 cm high) sealed at the bottom with nylon mesh (53-µm aperture). Half of the tubes were placed upright in the wells of a 24-well plate to which 1 mL of distilled water was added, submerging the granules. The plate was covered, sealed with parafilm and incubated at 5°C (to minimise potential degradation). The other half was similarly treated and incubated at 20°C. After 1 h of incubation, each tube was carefully lifted, and the entire water was removed by a pipette and transferred to 100 mL conical flasks. The well was rinsed trice, and the water added to the flask. Fresh distilled water was added to the well and the plates incubated as before. The water in the flask was made up to the 100 mL mark (1/100

dilutions), and 1 mL subsample was filtered through 0.2 µm sieves to analyse for fluensulfone. Sampling and analysis for fluensulfone was repeated at 12h and 24h, and then at 3.5, 7, 17, 23, 35 and 41 days after incubation.

4.4.5 Analyses of data

The dissipation rate constants (*k*) and the time to 50 % dissipation (DT_{50}) were determined following the method described in Osborn *et al*., (2010). Briefly, the measured concentrations from each replicate plot were regressed against time in days after application, and the parameters *k* and DT_{50} estimated from the curves which best fitted the data. Curve fitting and parameter estimations were carried out using SigmaPlot V.12 (Systat Software, Inc. London, UK). The parameters obtained were analysed by one-way ANOVA using Genstat v.14 (VSN International Ltd., Hemel Hempstead, UK). In this study, however, there were instances when the day zero samples contained lower concentrations than were detected at 7 DAA (Appendixes 4 - 7). In these instances, the starting concentration for calculating the DT_{50} value was estimated from the fitted curve as recommended by FOCUS (2006).

4.5 Results

4.5.1 Soil properties

The organic matter content was lower at Woodcote (1.87 %) than at Howle (2.30 %) but varied greatly between the plots at the former (0.44 – 4.04%) than at the later site (2.10 – 2.46 %). Soil pH at Woodcote ranged from 6.45 to 7.01 with a mean of 6.73, and at Howle, where it varied from 4.93 – 6.30 with a mean of 5.34. The soils used for the polytunnel

experiment varied widely in terms of the texture (ranged from loamy peat – sandy loam) and organic matter contents (2.2 – 45.4%; Table 4.1).

4.5.2 Precipitation/rainfall and soil temperature

The mean soil temperature at 15 cm depth and the total precipitation/rainfall received, during the experiments at Woodcote and Howle, are shown in Figure 4.1. No precipitation/rainfall was received at either site until the first 7 DAA. Total amounts differed only slightly between the two sites (378.8 mm and 370.0 mm at Woodcote and Howle, respectively). The soil was somewhat warmer at Woodcote (16.6 ºC) than at Howle (15.3ºC).

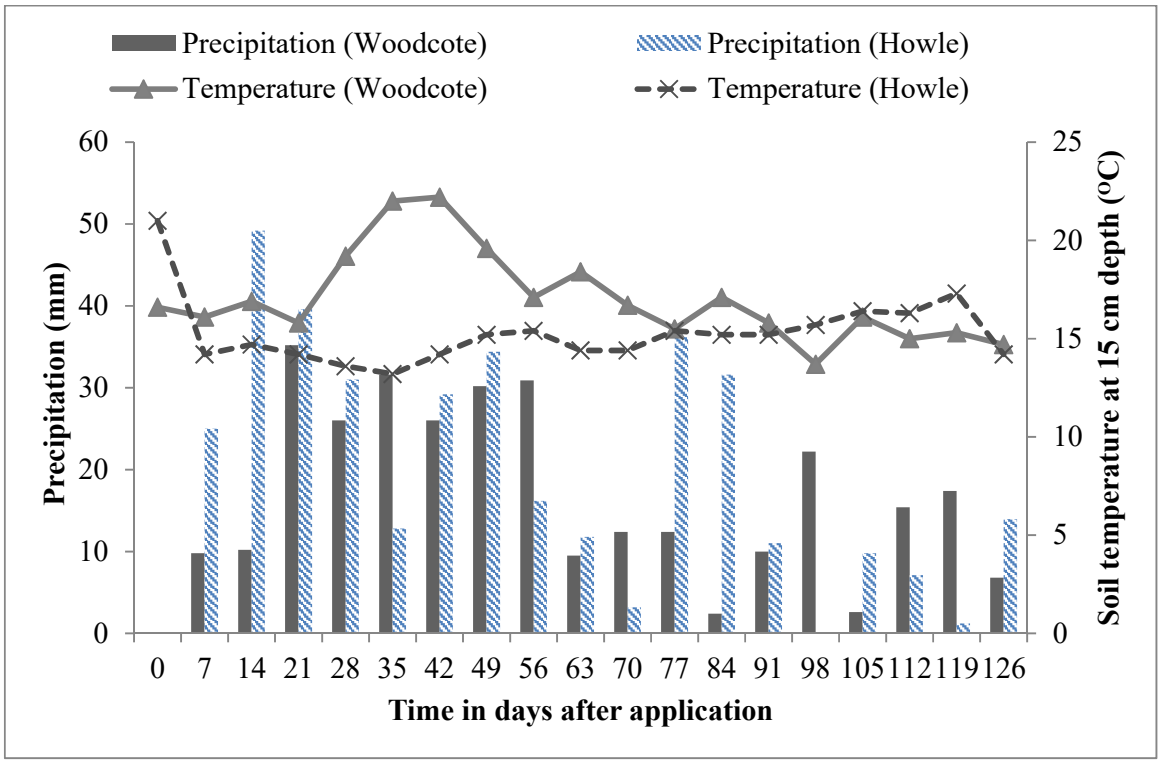


Figure 4.1. Mean soil temperature at 15 cm depth and precipitation recorded during the experiments at Woodcote in 2010 and Howle in 2011.

4.5.3 Release of fluensulfone from the granular formulation

Fluensulfone extracted by acetonitrile and the release from the granular formulation into water as functions of time are shown in Figure 4.2. Assuming no degradation had occurred during the test, the extraction with acetonitrile showed that ca. 91% (3.67 mg) of the expected fluensulfone (4.05 mg) was available for extraction by 12h of shaking, and much of this (3.66 mg) was extracted by the 4th hour. Water, on the other hand, induced a gradual, but incomplete, release of fluensulfone from the formulation within 41 days of incubation. The amount and rate of release depended mainly on the temperature and duration of incubation. Except for the samplings at 1 and 12h, when as much fluensulfone was released at 5ºC as at 20ºC incubation temperature, the amount released at respective sampling times was greater at 20ºC than at 5ºC. The percentage cumulative release, as of 41 days of incubation, was significantly lower (2.93 mg) for material incubated at 5°C than that seen at 20°C (3.53 mg) ($P < 0.001$), and the amounts released at either incubation temperature correlated positively ($r^2 = 0.99$; $P < 0.001$) with the duration of incubation.

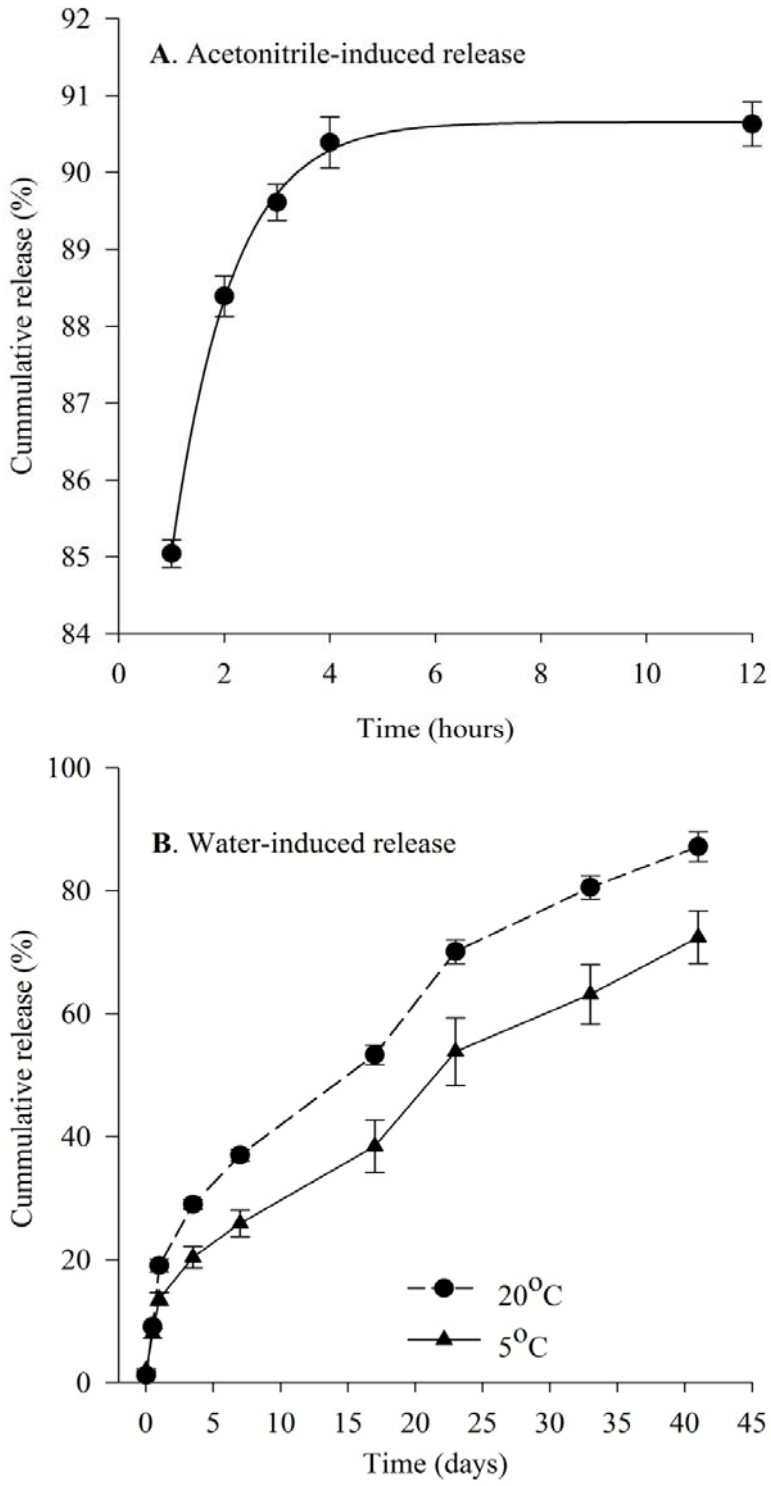


Figure 4.2. (A) Fluensulfone extracted from the granular formulation with acetonitrile over 12h and (B) water-induced release kinetics of fluensulfone from the granular formulation over 41 days. Percentage cumulative release was plotted against duration of incubation. Bars show the standard error of the mean (n = 3).

4.5.4 Dissipation of fluensulfone in the field plots

Figure 4.3 shows the dissipation of fluensulfone and fosthiazate within 126 days in the 20 cm soil depth of the field plots at Woodcote and Howle in 2010 and 2011, respectively. The mean concentration of fluensulfone on the day of application (0 DAA) at Woodcote was 2.35 mg Kg^{-1} soil and was slightly higher by 7 DAA (2.69 mg kg^{-1} soil) before dissipating quite rapidly through days 14 and 21 after application to 0.54 mg Kg^{-1} soil (ca. 63% dissipation) by 28 DAA. Losses thenceforth occurred rather slowly, with the concentrations fluctuating between 0.27 and 0.12 mg Kg^{-1} soil. The loss of fluensulfone from the plots at Howle followed a similar trend. The mean concentration at 0 DAA was 1.90 mg kg^{-1} and was slightly higher (2.10 mg Kg^{-1} soil) by 7 DAA before dropping rapidly through days 14, 21 and 28 after application to 0.27 mg Kg^{-1} soil (ca. 75 % dissipation) by 35 DAA. Further losses appeared rather slowly, with fluctuating concentrations being detected up until the final sampling at 126 DAA. Overall, the dissipations of fluensulfone in both fields were described adequately by sigmoidal model (Table 4.3). The DT_{50} values obtained as per the replicate plots at Woodcote varied from 19.6 to 30.0 days, with a mean of 24.3 days, and from 13.9 to 31.5 days, with a mean of 23.7 days at Howle. The loss of fosthiazate at Woodcote was exponentially described (r^2 = 95%; SE = 0.11), with 34 % dissipation occurring within the first 7 DAA. This was then followed by a period of no significant loss in concentration until 42 DAA when 0.23 mg kg^{-1} soil remained (ca. 87% dissipation). The dissipation of fosthiazate at Howle followed a sigmoidal model (r^2 = 97.16; SE = 0.12), with no losses in the measured concentration until after 7 DAA when the 0 DAA concentration (1.80 mg kg^{-1} soil) dissipated significantly ($P < 0.001$) to 0.52 mg Kg^{-1} soil by 21 DAA (ca. 71% dissipation). The DT_{50} values obtained for fosthiazate at Woodcote ranged from 10.4 to 35.2 days, with a mean of 21.3 days, and were more varied than was seen at Howle (16.3 – 21.1 days) with a mean of 18.8 days. No significant differences were seen for comparisons between the DT_{50} for fluensulfone and fosthiazate at either site (Figure 4.4).

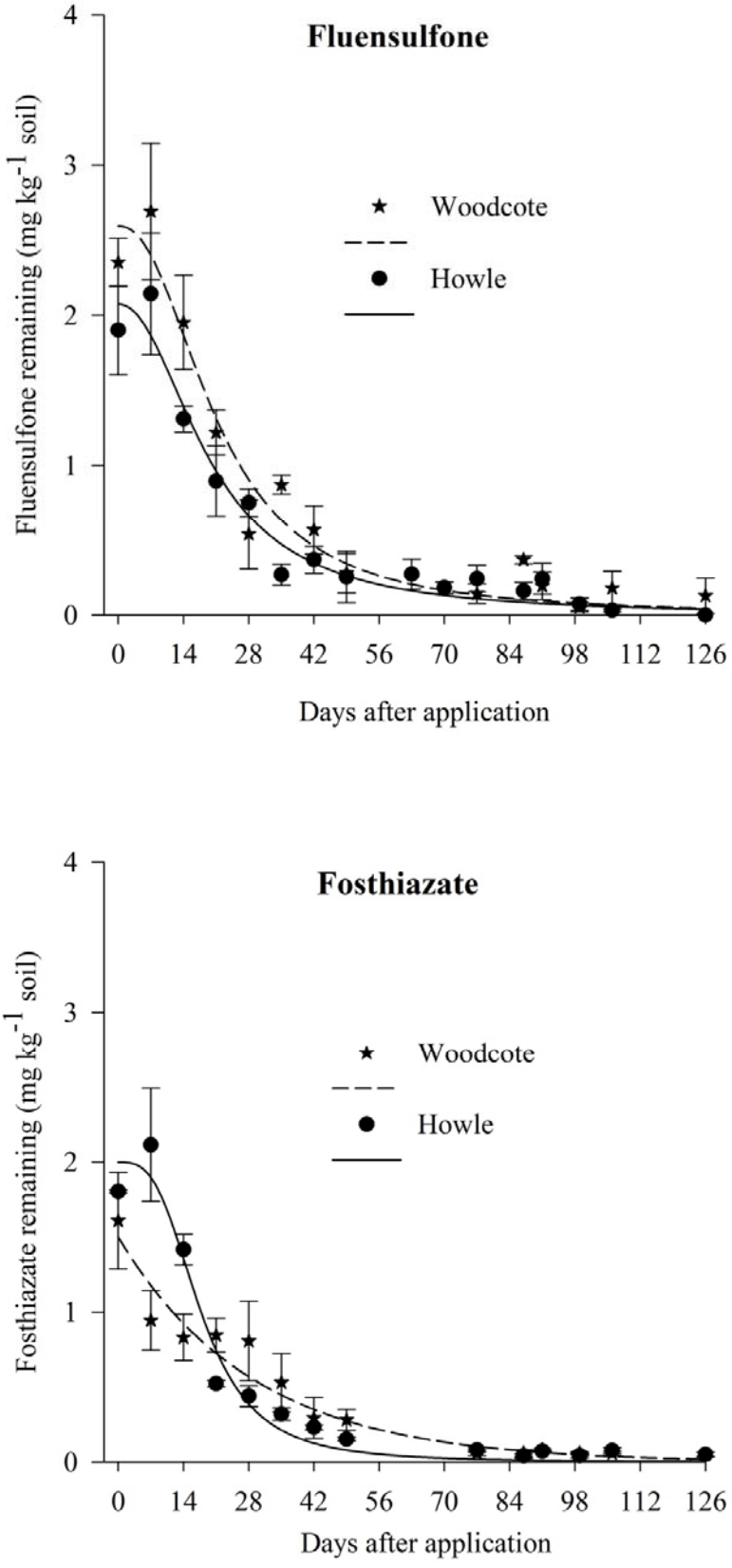


Figure 4.3. Dissipation of (A) fluensulfone and (B) fosthiazate in the top 15-20 cm soil layer of field plots at Woodcote in 2010 and at Howle in 2011.

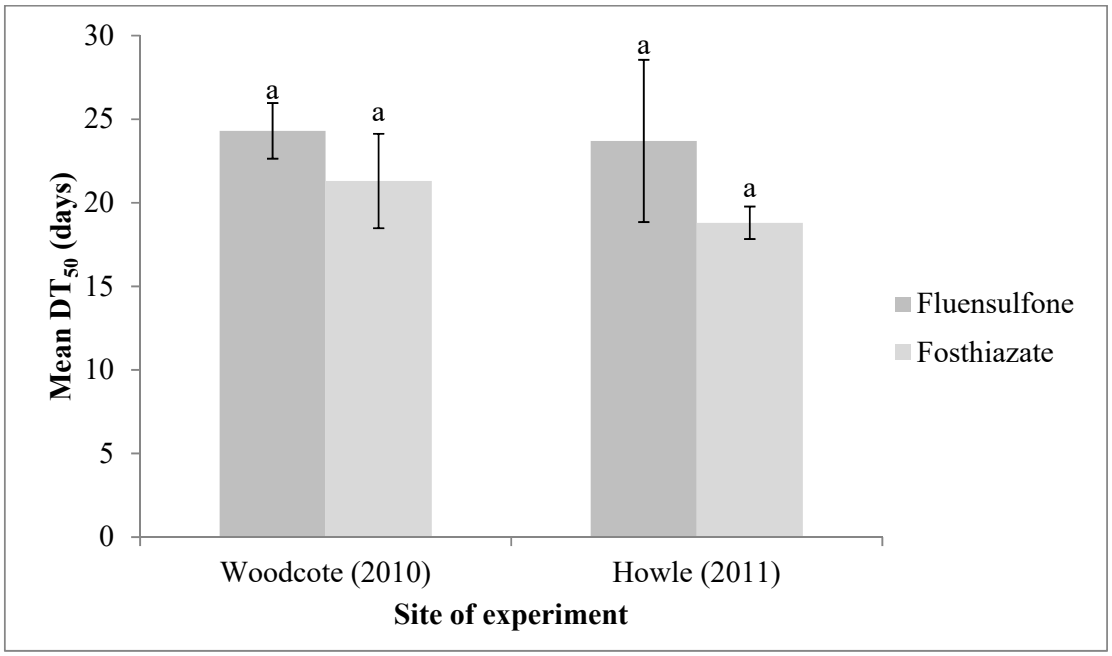


Figure 4.4. Comparisons of half-lives (DT_{50}) observed for fluensulfone and fosthiazate in field plots at Woodcote (2010) and Howle (2011). Bars represent standard error values (n = 5).

4.5.5 Dissipation of fluensulfone in soil under polytunnel conditions

Figure 4.5 shows the dissipation of fluensulfone within 56 days of application to the soils investigated in the polytunnel study. The analysis of the samples collected beyond the 56th day after application mostly, did not yield detectable concentrations and are therefore, not presented. Fluensulfone detected in the day zero samples varied widely between the soils; it was highest in the soil collected from Cherrington (3.1 mg kg^{-1} soil) and lowest in that collected from Tibberton (1.7 mg kg^{-1} soil). Just as was observed in the field experiments, all four soils contained slightly higher fluensulfone at 7 DAA than at 0 DAA. Although the rate of loss (*k*) of fluensulfone from the soil differed between the soils, the trend of loss was essentially sigmoidal (Table 4.3). A significant decline in fluensulfone became apparent only beyond 14 DAA, with more than 95% dissipation across the soils by 28 DAA. The DT_{50}

ranged from 19.3 – 21.8 days (Figure 4.6) and did not differ significantly between the soils (see Figure 4.5). Despite differing soil properties, neither the DT_{50} nor the rate of loss correlated significantly with any single soil property (Table 4.4).

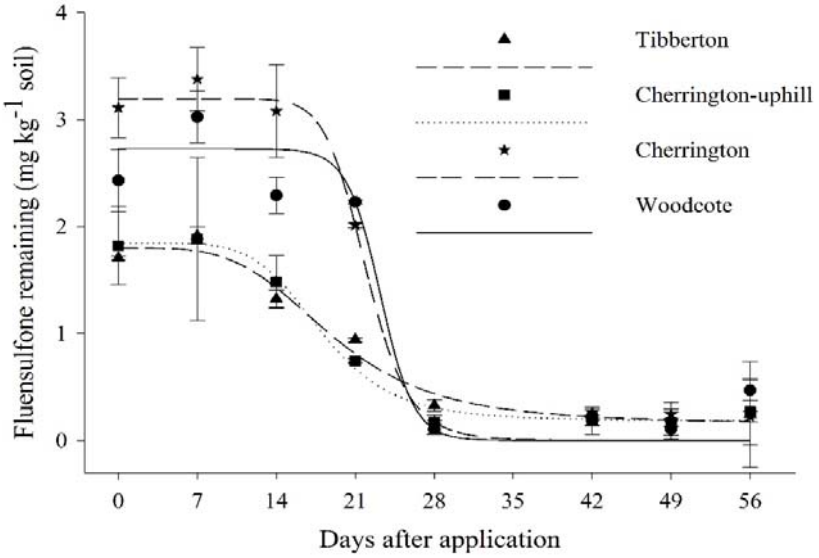


Figure 4.5. Dissipation of fluensulfone in four arable soils in pots under polytunnel conditions. Bars represent standard error values (n =5).

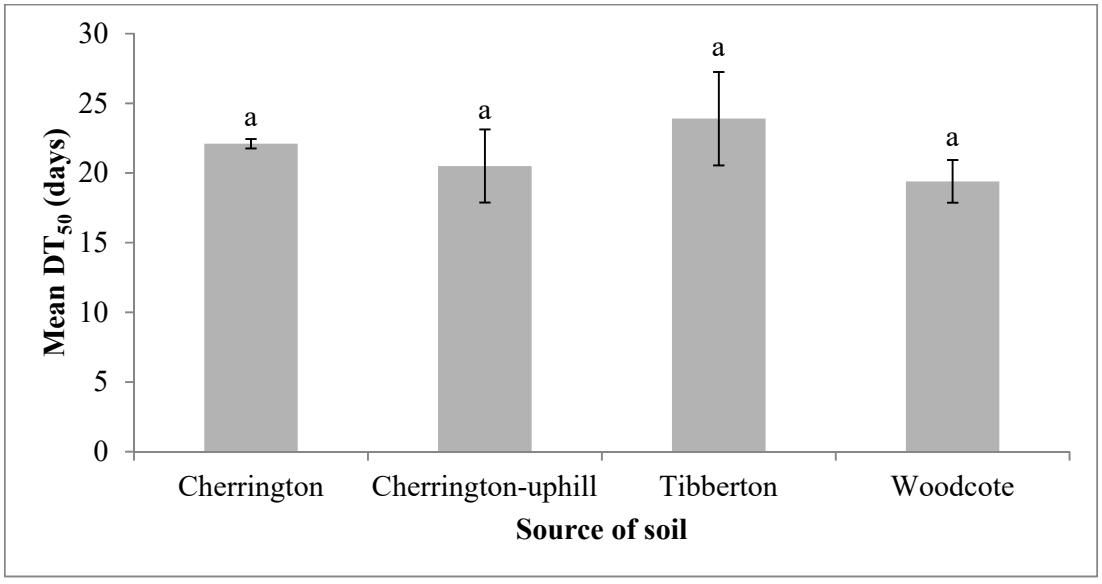


Figure 4.6. The half-lives (DT_{50}) observed for fluensulfone following application of granular product (Fluensulfone 15G) to four contrasting arable soils in a polytunnel pot study. Bars represent standard error values.

Table 4.3. Dissipation rates constant (k, day^{-1}), coefficient of determination (r^2) and half-lives (DT_{50}) obtained for fluensulfone and fosthiazate in the field and polytunnel soils. Values are means ± standard error.

	Nematicide	k, day^{-1}	r^2	Significance
Field Study				
Howle	Fluensulfone	2.12 ± 0.28	96.45 ± 0.13	$P < 0.0001$
Woodcote	Fluensulfone	2.36 ± 0.40	95.45 ± 0.20	$P < 0.0001$
Howle	Fosthiazate	3.13 ± 0.54	97.15 ± 0.20	$P < 0.0001$
Woodcote	Fosthiazate	0.03 ± 0.01	94.87 ± 0.18	$P < 0.0001$
Polytunnel Study				
Cherrington	Fluensulfone	2.08 ± 0.77	98.57 ± 0.21	$P < 0.0001$
Cherrington-uphill	Fluensulfone	3.90 ± 1.31	96.29 ± 0.17	$P = 0.0003$
Woodcote	Fluensulfone	1.33 ± 1.11	94.80 ± 0.41	$P = 0.0006$
Tibberton	Fluensulfone	5.79 ± 1.43	94.92 ± 0.13	$P = 0.0006$

Table 4.4. Correlations between the rate constant (k) and the half-life (DT_{50}) and the properties of the soils investigated in the polytunnel study

	k	Soil OM	Soil pH	Sand	Clay	Silt
k	1	-0.356	-0.475	0.369	-0.225	-0.452
DT_{50}	0.344	0.461	-0.157	-0.503	0.492	-0.409

OM = Organic matter

4.6 Discussion

4.6.1 Release of fluensulfone

The release kinetics of fluensulfone from the formulation into water suggest that the active substance may be readily available for entry into water once the granules become hydrated and that the carrier material is unlikely to be a limiting factor to its availability to soil water. The gradual release from the formulation implies that the granules may act as a controlled-release formulation. This may enhance availability in soil water since at the current rate it is plausible that the formulation will sustain continued release into surrounding water. Furthermore, longer persistence of granular forms of pesticides, for instance, the insecticide isazofos (Bowman, 1991, Bowman 1992) and the triconazole fungicide (Biedel *et al*., 1999) has been ascribed to controlled release of active substances. Even though the incubation test was made under conditions not comparable to field situations, gradual release of fluensulfone from the granular formulation is plausible in the soil and may influence the persistence of fluensulfone by retarding its availability to degradation and/or leaching processes, which are concentration dependent. It is impossible though, to ascertain the effects of the release characteristics on the persistence of fluensulfone in the current experiment. However, the EC and the capsule suspensions (CS) forms of fluensulfone are available and studies comparing persistence in these forms to the granules will be useful.

The results of the preliminary test involving the extraction of fluensulfone from the formulation with acetonitrile (Test 1) have proven useful in improving the efficiency of the extraction method. Indeed, the extraction method provided by ADAMA Agricultural Solution Ltd, Airport City Israel was developed using the technical-grade fluensulfone and the recovery of 98.4 ± 3.1% seen after shaking for 1h in acetonitrile is close to that reported (103 ± 6 %). It became apparent from this test that a longer shaking time was required to enable sufficient extraction of fluensulfone from the formulation and may explain the poor

recovery after the initial shaking. The implications of these to the current study could have been that of erroneous estimation of the dissipation of fluensulfone, and perhaps, its persistence in the soil.

4.6.2 Persistence of fluensulfone

The loss of fluensulfone from the field plots at Woodcote and Howle followed similar patterns suggesting similar dissipation kinetics of fluensulfone in both fields. This similarity in behaviour may be explained by the identical conditions under which the experiments were conducted; the two fields varied very little in their soil properties and the weather conditions. Because of the similarity in behaviour, the identification of factors that might have influenced the dissipation of fluensulfone could not be made. The lag phase of seven days preceding the dissipations of fluensulfone in both fields may be due partly to unavailability of fluensulfone in the soil during this period. A general property of non-fumigant nematicides is that their availability and subsequent redistribution in the soil are achieved by rainfall or irrigation water (Noling, 2003; Smelt and Leistra, 1992). As illustrated in Figure 4.1, no precipitations were received at either site until the first seven days after application. Therefore, the release of fluensulfone from the formulation, hence availability in the soil for degradation, could most likely be limited during this period. Indeed, the loss of fluensulfone from the plots in both fields coincided with the onset of precipitation/rainfall and the rapid decline in concentration, thereof, occurred during the period of highest precipitations. This highlights the likely influence of soil moisture on the overall persistence of fluensulfone. As discussed earlier, the release kinetics from the granular formulation may partly explain the initial delay in dissipation inform the field plots. The positive correlation between amount released and duration of incubation may imply that immediate availability of fluensulfone after application is very unlikely, even when soil moisture were adequate to trigger release.

An alternative explanation to the initial delay in dissipation may probably have to do with fluensulfone adsorption when it eventually becomes available in the soil. Adsorption has often been considered a limiting factor to microbial degradation since it limits pesticide availability to the soil aqueous phase where microbial activities are most preferable (Manuel *et al*., 2008; Kah and Brown, 2007; Guo *et al*., 2000; Biedel *et al*., 1999).

The dissipation of fluensulfone did not differ between the soils in the polytunnel experiment despite the variations in soil properties, particularly soil organic matter content; dissipation across the soils was sigmoidal and appeared to be characterized a lag phase of up to 14 days. Lag phases in pesticide degradation kinetics have been associated with microbial degradations, and have been suggested (Arbeli and Fuentes, 2007; Fenlon *et al*., 2007) to represent the period of adaption of microbial population capable of metabolizing newly encountered molecule. Even if fluensulfone dissipation in this experiment involved microbial activities, the lack of correlation between the parameters *k* and DT50, and the soil properties would suggest that this may have had limited effects on the overall dissipation. As suggested above, adsorption limits the availability of pesticide for degradation and has been shown to correlate positive and strongly with soil organic matter content (Pantelelis *et al*., 2006). In the exception of the soil from Woodcote, the duration of the lag phase was somewhat related to the organic matter content of the soils, with the longest delays associated with the soil highest in organic matter content. The involvement of sorption processes in the dissipation of fluensulfone is probable owing to the hydrophobic properties of the molecule. Sorption is known generally for limiting availability of pesticides for degradation (Guo *et al*., 1999) and sorbed fluensulfone may perhaps be unavailable for degradation. Studies involving sorption of fluensulfone might therefore be useful in determining its likely involvement in the persistence of fluensulfone in soil.

The DT_{50} obtained for fluensulfone in the field plots, as well as in the soils under polytunnel condition suggest persistence of no longer than 24 days and was similar to that observed for fosthiazate. This may be considered too short a period to providing effective control of *G. pallida* since at this persistence, half the concentration of both nematicides may have degraded as of peak J2 hatch at between 6 - 8 weeks suggested by Haydock and Evans, (1998). It is worth noting though that the DT_{50} only indicates the time it takes for half the initially applied concentration to disappear from the soil; it does not provide information on the biological activity of the remaining concentration. Deliopoulos *et al*., (2009) showed *in vitro* that J2 *G. pallida* hatching was inhibited and juvenile mobility reduced by fluensulfone at 0.00425 mg L^{-1}. The concentration of fluensulfone remaining in the field plots beyond 24 DAA ranged from 0.6 to 1.2 mg kg^{-1} soil at Woodcote and from 0.4 to 0.9 mg kg^{-1} soil at Howle. Clearly, these concentrations were higher than was suggested above for the biological activities of fluensulfone. Relating these concentrations to the control of root invasion seen for fluensulfone, as well as, for fosthiazate in both field, may suggest that even at the current short persistence, fluensulfone remained in the soil in effective concentrations to give control of *G. pallida*. Further studies involving the effects of sorption processes on the fates of fluensulfone in the soil will be useful in complementing the current findings. For instance, the effects of sorption processes on the availability of fluensulfone in the soil water may be useful, since availability in the soil aqueous is essential for controlling PCN.

CHAPTER 5

SORPTION OF FLUENSULFONE TO SOIL AND ITS IMPLICATIONS FOR EFFICACY AGAINST *GLOBODERA PALLIDA*

5.1 Introduction

Sorption is one of the processes that soil-applied pesticides are subjected to, and it has been widely suggested as the key process regulating pesticide availability in the soil's aqueous phase (Yu *et al*., 2006; Li *et al*., 2005; Cooke *et al*., 2004; Gevao *et al*., 2000), thereby, influencing pesticide fates such as mobility and leachability (Singh, 2008; Koskinen and Harper, 1990). Control achievable by nematicides is partly due to their distribution in the soil (Woods and Haydock, 2000; Garabedian and Hague, 1982; Whitehead *et al*., 1980), and for non-fumigant nematicides, redistribution in soil following the initial application occurs as rain and/or irrigation water infiltrates the soil (Noling, 2003). Furthermore, the uptake of non-fumigant nematicides into soil water is important for effective control (Smelt and Leistra, 1992), since nematodes are, essentially, aquatic organisms and, therefore, present in the soil water. Consequently, sorption may influence the efficacy of non-fumigant nematicides by directly limiting availability in the soil solution. On the other hand, however, sorption may prolong pesticide persistence (Kravvariti *et al*., 2010; Fernades *et al*., 2006; Barriuso *et al*., 1997) by limiting the availability of active substances to microbial degraders found in the soil (Guo *et al*., 2000, Berger, 1999; Ogram *et al*., 1985). The possibility of shortened persistence through sorption has also been proposed (Villaverde *et al*., 2008; Park *et al*., 2003). Presumably, sorption could variably influence the fate of fluensulfone in the soil, thus its assessment will be useful in making logical conclusions about its likely impacts on efficacy against *G. pallida*.

Whilst influencing the behaviour of pesticides in the soil, sorption is itself dictated by the interaction between pesticide, soil and environmental factors (Cao *et al*., 2008; Boivin *et al*.,

2005; Dell Site, 2001; Dec and Bollg, 1997; Zheng and Cooper, 1996; Chiou *et al*., 1983), and significant correlations between nematicide sorption and soil properties have been widely reported (Karpouzas *et al*., 2007; Pantelelis *et al*., 2006; Qin *et al*., 2004; Simon *et al*., 1992; Gerstl, 1984).

This chapter presents the results of three laboratory sorption experiments and that of a polytunnel pot experiment involved with the effects of granular fluensulfone application at the full rate to a peat-amended sandy clay loam.

5.2 Aim

The aim was to determine how sorption could influence the efficacy of fluensulfone in controlling *G*. *pallida*.

5.2 Objectives

The laboratory experiments

i. Determined fluensulfone sorption coefficient (K_D) as a function of soil type and established relationships between this parameter and soil's physical and chemical properties.
ii. Compared the sorption of technical-grade fluensulfone with that of the granular product in order to determine if the formulation could influence sorption to soil.
iii. Determined the effects of soil amendments with moss peat on the sorption and desorption of fluensulfone.

The polytunnel pot experiment determined

i. The effect of soil amendments with peat on the availability of fluensulfone for control of *G*. *pallida* by comparing concentration detected in the soil as per acetonitrile and water extractions

ii. The effects of fluensulfone on *G*. *pallida* J2 viability, hatching and invasion of potato roots in peat-amended soil.

5.3 Hypothesis

The hypothesis tested was that sorption will reduce the availability of fluensulfone in the soil to be effective and that the soil's organic fraction will constitute the main soil factor limiting the efficacy of fluensulfone.

5.4 Materials and methods

5.4.1 Laboratory sorption experiments

The laboratory sorption experiments were conducted using the batch equilibrium method following the Organization for Economic Co-operation and Development (OECD) guideline 106 (OECD, 2000).

5.4.2 Chemicals, reagents and analytical method

Table 5.1 shows selected properties of fluensulfone. Solvent for preparing stock and working solutions of fluensulfone, throughout the study, comprised 0.01M calcium chloride ($CaCl_2$) solution prepared in deionised water. All fluensulfone analyses were undertaken by HPLC as per the chromatographic conditions detailed in section 4.3.3.2 (see Chapter 4).

Table 5.1. Selected properties of fluensulfone

Chemical formula	Water solubility at 20ºC	Log P_{ow} at pH 7.5	Water vapour
$C_7H_5ClF_3NO_2S_2$	622 mg L^{-1}	2.6	3.1 x 10^{-3} Pa

5.4.3 Substrates studied

The soils investigated (Table 5.2) were collected at 20 cm depth from commercial potato fields in Cambridgeshire, Lincolnshire, Nottinghamshire and Shropshire in England between June 2010 and May 2011. The samples were used after drying for at least seven days at a room temperature of 20 °C and sieving to < 2mm size. Moss peat was purchased from Clover Peat Products (Dungannon, Northern Ireland) and was used to amend soil organic matter content. Soil pH, residual water content and soil texture were determined according to standard methods (MAFF, 1986). Soil organic carbon content was determined using a LECO sulphur-carbon analyser (LECO®Hazer Grove, Stockport, UK. Maximum water-holding capacity (MWHC) was determined using a model 1600 Pressure Plate Extractor (ELE International, Bedfordshire, UK).

Table 5.2. Sources and properties of the soils used in the sorption experiments

Soil source	UK Ordinance Survey map reference	% Sand	% Silt	% Clay	Soil pH	% Soil OC	% MWHC	Textural class
Gosberton, Lincolnshire (GL)	TF 2458430830	83.11	8.90	8.03	6.54	1.19	14.03	LS
Howle, Shropshire (HS)	SJ 6948523830	69.10	5.50	24.70	5.30	1.54	13.89	SCL
Mattersey, Nottinghamshire (MN)	SK 7014289453	83.80	15.00	11.00	7.20	1.14	13.50	LS
Wisbech, Cambridgeshire (WC)	TF 4497113241	33.33	25.60	31.10	6.90	1.38	14.80	CL
Kirkby, Lincolnshire (KL)	TF 3286961538	83.21	9.70	7.10	7.50	1.23	10.20	LS
West Pinchbeck, Lincolnshire (WL)	TF 2050427108	55.50	32.60	11.80	6.70	1.44	16.10	SL

OC: organic carbon content; MWHC: maximum water holding capacity; SCL= sandy clay loam; LS= loamy sand; SL= sandy loam; CL = clay loam

5.4.4 Preliminary experiment: Determination of optimal soil to solution ratio and time to sorption equilibration

Optimal soil to solution ratio for measuring sorption and the time to sorption equilibration were determined in preliminary tests performed in duplicate on the soil HS (amended with peat at 9.6%) and the soil WL, and at soil:solution ratios of 1/1 (w/w) and 1/2 (w/w). In each test, 10 g dried soil samples were weighed into 50 mL polytetrafluoroethylene (PTFE) centrifuge tubes and their moisture content adjusted to 90% of their respective water-holding capacity according to Table 5.2. The tubes were allowed to stand for ca. 1h when 9 and 18 mL of 0.01 M $CaCl_2$ solution was added respectively, for the 1/1 and 1/2 soil/solution ratios. The suspensions were shaken on a HS 501 digital reciprocal shaker (IKA®Werke GmbH & Co. KG, Staufen, Germany) at 200 oscillations min^{-1} for ca. 24h at room temperature of 20 ºC. Afterwards, the suspensions were spiked with 1 mL (1/1 soil:solution ratio) and 2 mL (1/2 soil:solution ratio) of a 50 mg L^{-1} stock solution of fluensulfone in 0.01M $CaCl_2$ solution to give a concentration of 5 mg L^{-1} (C_I). The suspensions were returned to the shaker and at 2, 4, 6, 8, 12 and 24 h, they were centrifuged at 3000 g for 15 minutes, and 0.1 mL of the supernatant was pipetted, passed through a 0.2 µm pore sized Polyvinylidene Difluoride (PVDF) syringe filters (GE Healthcare UK Ltd) into 2 mL HPLC vials to analyse for fluensulfone. Each test included four extra tubes for the following: (i) assess stability of fluensulfone within the duration of the test (tube contained 9 mL $CaCl_2$ solution without soil but spiked with 1 mL of stock fluensulfone solution) (ii) blank control $CaCl_2$ solution (tube contained 10 mL $CaCl_2$ solution without soil) and (iii) control soil suspensions (one tubes of 1/1 soil/solution for each soil type). Fluensulfone sorbed to soil, as percentage of C_I, was determined according to the equation in (6)

$$\text{Sorbed fluensulfone (\%)} = [(C_I - C_T)/C_I]*100 \quad (6)$$

where, C_I and C_T are, respectively, the initial solution concentration and the concentration measured at the respective sampling times.

5.4.5 Preliminary results: Optimum soil/solution ratio and time to sorption equilibration

Figure 5.1A shows the fluensulfone sorption kinetics at the 1/1 and 1/2 soil/solution ratios and the changes in fluensulfone in the $CaCl_2$ solution without soil during the 24h equilibration at room temperature of 20 °C. Sorption of fluensulfone was quite fast, with ca. 43 and 77% of fluensulfone sorbed respectively, by soils WL and HS within 2h. Sorption, thereafter, proceeded slowly to levelling off by the 4th hour. The soil/solution ratio did not influence the sorption kinetics but had affected the amount of fluensulfone sorbed; greater sorption occurred at the 1/1 than at the 1/2 soil/solution ratio. Both ratios, however, gave more than 20% sorption, which is the lowest suggested by OECD (2000) to settle for in selecting a soil/solution ratio for the batch equilibrium method. Either ratio could provide reliable estimates of fluensulfone sorption, but other in example, investigators, for example Bermúdez-Couso *et al*., (2012), Kah and Brown, (2007) and Boesten, (1990) have recommended that sorption measurements be made at the highest soil/solution ratio since this may be closer to a field situation. The 1/1 soil/solution ration was therefore, selected for this study.

It was apparent that sorption equilibrated earlier on the peat-amended soil HS (2h) than soil WL (4h), and the variation could partly be due to greater sorptive capacity of soil HS owing to its higher organic matter contents. Figures 5.1B however, showed that significant losses in fluensulfone in solution were very likely by 4h; therefore, the sorption measurements were made using 2-hour equilibration.

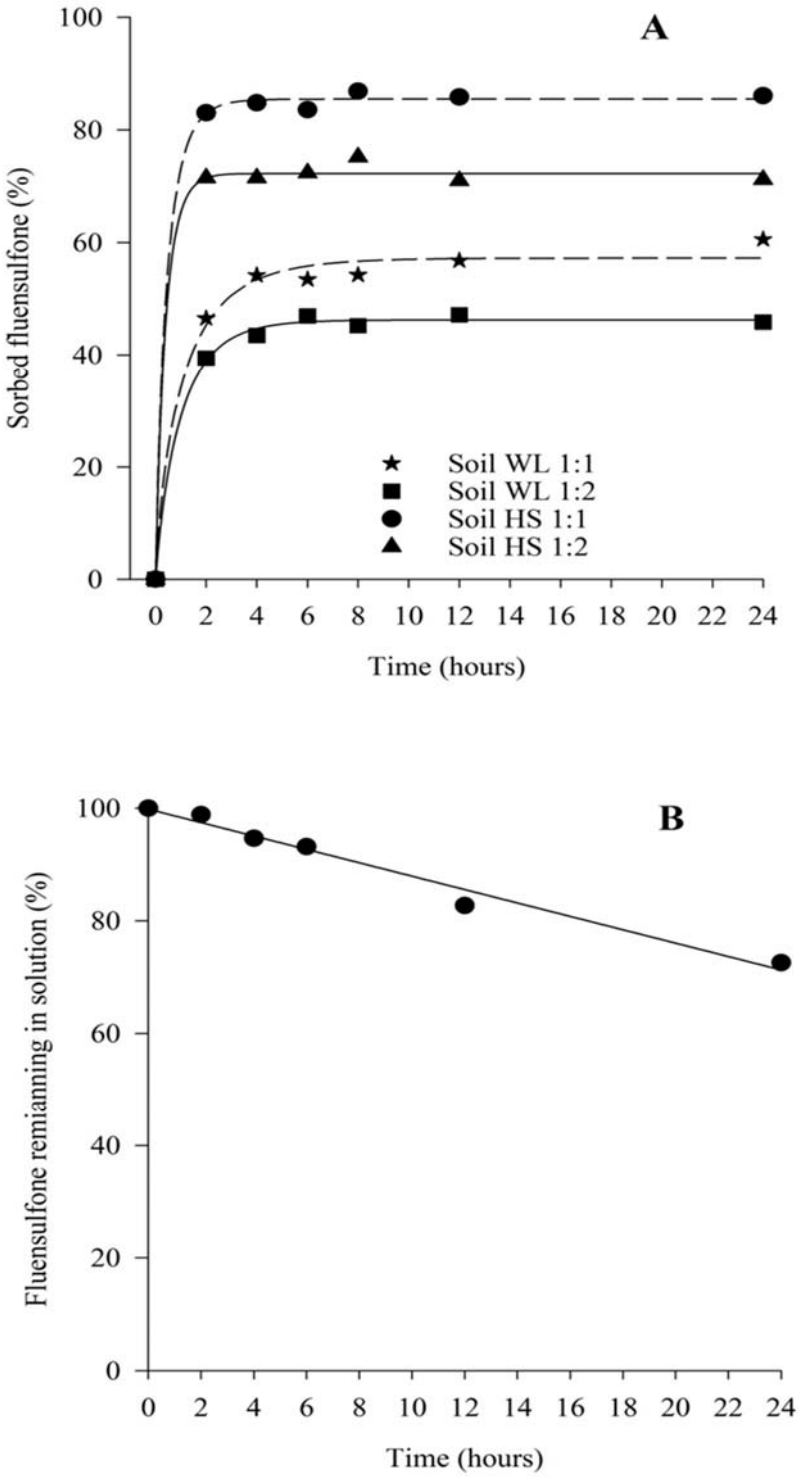


Figure 5.1. (A) Fluensulfone sorption equilibration on two arable soils: a Shropshire sandy clay loam (soil HS) and a 9.6% peat-amended Lincolnshire sandy loam (soil WL) at soil:solution ratios of 1:1 and 1:2 and (B) changes in fluensulfone in 0.01M $CaCl_2$ solution without soil during sorption equilibration at room temperature of 20 ºC.

5.4.6 Laboratory experiment 1: Determination of sorption isotherms

Fluensulfone sorption isotherms were determined in triplicate experiments at four initial concentrations of fluensulfone (C_I = 1.25, 2.5, 5.0 and 7.5 mg L^{-1}) using the original soils (Table 5.2) and soil HS amended with moss peat at five levels (0.0, 1.2, 2.4, 4.8 and 9.6%). The soil suspensions (1/1 soil/solution) were pre-equilibrated over a period of ca.16 h and were spiked with the appropriate volume of 50 mL stock fluensulfone to achieve the above initial concentrations. After shaking for 2h, the suspensions were centrifuged at 3000g for 15 minutes when 1 mL of the supernatant was removed to analyse for the equilibrium concentration (C_E mg L^{-1}). The quantity of fluensulfone sorbed on soil (C_S mg Kg^{-1}soil) was calculated as the difference between C_I and C_E. Sorption isotherms were described by fitting the linear form of the Freundlich sorption model to C_S and C_E (as in Equation 7).

$$\text{Log}\,(C_S) = \text{Log}\,(K_F) + 1/n\ \text{Log}\,(C_E) \qquad (7)$$

where K_F and $1/n$ respectively, are the Freundlich sorption coefficient (sorption capacity) and sorption exponent (sorption intensity). These parameters were determined by regressing Log C_S against Log C_E, with K_F and $1/n$ represented respectively, by the intercept and the slope of the regression equation. Sorption was normalised to soil organic carbon (K_{OC}) using the equation shown in (8) where OC is the organic carbon content of soil.

$$K_{FOC} = K_F \times 100 / \text{OC} \qquad (8)$$

5.4.7 Laboratory experiment 2: Sorption of technical grade and granular fluensulfone

This experiment compared the sorption of fluensulfone in the technical grade and granular form in order to determine if the formulation could affect fluensulfone sorption to soil. The equilibrium sorption coefficients (K_D) were determined for both forms at a single initial concentration of ca. 5.0 mg L^{-1}. The experiments utilised the original soils (see Table 5.2) in the exception of that collected from Gosberton in Lincolnshire. Sample processing and fluensulfone analysis followed the procedure as per Section 5.3.5. After a pre-equilibration of ca. 16h, the soil suspensions were either spiked with 1 mL of 50 mg mL^{-1} stock fluensulfone solution (5.0 mg L^{-1}) or 34 mg of Fluensulfone 15G were added (5.1 mg L^{-1}). In addition to the test suspensions, the setup included two additional samples, both without soil, one of 9 mL $CaCl_2$ spiked with 1 mL of 50 mg mL^{-1}, the other of 10 mL $CaCl_2$ treated with 34 mg of Fluensulfone 15G. These were used to monitor changes in solution concentration of fluensulfone within the period of the experiment since the earlier test in Chapter 4 (Section 4.4.3) showed a gradual release of fluensulfone from the formulation. The samples were shaken for 2 (equilibrium sorption), 4, 6, and 8h when the liquid phase fluensulfone was quantified by HPLC. Sorption at the respective sampling times was determined according to the equation in (6), and K_D was determined by the equation in (9) shown below.

$$K_D = C_S/C_E \quad (9)$$

5.4.8 Laboratory experiment 3: Effects of soil amendment with moss peat on the sorption and desorption of fluensulfone.

This experiment determined the sorption and desorption of fluensulfone in the technical grade and granular form on soil HS amended with moss peat at five levels (0.0, 1.2, 2.4, 4.8, and 9.6%). The procedure for sorption measurements followed that described under Section 5.3.6. Desorption was determined after the last centrifugation and sampling at 8h. The supernatant was removed carefully, and replaced with fresh 10 mL of 0.01M $CaCl_2$ solution. However, due to laboratory time schedule, the suspensions were stored at 4 ºC overnight and returned to the shaker the following day (i.e. approximately 16h after the sorption experiment). Sampling for desorption was made after shaking for 2, 4, and 6h (18, 20 and 22h after sorption experiment). The amount of fluensulfone desorbed was calculated by the equation shown in (10) where, C_S and C_D respectively are sorbed fluensulfone and solution concentrations at sampling.

$$\text{Fluensulfone desorbed (\%)} = [(C_S - C_D)/C_D] * 100 \quad (10)$$

5.5 Polytunnel pot experiment 2 (2012): Effects of organic soil amendments on availability and efficacy of fluensulfone

This experiment was conducted, concurrently, with the Polytunnel experiment 1 (see Chapter 3, section 3.4.1) from May 16 to 28 June, 2012 (ca. 44 DAP). The polytunnel conditions regarding soil and air temperatures, therefore, are same as reported in Section 3.3.4.1 (Chapter 3). The experiment evaluated the effects of soil amendment with moss peat on the control of *G. pallida* by fluensulfone application at the full rate (4.05 kg a.s. ha^{-1}). The assumption was that the peat amendments could enhance sorption of fluensulfone by

the soil and, therefore, could limit availability in the soil water to be effective. This hypothesis was tested by quantifying fluensulfone in the soil using two extraction solvents (water and acetonitrile), with an aim of establishing a relationship between fluensulfone extracted with water and control of *G. pallida*.

5.5.1 Methodology

5.5.1.1 Treatments and experimental design

The experiment had two factors, with peat amendment at four levels (0.0, 1.2, 2.4, and 4.8 %) and fluensulfone treatments at two levels (soil treated with Fluensulfone 15G at 4.05 kg a.s. ha^{-1} or left untreated). The treatments were replicated four times in randomised blocks. The soil amendments were made by mixing the required amount of peat and the soil in a Belle mini 140 cement mixer (Belle Engineering [Sheen] Ltd, Sheen, Derbyshire). The amendments were made seven days prior to soil treatments with fluensulfone and planting of potatoes on May 23, 2012.

5.5.1.2 Analysis of fluensulfone concentration in soil

Soil samples were taken for fluensulfone analysis at 0, 7, 14, 21, 28, and 35 DAP. At each sampling, five random soil cores were taken (ca. 100 g soils) with a cheese corer (2.5 × 30 cm) from the entire depth of the soil and transferred to polypropylene bags. The samples were either extracted shortly afterwards or stored at -20 ºC for future analysis. At each extraction, the samples (thawed overnight at room temperature if frozen) were mixed thoroughly and duplicate 20 g sub-samples transferred to 100 mL shaking bottles to which 20 mL of deionised water (water extraction) or 20 mL acetonitrile (acetonitrile extraction)

was added. The procedure for acetonitrile extraction followed that detailed previously according to section 4.3.3.5 (Chapter 4). For water extraction, the bottles were allowed to stand undisturbed for ca.15 min when 1 mL of the supernatant was removed and passed through a 0.2 µm pore size Polyvinylidene Difluoride (PVDF) syringe filters (GE Healthcare UK Ltd) into HPLC vials to analyse for fluensulfone.

5.5.1.3 Control of *G. pallida* by fluensulfone

Root invasion by *G. pallida* were estimated in a 2-g sub-root-sample as per Section 2.4 (Chapter 2). The number of viable eggs remaining within the cysts and the hatching of J2 were determined as per Section 3.3.5.2 (Chapter 3). The experiment was terminated at 35 DAP when plant growth measurements such as fresh shoot and root weights; numbers of stem and stolon were determined.

5.6 Results

5.6.1 Sorption isotherms and sorption coefficients

Figure 5.2 shows the isotherms obtained for fluensulfone sorption on the six original soils and the peat-amended soil HS. The Freundlich sorption parameters (K_F and $1/n$), the coefficients of determination (r^2) and sorption normalised to soil OC (K_{FOC}) are presented in Tables 5.3 and 5.4. The isotherms describing fluensulfone sorption on the original soils were nonlinear S-type isotherms ($1/n > 1$), according to the classification of Giles *et al*., (1960). The extent of sorption on these soils, expressed by the values of the K_F, varied quite markedly, and was lowest after soil MN (0.72 mg Kg^{-1}) and highest in the HS (1.72 mg Kg^{-1}). Likewise, sorption normalised to soil OC, K_{FOC}, which was lowest at 63 mL Kg^{-1} soil and

highest at 114 mL Kg^{-1} soil. A significant positive correlation (Table 5.5) was observed between K_F and soil OC (r^2 = 0.907; P = 0.013). Good correlations were also seen between K_F and soil clay (r^2 = 0.554) and soil pH (r^2 = -0.744) but these were not significant. The moss peat amendments to soil HS had affected both parameter K_F and $1/n$, both of which, varied according to the level of the amendments. $1/n$ was inversely, but not significantly (r^2 = -0.777; P = 0.123), related to the amendments, with the S-type isotherm, describing sorption on the original soil HS (moss peat at 0.0%) being transformed to an L-type isotherm ($1/n$ <1) on the additions of moss peat at 2.4, 4.8 and 9.6%. The K_F correlated positively and significantly (r^2 = 0.897; P = 0.033) with the amendments, with sorption in the original soil SH been increased 1.4, 2.6, 3.4 and 4 times respectively, by the amendments at 1.2, 2.4, 4.8, and 9.6%.

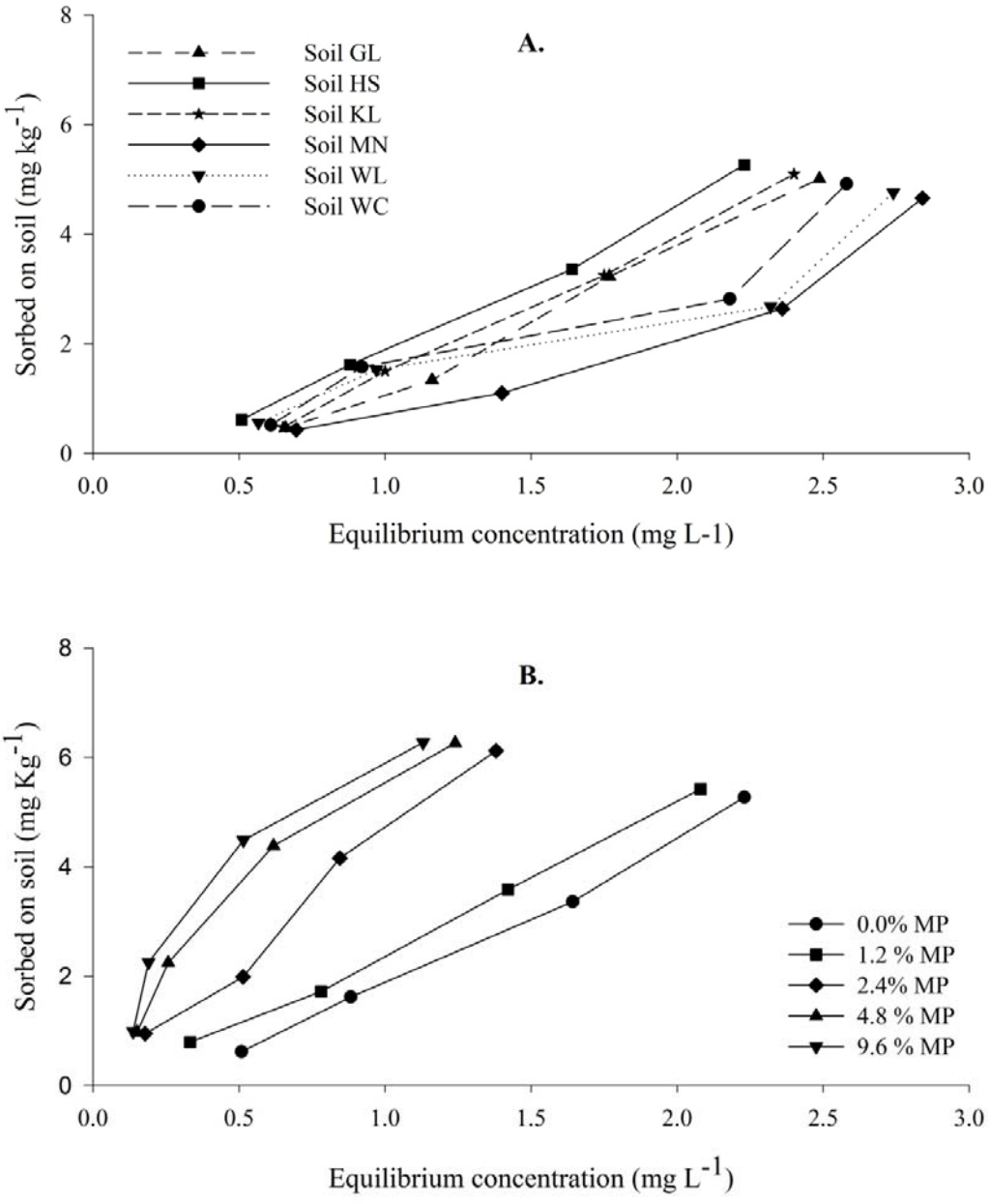


Figure 5.2. Fluensulfone sorption isotherms (A) for six UK arable soils collected from Cambridgeshire (Soil WC), Lincolnshire (Soils GL, WL, and KL), Nottinghamshire (Soil MN), and Shropshire (Soil HS) and (B) for Soil HS amended with moss peat (MP) at five levels.

Table 5.3. Freundlich sorption parameters (K_F and $1/n$), coefficient of determination (r^2) and sorption normalised to soil organic carbon content (K_{FOC}) obtained for fluensulfone sorption by six UK arable soils. Mean parameters are presented with standard error values (n = 3).

Soil	K_F (mL Kg^{-1})	$1/n$	r^2	K_{FOC}
GL	1.02 ± 0.02	1.83 ± 0.08	0.986	87.2
HS	1.72 ± 0.02	1.42 ± 0.08	0.982	114.6
KL	1.19 ± 0.05	1.76 ± 0.20	0.952	98.4
MN	0.72 ± 0.05	1.64 ± 0.16	0.965	63.9
WL	1.25 ± 0.07	1.19 ± 0.21	0.899	88.1
WC	1.25 ± 0.08	1.34 ± 0.28	0.863	92.0

GL: Gosberton, Lincolnshire; HS: Howle, Shropshire; KL: Kirkby, Lincolnshire; MN: Mattersey, Nottinghamshire; WL: West Pinchbeck, Lincolnshire; WC: Wisbech, Cambridgeshire.

Table 5.4. Freundlich sorption parameters (K_F and $1/n$), coefficient of determination (r^2) obtained for fluensulfone sorption on a Shropshire sandy clay loam amended with moss peat (MP). Mean parameters are presented with standard error values (n = 3).

Amendment (%)	K_F (mL Kg^{-1})	$1/n$	r^2
0.0	1.72 ± 0.022	1.42 ± 0.086	0.989
1.2	2.43 ± 0.012	1.06 ± 0.052	0.993
2.4	4.43 ± 0.041	0.93 ± 0.100	0.966
4.8	5.90 ± 0.072	0.86 ± 0.138	0.926
9.6	6.55 ± 0.111	0.81 ± 0.191	0.849

Table 5.5. Correlations between Freundlich sorption coefficient, K_F, and properties of six UK arable soils collected from Cambridgeshire, Lincolnshire, Nottinghamshire and Shropshire.

	% OC	%Clay	%Silt	%Sand	Soil pH
K_F	0.907*	0.554	-0.169	-0.366	-0.744

*: Significant at $P < 0.05$; OC: organic carbon content.

5.6.2 Sorption of fluensulfone in the technical grade and granular forms

Figure 5.3 shows an 8-hour sorption of fluensulfone in the technical grade and granular forms. Sorption of both forms followed similar kinetics, reaching equilibrium within 2h, and was in agreement with the preliminary test. Figure 5.3a shows changing concentrations of fluensulfone in $CaCl_2$ solution without soil. Concentrations of fluensulfone were, generally, higher after the technical grade than the granular formulation. Therefore, sorption estimates were corrected for these differences, accordingly. The equilibrium sorption coefficients, K_D, are shown in Figure 5.4. Regardless of soil type, fluensulfone was sorbed to significantly greater extent ($P < 0.001$) when in the technical-grade form (K_D = 1.37 mg Kg^{-1} soil) than when formulated as granules (K_D = 0.32 mg Kg^{-1} soil). Soil effects on sorption of both form of fluensulfone were also highly significant ($P < 0.001$); K_D was lowest on soil MN and highest on soil WL. The K_D values correlated positively with soil OC (Table 5.6) but significantly only when the technical-grade fluensulfone was used ($r^2 = 0.88$; $P = 0.05$).

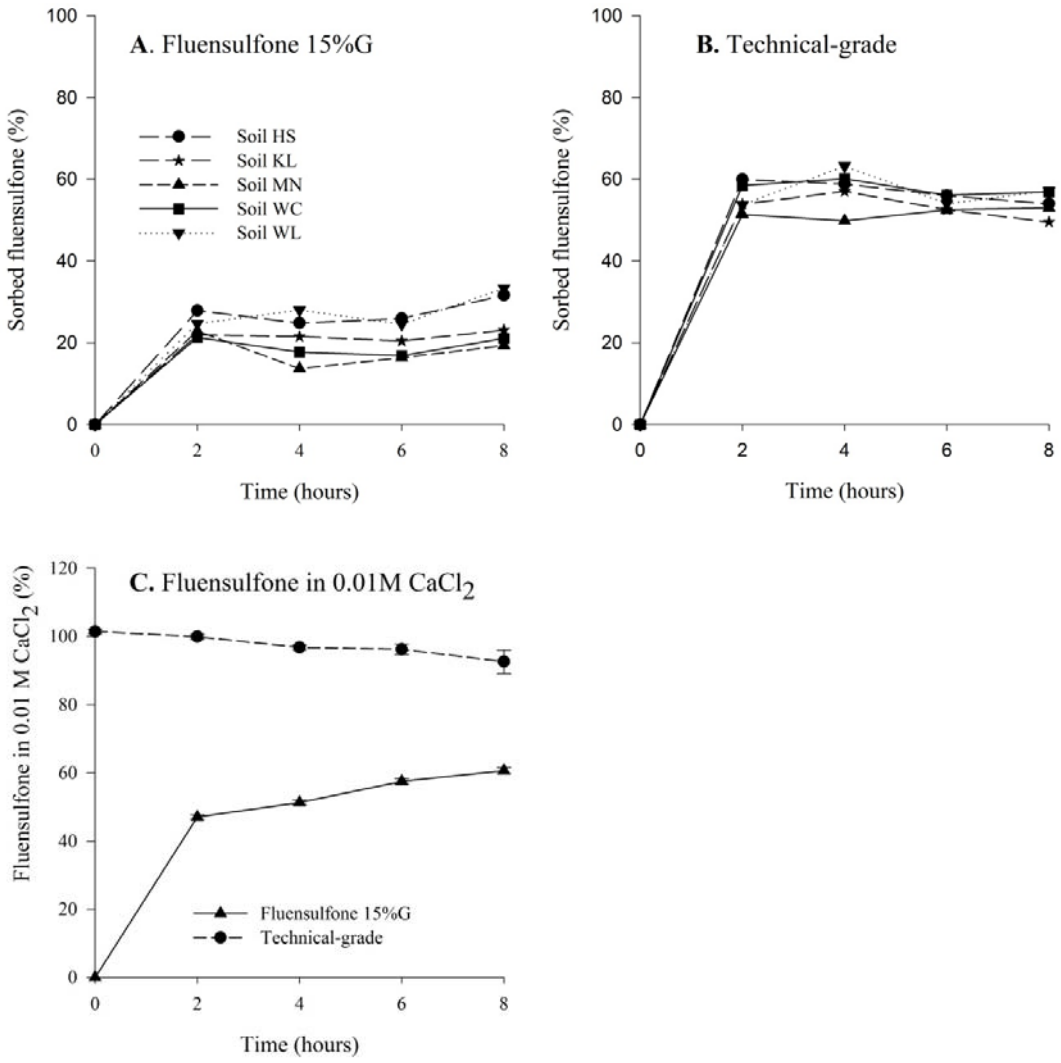


Figure 5.3. An 8-hour sorption of (A) granular and (B) technical-grade fluensulfone by five UK arable soils and (C) changing fluensulfone concentration in 0.01 M $CaCl_2$ solution without soil at room temperature of 20 ºC.

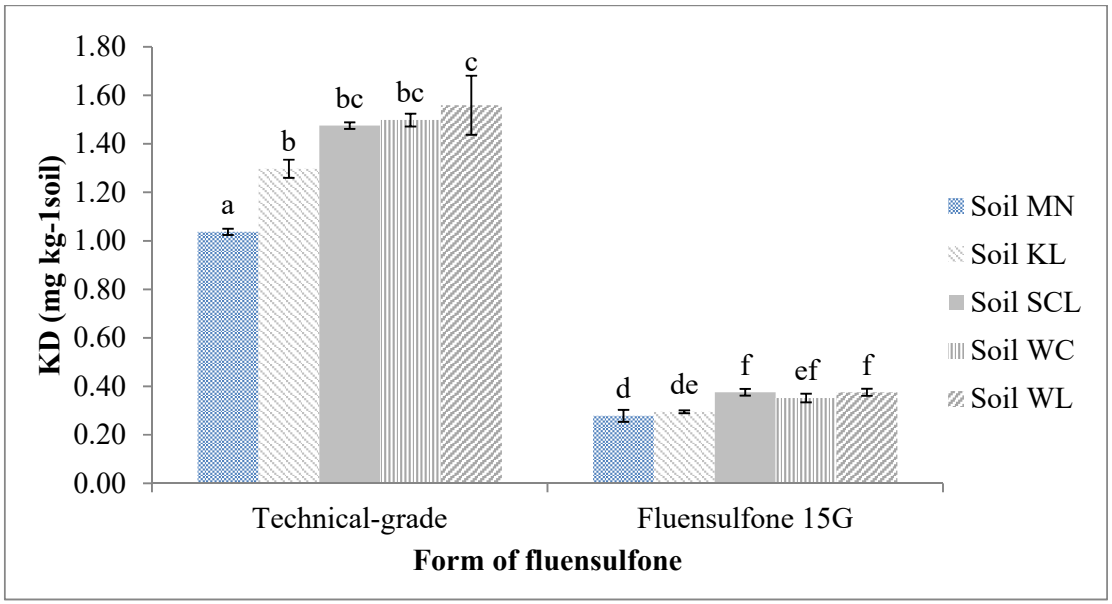


Figure 5.4. Comparisons of equilibrium sorption coefficient, (K_D) obtained for sorption of technical-grade and granular fluensulfone by five UK arable soils. Mean coefficients are presented with standard error values (n = 3).

Table 5.6 Correlations between equilibrium sorption coefficients, K_D, as per sorption of technical grade or granular form of fluensulfone and the properties of five UK arable soils collected from Cambridgeshire, Lincolnshire, Nottinghamshire and Shropshire

	Soil OC	Clay	Sand	Silt	Soil pH
Technical grade	0.88*	0.50	-0.73	0.42	-0.48
Fluensulfone 15G	0.72	-0.09	0.08	-0.01	-0.70

*: significant at $P < 0.05$

5.6.3 Effects of peat amendments on the sorption and desorption of fluensulfone

Figure 5.5 shows the sorption and desorption of fluensulfone on soil HS following amendments with moss peat. Apparently, the kinetic of sorption was similar at all levels of the amendments. As it was observed earlier, sorption was fast and reached equilibrium within 2h. The equilibrium sorption coefficients obtained are presented as plots of K_DT (technical grade) or K_DG (formulation) against percentage peat amendment in Figure 5.6; it shows that K_D significantly increased ($P < 0.05$) on the peat amendments to the soil. The correlations between K_D and amendments were significantly positive ($r^2 = 0.752$; $P = 0.036$ for K_DT and $r^2 = 0.996$; $P < 0.001$ for K_DG). Again, sorption across the soils was significantly greater ($P < 0.001$) after the technical grade than after the granular product. The proportions of fluensulfone desorbed after three desorption cycles are presented in Figure 5.7. Desorption coefficient could not be estimated because desorption equilibrium was not reached within the duration of the experiment. Amounts of fluensulfone desorbed, within a 6 hour-period, are plotted against percentage amendments in Figure 5.8, and it can be seen that amount desorbed, related inversely to the level of amendment.

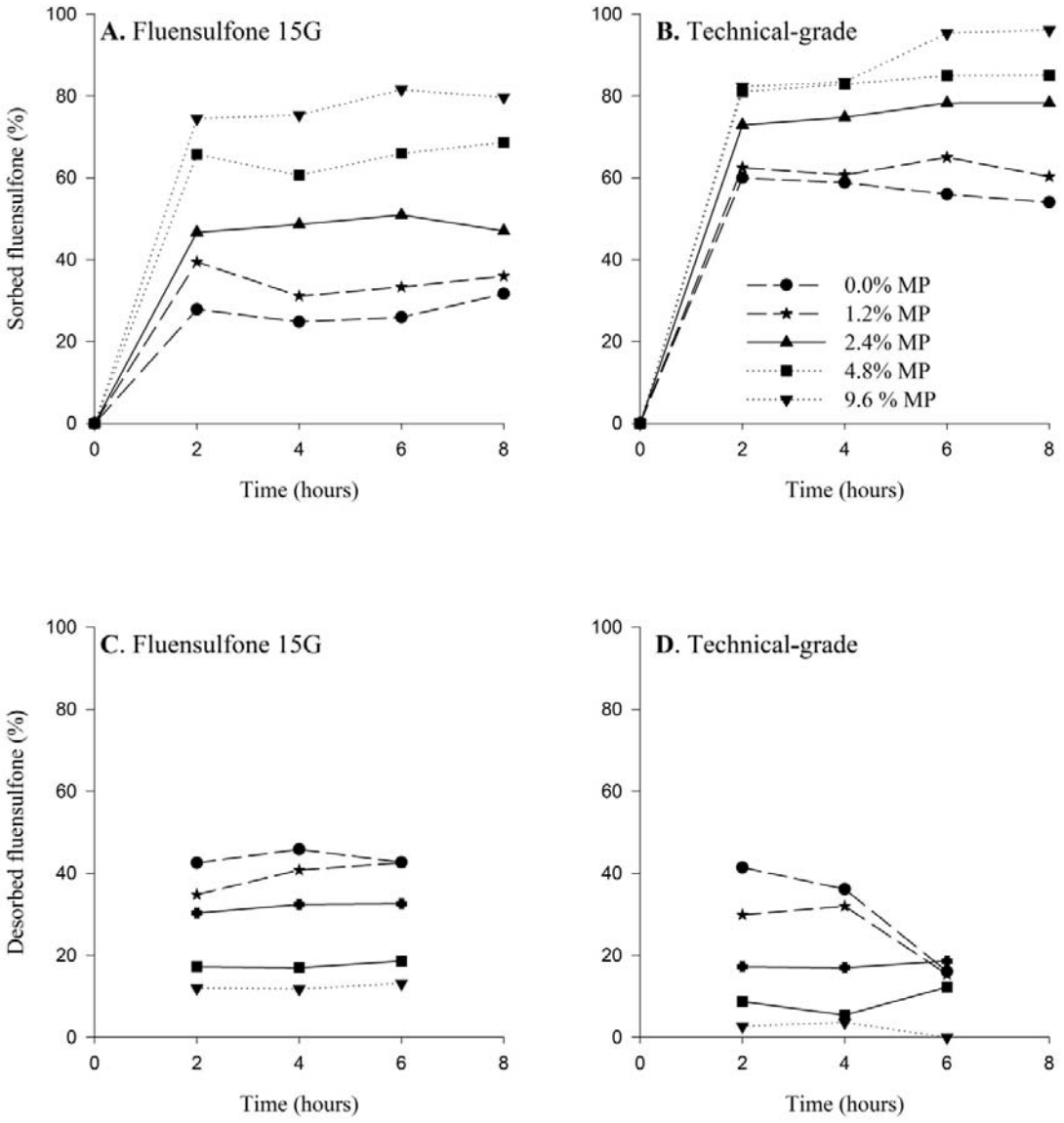


Figure 5.5. Percentage sorption (A and B) and desorption (C and D) of granular and technical-grade fluensulfone on a peat-amended Shropshire sandy clay loam amended with moss peat (MP) at room temperature of 20 ºC.

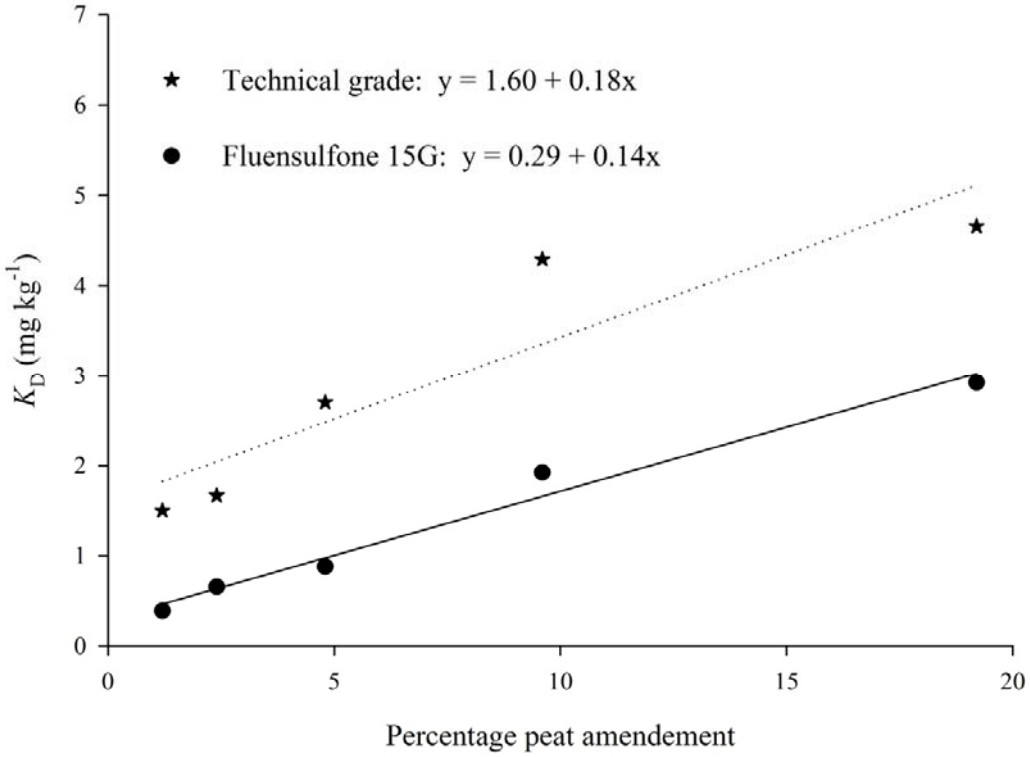


Figure 5.6. Plots of equilibrium sorption coefficient (K_D) determined for fluensulfone sorption in the technical-grade or the granular form (Fluensulfone 15G) against levels of moss peat amendments (MP). Data point are means (n = 3).

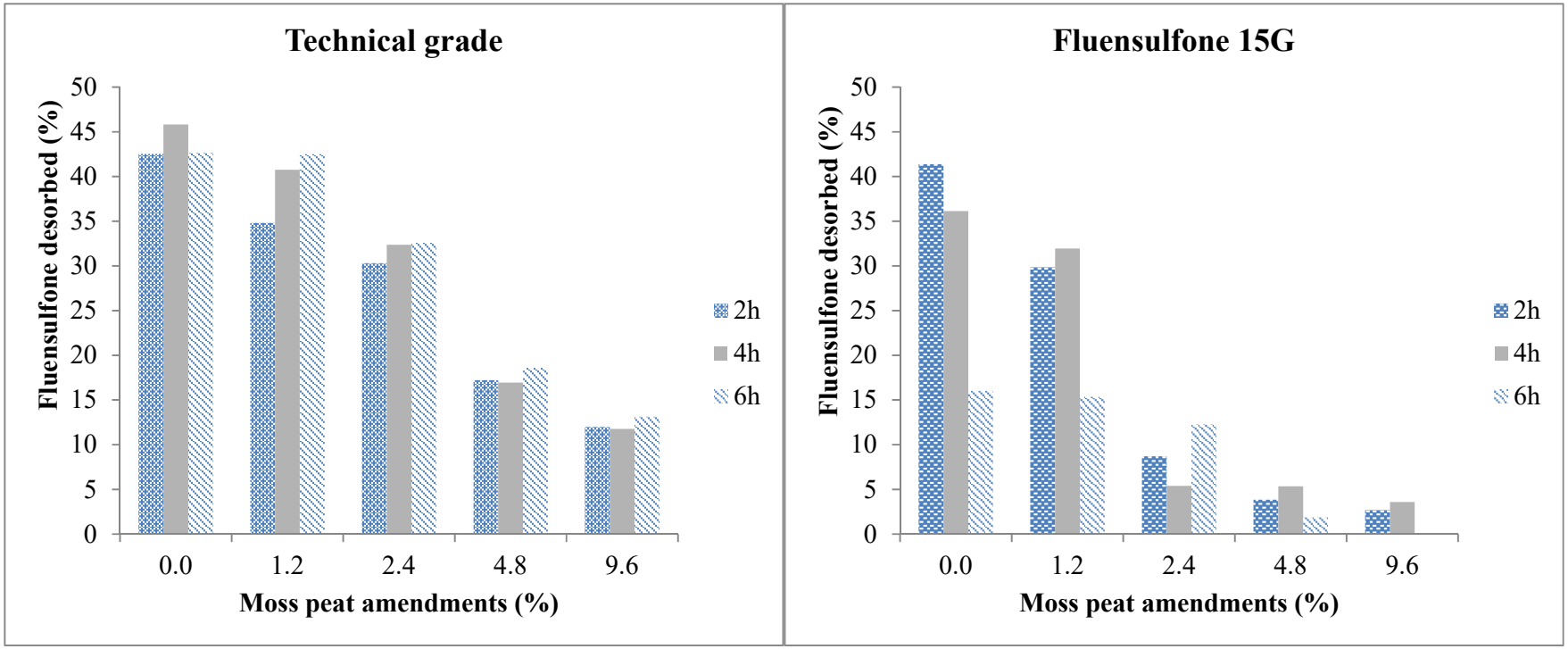


Figure 5.7. Percentage desorbed fluensulfone over a 6-hour period as a function of moss peat amendment. Bars are meansages desorption (n = 3).

5.6.2 Polytunnel experiment

5.6.2.1 Effects of soil amendment with peat on the availability of fluensulfone in soil

Figure 5.8 shows fluensulfone quantified in soil at 7-day intervals over 35 days according to water and acetonitrile extractions. Table 5.7 shows that extraction method and sampling times, but not moss peat amendment, significantly affected the amounts of fluensulfone extracted from soil ($P < 0.001$). Water and acetonitrile extracted fluensulfone throughout the duration of the experiment, but the former gave significantly lower ($P < 0.001$) concentrations at all sampling times. When averaged over the soils, the acetonitrile extraction showed that total fluensulfone available immediately after application averaged 2.24 mg Kg^{-1} soil and was found to have dissipated significantly ($P < 0.001$) within the 35-day duration of the experiment. When averaged over the soils, the initial concentration did not decline significantly until 28 days after application (0.99 mg Kg^{-1} soil). Table 5.8 shows no significant differences between the dissipation rate constant (k) and the DT_{50} values obtained. Nonetheless, there appeared a trend towards decreasing k with increasing level of the amendments. The concentration measured, as per the water extraction, did not differ significantly between sampling times. When averaged over the duration of 35 days, water extracted slightly higher fluensulfone, from the peat-amended soils than from the original soil (0.10, 0.13, 0.11, 0.19 mg L^{-1}, respectively, for MP 4.8%). amendments at 0.0, 1.2, 2.4 and 4.8%).

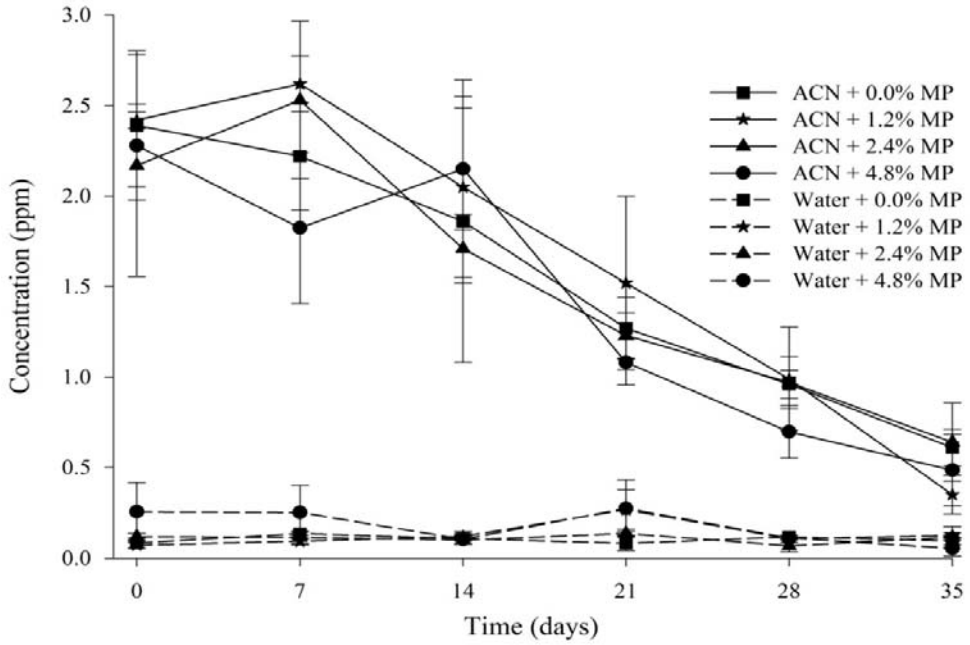


Figure 5.8. Fluensulfone concentration measured in a peat-amended Shropshire sandy clay loam as per acetonitrile and water extractions. Mean concentrations are presented with standard error values.

Table 5.7. Analysis of variance for the effect of soil amendment with peat on the availability of fluensulfone in soil

Mean	Significance	$SED_{DF=94;CV=42.5}$
Soil	$P = 0.763$	0.05
Extraction method	$P < 0.001$	0.03
Time	$P < 0.001$	0.06
Soil*extraction method	$P = 0.315$	0.07
Soil*time	$P = 0.146$	0.11
Extraction method*time	$P < 0.001$	0.08
Soil*extraction method*time	$P = 0.105$	0.16

Table 5.8. The rates of degradation (k) and the time to 50% degradation (DT_{50}) of fluensulfone in peat-amended and non-amended sandy clay loam

MP (%)	Rate of decline *k*, (day^{-1})	DT_{50} (days)
0.0	2.38 ± 0.31	20.4 ± 1.95
1.2	2.54 ± 0.07	20.4 ± 3.69
2.4	2.32 ± 0.41	17.0 ± 3.72
4.8	2.16 ± 0.22	21.8 ± 2.36

5.6.2.2 Control of *G. pallida* by fluensulfone

5.6.2.2.1 Effects of fluensulfone treatment on *G. pallida* egg viability and juvenile hatching

The data did not show significant differences between the numbers of viable eggs that were found in the cysts extracted from the fluensulfone treated-soils and the untreated controls at 44 DAP (Table 5.9). The hatching of J2 from the two group of cysts, over a five-week incubation period in potato root leachate (Figure 5.9), did not vary significantly with the fluensulfone and peat amendments, but varied significantly with the duration of incubation ($P < 0.001$); J2 hatched mostly in the first three weeks. Figure 5.10 shows total juvenile hatch from the cysts.

Table 5.9. Numbers of viable *G. pallida* (eggs and juveniles) remaining in cysts extracted from moss peat-amended (MP) soil treated with fluensulfone compared with the untreated.

Amendment (%)	Fluensulfone		Amendment mean
	Untreated	Treated	
0.0	8.16	8.09	8.129
	(3295)	(3540)	(3418)
1.2	8.2	7.71	7.955
	(2455)	(3966)	(3211)
2.4	7.78	7.78	7.779
	(2735)	(3109)	(2922)
4.8	8.42	8.25	8.336
	(3872)	(4604)	(4238)
Fluensulfone mean	7.958	8.141	
	(3089)	(3805)	

MEAN	Significance	SED	CV%	DF
Amendment	$P = 0.13$	0.23	5.8	21
Fluensulfone	$P = 0.27$	0.16	5.8	21
Amendment*fluensulfone	$P = 0.73$	0.33	5.8	21

Back transformed numbers are shown in parentheses

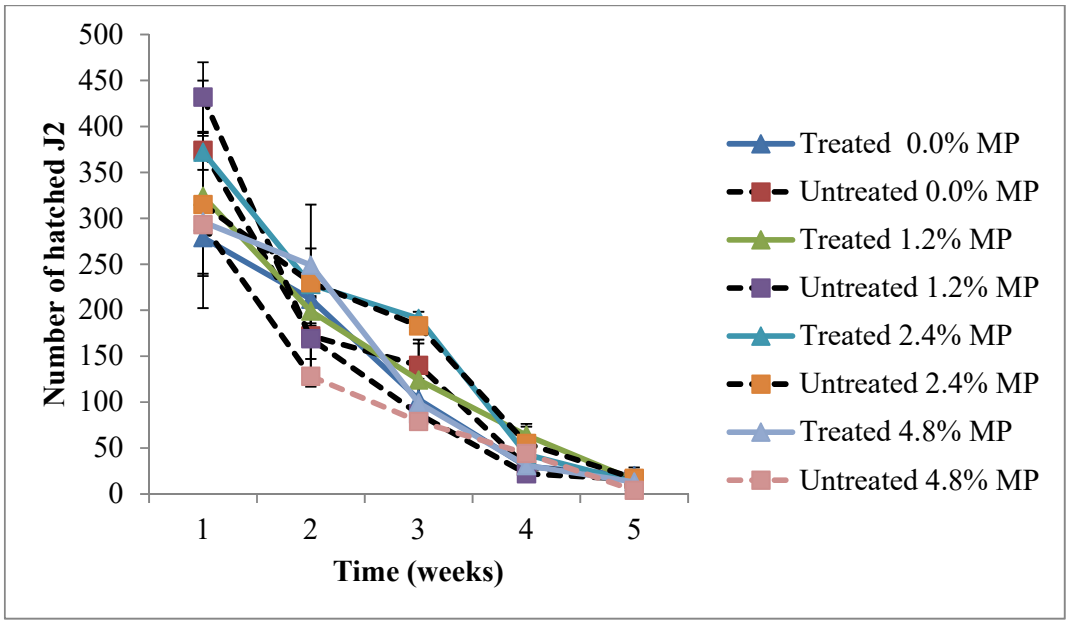


Figure 5.9. Emergence of *G. pallida* from cysts extracted from peat-amended soil either treated with fluensulfone or left untreated for 35 days. Error bars are the standard error of the mean (n = 4)

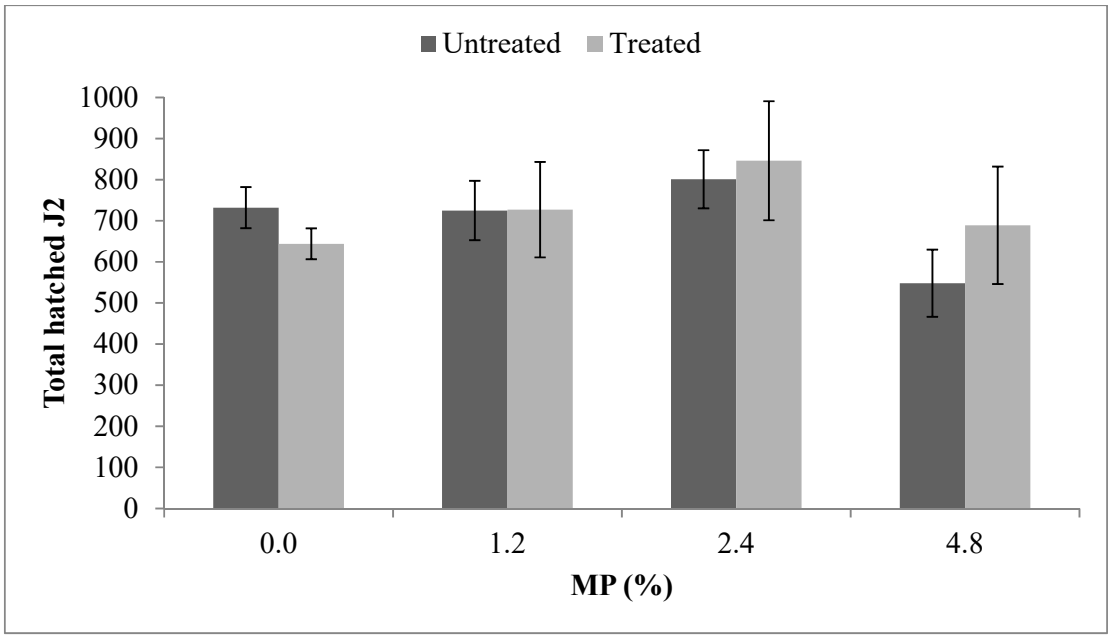


Figure 5.10. Total *G. pallida* hatch from cysts extracted from peat-amended soil either treated with fluensulfone or left untreated for 35 days. Error bars are the standard error of the mean (n = 4)

5.6.2.2.2 Root invasion by *G. pallida*

Figure 5.11 and Table 5.10 respectively, show the total numbers of *G. pallida* and juvenile stages per gram root, at 35 DAP. Root invasions were reduced in all fluensulfone treated soils but significantly ($P = 0.002$) just in the unamended soil and that amended with MP at 9.6% when compared with the untreated controls. No significant effect was found for either the peat amendments or the treatment interactions on root invasion. Nonetheless, when considered across the fluensulfone treated soils, there appeared a trend towards increasing fluensulfone effect on total root invasion (Figure 5.11) with increasing level of the amendment. Significantly reduced numbers of J2 ($P < 0.001$), J3 male ($P = 0.046$), J5 female ($P = 0.017$) and J5 male ($P = 0.012$) stages per g^{-1} root were recorded after the fluensulfone treatment in comparison to the untreated control (Table 5.10). The soil amendment significantly decreased the numbers of J2 ($P < 0.001$) and J5 female ($P = 0.015$) stages per gram root and an inverse relationship was apparent between the number of J2 and level of peat amendments.

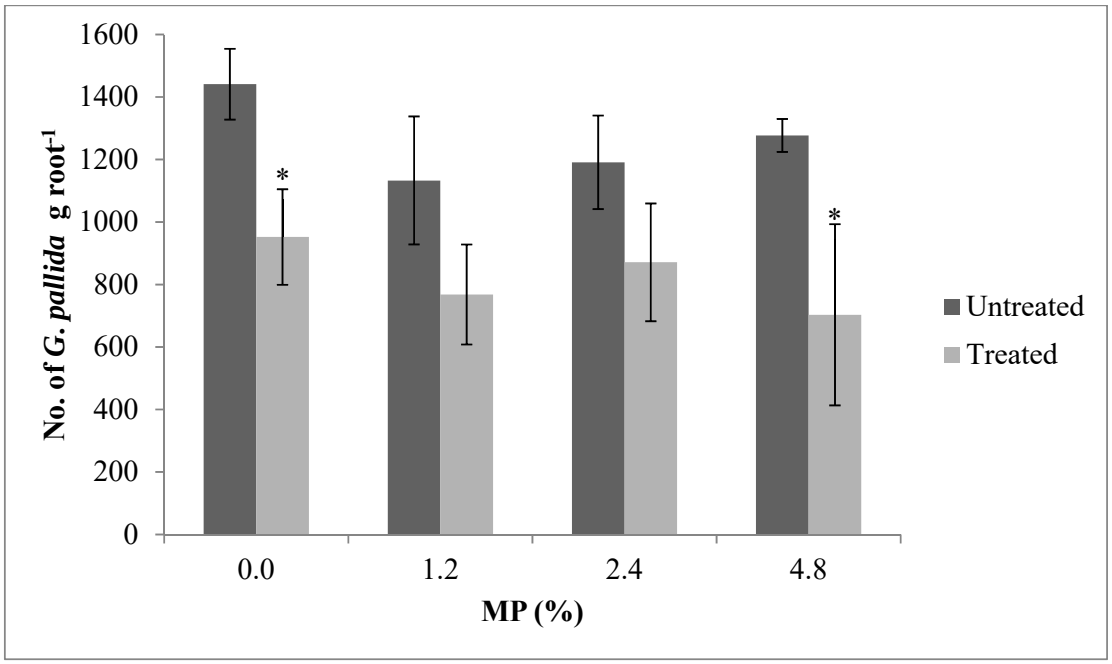


Figure 5.11. Invasion of potato (*cv* Estima) by *G. pallida* in peat-amended or unamended soil treated with fluensulfone or left untreated for 35 days. Error bars are the standard error of the mean

Table 5.10. Number of *G. pallida* stages per gram root (back transformed data) of potato (*var*. Estima) after 35 days of growing in soil amended or unamended with peat and treated with fluensulfone or soil left untreated.

Treatment	J2	J3		J4		J5	
		Female	Male	Female	Male	Female	Male
Amendment (%)							
0	371.0	88.3	65.5	195.0	116.0	296.0	82.0
1.2	179.0	101.5	37.0	188.0	97.0	233.0	154.0
2.4	161.0	93.7	55.1	188.0	169.0	162.0	123.0
4.8	125.0	111.1	52.0	184.0	223.0	159.0	178.0
$SED_{DF=21}$	38.5***	27.78	13.07	58.4	44.1	33.7*	39.9
FLUENSULFONE							
Untreated	274.0	112.4	61.4	221.0	170.0	252.0	175.0
Treated	144.0	84.9	43.4	156.0	132.0	173.0	94.0
$SED_{DF=21}$	27.2***	19.64	9.24*	41.3	31.2	23.8*	28.2*
FLUENSULFONE X AMENDMENT INTERACTION							
Untreated MP 0.0%	458.0	104.2	79.5	229.0	143.0	333.0	115.0
Treated MP 0.0%	283.0	72.5	51.5	161.0	89.0	258.0	50.0
Untreated MP 1.2%	240.0	120.8	44.8	227.0	118.0	310.0	217.0
Treated MP 1.2%	119.0	82.2	29.1	149.0	77.0	156.0	92.0
Untreated MP 2.4%	215.0	108.3	66.7	235.0	173.0	177.0	158.0
Treated MP 2.4%	108.0	79.0	43.5	140.0	164.0	146.0	87.0
Untreated MP 4.8%	183.0	116.3	54.5	194.0	248.0	186.0	209.0
Treated MP 4.8%	66.0	105.9	49.5	173.0	197.0	132.0	148.0
$SED_{DF=21}$	54.5	39.29	18.48	82.6	62.4	47.6	56.4

***; ***:** Significant at $P < 0.05$and $P < 0.001$, respectively

5.6.3 Plant growth

The growth of Estima did not differ significantly in fluensulfone-treated soils and those left untreated (Tables 5.11 – 5.14), except for stem number (Table 5.14), which was significantly affected by amendment x fluensulfone interaction ($P < 0.001$).

Table 5.11. Effects of soil amendments with peat and fluensulfone treatment on fresh shoot weight (Log_e transformed) of potato (*cv*. Estima) grown for 44 days in *G*. *pallida* soil infected.

Amendment (%)	Treated	Untreated	Amendment mean
0.0	4.14	4.12	4.13
	(65.7)	(62.70)	(64.2)
1.2	4.06	3.76	3.91
	(60.4)	(51.30)	(55.8)
2.4	4.20	3.97	3.99
	(56.3)	(54.9)	(55.6)
4.8	4.13	3.68	3.91
	(66.0	(54.9)	(53.0)
Grand mean	4.13	3.88	
	(62.1)	(55.20)	

MEAN	Significance	SED	DF	CV%
Amendment	$P = 0.604$	0.19	21	9.6
Fluensulfone	$P = 0.332$	0.14	21	9.6
Amendment*fluensulfone	$P = 0.965$	0.27	21	9.6

Back transformed means are in parentheses

Table 5.12. Effects of soil amendments with peat and fluensulfone treatment on fresh root weight (Log_e transformed) of potato (*cv. Estima*) grown for 44 days in *G. pallida* soil infected.

Amendment (%)	Treated	Untreated	Amendment mean
0.0	2.42	2.33	2.38
	(11.54)	(10.66)	(11.10)
1.2	2.03	1.97	2.00
	(7.99)	(7.40)	(7.70)
2.4	2.36	2.08	2.22
	(11.30)	(10.18)	(10.74)
4.8	2.40	1.87	2.14
	(11.07)	(6.92)	(8.99)
Fluensulfone mean	2.30	2.06	
	(10.47)	(8.79)	

MEAN	Significance	SED	D.F.	CV%
Amendment	$P = 0.259$	0.23	21	20.9
Fluensulfone	$P = 0.216$	0.16	21	20.9
Amendment*fluensulfone	$P = 0.758$	0.32	21	20.9

Back transformed means are in parentheses

Table 5.13. Effects of soil amendments with moss peat (MP) and fluensulfone treatment on stolon number (Log_e transformed) of potato (*cv.* Estima) grown for 44 days in *G. pallida* soil infected.

Amendment (%)	Treated	Untreated	Amendment mean
0.0	1.75	1.89	1.82
	(6.50)	(6.83)	(6.67)
1.2	1.95	1.51	1.73
	(7.54)	(6.06)	(6.80)
2.4	2.14	2.22	2.18
	(8.55)	(9.56)	(9.06)
4.8	2.13	1.02	1.57
	(9.15)	(3.37)	(6.26)
Fluensulfone mean	1.99	1.66	
	(7.93)	(6.46)	

MEAN	Significance	SED	D.F.	CV%
Amendment	$P = 0.394$	0.30	21	33.5
Fluensulfone	$P = 0.245$	0.22	21	33.5
Amendment*fluensulfone	$P = 0.456$	0.43	21	33.5

Back transformed means are in parentheses.

Table 5.14. Effects of soil amendments with moss peat (MP) and fluensulfone treatment on stem number (Log_e transformed) of potato (c*v.* Estima) grown for 44 days in *G*. *pallida* soil infected. Untransformed means are in parentheses.

Amendment (%)	Treated	Untreated	Amendment mean
0.0	1.43	1.24	1.33
	(4.25)	(3.50)	(3.88)
1.2	1.68	0.80	1.24
	(5.50)	(2.25)	(3.88)
2.4	1.07	1.56	1.31
	(3.25)	(5.00)	(4.12)
4.8	1.14	1.56	1.35
	(3.25)	(5.00)	(4.12)
Fluensulfone mean	1.33	1.29	
	(4.06)	(3.94)	

MEAN	Significance	SED	DF	CV%
Amendment	$P = 0.964$	0.17	21	25.3
Fluensulfone	$P = 0.797$	0.12	21	25.3
Amendment*fluensulfone	$P = 0.003$	0.23	21	25.3

5.7 Discussion

5.7.1 Sorption kinetics

The sorption of fluensulfone in the technical grade and granular forms, apparently, occurred in two phases; an initially fast sorption accounting for most sorption was followed by slower sorption. Fluensulfone was sorbed, mostly within the first two hours of making contacts with soil. This fast sorption of fluensulfone, as observed here, would imply that a rapid removal from soil solution is likely as fluensulfone becomes available in the soil. Sorption, in this case, may become a limiting factor to availability in the soil solution if it manifests strongly and is not followed by desorption. Otherwise, the fast sorption could probably retain fluensulfone in the soil for longer by guarding against loss due to processes such as leaching. Two-phase sorption has been widely reported in the literature (E.g. Kookana *et al*., 1992; Ball *et al*., 1991; Cancela *et al*., 1990). The initially fast sorption has been ascribed to physical attachment of compound onto the external surfaces (adsorption) of sorbing materials (Hung *et al*., 1996), and the slower phase is thought to be due to slow intra-particle diffusion of chemical species (Alexander, 1995). If sorption of fluensulfone on the soils were by adsorption, which accumulates compounds at the soil-liquid interface (Koskinen and Harper, 1990), then even when sorbed, fluensulfone may be easily accessible to percolating soil water, and plausibly, be available readily for distribution in the soil. Barbercheck and Duncan, (2004) and Koenning and Snipes, (1998) alluded to the fact that movement of nematodes through soil is most effective when soil moisture content is low and soil particles are covered with a film of water. Perhaps, an adsorption driven sorption could concentrate fluensulfone around sorbing soil particles, thereby exposing the host seeking J2 to higher dosage. On the other hand, however, easy availability to soil water may suggest vulnerability to leaching. Furthermore, Scow and Johnson (1996) suggested that most aerobic organisms and activity are mostly associated with outer surface of soil particles. It could as well be that

adsorption could shorten persistence in the soil by enhancing availability to microbial degraders.

5.7.2 Sorption isotherms, sorption coefficients and desorption of fluensulfone

Isotherms are commonly used to assess the sorption of organic compounds into soil (Thompson and Goyne, 2012) and may be useful indicators of the underlying sorption mechanism (Calvet, 1989). Sorption of compounds, exhibiting low water solubility, have been suggested to manifest mainly by hydrophobic partitioning on soil organic matter (Murphy *et al*., 1990) and, yielding linear isotherms (Chiou *et al*., 1998;). Even though the soil organic matter explained most of the variations seen in sorption of fluensulfone, the isotherms observed were essentially, nonlinear ($1/n \neq 1$), and could suggest that the process may not have been predominantly by hydrophobic partitioning. An S-type isotherm denotes easier sorption as solution concentration increases (Dell Site, 2001), and is characteristic of sorption of organic compounds onto a surface with low sorbing potential (Sposito, 1984). This condition relates, more often, to the adsorption of organic compounds onto clay surfaces (Patakioutas and Albanis, 2002; Torrents and Jayasundera, 1997; Weber *et al*., 1986). Consequently, the clay fraction may be important in the sorption of fluensulfone. Even though the K_F and soil clay were not significantly correlated, 55% of the total variation seen in sorption were due to the soil clay (see Table 5.5) indicating probable involvements of the soil's mineral fraction in the sorption of fluensulfone. The transformation of the S-type ($1/n >1$) isotherm, describing sorption on the original soil HS, to an L-type isotherm ($1/n < 1$) on the peat amendments to the soil provided greater evidence of probable contribution of the soil clay to fluensulfone sorption onto the soils. The addition of peat may have had a diminishing effect on the contribution of clay to the sorption process, which could be expected as the proportion of clay in the soils is diluted on the additions of peat. The

suggestion, therefore, is that sorption of fluensulfone may not be entirely due to hydrophobic partitioning but also by adsorption onto the mineral fraction, mainly clay.

The extent of fluensulfone sorption varied widely between the original soils, and the differences were due mainly to their organic matter content. Nonetheless, sorption was generally low across the soils suggesting low sorbing potential for fluensulfone to soil. The extent to which organic compounds sorbed onto soil are known to depend on both pesticide and soil properties (Zheng *et al*., 2010; Spark and Swift, 2002; Kile and Chiou, 1999; Johnson *et al*., 1995; Lafrance *et al*., 1994). The agreement between fluensulfone sorption and soil organic matter emphasises the influence hydrophobicity of fluensulfone could have on its affinity for soil. Inferences from the properties of fluensulfone (Table 5.1) would suggest moderate hydrophobicity and could be related to the low sorption across the soils. Amending soil HS with peat significantly increased this soil's capacity for sorbing fluensulfone. Even then, it is worth noting that the increases in sorptivity, with respect to the levels of the amendment, were not particularly high, and may be a further indication of potentially low affinity of fluensulfone for the soils. This low sorption implies that substantial amounts of fluensulfone might remain in solution for distribution and biological activity. Furthermore, the generally low K_{FOC} values would indicate that fluensulfone may be mobile in the soil and therefore, may not be a difficult nematicide to distribute by percolating soil water. The assumption, therefore, is that sorption, as measured under the current conditions, may not constitute a limiting factor to the efficacy of fluensulfone in controlling *G. pallida*. The desorption data showed that reversibility of fluensulfone sorption was very likely and suggested probable weak sorption of fluensulfone to the soils. Clearly, the proportion of desorbed fluensulfone was inversely related to the levels of the peat amendments, and as far as control of *G. pallida* is concerned, a direct relationship between fluensulfone sorption, as well as desorption and organic matter would suggest that soil organic fraction may mainly influence fluensulfone efficacy.

It was evidently clear from the comparative study of sorption of the technical-grade, and the granular product that the formulation had decreased fluensulfone sorption by the soils, and was attributable to reduced availability of fluensulfone in solution after the formulation. Indeed, granular formulations release active substances gradually (Flury, 1996), and is evidently the case for this formulation (see Chapter 4, section 4.3.2). The lower sorption exhibited by the granular product would suggest that studies involving the technical-grade fluensulfone could over-estimate sorption in potato beds receiving treatments of this formulation. Coupling effects of limited availability and fast sorption may further suggest how low soil solution concentrations could be expected when the granules are applied to potato beds.

5.7.3 Effects of soil amendments with peat on availability and efficacy of fluensulfone

5.7.3.1 Availability of fluensulfone in soil

The soil concentrations of fluensulfone, according to the acetonitrile extraction, indicate that fluensulfone dissipated within the period of the experiment, but not at significantly different rates across the soils. However, a trend towards a decreasing rate of loss with increasing additions of peat was apparent and could be ascribed to the retention of fluensulfone in the amended soil because of sorption. Sorption is reported for limiting bioavailability of pesticides in soil (Guo *et al*., 2000; Guo *et al*., 1999; Barriuso *et al*., 1997) and so could the proportions of fluensulfone available, for dissipation, decrease as sorption is enhanced on the addition of peat. Fluensulfone measured, as per the water extraction, is presumed to represent that which was available in the soils' aqueous phases, and the data as presented herein, did not provide evidence to suggest limiting effects of sorption on the availability of fluensulfone in the soil solution. Instead, slightly higher concentrations were measured in the peat-amended than in the unamended control soil. Many studies, for example, Chen *et*

al., (2010), Ding *et al*., (2011), Song *et al.,* (2008) and Cox *et al*., (2007) have suggested that organic amendments introduce soluble and colloidal organic matter into soil, which may favour the entry of sorbed compounds into solution. Since fluensulfone sorbed, mainly on organic matter, the presence of such materials upon the amendment could enhance its availability in the soil solution. Generally, however, extractable fluensulfone by water throughout the period of the experiment was limited to substantially low concentrations (11 - 17% of total available concentration), which, as suggested earlier, could most probably be due to coupling effect of regulated release by the formulation, sorption onto soil and, perhaps, losses due to degradation.

5.7.3.2 Control of *G. pallida*

The treatment with Fluensulfone 15G at 27 kg ha^{-1} reduced the invasions of Estima by *G. pallida,* but satisfactory controls were recorded in just the original soil SH (non-amended control) and that amended with peat at 9.6%. Apparently, the treatment did not significantly affect hatching activities of *G. pallida* that remained within the cysts extracted from the soils at 35 DAP. Overall, the data did not provide any strong evidence to suggest that the peat amendments limited the activities of fluensulfone against *G. pallida*. With the effects of the fluensulfone treatment on root invasion, the results agree, somewhat, with the concentration of fluensulfone measured in the soil as per water extraction; slightly greater fluensulfone was available in the amended than the original soil. This greater availability is probably due to retention of fluensulfone by sorption. Since a direct relationship exists between nematicide efficacy and dosage (Hague and Gowen, 1987), greater fluensulfone activity in the amended soils was a possibility. The average soil solution concentrations measured across the soils within the 35-day period of the experiment (0.10 – 0.19 mg L^{-1}) was well within the range of 0.0078 - 1.0 mg L^{-1} suggested by Deliopoulos *et al*., (2009) for immobilizing J2 *G. pallida*

in vitro. This would suggest that the additions of peat to soil HS did not limit availability of fluensulfone in the soil for control root invasion by the J2. Besides reducing invasion, there were significant reductions in J3 and J5 stages as well, which is in agreement with the results from the field and Polytunnel experiment in Chapter 3, supporting the earlier suggest that fluensulfone soil treatments could interfere with the root invasion activities of J2, as well as development thereof within the root. There was a notably decrease in the numbers of J2 and J5 by the peat amendments. This may well be associated with the addition of organic matter to the soil. Evidence in the literature suggest that addition of organic material to soil introduces nematode antagonists or provide a substrate for the development of these organisms (Oka, 2007; Thoden *et al*., 2011; Viaene *et al*., 2006; Bulluck *et al*., 2002; Chen *et al*., 2000). Others such as Oka, (2010) and Akhtar and Malik, (2000) suggested that the decomposition of organic material may yield compounds with nematicidal properties.

5.8 Conclusion

The sorption of fluensulfone was generally low on soils investigated in this study, despite contrasting soil properties. This may suggest that sorption may not be a limiting factor to the efficacy of fluensulfone in controlling of *G*. *pallida*, at least in soils with properties similar to the ones investigated here. Soil organic matter was identified as the main factor influencing fluensulfone sorption under the conditions of this study. However, the study could not demonstrate limiting effects of the organic amendments on the efficacy of fluensulfone in controlling *G*. *pallida* under the polytunnel conditions.

CHAPTER 6

GENERAL DISCUSSION AND RECOMMENDATIONS FOR FUTURE STUDIES

6.1 General discussion

This study has demonstrated that fluensulfone applied to soil can prevent the invasion of potato roots by *G. pallida* and that the treatments may suppress population increases. This finding is evidence in support of acceptance of the hypothesis that fluensulfone possess sufficient nematicidal activity for control of *G. pallida* in-field. Although the field experiments did not determine effects of fluensulfone on the encysted *G. pallida,* the polytunnel experiments suggested no significant treatment effects on hatching of the J2. Therefore, fluensulfone is more effective at suppressing the activities of the J2 in the soil than within the cyst. The attributes demonstrated for fluensulfone *in vitro* (Oka *et al*., 2013; Oka *et al* 2012; Oka *et al*., 2009 Holden-Dye and O'Connor, 2010 and Deliopoulos *et al*., 2009) implied that activities against host finding and root invasion were probable. Thus, impairments of the J2's perception of root stimuli and ability to migration towards root and to invade are expected. It is not clear though if at the current rates, fluensulfone would be available in doses deemed lethal to the J2 in the soil. However, the fact that the crop was invaded in the fluensulfone-treated soil, and there was increased invasion by the second assessment at six weeks after planting, indicated that the hatching J2s had survived the available concentrations to, successfully, invade the roots. Thus, implying nematistatic rather than nematicidal effects suggested *in vitro* (Deliopoulos *et al*., 2009). The polytunnel experiment 3, for instance, provided clues as to what concentrations could be expected in solution when granules are mixed into soil, when soil moisture was near the field capacity; it suggested, substantially, low concentrations, which, may be lowered further when subjected to degradation and leaching processes, as well as dilution by percolating rain and/or irrigation water, for instance, in field plots. Presumably, therefore, exposure to lower than lethal dosage is most likely.

Reducing the size of potential inoculum, with respect to the number of J2 that invades host plants, is one, if not the main principle underlying soil treatments with nematicides (Schomaker and Been, 2013; Trudgill *et al*., 2003). The rational being the direct relationship between the invasion activities of the J2 and yield losses (Whitehead, 1998; Elston *et al*., 1991). If fluensulfone could limit the chances of the J2 in finding and infecting developing potato roots, then, its application to soil would serve the purpose of protecting the developing potato from invasion damages by the PCN, and thus, could improve tuber yield. This suggestion was not supported by the experiment at Woodcote, but at Howle, where there was increased in yield following the fluensulfone, as well as the fosthiazate and oxamyl treatments. The mean ware yield for the fluensulfone treated-Estima in the field experiments 2 and 3, for instance, increased by 61.5 and 57.7% vis-à-vis decreases in root invasion of 44 and 43%, respectively. Similarly, the yield of fluensulfone treated-Santé was about two-fold (107.5%) greater in relation to 52.5% reductions in the root invasion. Furthermore, the data for the polytunnel experiment 1 and 3 showed improved shoot and root biomasses in the highly infested soil. There is, therefore, reasons to accept the suggestion that, fluensulfone soil treatments could protect the potato from infections by PCN. The question, however, as to which dosage of granular fluensulfone would provide a viable control of *G. pallida* could not be answered with certainty due to variable treatment effects. Nonetheless, the application of the full rate in the granular form, appear to be a more robust treatment than the lower rates of 1.95 and 3.00 kg a.s ha^{-1}. When applied in the granular form, for instance, the full rate had decreased the invasion of the potatoes grown in the moderately infested fields (mean *Pi* ranged from 9.6 – 16.2 eggs g^{-1} soil) at Woodcote (*cv* Estima) and Howle (*cv*s Estima, Santé and Vales Everest), by both sampling times of four and six weeks after planting. The same treatment was effective in reducing invasion of Estima in the highly infested-soil (*Pi* = 50 eggs g^{-1} soil) in the Polytunnel experiment 1 in Chapter 3, and the control seen in this experiment was consistent with that achieved in the same soil in the Polytunnel experiment

2 in Chapter 5. Furthermore, the full rate in the EC form, significantly and consistently reduced the invasion of Estima at the Woodcote and Howle sites (see Chapter 3). Regarding suppression of the build-up of the population, the current data suggested only partial control on the susceptible Estima by the application of the full rate. Even though this control was not, consistently achieved, no evidence was shown for significantly greater efficacy at the application rates higher than the full rate. Therefore, the 5.05 and 6.00 kg a.s. ha^{-1} treatments cannot be justified.

The treatments of fluensulfone in this study, more specifically, the 4.05 kg a.s. ha^{-1} rate, gave controls of *G. pallida*, which paralleled those obtained from the application of either of the two currently available commercial nematicides for PCN, suggesting that fluensulfone has comparable efficacy. Even though the treatment of fluensulfone did not significantly increased tuber yield across the experiments, there was, generally, no better performance from fosthiazate and oxamyl as well. It has been acknowledged that the current UK potato production system hinges on the availability of nematicides for PCN. The uncertainty surrounding the future availabilities of fosthiazate and oxamyl, as per EU reviews regarding health and environment hazards associated with their usage however, coupled with the fact that there are, as at yet, alternative treatments to protect the potato from PCN damages, will increase demand for new molecules, such as fluensulfone with far lower environmental toxicity. Even though the research into the nematicidal efficacy of fluensulfone is at its early stages, there is evidence in this study to suggest that soil treatment with fluensulfone for control PCN is feasible. Even if the use of fosthiazate and oxamyl continues, an addition of fluensulfone to the list of nematicides for PCN would provide growers with more options, and could, perhaps, help in curbing the problems of accelerated degradation associated with repeated applications of the current products, for example, oxamyl (Osborn *et al.*, 2009).

This study demonstrated significant interaction between fluensulfone and partially resistant potatoes on the control of *G. pallida* population increases and suggested that similar controls could be achieved by combining Santé with fluensulfone application in the granular form at 4.05 kg a.s. ha^{-1} as would be obtained from treating the crop with oxamyl or fosthiazate at their commercial rates. Indeed, the traditional method of treating resistant varieties with nematicides is based on the principle that resistant cultivars, like their susceptible counterparts, stimulates hatching of the J2, thus, must be protected. Even though Vales Everest has a higher resistance score of 6, as against 4 for Santé (http://www.potato.org.uk/), and as expected, had more pronounced effects on the population development, it did not benefit from the fluensulfone, as well as the oxamyl and the fosthiazate treatments. In contrast, there were improvements in the growth and tuber yields, as well as enhancements in the population control when Santé received a nematicide treatment. The combined treatment, in each case, decreased the already fully controlled population to below the damage threshold of 5 eggs g^{-1} soil, and would imply that, the application of fluensulfone would better serve its purpose in the long term management of *G. pallida*, when combined with partially resistant cultivars, for example, Santé.

The suggestion above that fluensulfone would control *G. pallida,* mainly by suppressing the activities of the J2 in the soil, denotes that, likewise the currently available molecules, the efficacy will be subjected to delay hatching, and thus, underpins the significance of persistence in the soil for effective control. The DT_{50} observed in this study, ranged from 17 to 24 days and were similar in-field (Chapter 4) as in the soils in-polytunnel (Chapters 4 and 5). In numeric term, it indicated persistence that is short of 4 – 8 weeks that may be required for effective control (Rich *et al*., 2003). Nonetheless, there is strong evidence that controls of *G. pallida* were achieved at the current persistence. Indeed, the concentrations of fluensulfone detected in the soils, during most parts of the experiments, were higher than were shown *in vitro* for reducing the hatching and mobility of the J2 *G. pallida* (Deliopoulos

et al., 2009). As noted earlier, the DT_{50} is an important index for environmental persistence, but in effect, may convey limited, if any, information on the minimum effective fluensulfone dosage for *G*. *pallida*. The same could be said for fosthiazate, which had a similar persistence as fluensulfone in this study, but still gave control of *G*. *pallida*. The trend may not be different for oxamyl, which was shown to have a short persistence in the soil (Haydock *et al*., 2012; Osborn *et al*., 2010; Osman *et al*., 2009; Giannakou *et al*., 2005), but still has a wide usage in the UK. The suggestion, therefore, is that fluensulfone may control *G*. *pallida* though it may persist shortly in the soil.

The sorption studies (Chapter 5) identified soil organic matter contents as the most influential of fluensulfone sorption, signifying that this soil property may greatly influence environmental fates, and most likely, determine efficacy against *G*. *pallida*. However, altering the soil organic matter contents in the Polytunnel experiments 3 (Chapter 5) had no significant effects on the efficacy of fluensulfone at controlling the *G*. *pallida* root invasion of Estima. This is thought to be due to low sorption of fluensulfone, which was evident from the parameters K_F and K_D. Certainly, there was ample availability of the fluensulfone in the soil solution to be effective. Furthermore, the generally low K_{OC} indicated that fluensulfone be mobile in the soil and, therefore, may not be a difficult nematicide to redistribute by percolating soil water following initial mixing into potato beds. Therefore, it is highly unlikely that sorption would constitute a limiting factor to the efficacy of fluensulfone. Having low affinity for soil, would imply that fluensulfone could offer effective control of *G*. *pallida* in a wide range of soils. Nonetheless, there is considerable literature, suggesting that molecules sorbing weakly on soil are, most likely, to leach (E.g. Karpouzas *et al*., 2007; Cooke *et al*., 2004), and so may fluensulfone. If the laboratory results could be extrapolated to field situations, then both the amount and frequency of irrigation/rainfall, following application of fluensulfone to potato beds, could determine persistence in the rhizosphere of the developing potato. If so, it could provide some explanations to the dissipation of

fluensulfone from the field plots at Woodcote and Howle, where the significant dissipation in both experiments occurred during the periods of highest precipitations. Soil sampling for the persistence studies in Chapter 4, were made from the depth of incorporation (topmost 15 – 20 cm) and, assuming that there was leaching during the sampling period, it could be that fluensulfone was flushed to deeper layer and, therefore, beyond the sampling depth. The same could be suggested for the loss of fosthiazate, and perhaps oxamyl, which are already known for sorbing weakly on soil (Pantelelis *et al*., 2006; Qin *et al*., 2004) and are prone to leaching (Karpouzas *et al*., 2007; Gerstl, 1984). Leaching, however, could not have played a significant role in the loss of fluensulfone from the soils in the polytunnel pot experiments in Chapters 4 and 5, where the soil moisture was maintained at just about the maximum field capacity and water leaching out of the pot during irrigation was negligible. Furthermore, sampling in these experiments was done from the entire depth of the soil. This may indicate that multiple processes may be involved in the dissipation of fluensulfone from the soil

In summary, the experiments reported in this thesis had indicated that fluensulfone soil treatment is a likely control option for *G*. *pallida* and a potential addition to the nematicides for PCN. The incorporation of the 15% granular formulation at the full rate of 4.05 kg a.s ha^{-1} at 15-20 cm depth at planting could be as robust as current treatments of oxamyl and fosthiazate, in controlling PCN root invasion and suppressing building-up of the population. For production systems intending to apply fluensulfone, the timing and amount of irrigation/rainfall, as well as soil organic matter contents are most likely predictors of control. The weak sorption and short persistence demonstrated for fluensulfone, in this study, are attributes, which are desirable and also limiting; fluensulfone may be readily available in the soil's aqueous phase to be effective at controlling *G*. *pallida*, but then easily leached, whereas its short persistence may suggest that it may not pose a hazard to the environment but may not persist enough.

6.2 Recommendations for future studies

Activity of fluensulfone against *G. pallida*

Considering that the fluensulfone soil treatments did not significantly affect the viability and hatching of J2 would suggest that fluensulfone may reduce root invasion, mainly by suppressing J2 activity in the soil. Further studies should therefore investigate whether at the current applications; fluensulfone could be toxic to J2 in the soil or merely interfere with J2 activities such as perception of exudates from developing potato roots, mobility and orientation towards the potato roots. This could be investigated *in vitro* by using electrophysiological techniques as demonstrated by Twomey *et al*., (2000) for the nematicide DiTera®.

Even though this study did not provide evidence of significant fluensulfone effects on hatching of the J2 at the current application rates, the number of viable J2 remaining unhatched in the polytunnel experiments in Chapter 3 and 5, were generally more in the fluensulfone treated-soil than in the untreated control. This could be indicative of delay hatching of J2 and should be investigated. This could be done by sampling soil at 2, 4, 6, and 8 weeks after planting and determining the cyst content in comparison to that of the *Pi*.

Efficacy of fluensulfone

Even though the study suggested that fluensulfone could provide control of *G. pallida* comparable to the currently available nematicides, between field variations in the controls were apparent despite the fields being similar. Therefore, repeated experiments are needed

in order to confirm the viability of the fluensulfone treatments in controlling field infestations of *G. pallida*.

Factors affecting efficacy of fluensulfone

The weak sorption of fluensulfone on the soils in this study would make field mobility studies very useful in determining whether leaching could constitute a limiting factor to efficacy.

Although the soil organic matter may influence the fate of fluensulfone, mostly in the soil, the sorption studies showed good correlation for the soil clay and soil pH as well. Further studies modifying these factors could determine their likely influence on the efficacy of fluensulfone.

LIST OF REFERENCES

Accardi-Dey A. and Gschwend, P. M. 2003. Reinterpreting literature sorption data considering both absorption into organic carbon and adsorption onto black carbon. *Environmental Science and Technology*, 37: 99-106.

Agrios, G. N. 2005. Plant Pathology. Fifth Edition. Elsevier Inc., US. 948 pp.

Akhtar, M., and Malik, A. 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology*, 74: 35-47.

Alexander, M. 1995. How Toxic Are Toxic Chemicals in Soil? *Environmental Science and Technology*, 29: 2713-2717.

Allen, R. and Walker, A. 1987. The influence of soil properties on the rates of degradation of metamitron, metazachlor and metribuzin. *Pesticide Science*, 18: 95-111.

Ambrose, E., Haydock, P. P. J. and Wilcox, A. 2000. Degradation of the nematicide oxamyl in field conditions. *Aspects of Applied Biology*, 59: 41-51.

Anon., 1986. *The analysis of Agricultural Materials*, 3rd edition. ADAS, HMSO, London, UK.

Anon., 1996. EXTOXNET Extension Toxicology Network, Pesticide Information Profiles. [Online] NPIC. Available from: http://extoxnet.orst.edu/pips/oxamyl.htm. [ACCESSED]. 19 February 2014.

Anon., 2002. Interim Reregistration Eligibility Decision (IRED) Fenamiphos. [On-line]. EPA. Available from: http://www.epa.gov/oppsrrd1/REDs/fenamiphos_ired.pdf. [ACCESSED] 15 January 2014.

Anon., 2004. Conditional Registration, Fosthiazate. [Online]. EPA. Available from: http://www.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-129022_01-Jan-04.pdf. [ACCESSED]. 15 January 2014.

Anon., 2007. Reregistration Eligibility Decision for Aldicarb. Chapter 2006. [Online]. EPA. Available from: http://www.epa.gov/oppsrrd1/REDs/aldicarb_red.pdf. [ACCESSED] 15 January 2014.

Anon., 2012. GB Potatoes: *Market Intelligence* 2011-12. [On-line]. Potato Council. Available from: *http://www.potato.org.uk/publications/gb-potatoes-market-intelligence-*2011-12. [ACCESSED] 15 February 2013.

Anon., 2013a. GB Potatoes: Market Intelligence 2012-13 [On-line]. Potato Council. Available from: http://www.potato.org.uk/publications/gb-potatoes-market-intelligence-2012-13. [ACCESSED] 17 April 2013.

Anon., 2013b. Improving potato nematicide application. [On-line]. AGRII. Available from: http://www.agrii.co.uk/blog/2013/03/26/potato-nematicide-application/. [ACCESSED] 11 May 2013.

Arbeli, Z. and Fuentes, C. L. 2007. Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. *Crop Protection*, 26: 1733-1746.

Arntzen, F. K., Visser, J. H. M. and Hoogendoorn, J. 1993. Hatching of *Globodera pallida*juveniles by diffusate of potato genotypes, differing in tolerance to *G. pallida*. *Annals of Applied Biology*, 123: 83-91.

Arntzen, F. K., Visser, J. H. M. and Hoogendoorn, J. 1994. The effect of the potato cyst nematode *Globodera pallida* on *in vitro* root growth of potato genotypes, differing in tolerance. *Annals of Applied Biology*, 124: 59-64.

Back, M. A., Haydock, P. P. J. and Jenkinson, P. 2002. Disease complexes involving plant parasitic nematodes and soilborne pathogens. *Plant Pathology*, 51: 683-697.

Back, M., Haydock, P. and Jenkinson, P. 2006. Interactions between the potato cyst nematode *Globodera rostochiensis* and diseases caused by *Rhizoctonia solani* AG3 in potatoes under field conditions. *European Journal of Plant Pathology*, 114: 215-223.

Baldwin, J. G. and Mundo-Ocampo, M. 1991. *Hetroderinae* cyst and non-cyst forming nematodes. *In*: W. R. Nickle, (ed.), Manual of Agricultural Nematology. M. Decker Inc., New York. pp. 275-362.

Ball, W. P. and Roberts, P. V. 1991. Long-term sorption of halogenated organic chemicals by aquifer material. 2. Intraparticle diffusion *Environmental Science Technology*, 25: 1237-1249.

Barbercheck, M. E. and Duncan, L. 2004. Abiotic factors *In*: R. Gaugler and A. L. Bilgrani (eds.), Nematode Behaviour. CABI Wallingford, UK. pp. 309-343.

Barriuso, E., Houot, S. and Serra-Wittling, C. 1997. Influence of Compost Addition to Soil on the Behaviour of Herbicides. *Pesticide Science*, 49: 65-75.

Bates, J. A., Taylor, E. J. A., Gans, P. T. and Thomas, J. E. 2002. Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. *Molecular Plant Pathology*, 3: 153-161.

Been, T. H. and Schomaker, C. H. 2001. Ways to improve the accuracy of hatching tests for *Globodera* spp. with special emphasis on nematicide trials. *Nematology*, 3: 773-795.

Been, T. H. and Schomaker, C. H. 2006. Distribution Patterns and Sampling. *In*: R. N. Perry and Moens, M. (eds.), Plant Nematology. CABI Wallingford, UK. pp. 302-326.

Behrens, E. 1975. *Globodera* Skarbilovich, 1959. Eine selbständige Gattung in der Unterfamilie Heteroderinae Skarbilovich, 1947 (Nematoda: *Heteroderidae*). *In*: Vortragstagung zu Aktuellen Problemen der Phytonematologie, Rostock, Germany. pp. 12-26.

Beigel C., Charnay M. and Barriuso E. 1999. Degradation of formulated and unformulated triticonazole fungicide in soil: effect of application rate. *Soil Biology and Biochemistry*, 31: 525-534.

Berger, B. M. 1999. Factors influencing transformation rates and formation of products of phenylurea herbicides in soil. *Journal of Agricultural and Food Chemistry*, 47: 3389-3396.

Bermúdez-Couso, A., Fernández-Calviño, D., Rodríguez-Salgado, I., Nóvoa-Muñoz, J. C. and Arias-Estévez, M. 2012. Comparison of batch, stirred flow chamber, and column experiments to study adsorption, desorption and transport of carbofuran within two acidic soils. *Chemosphere*, 88: 106-112.

Bhattarai, S. and Haydock, P. P. J. 2010. The efficacy of the nematicide MCW2 against potato cyst nematode *Globodera pallida* in potato in an ambient temperature polytunnel. Study report prepared for Makhteshim-Agan Industries Ltd., Beer Sheva, Israel. 17 pp.

Biffinger, J. C., Kim, H. W. and DiMagno, S. G. 2004. The polar hydrophobicity of fluorinated compounds. *ChemBioChem*, 5: 622-627.

Boesten, J. J. T. I. 1990. Influence of solid/liquid ratio on the experimental error of sorption coefficients in pesticide/soil systems. *Pesticide Science*, 30: 31-41.

Boesten, J. J. T. I. and Van Der Pas, L. J. T. 1988. Modeling adsorption/desorption kinetics of pesticides in a soil suspension. *Soil Science*, 146: 215-295.

Boivin, A., Cherrier, R. and Schiavon, M. 2005. A comparison of five pesticides adsorption and desorption processes in thirteen contrasting field soils. *Chemosphere*, 61: 668-676.

Bowman, B, B. T. 1991. Use of field lysimeters for comparison of mobility and persistence of granular and EC formulations of the soil insecticide isazofos. *Environmental Toxicology and Chemistry*, 10: 873-879.

Bowman, B. T. 1992. Mobility and persistence of isazofos in granular and microencapsulated formulations in two soils, using field lysimeters. *Pesticide Science*, 36: 181-188.

Brodie, B. B. Evans, K. and Franco, F. 1993. Nematode parasites of potatoes, *In*: K. Evans, D. L. Trudgill, and J. M. Webster. (eds.), Plant-Parasitic Nematodes in Temperate Agriculture. CABI, Wellingford, UK, pp. 87-132.

Bromilow, R. H., Baker, R. J., Freeman, M. A. H. and Görög, K. 1980. The degradation of aldicarb and oxamyl in soil. *Pesticide Science*, 11: 371-378.

Brown, E. B. 1969. Assessment of the damage caused to potatoes by potato cyst eelworm, *Heterodera rostochiensis* Wol I. *Annals of Applied Biology*, 63: 493-502.

Brown, E. B. 1970. The behaviour of populations of potato cyst eelworm *Heterodera rostochiensis* Woll. Towards resistant potato varieties derived from *Solarium tuberosum* ssp. *andigena* Juz and Buk. *Annals of Applied Biology*, 65: 377-383.

Bullock, L. R., Barker, K. R. and Ristaino, J. B. 2002. Influences of organic and synthetic soil fertility amendments on nematode trophic groups and community dynamics under tomatoes. *Applied Soil Ecology*, 21: 233-250.

Burstall, L. and Harris, P. M. 1983. "The estimation of percentage light interception from leaf area index and percentage ground cover in potatoes." *Journal of Agricultural Science*, *Cambridge* 100: 241-244.

Burton, W. G. 1989. The Potato. Third edition. Longman Group, UK.753 pp.

Byrne, J. T., Maher, N. J. and Jones, P. W. 2001. "Comparative responses of *Globodera rostochiensis* and *G. pallida* to hatching chemicals." *Journal of Nematology*, 33: 195-202.

Calvet, R. 1989. Adsorption of Organic Chemicals in Soils. *Environmental Health Perspectives*, 83: 145-177.

Cancela, G. D., Taboada, E. R. and Sanchez-Rasero, F. 1990. Adsorption of cyanazine on peat and montmorillonite clay surfaces. *Soil Science*, 150: 831-907.

Cao, J., Guo, H., Zhu, H. M., Jiang, L. and Yang, H. 2008. Effects of SOM, surfactant and pH on the sorption–desorption and mobility of prometryne in soils. *Chemosphere*, 70: 2127-2134.

Chen, B., Johnson, E. J., Chefetz, B., Zhu. L. and Xin, B. 2005. Sorption of polar and nonpolar aromatic organic contaminants by plant cuticular materials: the role of polarity and accessibility. *Environmental Science and Technology*, 39: 6138-6146.

Chen, G., Lin, C., Chen, L. and Yang, H. 2010. Effect of size-fractionation dissolved organic matter on the mobility of prometryne in soil, *Chemosphere*, 79: 1046-1055.

Chen, J., Abawi, G. S. and Zuckerman, B. M. 2000. Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii* and *Streptomyces costaricanus* with and without organic amendments against *Meloidogyne hapla* infecting lettuce. *Journal of Nematology*, 32: 70-77.

Chiou, C. T., McGroddy, S. E. and Kile, D. E. 1998. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments. *Environmental Science and Technology*, 32: 264-269.

Chiou, C. T., Porter, P. E. and Schmedding D. W. 1983. Partition equilibriums of nonionic organic compounds between soil organic matter and water. *Environmental Science and Technology*, 17: 227-231.

Clayton, R., Parker B, Ballingall, M. and Davies, K. 2008. Impact of reduced pesticide availability on control of potato cyst nematodes and weeds in potato crops. [On-line]. Potato Council Ltd, Oxford, UK. http://www.potato.org.uk/sites/default/files/%5Bcurrent-page%3Aarg%3A%3F%5D/Reduced%20pesticide%20PCN%20case%20report%20Sept%2008_0.pdf. [ACCESSED] 14 February 2012.

Cooke, C. M., Shaw, G. and Collins, C. D. 2004. Determination of solid–liquid partition coefficients (K_d) for the herbicides isoproturon and trifluralin in five UK agricultural soils. *Environmental Pollution*, 132: 541-552.

Cornelissen, G., Gustafsson, O., Bucheli, T. D., Jonker, M. T. O., Koelmans, A. A. and Van Noort, P. C. M. 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environmental Science and Technology***,** 39: 6881-6895.

Cox, L., Velarde, P., Cabrera, A., Hermosín, M. C. and Cornejo, J. 2007. Dissolved organic carbon interactions with sorption and leaching of diuron in organic-amended soils. *European Journal of Soil Science*, 58: 714-721.

Cox, L., Walker, A. and Welch, S. J. 1996. Evidence for the accelerated degradation of isoproturon in soils. *Pesticide Science*, 48: 253-260.

Davis, E. L., Hussey, R. S., Mitchum, M. G. and Baum, T. J. 2008. Parasitism proteins in nematode–plant interactions. *Current opinion in plant biology*, 11: 360-366.

Davis, R. F., Wauchope R. D., Johnson A. W., Burgoa B. and Pepperman A. B. 1996. Release of fenamiphos, atrazine, and alachlor into flowing water from granules and spray deposits of conventional and controlled-release formulations. *Journal of Agricultural and Food Chemistry*, 44: 2900-2907.

De Boer, J. M., Overmars, H. A., Pomp, H., Davis, E. L., Zilverentant, J. F., Goverse, A., Smant, G., Stokkermans, J. P. W. G., Hussey, R. S., Gommers, F. J., Bakker, J. and Schots, A. 1996. Production and characterization of monoclonal antibodies to antigens from second stage juveniles of the potato cyst nematode, *Globodera rostochiensis*. *Fundamental of Applied Nematology*, 19: 545-554.

De Boer, J. M., Yan, Y., Wang, X., Smant, G., Hussey, R. S., Davis, E. L. and Baum, T. J. 1999. Developmental expression of secretory β-1, 4-endoglucanases in the subventral esophageal glands of *Heterodera glycines*. *Molecular Plant-Microbe Interactions*, 12: 663-669.

De Ruijter, F. J. and Haverkort, A. J. 1999. Effects of potato-cyst nematodes (*Globodera pallida*) and soil pH on root growth, nutrient uptake and crop growth of potato. *European Journal of Plant Pathology*, 105: 61-76.

Dec, J. and Bollag, J. 1997. Determination of covalent and non-covalent binding interactions between xenobiotic chemicals and soil. *Soil Science*, 162: 858-874.

Deliopoulos, T., Danquah, W. B. and Haydock, P. P. J. 2009. Efficacy studies of the MCW-2 nematicide against the potato cyst nematode *Globodera pallida.* Study report prepared for Makhteshim-Agan Industries Ltd, Beer Sheva Israel. 8 pp.

Deliopoulos, T., Minnis, S. T., Jones, P. W., and Haydock, P. P. J. 2010. Enhancement of the efficacy of a carbamate nematicide against the potato cyst nematode, *Globodera pallida*, through mycorrhization in commercial potato fields. *Journal of nematology*, 42: 22.

Delle Site, A. 2001. Factors Affecting Sorption of Organic Compounds in Natural Sorbent Water Systems and Sorption Coefficients for Selected Pollutants. A Review. *Journal of Physical and Chemical Reference Data*, 30: 187-439.

Den Nijs L. J. M. F. 2007. The Canon of Potato Science: 14. Cyst Nematodes. *Potato Research*, 50: 259-262.

Devine, K. J., Byrne, J., Maher, N. and Jones, P. W. 1996. Resolution of natural hatching factors for golden potato cyst nematode, *Globodera rostochiensis*. *Annals of Applied Biology*, 129: 323-334.

Ding, Q., Wu, H. L., Xu, Y., Guo, L. J., Liu K., Gao H. M. and Yang, H. 2011. Impact of low molecular weight organic acids and dissolved organic matter on sorption and mobility of isoproturon in two soils. *Journal of Hazardous Materials*, 190: 823-832.

Dinoiu, V. 2006. Fluorine Chemistry: Past, Present and Future *Review. Roumaine de Chimie* 51: 1141-1152.

Elston, D. A., Phillips, M. S. and Trudgill, D. L. 1991. The relationship between initial population density of potato cyst nematode *Globodera pallida* and the yield of partially resistant potatoes. *Revue de Nématologie*, 14: 213-219.

EPA Oxamyl IRED 2000. Reregistration Eligibility Decision for Oxamyl. [On-line]. EPA. http://www.epa.gov/oppsrrd1/REDs/oxamyl_red.pdf. [ACCESSED]. 15 January 2014.

Evan, K. and Rowe, J. A. 1998. Distribution and economic importance. *In*: S. B. Sharma (ed.), the Cyst Nematodes. Kluwer Academic Publishers, The Netherlands. pp. 1-30.

Evans, K. 1982. Effects of infestation with *Globodera rostochiensis* (Wollenweber) Behrens Rol on the growth of four potato cultivars. *Crop Protection*, 1: 169-179.

Evans, K. 1987. The interactions of potato cyst nematodes and *Verticillium dahliae* on early and maincrop potato cultivars. *Annals of Applied Biology*, 110: 329-339.

Evans, K. 1993. New approaches for potato cyst nematode management. *Nematropica* 23: 221-231.

Evans, K. and Haydock, P. P. J. 1990. A review of tolerance by potato plants of cyst nematode attack, with consideration of what factors may confer tolerance and methods of assaying and improving it in crops. *Annals of Applied Biology*, 117: 703-740.

Evans, K. and Haydock, P. P. J. 1993. Interactions of nematodes with root-rot fungi. *In* M. W. Khan (ed.), Nematode Interactions. Chapman and Hall, New York. pp. 104-133.

Evans, K. and Haydock, P. P. J. 2000. Potato cyst nematode management – present and future. *Aspects of Applied Biology* 59, *Potato Cyst Nematode Management* 91-98.

Evans, K., and Stone, A. R. 1977. "A review of the distribution and biology of the potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*." *International Journal of Pest Management*, 23: 178-189.

Evans, K., Franco, J. and De Scurrah, M. M. 1975. Distribution of species of potato cyst nematodes in South America. *Nematologica*, 21: 365-369.

Evans, K., Webster, R., Barker, A., Halford, P., Russell, M., Stafford, J. and Griffin, S. 2003. Mapping Infestations of potato cyst nematodes and the potential for spatially varying application of nematicides. *Precision Agriculture*, 4: 149-162.

Evans, S. G. and Wright, D. J. 1982. Effects of the nematicide oxamyl on life cycle stages of *Globodera rostochiensis*. *Annals of Applied Biology*, 100: 511-519.

Extoxnet oxamyl. Available online at http://extoxnet.orst.edu/pips/oxamyl.htm. Retrieved on January 15, 2014.

FAO, 2009. Available online at: ftp://ftp.fao.org/docrep/fao/012/i1127e/i1127e00.pdf. Retrieved on March 4, 2013.

FAO, 2012. "FAOSTAT" Faostat.fao.org. Retrieved on May 24, 2013.

Fenlon, K. A., Jones, K. C. and Semple, K. T. 2007. Development of microbial degradation of cypermethrin and diazinon in organically and conventionally managed soils. *Journal of Environmental Monitoring*, 9: 510-515.

Fenwick, D. W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology*, 18: 155-172.

Fernandes, M. C., Cox, L., Hermosín, M. C. and Cornejo, J. 2006. Organic amendments affecting sorption, leaching and dissipation of fungicides in soils. *Pest Management Science*, 62:1207-1215.

Fleming, C. C. and Powers, T. O. 1998. Potato cyst nematode diagnostics: morphology, differential hosts and biochemical techniques. *In*: R. J. Marks and B. B. Brodie (eds.), Potato cyst nematodes, Biology, Distribution and Control. CABI Wallingford, UK. pp. 91-114.

Flury, M. 1996. Experimental evidence of transport of pesticides through field soils—a review. *Journal of Environmental Quality*, 25: 25-45.

FOCUS, 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference SANCO/10058/2005 version 2.0, June 2006. Available at: http://focus.jrc.ec.europa.eu/dk/docs/finalreportFOCDegKin04June06linked.pdf. Retrieved 02 March, 2012.

Garabedian, S. and Hague, N. 1982. Distribution of nematicides in soil and its influence on the control of sugarbeet cyst nematode *Heterodera schachtii* and root-knot nematode *Meloidogyne incognita*. *Nematropica*, 12: 313-318.

Gerstl, Z. 1984. Adsorption, decomposition and movement of oxamyl in soil. *Pesticide Science*, 15: 9-17.

Gevao, B., Semple, K. T. and Jones, K. C. 2000. Bound pesticide residues in soils: a review. *Environmental pollution*, 108: 3-14.

Gheysen, G. and Jones, J. T. 2006. Molecular Aspects of Plant Nematode Interactions. *In*: R. N. Perry and M. Moens (eds.), Plant Nematology. CABI, Wallingford. pp. 234-254.

Gheysen. G. and Mitchum, M. G. 2009. Molecular Insights in the Susceptible Plant Response to Nematode Infection. *Plant Cell Monographs,* 15: 45-81.

Giannakou, I. O., Karpouzas, D. G., Anastasiades, I., Tsiropoulos, N. G. and Georgiadou, A. 2005. Factors affecting the efficacy of non-fumigant nematicides for controlling root-knot nematodes. *Pest Management Science*, 61: 961-972.

Giles, C. H., MacEwan, T. H., Nakhwa, S. N. and Smith, D. 1960. Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms and its use in diagnosis of adsorption mechanisms and in measurement of specific surface areas of solids. *Journal of the Chemical Society*, 3: 3973-3993.

Golden, A. M. and Ellington, D. M. S. 1972. Redescription of *Heterodera rostochiensis* (Nematoda: *Heteroderidae*) with a key and notes on closely related species. *Proceedings of the Helminthological Society of Washington*, 39: 64-78.

Gourd, T. R., Schmitt, D. P. and Barker, K. R. 1993. Differential Sensitivity of *Meloidogyne* spp. and *Heterodera glycines* to selected nematicides. *Journal of Nematology*, 25(supplement No. 4): 746-751.

Gratwick, M. 1992. Crop Pests in the UK: Collected edition of MAFF leaflets. Chapman and Hall, London. 490 pp.

Greco, N. and Thomason, I. J. 1980. Effect of Phenamiphos on *Heterodera schachtii* and *Meloidogyne javanica. Journal of Nematology*, 12: 91-96.

Grove, I. G., Haydock, P. P. J., Evans, K. and Lewis, D. J. 1999a. Supplementary foliar N, P and K, applied individually or in combinations, and the tolerance of potatoes to infection by the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Annals of Applied Biology*, 134: 193-204.

Grove, I. G., Haydock, P. P. J., Evans, K. and Lewis, D. J. 1999b. Basal fertiliser application method, tuber initiation nitrogen, foliar NPK and the tolerance of potatoes to infection by the potato cyst nematodes *Globodera rostochiensis* and *Globodera pallida*. *Annals of Applied Biology*, 134: 205-214.

Guo, L., Jury, W. A., Wagenet, R. J. and Flury, M. 2000. Dependence of pesticide degradation on sorption: non-equilibrium model and application to soil reactors. *Journal of Contaminant Hydrology*, 43: 45-62.

Guo, L., Wagenet, R. J. and Jury, W. A. 1999. Adsorption effects on kinetics of aldicarb degradation: equilibrium model and application to incubation and transport experiments. *Soil Science Society American Journal*, 63: 1637-1644.

Hague, N. G. M. and Gowen, S. R. 1987. Chemical control of nematodes *In*: R. H. Brown and B. R. Kerry (eds.), Principles and practices of nematode control in crops. Academic Press, Australia. pp. 131-178.

Halford P. D., Russell M. D. and Evans K. 1999. Use of resistant and susceptible potato cultivars in the trap cropping of potato cyst nematodes, *Globodera pallida* and *G. rostochiensis*. *Annals of Applied Biology*, 134: 321-327.

Haverkort, A. J., Mulder, A. and Van De Waart, M. 1993. The effect of soil pH on yield losses caused by the potato cyst nematode *Globodera pallida. Potato Research*, 36: 219-226.

Hawkes, J. G. and Francisco-Ortega, J. 1993. The early history of the potato in Europe. *Euphytica*, 70: 1-7.

Haydock, P. P. J and Evans, K. 1998. Management of potato cyst nematodes in the UK: an integrated approach? *Outlook on Agriculture*, 27: 253-260.

Haydock, P. P. J. 1989. Seed tuber physiological age and the growth of potato cultivars with partial resistance to the potato cyst nematode *Globodera pallida.* Tests of Agrochemicals and Cultivars 10, *Annals of Applied Biology*, 114: 158-159.

Haydock, P. P. J., Ambrose, E. L., Wilcox, A. and Deliopoulos, T. 2012. Degradation of the nematicide oxamyl under field and laboratory conditions. *Nematology*, 14: 339-352.

Haydock, P. P. J., and Evans, K. 1998. Management of potato cyst nematodes in the UK: an integrated approach? *Outlook on Agriculture*, 27: 253-260.

Haydock, P. P. J., Woods S. R., Grove I. G. and Hare, M. C. 2006. Chemical control of nematodes. *In*: Perry, R. N. and Moens, M. (eds.), Plant *Nematology*, CABI Wallingford, UK. pp. 393-431.

Hijmans, R. J. and Spooner, D. M. 2001. Geographic distribution of wild potato species. *American Journal of Botany*, 88: 2101-2112.

Hillocks, R. J. 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, 31: 85-93.

Hockland, S., Inserra, N., Mollar, L. and Lehman, P. S. 2006. International plant health-putting legislation into practice. *In*: R. N. Perry and M. Moens (eds.), Plant Nematology. CABI Wallingford, UK. pp. 327-345.

Hockland, S., Niere, B., Grenier, E., Blok, V., Phillips, M., Den Nijs, L., Anthoine, G., Pickup, J. and Viaene, N. 2012. An evaluation of the implications of virulence in non-

European populations of *Globodera pallida* and *G. rostochiensis* for potato cultivation in Europe. *Nematology*, 14: 1-13.

Holden-Dye, L. and O`Connor V. 2010. Mode of action of fluensulfone on *Caenorhabditis elegans*. Study report prepared for Makhteshim-Agan Industries Ltd. Beer Sheva, Israel. 44 pp.

Hooper, D. J. 1986. Extraction of free-living stages from soil. *In*: J. F. Southey, (ed.), Laboratory Methods for Work with Plant and Soil Nematodes. Ministry of Agriculture, Fisheries and Food, Reference Book No. 402. London, UK: HMSO; pp. 5-30.

Huang, W., Schlautman, M. A. and Weber, W. J. 1996. A distributed reactivity model for sorption by soils and sediments. 5. The influence of near-surface characteristics in mineral domains. *Environmental Science Technology*, 30: 2993-3000.

Ibrahim, S. K. and Haydock, P. P. J. 1992. Cadusafos inhibits hatching, invasion, and movement of the potato cyst nematode *Globodera pallida. Journal of Nematology*, 31: 201-206.

Ichino, T., Sasaki, S., Matsuura, T. and Nishi, S. 1990. Synthesis and properties of new polyimides containing flourinated alkoxy side chains. *Journal of Polymer Science. A Polymer Chemistry*, 28: 323-331.

Inagaki, Haruo and Kazuo, K. 1973. Discovery of the potato cyst nematode, *Heterodera rostochiensis* Wollenweber, 1923, (Tylenchida: *Heteroderidae*) from Peru guano. *Applied Entomology and Zoology*, 8: 97-102.

Jeschke, P. 2004. The unique role of fluorine in the design of active ingredients for modern crop protection. *ChemBioChem*, 5: 570-589.

Jeschke, P. 2010. The unique role of halogen substituents in the design of modern agrochemicals. *Pest Management Science*, 66: 10-27.

Johnson, W. G., Lavy, T. L. and Gbur, E. E. 1995. Sorption, mobility and degradation of trichlopyr and 2, 4, 4-D on 4 soils. *Weed Science*, 43: 678-684.

Jurado-Exposito, M and Walker, A. 1998. Degradation of isoproturon, propyzamide and alachlor in soil with constant and variable incubation conditions. *Weed Research*, 38: 309-318.

Kah, M. and Brown, C. D. 2006. Adsorption of ionisable pesticides in Soils. *Reviews of Environmental Contamination and Toxicology*, 188: 149-217.

Kah, M. and Brown, C. D. 2007. Changes in pesticide adsorption with time at high soil to solution ratios. *Chemosphere*, 68: 1335-1343.

Karpouzas, D. G. and Walker, A. 2000. Aspects of the enhanced biodegradation and metabolism of ethoprophos in soil. *Pest Management Science*, 56: 540-548.

Karpouzas, D. G., Giannakou, I. O., Walker, A. and Gowen, S R. 1999. Reduction in biological efficacy of ethoprophos in a soil from Greece due to enhanced biodegradation: comparing bioassay with laboratory incubation data. *Pesticide Science*, 55: 1089-1094.

Karpouzas, D. G., Pantelelis, I., Menkissoglu-Spiroudi, U., Golia, E. and Tsiropoulos, N. G. 2007. Leaching of the organophosphorus nematicide fosthiazate. *Chemosphere*, 68: 1359-1364.

Key, B. D., Howell, R. D. and Criddle, C. S. 1997. Fluorinated Organics in the Biosphere. *Environmental Science and Technology*, 31: 2445-2454.

Key, B. D., Howell, R. D. and Criddle, C. S. 1998. Defluorination of organofluorine sulfur compounds by *Pseudomonas* spp. Strain D2. *Environment Science and Technology*, 32: 2283-2287.

Kile, D. E. and Chiou, C. T. 1999. Correlation of soil and sediment organic matter polarity to aqueous sorption of non-ionic compounds. *Environmental Science and Technology*, 33: 2053-2056.

Kirk, K. L. 2006. Fluorine in medicinal chemistry: Recent therapeutic applications of fluorinated small molecules. *Journal of Fluorine Chemistry*, 127: 1013-1029.

Koenning, S. R. and Sipes, B. S. 1998. *In*: S.B. Sharma, (ed.), The Cyst nematode. Kluwer Academic Publishers, Great Britain. pp. 156-190.

Kookana, R. S., Aylmore, L. A. G., and Gerritse, R. G. 1992. Time-dependent sorption of pesticides during transport in soils. *Soil Science*, 154: 171-255.

Kort, J., Ross, H., Rumpenhorst, H. J. and Stone, A. R. 1977. An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica*, 23: 333-339.

Koskinen, W. C. and Harper, S. S. 1990. The retention process: mechanisms. *In*: Cheng, H.H. (ed.), Pesticides in the soil environment: processes, impacts, and modeling. pp. 51-77.

Kravvariti, K., Tsiropoulos, N. G. and Karpouzas, D. G. 2010. Degradation and adsorption of terbuthylazine and chlorpyrifos in biobed biomixtures from composted cotton crop residues. *Pest Management Science*, 66:1122-1128.

Lafrance, P., Marineau, L. Perreault, L. and Villeneuve, J. P. 1994. Effect of natural dissolved organic matter found in groundwater on soil adsorption and transport of pentachlorophenol, *Environmental Science and Technology*, 28: 2314-2320.

Lee, C., Chronis, D., Kenning, C., Peret, B., Hewezi, T., Davis, E. L., Baum, T. J., Hussey, R., Bennett, M. and Mitchum, M. G. 2011. The novel cyst nematode effector protein 19C07 interacts with the arabidopsis auxin influx transporter LAX3 to control feeding site development. *Plant Physiology,* 155: 866-880.

Leistra, M. and Green, R. E. 1990. Efficacy of soil applied pesticides. *In*: H. H. Chen (ed), Pesticides in the soil environment: Processes, Impacts, and Modelling. pp. 401-428.

Li, Z., Xu, J., Muhammad, A. and Ma, G. 2005. Effect of bound residues of metsulfuron-methyl in soil on rice growth. *Chemosphere*, 58: 1177-1183.

Lilley, C. J., Urwin, P. E., Johnston, K. A. and Atkinson, H. J. 2004. Preferential expression of a plant cystatin at nematode feeding sites confers resistance to *Meloidogyne incognita* and *Globodera pallida*. *Plant Biotechnology Journal*, 2:3-12.

Lutaladio, N., Ortiz, O., Haverkort, A. and Caldiz, D. 2009. Sustainable potato production. Guidelines for developing countries. Available online at online at FAO.org. Retrieved on April 17, 2012.

Manuel, A. E., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J. C. and García-Río, L. 2008. "The mobility and degradation of pesticides in soils and the pollution of groundwater resources." *Agriculture, Ecosystems & Environment* 123: 247-260.Anon 2009. EC Regulation No 1107/2009. Concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union 52: 1–50. [On-line]. EU-LEX. http://eur-lex.europa.eu/LexUriServ/. [ACCESSED]. 16 January 2014.

Mehrdad, M., Subbotin, S. A. and Moens, M. 2005. Quantitative detection of the potato cyst nematode, *Globodera pallida* and the beet cyst nematode, *Heterodera schachtii*, using real-time PCR with SYBR green I dye. *Molecular and Cellular Probes*, 19: 81-86.

Ministry of Agricultural, Fisheries and Food (MAFF) 1985. Soil Texture System and Pesticide Use. Pamphlet 3001. 4 pp.

Ministry of Agriculture, Fisheries and Food (MAFF) 1986. *The analysis of agricultural materials*, 3rd edition. *Reference book 427*. Her Majesty's office, London, UK.

Minnis, S. T., Haydock, P. P. J., Ibrahim, S. K., Grove, I. G., Evans, K. and Russell, M. D. 2002. Potato cyst nematodes in England and wales - occurrence and distribution. *Annals of Applied Biology*, 140: 187-195.

Mugniery, D. 1978. Vitesse de développement, en fonction de la température, de *Globodera rostochiensis* et *G. pallida* (Nernatoda: *Heteroderidae*). *Revue de Nérnatologie*, 1: 3-12.

Mulder, A. and Van Der Wal, A. F. 1997. Relationship between potato cyst nematodes and their principal host. I. A literature review. *Potato Research*, 40: 317-326.

Mullin, B. A. and Brodie, B. B. 1988. Effects of Host Resistance on the Fecundity of *Globodera rostochiensis. Journal of Nematology*, 21: 109-112.

Murphy, E. M., Zachchara, J. M. and Smith, S. C. 1990. Influence of mineral-bound humic substances on the sorption of hydrophobic organic compounds. *Environment Science and Technology*, 24: 1507-1516.

Natarajan, R., Azerad, R., Badet, B. and Copin, E. 2005. Microbial cleavage of C-F bond *Journal of Fluorine Chemistry*, 126: 424-435

Noe, J. P. 2008. Plant-Parasitic Nematodes. *In*: G. N. Agrios (ed), Plant Pathology. Sixth edition. Taylor and Francis Group, Boca Raton, USA. pp. 65-77.

Noling, J. W. 2003. Movement and toxicity of nematicides in the plant root zone. Fact Sheet ENY-041 (formerly RF-NG002). Available at online at http://edis.ifas.ufl.edu/ng002. Retrieved on 05 May, 2011.

North-Western European Potato Growers (NEPG), 2013. News: North-West European Potato Grower's issues first potato area estimate Available on-line at http://www.potatopro.com/Lists/News/DispForm.aspx?ID=7237. Retrieved on August 9, 2013.

O'Brien, P. J., Allen, E. J., Bean, J. N., Griffith, R. L., Jones, S. A. and Jones, J. L. 1983. Accumulated day-degrees as a measure of physiological age and the relationships with growth and yield in early potato varieties. *Journal of Agricultural Science, Cambridge*, 101: 613-631.

OECD Guidelines for the testing of chemicals, 2000. Test No. 106: adsorption–desorption using a batch equilibrium method. OECD Organisation for Economic Co-operation and Development. Available online at http://www.oecd-ilibrary.org. Retrieved on 02 July, 2011.

Ogram, A.V., Jessup, P. P., Ou, L.T. and Rao, P. S. C. 1985. Effects of sorption on biological degradation rates of 2, 4-dichlorophenoxy acetic acid in soils. *Applied and Environmental Microbiology*, 49: 582-587.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments - a review. *Applied Soil Ecology*, 44: 101-115.

Oka, Y., Shapira, N. and Fine, P. 2007. Control of root-knot nematodes in organic farming systems by organic amendments and soil solarization. *Crop Protection*, 26: 1556-1565.

Oka, Y., Shuker, S. and Tkachi, N. 2009. Nematicidal efficacy of MCW-2, a new nematicide of the fluoroalkenyl group, against the root-knot nematode. *Meloidogyne javanica. Pest Management Science*, 65: 1082-1089.

Oka, Y., Shuker, S. and Tkachi, N. 2012. Systemic nematicidal activity of fluensulfone against the root-knot nematode *Meloidogyne incognita* on pepper. *Pest Management Science*, 68: 268-275.

Oka, Y., Shuker, S. and Tkachi, N. 2013. Influence of soil environments on nematicidal activity of fluensulfone against *Meloidogyne javanica*. *Pest Management Science*, 69:1225-1234.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Mededelingen van de Landbouwhogeschool, Wageningen 66/4: 46.

Opperman, C. H. and Bird, D. M. 1998. The soybean cyst nematode, *Heterodera glycines*: a genetic model system for the study of plant-parasitic nematodes. *Current Opinion in Plant Biology*, 1: 342-346.

Opperman, C. H. and Chang, S. 1990. Plant-parasitic nematode acetylcholinesterase inhibition by carbamate and organophosphate nematicides. *Journal of Nematology*, 22: 481-488.

Osamn, K. A., Al-Rehiayani, S. M., Al-Deghairi, M. A. and Salama, A. K. 2009. Bioremediation of oxamyl in sandy soil using animal manures. *International Biodeterioration and Biodegradation*, 63: 341-346.

Osborn, R. K., Edwards S. G., Wilcox, A. and Haydock, P. P. J. 2010. Potential enhancement of degradation of the nematicides aldicarb, oxamyl and fosthiazate in UK agricultural soils through repeated applications. *Pest Management Science*, 66: 253-261.

Osborne, P. 1973. The effect of aldicarb on the hatching of *Heterodera rostochiensis* larvae. *Nematologica*, 19: 7-14.

Pantelelis, I., Karpouzas, D. G., Menkissoglu-Spiroudi, U. and Tsiropoulos, N. 2006. Influence of soil physicochemical and biological properties on the degradation and adsorption of the nematicide fosthiazate. *Journal of Agricultural and Food Chemistry*, 54: 6783-6789.

Park, B. K., Kitteringham, N. R. and O'Neill, P. M. 2001. Metabolism of fluorine-containing drugs. *Annual Review Pharmacology Toxicology*, 41: 443-70.

Park, J. H., Feng, Y., Ji, P., Voice, T. C. and Boyd, S. A. 2003. Assessment of bioavailability of soil-sorbed atrazine. *Applied Environmental Microbiology*, 69: 3288-3298.

Patakioutas, G. and Albanis, T. A. 2002. Adsorption–desorption studies of alachlor, metolachlor, EPTC, chlorothalonil and pirimiphos-methyl in contrasting soils. *Pest Management Science*, 58: 352-362.

Phillion, D. P., Yalamanchili, G. and Ruminski, P. G. 1999. Fluoroalkenyl compounds and their use as pest control agents United States Patent NO. 5968916.

Phillips, M. S. and Blok, V. C. 2008. Selection for reproductive ability in globodera*Globodera pallida* populations in relation to quantitative resistance from *Solanum vernei* and *S. tuberosum* ssp. *andigena* CPC2802. *Plant Pathology*, 57: 573-580.

Phillips, M. S. and Trudgill, D. L. 1998. Population modelling and integrated control option for potato cyst nematodes. *In*: R. J. Marks and B. B. Brodie (eds.), Potato cyst nematodes, Biology, distribution and control. CABI Wallingford, UK. pp. 153-164.

Pickup, J., Cole, Y. Reid, A., Kerr, J., Speirs, J. and MaCRae, D. 2012. Potato Cyst Nematodes: initial impressions of the impact of the new EU Directive in Scotland. *In*: Proceedings of the Conference held at the West Park Conference Centre Dundee, Scotland 28 – 29 February 2012. The Dundee Conference Crop Protection in Northern Britain 2012. 315 pages. [On-line]. SIPR. http://www.sipr.ac.uk/CPNB/Index_and_Proceedings_2012.pdf. [ACCESSED] 13 February, 2013.

Qin, S., Gan, J. Liu, W. and Becker, J. O. 2004. Degradation and adsorption of fosthiazate in soil. *Journal of Agricultural and Food Chemistry*, 52: 6239-6242.

Rice, S. L., Leadbeater, B. S. C. and Stone, A. R. 1985. Changes in cell structure in roots of resistant potatoes parasitized by potato cyst-nematodes. I. potatoes with resistance gene *H1* derived from *Solanum tuberosum* ssp. *andigena*. *Physiological Plant Pathology*, 27: 219-234.

Riga, E., Perry, R. N., Barrett, J. and *Johnston,* M. R. L. 1996. Electrophysiological responses of males of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida,* to their sex pheromones. *Parasitology*, 112: 239-246.

Rijnaarts, H. H. M., Bachmann, A., Jumelet, J. C. and Zehnder, A. J. B. 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of a-hexachlorocyclohexane in a contaminated calcareous soil. *Environmental Science and Technology*, 24: 1349-1354.

Robinson, M., Atkinson, H. and Perry, R. 1987a. The influence of soil moisture and storage time on the motility, infectivity and lipid utilisation of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Revue de Nematologie*, 10: 343-348.

Robinson, M., Atkinson, H. and Perry, R. 1987b. The influence of temperature on the hatching activity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Revue de Nematologie*, 10: 349-354.

Ryan, N. A., Deliopoulos, T., Jones P. and Haydock, P. P. J. 2003. Effects of a mixed-isolate mycorrhizal inoculum on the potato – Potato cyst nematode interaction. *Annals of Applied Biology*, 143: 111-119.

Ryan, N. A., Duffy, E. M., Cassells, A. C. and Jones, P. W. 2000. The effect of mycorrhizal fungi on the hatch of potato cyst nematodes. *Applied Soil Ecology*, 15: 233-240.

Schans, J. and Arntzen, F. K. 1991. Photosynthesis, transpiration and plant growth characters of different potato cultivars at various densities of *Globodera pallida*. *Netherlands Journal of Plant Pathology*, 97: 297-310.

Scholte, K. and Vos, J. 2000. Effects of potential trap crops and planting date on soil infestation with potato cyst nematodes and root-knot nematodes. *Annals of Applied Biology*, 137: 153-164.

Scholte, K., and J. Vos. 2000. Effects of potential trap crops and planting date on soil infestation with potato cyst nematodes and root-knot nematodes. *Annals of Applied Biology* 137, no. 2: 153-164.

Schomaker, C. H. and Been, T. B. 2013. Quantitative Nematology and Management. *In*: R. N. Perry and M. Moens (eds.), Plant Nematology. CABI Wallingford, UK. pp. 301-330.

Schomaker, C.H. and Been, T.H. 2006. Plant Growth and Population Dynamics. *In*: R. N. Perry and M. Moens (eds.), Plant Nematology. CABI, Wallingford UK. pp. 275-301.

Scow, K. M. and Johnson, C. R. 1996. Effect of sorption on biodegradation of soil pollutants. *Advances in Agronomy*, 58: 1-56.

Seinhorst, J. W. 1985. The development of individual s and populations of cyst nematodes on plants. *In*: F. Lambberti and C. E. Taylor (eds.), Cyst Nematodes. Plenum Press, New York. pp. 101-117.

Seinhorst, J. W. and Ouden, H. D. 1971. The relation between density of *Heterodera rostochiensis* and growth and yield of two potato varieties. *Nematologica*, 17: 347-369.

Shepherd, A. M. 1986. Extraction and estimation of cyst nematodes. *In*: J. F. Southey, (ed.), Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food, Reference Book No. 402. London, UK: HMSO; pp. 31-50.

Simon, L., Spiteller, M., Haisch, A. and Wallnöfer, P. R. 1992. Influence of soil properties on the degradation of the nematocide fenamiphos. *Soil Biology and Biochemistry*, 24: 769-773.

Singh, N. 2008. Biocompost from sugar distillery effluent: effect on metribuzin degradation, sorption and mobility. *Pest Management Science*, 64: 1057-1062.

Smant, G., Goverse, A., Stokkermans J. P. W. G., De Boer, J. M , Pomp H., Zilverentant, J. F., Overmars. H. A., Helder. J., Schots, A. and Bakker, J. 1997. Potato root difusate-induced secretion of soluble, basic proteins originating from the subventral oesophageal glands of potato cyst nematodes. *Phytopathology*, 87: 839-845.

Smart, B. E. 2001. Fluorine substituent effects (on bioactivity). *Journal of Fluorine Chemistry*, 109: 3-11.

Smelt, J. H. and Leistra, M. 1992. Availability, movement and (accelerated) transformation of soil-applied nematicide *In*: Gommers, F. J. and Maas, P. W. Th. (ed.), *Proceedings*: Second International Nematology Congress 11-17 August, 1990, Veldhoven, the Netherlands Scotland, UK: European Society of Nematologists. pp. 266-293.

Smelt, J. H., Crum, S. J. H., Teunissen, W. and Leistra, M. 1987. Accelerated transformation of aldicarb, oxamyl and ethoprophos after repeated soil treatments. *Crop Protection,* 6: 295-303.

Sobczak, M. and Golinowski, W. 2008. Structure of cyst nematode feeding site. *In*: R. H. Berg and C. G. Taylor (eds.), Cell biology of plant nematode parasitism. Springer, Berlin, pp. 153-187.

Song, N. H. Chen, L. and Yang, H. 2008. Effect of dissolved organic matter on mobility and activation of chlorotoluron in soil and wheat. *Geoderma*, 146: 344-352.

Spark, K. M. and Swift, R. S. 2002. Effect of soil composition and dissolved organic matter on pesticide sorption. *Science of the Total Environment*, 298: 147-161.

Sposito, G. 1984. *The Surface Chemistry of Soils:* Oxford University Press, New York, USA. 245 pp.

Sposito, G. 2008. *The Chemistry of Soils,* 2nd edition. Oxford University Press, New York, USA. 344 pp.

Starr, J. L. and Roberts, P. A. 2004. Resistance to plant-parasitic nematodes. *In*: Chen, Z., X.; Chen S. Y. and Dickson, D. W. (eds.), Nematology Advance and perspectives. Vol. 2. Nematode Management and Utilization. CABI Wallingford, UK. pp. 879-907.

Steele, A. E. 1982. Effects of selected nematicides on hatching of *Heterodera schachtii*. *Journal of Nematology*, 15: 467-473.

Sternesen, J. 2004. Chemical pesticides mode of action and toxicology. CRC Press, Florida, USA, 296 pp.

Stone, A. R. 1973. *Heterodera pallida* N. Sp. (Nematoda: *Heteroderidae*), a second species of potato cyst nematode *Nematologica*, 18: 591-606.

Stone, A. R. 1985. Co-evolution of potato cyst nematodes and their hosts: implications for pathotypes and resistance. *EPPO Bulletin*, 15: 131-137.

Stone, A. R., Fuller, J. M. and Howard, H. W. 1979. The presence of pathotype Pa2 of *Globodera pallida* in the United Kingdom. *Plant Pathology*, 28: 134-137.

Stone, A. R., Holliday, J. M., Mathias, P. L. and Parrott, D. M. 1986. A selective survey of potato cyst-nematode pathotypes in Great Britain. *Plant Pathology*, 35: 18-24.

Storey, G. W. 1984. The effect of oxamyl and the growing of susceptible and resistant potato cultivars on the population dynamics of *Globodera rostochiensis* throughout the soil profile. *Annals of Applied Biology*, 104: 131-141.

Storey, G. W. and Evans, K. 1987, Interactions between *Globodera pallida* juveniles, *Verticillium dahliae* and three potato cultivars, with descriptions of associated histopathologies. *Plant Pathology*, 36: 192-200.

Suett, D. L. 1986. Accelerated degradation of carbofuran in previously treated soils in the United Kingdom. *Crop Protection*, 5: 165-169.

Thoden, T. C., Korthals, G.W. and Termorshuizen, A. J. 2011. Organic amendments and their influences on plant-parasitic and free-living nematodes: a promising method for nematode management? *Nematology*, 13: 133-153.

Thompson, A. and Goyne, K. W. 2012. Introduction to the sorption of chemical constituents in soils. *Nature Education Knowledge*, 3: 15.

Timmermans, B. G. H., Vos, J., Stomph, T. J., Van Nieuwburg, J. and Van der Putten, P. E. L. 2006. Growth duration and root length density of *Solanum sisymbriifolium* (Lam.) as determinants of hatching of *Globodera pallida* (Stone). *Annals of Applied Biology*, 148: 213-222.

Tobin, J. D., Haydock, P. P. J., Hare, M. C., Woods, S. R. and Crump, D. H. 2008. Effect of the fungus *Pochonia chlamydosporia* and fosthiazate on the multiplication rate of potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) in potato crops grown under UK field conditions. *Biological Control*, 46: 194-201.

Torrents, A. and Jayasundera, S. 1997. The sorption of nonionic pesticides onto clays and the influence of natural organic carbon *Chemosphere*, 35: 1549-1565.

Trudgill, D. L. 1980. Effect of *Globodera rostochiensis* and fertilisers on the mineral nutrient content and yield of potato plants. *Nematologica*, 26: 243-254.

Trudgill, D. L. 1985. Potato cyst nematodes: A critical review of the current pathotyping scheme1. *EPPO Bulletin*, 15: 273-279.

Trudgill, D. L. 1986. Yield losses caused by potato cyst nematodes: a review of the current position in Britain and prospects for improvements. *Annals of Applied Biology*, 108: 181-198.

Trudgill, D. L. and Cotes, L. M. 1983. Tolerance of potato-to-potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in relation to the growth and efficiency of the root system. *Annals of Applied Biology*, 102: 385-397.

Trudgill, D. L., Elliott, M. J, Evans, K. and Phillips, M. S. 2003. The white potato cyst nematode (*Globodera pallida*) – a critical analysis of the threat in Britain. *Annals of Applied Biology*, 143: 73-80.

Trudgill, D. L., Phillips, M. S. and Alphey, T. J. W. 1987. Integrated control of potato cyst nematodes. *Outlook on Agriculture*, 16: 167-172.

Trudgill, D. L., Phillips, M. S. and Hackett, C. A. 1996. The basis of predictive modeling for estimating yield loss and planning potato cyst nematode management. *Pesticide Science*, 47: 89-94.

Turner, S. J. 1996. Population decline of potato cyst nematodes (*Globodera rostochiensis*, *G. pallida*) in field soils in Northern Ireland. *Annals of Applied Biology*, 129: 315-322.

Turner, S. J. and Evans, K. 1998. The origins, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* (Woll.) and *Globodera pallida* Stone). *In*: R. J. Marks

and B. B. Brodie (eds.), Potato Cyst nematodes: Biology, Distribution, and Control, CABI Wallingford, UK. pp. 7-26.

Turner, S. J. and Rowe. A. 2006. Cyst nematodes. *In*: Perry, R. N. and Moens, M. (eds.), Plant nematology. CABI Wallingford, UK. pp. 91-122.

Twining, S., Clarke, J., Cook, S., Ellis, S., Gladders, P., Ritchie, F. and Wynn, S. 2009. Research Report Pesticide availability for potatoes following revision of Directive 91/414/EEC: Impact assessments and identification of research priorities. *Project Report 2009/2*, [On-line]. POTATO Council. Available from: *http://www.potato.org.uk/sites/default/files/%5Bcurrentpage%3Aarg%3A%3F%5D/20092_Pesticide_availability_Report_R415_0.pdf*. [ACCESSED]. 4 March 2011.

Twomey, U., Rolfe, R. N., Warrior, P. and Perry, R. N. 2002. Effects of the biological nematicide, DiTera®, on movement and sensory responses of second stage juveniles of *Globodera rostochiensis*, and stylet activity of *Globodera rostochiensis* and fourth stage juveniles of *Ditylenchus dipsaci. Nematology*, 4: 909-915.

Urwin, P. E, Green, J. and Atkinson, H. J. 2003. Expression of a plant cystatin confers partial resistance to *Globodera*, full resistance is achieved by pyramiding a cystatin with natural resistance. *Molecular Breeding*, 12: 263-269.

Urwin, P. E. Green, J. and Atkinson, J. H. 2000. Resistance to *Globodera spp*. in transgenic *Solanum tuberosum cv*. Desiree that expresses protein inhibitors. *Aspects of Applied Biology*, 59: 27-32.

Urwin, P. E., Troth, K. M., Zubko, E. I., and Atkinson, H. J. 2001. Effective transgenic resistance to *Globodera pallida* in potato field trials. *Molecular Breeding*, 8: 95-101.

Vanholme, B., De Meutter, J., Tytgat, T., Van Montagu, M., Coomans, A. and Gheysen, G. 2004. Secretions of plant-parasitic nematodes: A molecular update. *Gene*, 332: 13-27.

Viaene, N., Coyne, D. L. and Kerry, B. R. 2006. Biological and cultural management. *In*: R. N. Perry and M. Moens (eds), Plant Nematology. CABI Wallingford, UK. pp. 346-369.

Villaverde, J., Kah, M. and Brown, C. D. 2008. Adsorption and degradation of four acidic herbicides in soils from southern Spain. *Pest Management Science*, 64: 703-710.

Wagenet, R. J. and Hutson, J. L. 1990. Quantifying pesticide behavior in soil. *Annual Review of Phytopathology*, 28: 295-319.

Walker, A. 2003. Adsorption and degradation: from laboratory to the real world. *In*: A.A.M. Del Re., E. Capri., L. Padovani and M. Trevisan (eds.), Pesticides in air, plant, soil and water system. *Proceedings of the XII Symposium Pesticide Chemistry*, 4-6 June, 2003, Piacenza, Italy. pp. 1-6.

Walker, T. S., Schmiediche, P. E. and Hijmans, R. J. 1999. World trends and patterns in the potato crop: An economic and geographic survey. *Potato Research*, 42: 241-264.

Wang, P. and Keller, A. A. 2009. Sorption and desorption of atrazine and diuron onto water dispersible soil primary size fractions. *Water Research*, 43: 1448-1456.

Wauchope, R. D., Yeh, S., Linders, J. B. H. J., Kloskowski, R., Tanaka, K., Rubin, B., Katayama, A., Kördel, W., Gerstl, Z., Lane, M. and Unsworth, J. B. 2002. Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. *Pest Management Science,* 58: 419-445.

White, R. E., Dyson, J. S., Gerstl, Z. and Yaron, B. 1986. Leaching of herbicides through undisturbed cores of a structured clay soil. *Soil Science Society of American Journal*, 50: 277-283.

Whitehead A. G., Nichols A. J. F. and Senior J. C. 1991. Control of potato pale cyst-nematode, *Globodera pallida*, with a granular nematicide and partially resistant potatoes. *Annals of Applied Biology*, 118: 623-636.

Whitehead A. G., Tite D. J., Fraser J. E. and Nichols A. J. F. 1984. Differential control of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* by oxamyl and the yields of resistant and susceptible potatoes in treated and untreated soils. *Annals of Applied Biology*, 105: 231-244.

Whitehead, A. G. 1986. Chemical and integrated control of cyst nematodes. *In*: Lamberti, F. and Taylor, C. E. (eds.), Cyst nematodes. Plenum Press, New York and London, pp: 413-432

Whitehead, A. G. 1991. Selection for virulence in the potato cyst-nematode, *Globodera pallida*. *Annals of Applied Biology*, 118: 395-402.

Whitehead, A. G. 1991. The resistance of six potato cultivars to English populations of potato cyst-nematodes, *Globodera pallida* and *G. rostochiensis*. *Annals of Applied Biology*, 118: 357-369.

Whitehead, A. G. 1992. Emergence of juvenile potato cyst-nematodes *Globodera rostochiensis* and *Globodera pallida* and the control of *G. pallida*. *Annals of Applied Biology*, 120: 471-486.

Whitehead, A. G. 1995. Decline of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, in spring barley microplots. *Plant Pathology*, 44: 191-195.

Whitehead, A. G. and Turner, S. J. 1998. Plant Nematode Control. CABI Wallingford, UK. 383 pp.

Whitehead, A. G., Nichols, A. J. F. and Senior, J. C. 1994. The control of potato pale cyst-nematode (*Globodera pallida*) by chemical and cultural methods in different soils. *The Journal of Agricultural Science*, 123: 207-218.

Whitehead, A. G., Tite, D. J. and Bromilow, R. H. 1981. Techniques for distributing non-fumigant nematicides in soil to control potato cyst-nematodes, *Globodera rostochiensis* and *G. pallida*. *Annals of Applied Biology*, 97: 311-321.

Whitehead, A. G., Tite, D. J., Fraser, J. E. and French, E. M. 1980. Effects of aldicarb and oxamyl in peaty loam soil on potato cyst-nematode, *Globodera rostochiensis,* and on resistant and susceptible potatoes. *The Journal of Agricultural Science*, 95: 213-217.

Whitehead, A. G., Tite, D. J., Fraser, J. E. and Nichols, A. J. F. 1984. Differential control of potato cyst-nematodes, *Globodera rostochiensis* and *G. pallida* by oxamyl and the yields of resistant and susceptible potatoes in treated and untreated soils. *Annals of Applied Biology*, 105: 231-244.

Whitehead, A. G., Webb, R. M. and Beane, J. 1991. Effects of rotation length and oxamyl on potato yield and the potato cyst-nematode, *Globodera rostochiensis*, in a sandy loam soil. *Annals of Applied Biology*, 118: 371-380.

Williamson, V. M. and Gleason, C. A. 2003. "Plant–nematode interactions." *Current Opinion in Plant Biology*, 6: 327-333.

Wollenweber, H. W. 1923. Krankheiten and Beschadigungen der Kartoffel. Arbeit der Forschungs Institut Kartoffel Berlin 7, 1-56.

Woods, S. R. and Haydock, P. P. J. 2000. The effect of granular nematicide incorporation depth and potato planting depth on potatoes grown in land infested with the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. *Annals of Applied Biology*, 136: 27-33.

Woods, S. R., Haydock, P. P. J. and Edmunds, C. 1999. Mode of action of fosthiazate used for the control of the potato cyst nematode *Globodera pallida*. *Annals of Applied Biology*, 135: 409-415.

Wright D. J., Blyth A. R. K. and Pearson P. E. 1980. Behaviour of the systemic nematicide oxamyl in plants in relation to control of invasion and development of *Meloidogyne incognita*. *Annals of Applied Biology*, 96: 323-334.

Wright, D. J. and Rowland, A. J. 1982. Susceptibility of different developmental stages of the root-knot nematode, *Meloidogyne incognita*, to the nematicide oxamyl. *Annals of Applied Biology*, 100: 521–525.

Wyss, U. and F. M. W. Grundler. 1992. "Feeding behavior of sedentary plant parasitic nematodes." *Netherlands Journal of Plant Pathology*, 98: 165-173.

Yu, Y. L., Wu, X. M., Li, S. N., Fang, H., Zhan, H. Y. and Yu, J. Q. 2006. An exploration of the relationship between adsorption and bioavailability of pesticides in soil to earthworm. *Environmental Pollution*, 141: 428-433.

Zaheer, K., Fleming, C. C. and Turner, S. J. 1993. Distribution and frequency of occurrence of potato cyst nematode pathotypes in Northern Ireland. *Plant Pathology*, 42: 609-616.

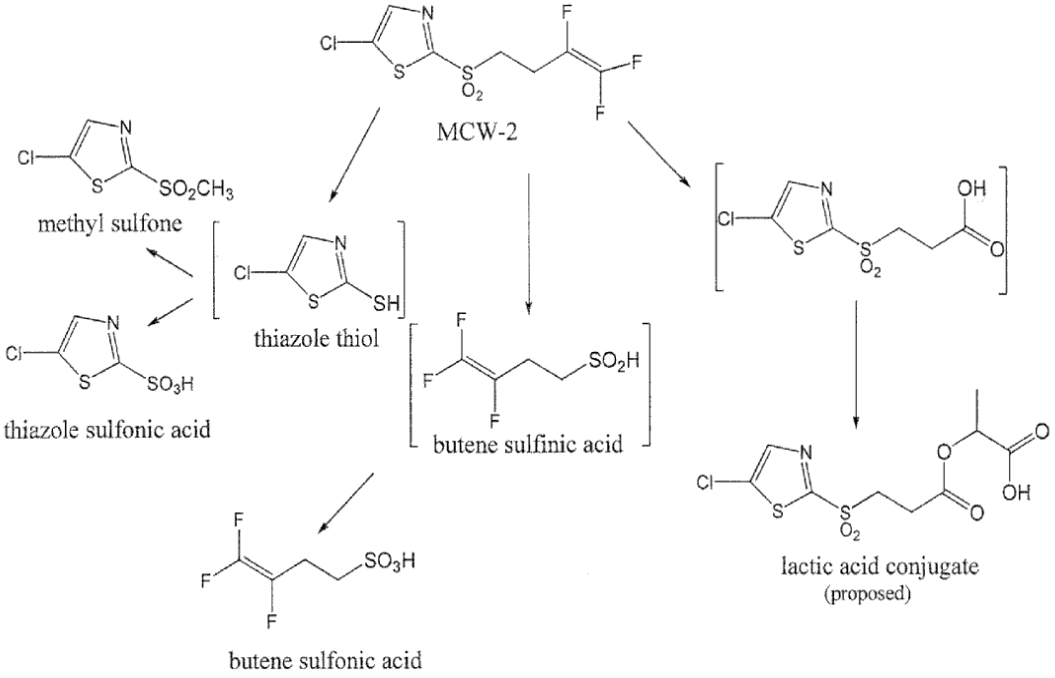
Zheng, S. Q. and Cooper, J. F. 1996. "Adsorption, desorption, and degradation of three pesticides in different soils." *Archives of Environmental Contamination and Toxicology*, 30: 15-20.

Zheng, W., Guo, M., Chow, T., Bennett, D. N. and Rajagopalan, N. 2010. Sorption properties of greenwaste biochar for two triazine pesticides. *Journal of Hazardous Materials*, 181: 121–126.

Zunke, U. and Eisenback, J. D. 1998. Morphology and ultrastructure. *In*: S. B. Sharma (ed.), The Cyst Nematodes. Kluwer Academic Publishers, Dordrecht, the Netherlands. pp. 31 – 56.

LIST OF APPENDIXES

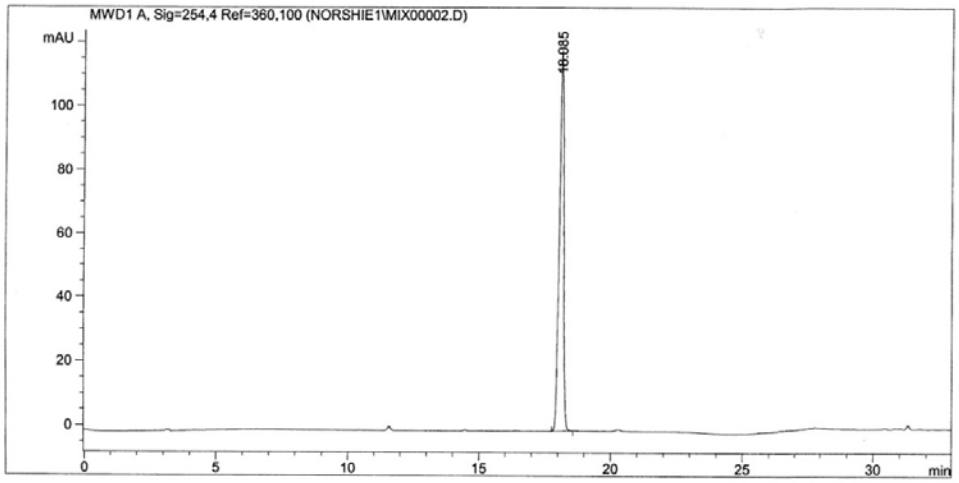
Appendix 1. Proposed metabolic pathway for fluensulfone



Appendix 2. Representative chromatogram for fluensulfone

Data File C:\HPCHEM\1\DATA\NORSHIE1\MIX00002.D Sample Name: MCW 1/5 dil

```
Injection Date  : 5/27/2011 1:35:15 PM          Seq. Line :   1
Sample Name     : MCW 1/5 dil                    Location : Vial 1
Acq. Operator   : Patrick Norshie                    Inj :   2
Acq. Instrument : Instrument 1                  Inj Volume : 10 µl
Sequence File   : C:\HPCHEM\1\SEQUENCE\MCW-2.S
Method          : C:\HPCHEM\1\METHODS\MCW-2.M
Last changed    : 5/27/2011 11:58:03 AM by Patrick Norshie
MCW-2
```




```
                        External Standard Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=254,4 Ref=360,100

                          Area Percent Report

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=254,4 Ref=360,100

Peak RetTime Type  Width     Area      Height     Area
  #   [min]       [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  18.085 BB    0.1810 1379.70496  119.19561 100.0000

Totals :                  1379.70496  119.19561

                          *** End of Report ***
```

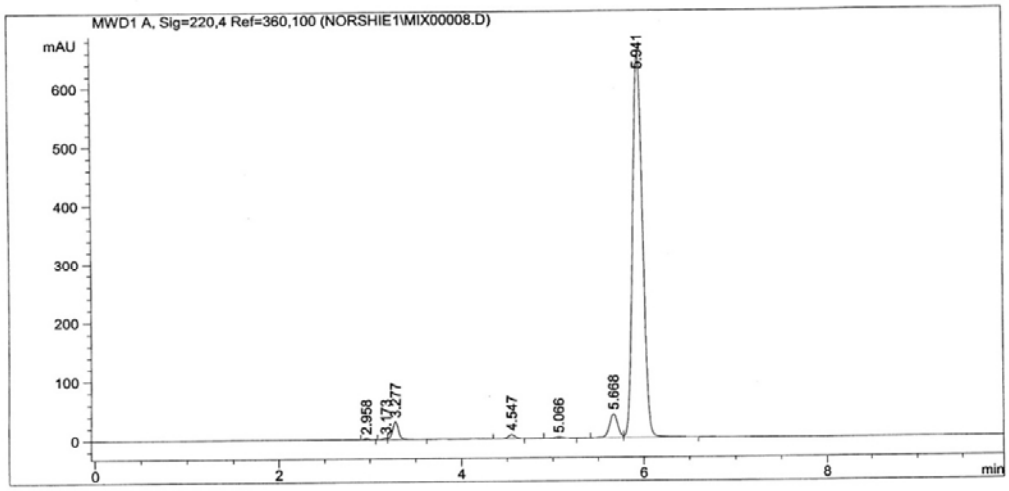
Instrument 1 5/27/2011 6:03:16 PM Patrick Norshie Page 2 of 17

Appendix 3. Representative chromatogram for fosthiazate

Data File C:\HPCHEM\1\DATA\NORSHIE1\MIX00008.D Sample Name: Fost1/2 dilution

Injection Date	: 5/27/2011 4:23:01 PM	Seq. Line	: 6
Sample Name	: Fost1/2 dilution	Location	: Vial 6
Acq. Operator	: Patrick Norshie	Inj	: 1
Acq. Instrument	: Instrument 1	Inj Volume	: 10 µl
Sequence File	: C:\HPCHEM\1\SEQUENCE\MCW-2.S		
Method	: C:\HPCHEM\1\METHODS\FOS.M		
Last changed	: 5/27/2011 12:50:18 PM by Patrick Norshie		

Fos



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Sig=220,4 Ref=360,100

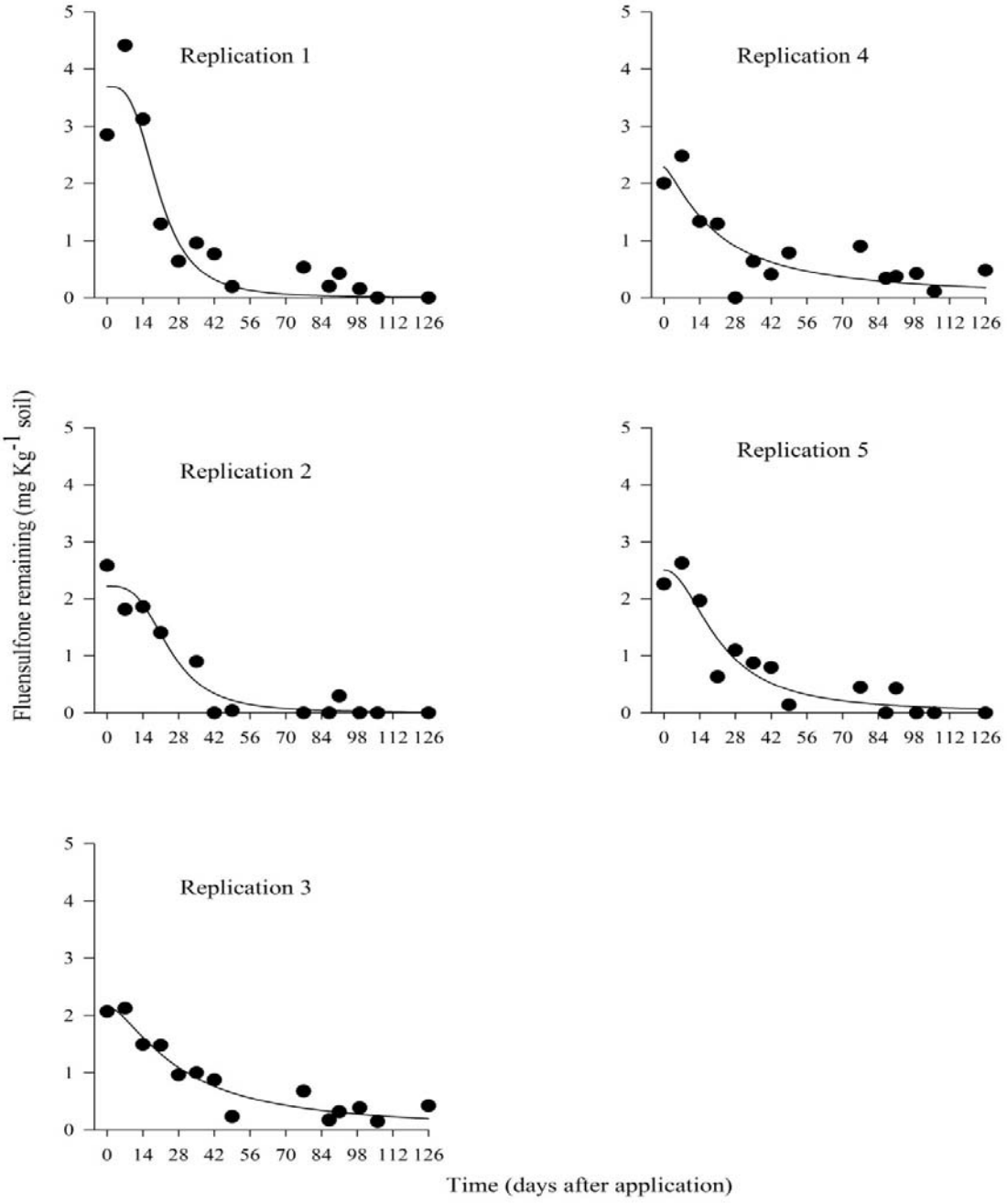
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.958	BB	0.0534	8.55280	2.41047	0.1574
2	3.173	BV	0.0521	8.61823	2.50959	0.1586
3	3.277	VB	0.0697	139.66452	30.31823	2.5700
4	4.547	VP	0.0800	34.80566	6.77098	0.6405
5	5.066	VB	0.1031	14.60238	2.15396	0.2687
6	5.668	PV	0.1012	261.55124	40.57749	4.8128
7	5.941	VB	0.1185	4966.67773	654.70306	91.3921
Totals :				5434.47256	739.44377	

Summed Peaks Report

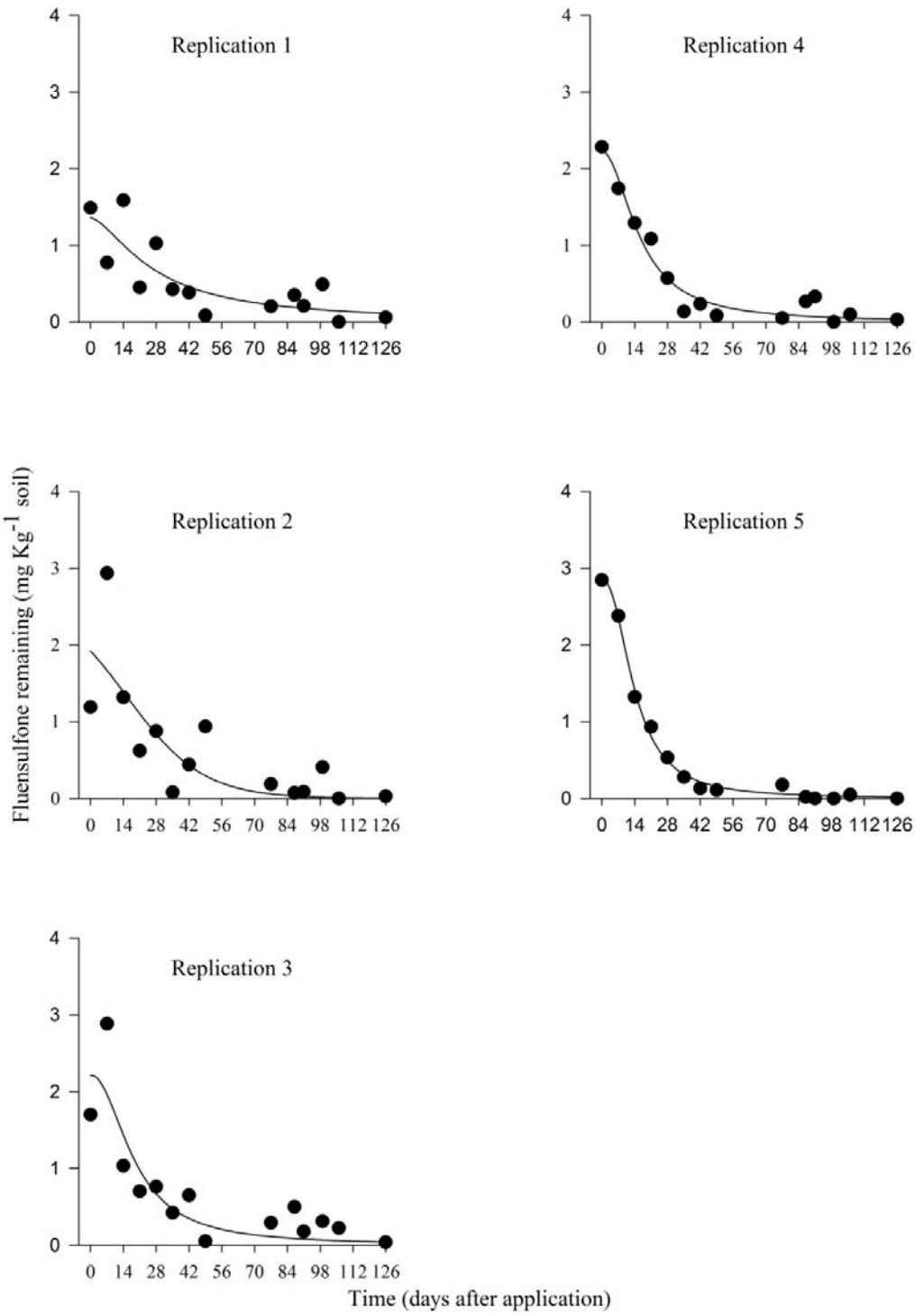
Signal 1: MWD1 A, Sig=220,4 Ref=360,100

Final Summed Peaks Report

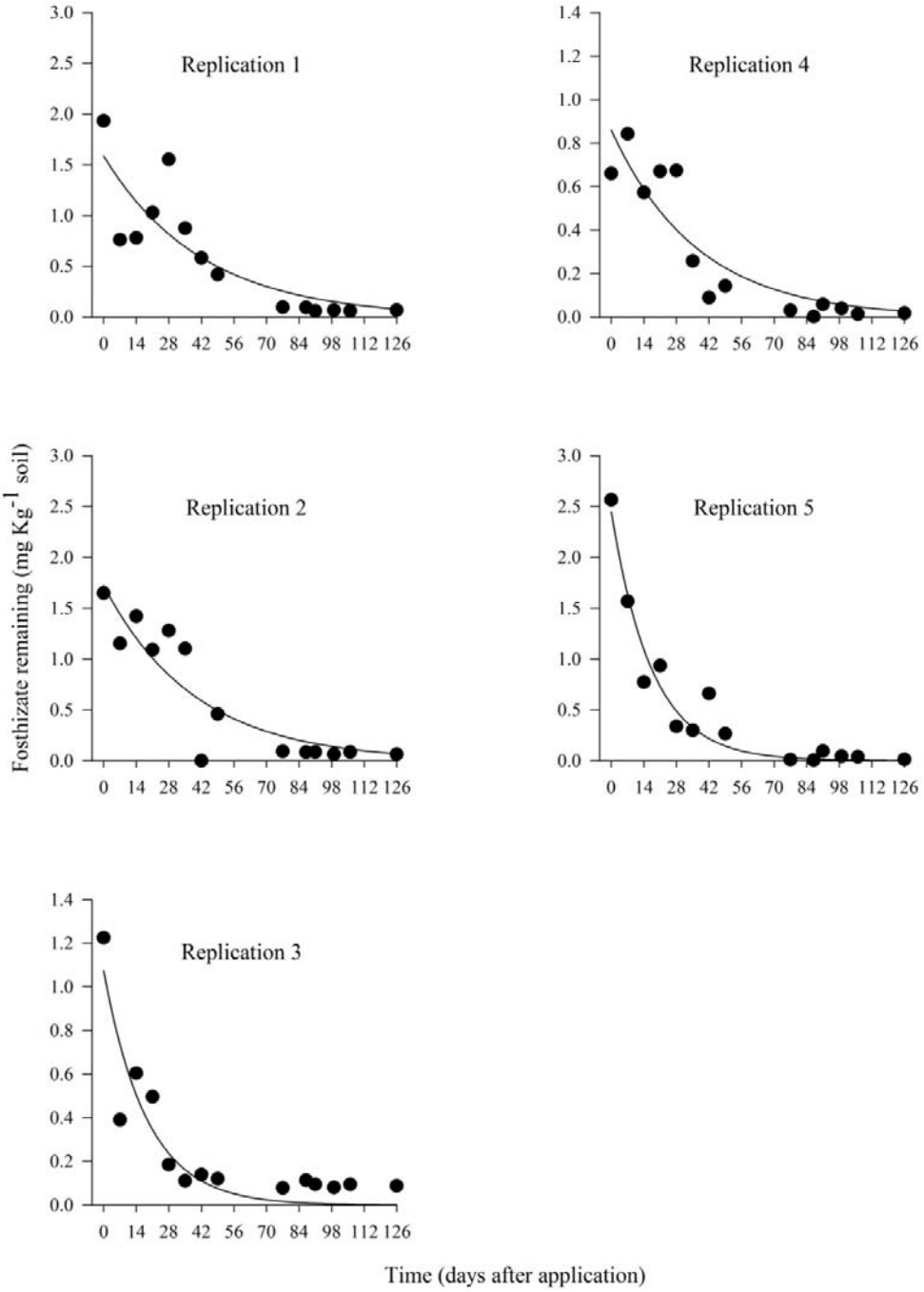
Signal 1: MWD1 A, Sig=220,4 Ref=360,100



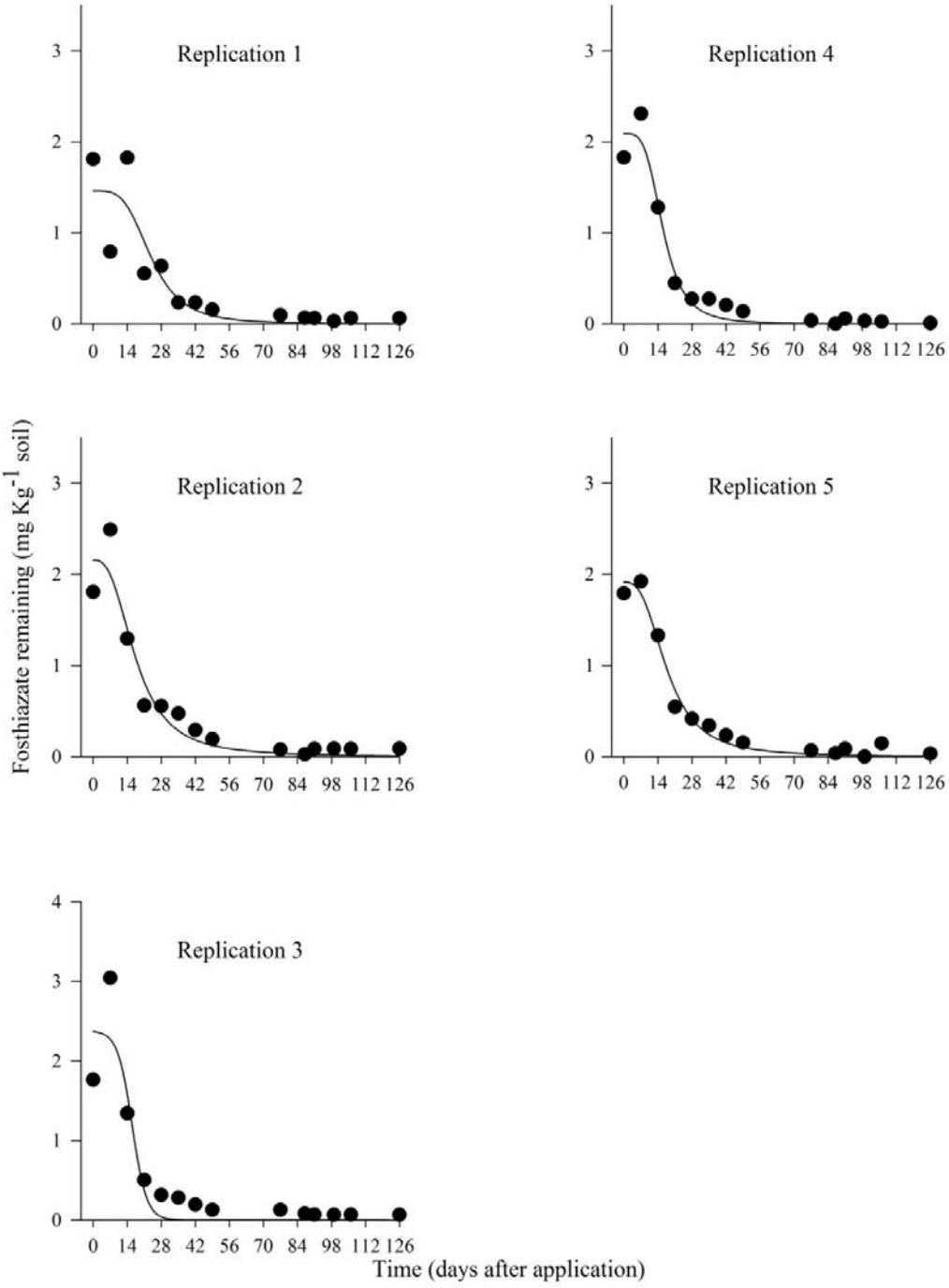
Appendix 4. Fluensulfone quantified in field soil over 126 days as per replicate plots at Woodcote (Shropshire, 2010).



Appendix 5. Fosthiazate quantified in field soil over 126 days as per replicate plots at Howle (Shropshire, 2010).



Appendix 6. Fluensulfone quantified in field soil over 126 days as per replicate plots at Howle (Shropshire, 2011).



Appendix 7. Fosthiazate quantified in field soil over 126 days as per replicate plots at Howle (Shropshire, 2011).