Persistence of the nematicide fluensulfone in potato (*Solanum tuberosum* ssp. *tuberosum*) beds under field conditions

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Title: Persistence of the nematicide fluensulfone in potato (*Solanum tuberosum* ssp. *tuberosum*) beds under field conditions

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Summary - As part of a broader study to evaluate the efficacy of fluensulfone for control of the potato cyst nematode *Globodera pallida*, two field experiments in Shropshire (at Woodcote and Howle in 2010 and 2011, respectively) England, were used to monitor the persistence of fluensulfone in potato beds treated with Nimitz 15G® (fluensulfone) at 27 Kg ha^{-1}. Fluensulfone dissipated at similar rates in the two fields, with a trend best described by a sigmoidal curve. The time to 50% dissipation (DT_{50}) was 24.3 days at Woodcote, and 23.7 days at Howle. No differences were found between the DT_{50} for fluensulfone and that observed for fosthiazate. The short DT_{50} demonstrated for fluensulfone in this study is an attribute in that it may pose a negligible hazard to the environment. However, its persistence at an effective dose may be long enough to be effective over the peak hatch period of *G. pallida*.

Keywords – Fosthiazate; *Globodera pallida*; half-life; management; Potato cyst nematodes

Nematicides are a vital component in the integrated management of potato cyst nematodes (PCN), *Globodera rostochiensis* (Wollenweber) Skarbilovich, (Woll.) and *G. pallida* (Stone) Behrens in the United Kingdom (Haddock & Evans, 1998). However, due to environmental concerns and the risk posed to human health a number of nematicides have been withdrawn. The limited remaining options (fosthiazate and oxamyl) may not qualify for reregistration under Regulation (EC) No 1107/2009, which is based on stringent hazard based criteria. The organophosphate, fosthiazate (as Nemathorin 10G; Syngenta Crop Protection Ltd, Cambridge, UK) and the carbamate, oxamyl (as Vydate 10G; DuPont Crop Protection Ltd, Stevenage, UK) are acetylcholine esterase inhibitors (Opperman & Chang, 1990), which are reported to control PCN by interfering with host finding abilities of the infective juvenile (Evans *et al.*, 1982), which ultimately reduces the extent of root damage during infection. However, fosthiazate and oxamyl have been reported as being ineffective for the control of *G. pallida* (Whitehead *et al.*, 1994). The challenge for nematicides to achieve effective control of *G. pallida* is commonly ascribed to their short term persistence in soil (Whitehead *et al.*, 1984; Whitehead *et al.*, 1991); Haydock *et al.*, 2012). The rapid degradation of fosthiazate and oxamyl combined with the slower hatching of *G. pallida* (Whitehead, 1992; Ryan *et al.*, 2003) means that the peak juvenile hatch can avoid effective nematicide concentrations (Evans, 1993), and potato roots are infected for a longer period. A study of the hatching behaviour of *G. pallida* in relation to persistence of the nematicide oxamyl, for instance, showed that an effective control will require it to extend for greater than 3 weeks (Haydock & Evans, 1998).

Fluensulfone is a relatively new nematicidal compound from ADAMA Agricultural Solutions Ltd., with activities against the root-knot nematode, *Meloidogyne species* (Oka *et al.*, 2009; Oka *et al.*, 2013; Morris *et al.*, 2015; Oka *et al.*, 2012), and the migratory nematodes, *Pratylenchus penetrans*, *P. thornei* and *Xiphinema index* (Oka, 2014). Fluensulfone is suggested (Kern *et al.*, *In Press*) to involve metabolic impairment, leading

to an inability to utilize lipid stores and death. Previously, we have presented findings on the efficacy of fluensulfone against *G. pallida* under field conditions in Shropshire, England (Norshie *et al.*,2016), where it was shown that a full rate application of fluensulfone (as Nimitz 15G at 27 kg ha^{-1}) at potato planting could be an option for the control of *G. pallida*. During these field experiments, the loss of fluensulfone from the potato beds was also determined. Since persistence influences nematicide efficacy, it was critical to establish whether fluensulfone will persist in soil for a sufficient period to reduce *G. pallida* effectively. The specific objectives were to determine the dissipation rate (*k*) and the time to 50% dissipation (DT_{50}) for the full rate application of fluensulfone in comparison to the field rate application of the nematicide fosthiazate (Nemathorin 10G).

Materials and methods

EXPERIMENTAL SITES, NEMATICIDE APPLICATION AND GENERAL AGRONOMY

The experiments used commercial potato growers' fields at Woodcote (UK Ordnance Survey Grid Reference: SJ 76901 15708) in 2010, and at Howle (UK Ordnance Survey Grid Reference: SJ 69485 23830) in 2011. The soils at both sites were sandy clay loam (1.8% organic matter, 6.6 pH, 14.8% moisture content at 5 KPa at Woodcote; 2.2% organic matter, 5.6 pH, 13.9% moisture content at 5 KPa at Howle). The growers cultivated the fields according to standard commercial practice. Both fields had received fosthiazate treatments five years prior to the experiments. Experimental plots measured 3.6 m wide and 6.0 m long, and comprised of four ridges (drills) with the outer two acting as guards. The experiments utilised certified potato (*Solanum tuberosum* ssp. *tuberosum*) seed (Super

Elite grade II) graded to 35 - 45 mm of the variety Estima (PCN susceptible). Nimitz 15G (ADAMA Agricultural Solutions Ltd., Airport City, Israel) and Nemathorin 10G (Syngenta Crop Protection Ltd., Cambridge, UK) were broadcasted on the beds at 27 kg ha^{-1} and at 30 kg ha^{-1} respectively, and incorporated to 0-20 cm depth using a tractor mounted Jones Bed-former (Jones Engineering Westwood Doncaster, UK). The Estima seed tubers were planted manually at a depth of 10-15 cm and 25 cm in-row spacing in the rows on 20 and 21 May 2010 at Woodcote, and 21 and 22 April 2011 at Howle. The experiments were randomized complete block designs with five replications.

SOIL SAMPLING, DETERMINATION OF SOIL TEMPERATURE AND AMOUNT OF PRECIPITATION

Soil sampling for fluensulfone laboratory analysis started within 4 hours of planting (i.e. zero days after application; 0 DAA), and then at 7-8 day intervals over the entire duration of the experiments. At each sampling point, 10 soil cores (2.5 cm diameter × 20 cm deep) were taken from each plot, bulked, thoroughly mixed and transferred to a 500 mL polypropylene bags, which were sealed and stored, within 1 hour after sampling, at -20ºC for analysis. Soil temperature was recorded with a pair of Tinytag Plus 2 temperature data loggers (Gemini data loggers, West Sussex, UK) buried at 15 cm depth. Rainfall records were taken at Harper Adams University, Newport in Shropshire (approximately 6.0 km from the experiments).

SOIL ANALYSIS

Technical-grade fluensulfone (> 95% purity, lot number 130291-PF-2) was supplied by ADAMA Agricultural Solutions Ltd, 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. Fluensulfone and fosthiazate were quantified in the soil samples by HPLC (High Performance Liquid Chromatography) analysis on an Agilent Technologies 1100 series apparatus (Agilent Technologies Ltd, Stockport, UK), which was equipped with an auto-sampler, a binary pump system, 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 protocol by ADAMA Agricultural Solutions Ltd. 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 comprised acetonitrile (eluent A) and 0.1% orthophosphoric acid in water (eluent B) mixture, and was set to flow at the gradient shown in Table 1. The injected volume (20 µL) was monitored at 254 nm peak area. Fosthiazate was analysed using the same column. , and a 20 µL aliquot was injected and but monitored at 230 nm peak area (Osborn *et al.*, 2010). The mobile phase was an 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 min and 5.9 min, respectively.

CALIBRATION OF HPLC EQUIPMENT

A stock solution 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 dilution of 100 µg mL^{-1}. Dilutions of fluensulfone and fosthiazate (0.001, 0.01, 0.1, 0.25, 0.50, 1.00, 2.5, 5 and 10 µg mL) were prepared from the stock solutions. Each dilution was injected

into the HPLC four times, starting with the lowest. The stock solutions were stored at 4°C until were needed for analysis where fresh working dilutions were prepared. The validities of the methods were determined by correlations between peak areas and concentrations. The correlations in each case were positively linear, and occurred over 0.1, 0.25, 0.50 and 1.0 µg mL for fluensulfone (a = 11.18b + 0.21, *r*= 0.996) and over 0.10, 1.0, 2.5 and 5.0 µg mL for fosthiazate (a = 14.56b + 0.25, *r* = 0.999).

VALIDATION OF EXTRACTION METHODS

The recovery of fluensulfone from field soil was determined by spiking the soil with either technical grade fluensulfone or Nimitz 15G. The field soil was collected from the plots left untreated at Woodcote and Howle in 2010 and 2011, respectively. The soil was 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 Nimitz 15G. The samples were allowed to stand for ca. 30 min before a 20 mL acetonitrile/water mixture (1/1, v/v) was added, and the mixture agitated at 300 rpm for 1h on a HS 501 Digital reciprocal shaker (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The samples were then allowed to stand for ca. 1h before 1 mL of the supernatant was removed with a 2 mL syringe (BD Plastics Ltd, UK) and sieved through a 0.2 µm pore size Polyvinylidene Difluoride (PVDF) syringe filter (GE Healthcare UK Ltd) into a screw cap 2 mL HPLC glass vial. A similar protocol was used to determine the recovery of fosthiazate from soil, but only analytical grade fosthiazate was used and the samples were shaken for a period of 3h (Osborn *et al.*, 2010). The mean recovered fluensulfone from the Nimitz 15G treated-soil was 85.8% (82.8 – 88.7%), which was lower than 98.2% (92.4 – 103%) for the technical grade fluensulfone spiked-soil. For this reason,

the Nimitz 15G treated-soil was agitated further, and analysed after 2, 3, 4 and 12h of agitation. Subsequent analysis showed that most fluensulfone (91%) was extracted after 3h of agitation. The recovered fosthiazate was 92 % (86 – 102%).

RELEASE OF FLUENSULFONE FROM THE GRANULAR FORMULATION

As part of the validation of the method, two tests were conducted to determine the release rates of fluensulfone from the granular formulation. The tests were made in order to optimise the extraction of fluensulfone from the soil samples, and to determine any effects of the formulation on the availability of fluensulfone upon application to the soil. Test 1, which was repeated three times, quantified total fluensulfone in the formulation. With this, 27 mg of granules (4.05 mg fluensulfone) were transferred in 100 mL glass bottles and shaken at 300 rpm for 1, 2, 3, 4 and 12 h in 20 mL of acetonitrile without soil. This was repeated three times. At each time point, 1 mL of the solvent was sampled and analysed for fluensulfone. Test 2 (repeated twice) was undertaken to determine a water-induced release of fluensulfone from the formulation. 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 a 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.

QUANTIFICATION OF FLUENSULFONE AND FOSTHIAZATE IN SOIL

Soil 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 20g sub-sample transferred in a 100 mL glass shaking bottle and agitated 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 30 minutes when 1mL of the supernatant was sampled, and was either analysed immediately or stored at -20°C for future analysis.

DATA ANALYSIS

The measured concentrations for each replicate plot were regressed against time (DAA), and the parameters *k* and DT_{50} estimated from curves which best fitted the data (Osborn *et al.*, 2010). 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). There were a few instances when the 0 DAA samples contained lower concentrations than were detected at 7 DAA. In these instances, the starting concentration for calculating the DT_{50} value was estimated from the fitted curve as recommended by Forum for the Co-ordination of pesticide fate models and their use, FOCUS. (FOCUS 2006).

Results

SOIL TEMPERATURE AND PRECIPITATION

Figure 1 shows the soil temperature and precipitation during the field experiments. The mean soil temperature was 17.04 ºC at Woodcote and 14.96ºC at Howle. No precipitation was recorded until 7 DAA. Total rainfall recorded over 126 days was 378.8 mm and 370.0 mm at Woodcote and Howle, respectively.

DISSIPATION OF FLUENSULFONE AND FOSTHIAZATE IN POTATO BEDS

Figure 2 shows the concentration of fluensulfone and fosthiazate in the 20 cm soil depth of potato beds over 126 days at Woodcote and Howle in 2010 and 2011, respectively. The mean concentration of fluensulfone at 0 DAA at Woodcote was 2.35 mg Kg^{-1} soil, and was slightly higher by 7 DAA (2.69 mg Kg^{-1} soil). It then dissipated quite rapidly through 14 DAA and 21 DAA to 0.54 mg Kg^{-1} soil (ca. 63% dissipation) by 28 DAA. Subsequent losses occurred rather slowly, with the concentrations fluctuating between 0.27 and 0.12 mg Kg^{-1} soil. The loss of fluensulfone from the potato beds at Howle followed a similar trend to that observed at Woodcote. 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 14, 21 and 28 DAA to 0.27 mg Kg^{-1} soil (ca. 75 % dissipation) by 35 DAA. Further losses appeared rather slowly, with fluctuating concentrations detected up until the final sampling at 126 DAA. Overall, fluensulfone dissipated at similar rates in the two fields (Table 2), and the trends followed a sigmoidal equation (Figure 2). The DT_{50} varied from 19.6 to 30.0 days, with a mean of 24.3 days at Woodcote, and varied from 13.9 to 31.5 days, with a

mean of 23.7 days at Howle. Fosthiazate dissipated at different rates in the two fields (Table 2). The trend was exponential at Woodcote (Figure 2), with 34 % dissipation occurring within the first 7 DAA. This was then followed by a period of no significant loss until 42 DAA when 0.23 mg Kg^{-1} soil remained (ca. 87% dissipation). The trend was exponential sigmoidal at Howle (Figure 2), with no losses 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} ranged from 10.4 to 35.2 days, with a mean of 21.3 days at Woodcote, and 16.3 – 21.1 days, with a mean of 18.8 days at Howle. The DT_{50} values obtained did not differ between the nematicides or the fields (Figure 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. 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 4^{th} 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 samples collected at 1 and 12h (first two points shown on figure 4b), when as much fluensulfone was released thereafter at 5ºC as at 20ºC incubation temperature, the amount released thereafter at later sampling times was greater at 20ºC than at 5ºC, figure 4b. 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.

Discussion

The DT_{50} for fluensulfone in this study suggests the half-life (persistence) is no longer than 24 days, and was similar to that observed for fosthiazate. This implies that half the concentration of both nematicides may have dissipated prior to peak J2 hatch at between 42 – 56 days (Haydock and Evans, 1998) Conversely, whilst the DT_{50} may be an important index for environmental persistence it may convey limited, if any, information on the minimum effective dosage for control of *G. pallida* or the length of that control. Indeed, control of *G. pallida* by the full rate fluensulfone treatments was evident in the field experiments (Norshie *et al*., 2016), which provided the soil for these laboratory analyses. This suggests that soil concentrations were at effective concentrations. Also, preliminary *in vitro* experiments (unpublished data) showed that fluensulfone acted against *G. pallida* hatching 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} 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. Similarly, the motility of J2 *G. pallida* was reduced following incubation in fluensulfone at 0.0078 – 32 mgL^{-1} for 24 – 72h. The concentration of fluensulfone remaining in the beds 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. Even with the calculated DT_{50} suggesting short persistence, fluensulfone may remain in the soil in sufficient concentrations to control *G. pallida*, and the same can be said of fosthiazate, which had a similar persistence to fluensulfone.

The dissipation kinetics for fluensulfone were similar for both fields. This could be explained by the similar soil and growing conditions under which the experiments were done. 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. Even though the incubation tests are made under laboratory conditions not directly comparable to field situations, gradual release of fluensulfone from the granular formulation in the soil is plausible, and may influence the persistence of fluensulfone by retarding its availability to degradation and/or leaching processes, which are concentration dependent. The lag phase of seven days preceding the dissipation of fluensulfone in both fields could be due to an initial delay in release which could limit availability 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 soil moisture as affected by rainfall or irrigation application (Noling, *2003*; Smelt & Leistra 1992; Rich *et al.*,2003). As shown in Figure 1, no precipitation occurred at either site until 7 DAA. Therefore, the release kinetics from the formulation may partly explain the initial delay in dissipation measured in the field plots. Indeed, the loss of fluensulfone from the plots in both fields coincided with the onset of precipitation/rainfall, and the subsequent rapid decline in concentration occurred during the period of highest precipitation. This highlights the likely importance of soil moisture on the overall persistence of 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 affect the persistence in the rhizosphere of the potato and, thus, its ability to protect roots from invasion. Soil samples in this study were only collected from the depth of incorporation (i.e. topmost 15 – 20 cm) and, assuming that there was leaching during the sampling period, it could be that fluensulfone was leached to deeper layers rather than being

degraded as such. The same could be suggested for the loss of fosthiazate, which is already known to be prone to leaching (Karpouzas *et al.*, 2007). Further laboratory/controlled environment studies modifying these factors could determine their likely influence on persistence, and therefore, the efficacy of fluensulfone.

In summary, the short DT_{50} demonstrated for fluensulfone in this study is an attribute in that it may pose a negligible hazard to the environment. However, its persistence at an effective dose may be long enough to be effective over the peak hatch period of *G. pallida.*

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Table 1. 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

*****: Time phase was provided by ADAMA Agricultural Solutions, Airport City, Israel.

Table 2. Dissipation rates constant (k, day^{-1}) and coefficient of determination (r^2) obtained for nematicides fluensulfone and fosthiazate in potato beds treated with Nimitz 15 at 27 kg ha^{-1} and Nemathorin 10G at 30 kg ha^{-1} at Woodcote in 2010 and at Howle in 2011. Values are means ± standard error.

Field Site	Nematicide	k, day^{-1}	r^2	Significance
Woodcote (2010)	Fluensulfone	2.36 ± 0.40	95.45 ± 0.20	$P < 0.0001$
Woodcote (2010)	Fosthiazate	0.03 ± 0.01	94.87 ± 0.18	$P < 0.0001$
Howle (2011)	Fluensulfone	2.12 ± 0.28	96.45 ± 0.13	$P < 0.0001$
Howle (2011)	Fosthiazate	3.13 ± 0.54	97.15 ± 0.20	$P < 0.0001$

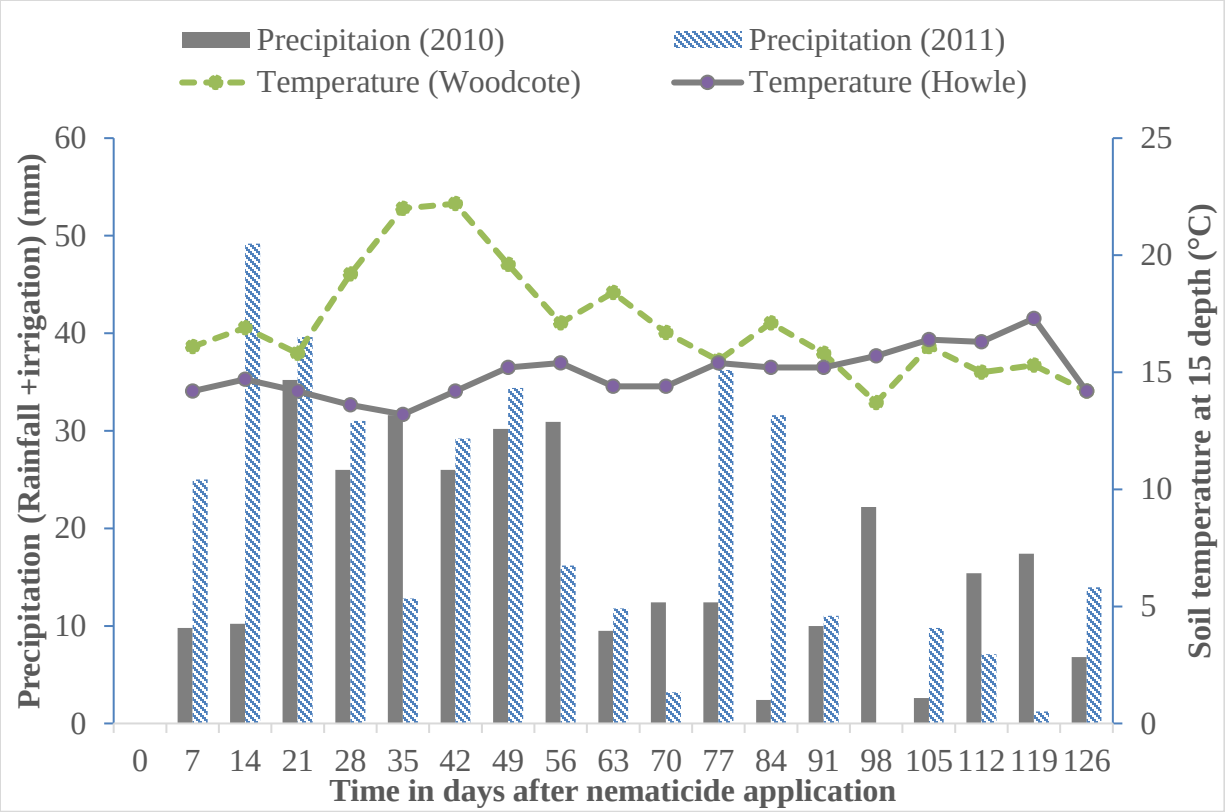

Figure 1. Mean soil temperature (°C) at 15 cm depth of potato beds and total precipitation (rainfall + irrigation) as recorded at Harper Adams University over 126 days of in-field dissipation studies of nematicides fluensulfone and fosthiazate at Woodcote in 2010 and at Howle in 2011.

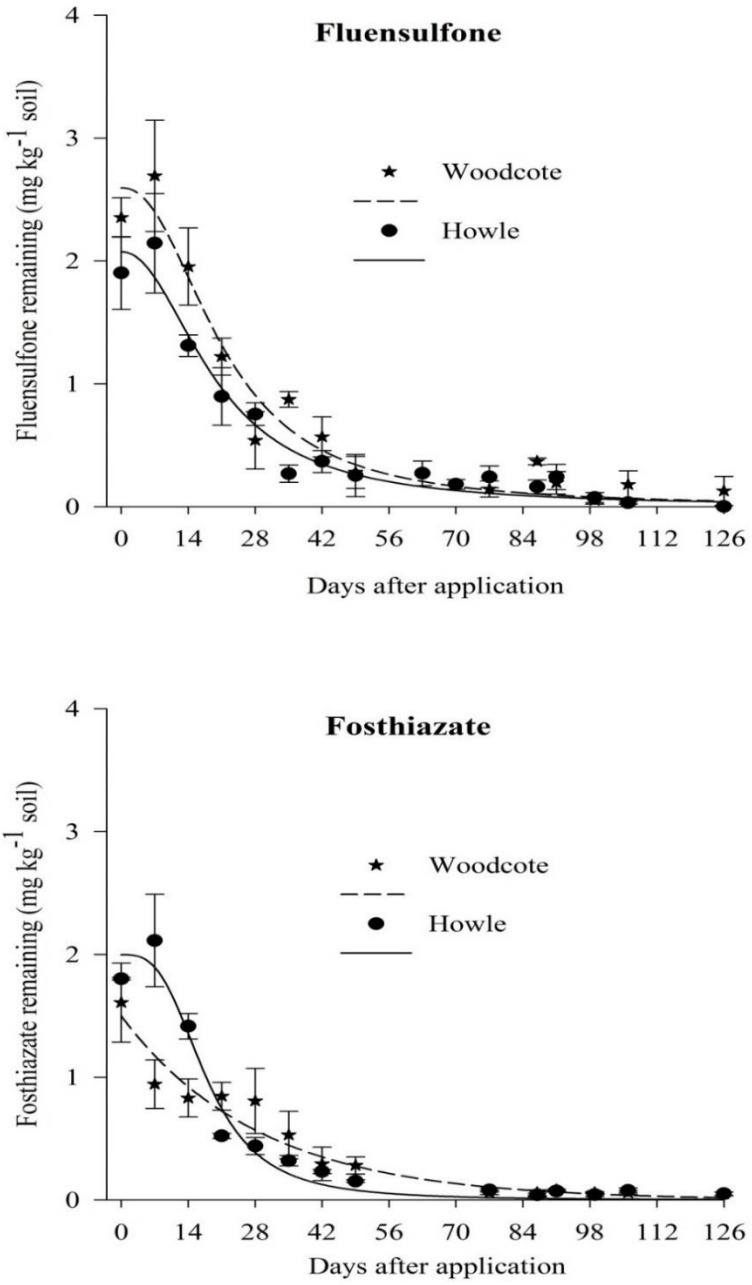

Fig. 2. Nematicides fluensulfone and fosthiazate quantified in the topmost 20 cm soil layer of potato beds following treatments with Nimitz 15G at 27 kg ha^{-1} and Nemathorin 10G at 30 kg ha^{-1} at Woodcote in 2010 and at Howle in 2011. The loss of fluensulfone followed the equations $Y= 4.04/(1+^{(-(X-10.38)/-16)})$ at Woodcote and $Y = 2.64/(1+^{(-(X-15.66)/-11.7)})$ at Howle. The loss of fosthiazate followed $Y = 1.47^{-0.03X}$ at Woodcote and $Y = 2.12/(1+^{(-(X-17.78)/6.08)})$ at Howle.

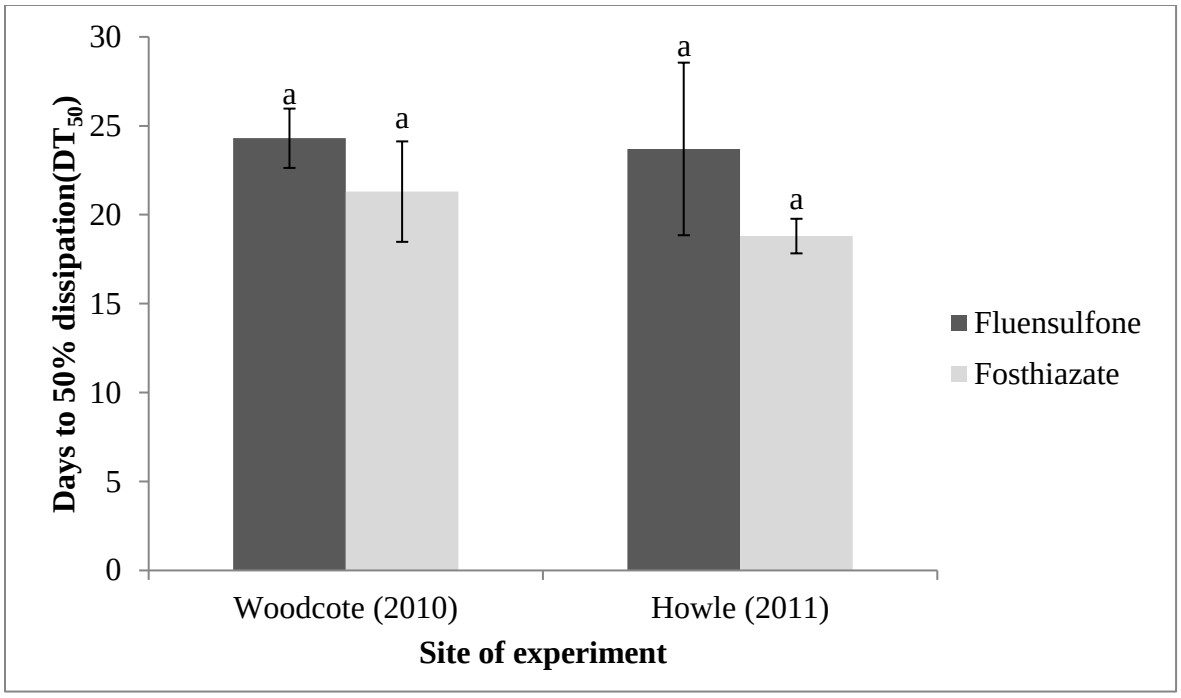

Figure 3. Comparisons of half-lives (DT_{50}) obtained for nematicides fluensulfone and fosthiazate at 20 cm depth of potato beds treated with Nimitz 15G at 27 kg ha^{-1} and Nemathorin 10G at 30 kg ha^{-1} at Woodcote in 2010 and at Howle in 2011. Bars represent standard error values (n = 5).

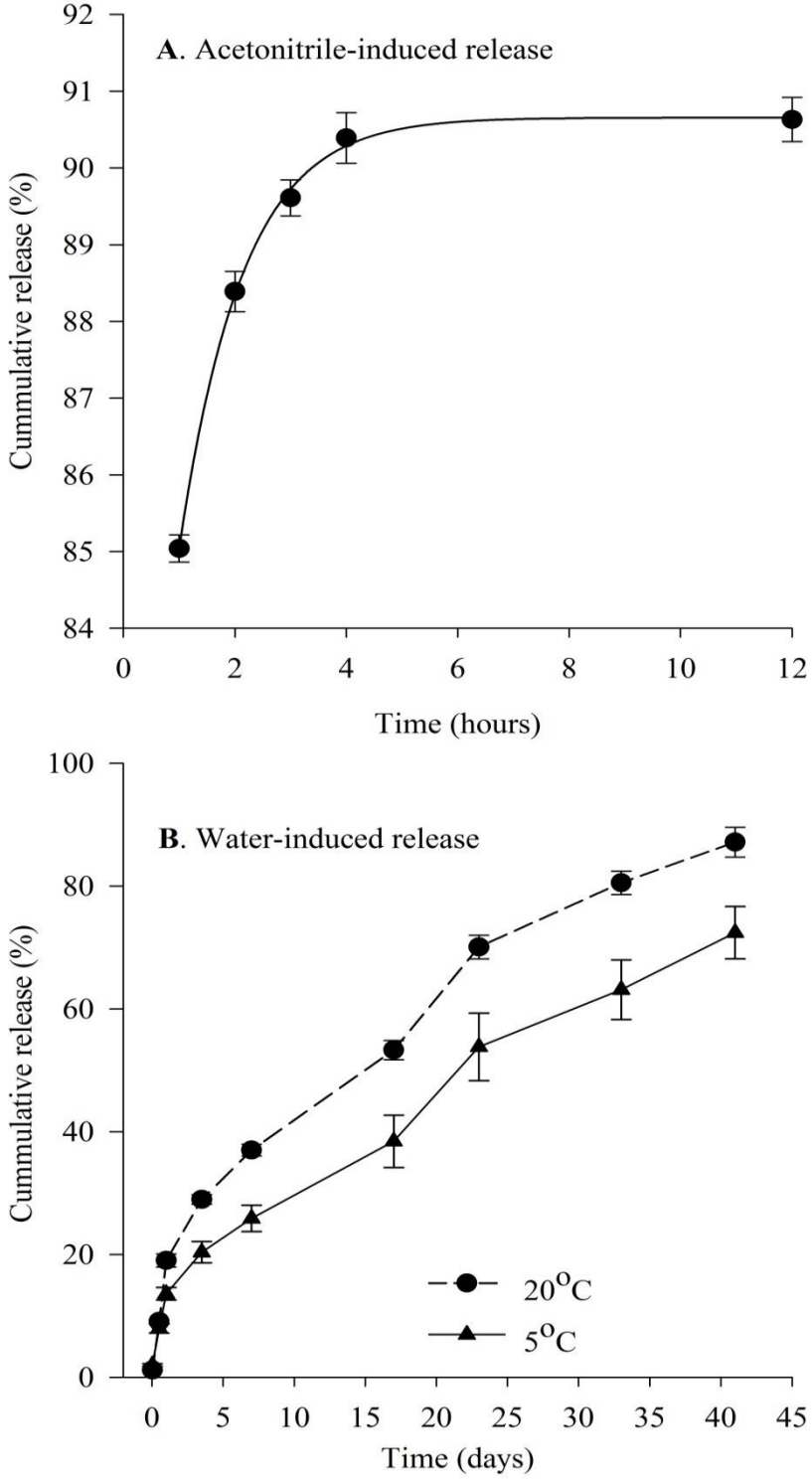

Figure 4. (A) Fluensulfone extracted from the granular formulation with acetonitrile over 12h and (B) water-induced release kinetics of fluensulfone from the granular formulation over 41 days. Percentage cumulative release was plotted against duration of incubation. Bars show the standard error of the mean (n = 3)