

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

by Norshie, P.M., Grove, I.G. and Back, M.A.

Copyright, Publisher and Additional Information: This is the author accepted manuscript. The final published version (version of record) is available via Brill Online Please refer to any applicable terms of use of the publisher.

DOI: [10.1163/15685411-00003085](https://doi.org/10.1163/15685411-00003085)



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

3

4

5

6 Patrick M Norshie^{1*}, Ivan G Grove² & Matthew A Back²

7

8

9 ¹ *Department of Agronomy, University College of Agriculture and Environmental Studies,*
10 *P. O. Box 27, Bunso, Eastern Region, Ghana.*

11

12

13

14 ²*Nematology and Entomology Group, Crop and Environment Research Centre, Harper*
15 *Adams University, Newport, Shropshire, TF10 8NB, UK*

16

17

18

19 *Correspondence: Patrick M Norshie (Ph.D.), Department of Agronomy, University*
20 *College of Agriculture and Environmental Studies, P. O. Box 27, Bunso, Eastern Region,*
21 *Ghana. E-mail: norshie@yahoo.com*

1 **Summary** - As part of a broader study to evaluate the efficacy of fluensulfone for control
2 of the potato cyst nematode *Globodera pallida*, two field experiments in Shropshire (at
3 Woodcote and Howle in 2010 and 2011, respectively) England, were used to monitor the
4 persistence of fluensulfone in potato beds treated with Nimitz 15G® (fluensulfone) at 27
5 Kg ha⁻¹. Fluensulfone dissipated at similar rates in the two fields, with a trend best
6 described by a sigmoidal curve. The time to 50% dissipation (DT₅₀) was 24.3 days at
7 Woodcote, and 23.7 days at Howle. No differences were found between the DT₅₀ for
8 fluensulfone and that observed for fosthiazate. The short DT₅₀ demonstrated for
9 fluensulfone in this study is an attribute in that it may pose a negligible hazard to the
10 environment. However, its persistence at an effective dose may be long enough to be
11 effective over the peak hatch period of *G. pallida*.

12

13

14

15

16

17

18

19

20

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

1 Nematicides are a vital component in the integrated management of potato cyst
2 nematodes (PCN), *Globodera rostochiensis* (Wollenweber) Skarbilovich, (Woll.) and *G.*
3 *pallida* (Stone) Behrens in the United Kingdom (Haddock & Evans, 1998). However, due
4 to environmental concerns and the risk posed to human health a number of nematicides
5 have been withdrawn. The limited remaining options (fosthiazate and oxamyl) may not
6 qualify for reregistration under Regulation (EC) No 1107/2009, which is based on stringent
7 hazard based criteria. The organophosphate, fosthiazate (as Nemathorin 10G; Syngenta
8 Crop Protection Ltd, Cambridge, UK) and the carbamate, oxamyl (as Vydate 10G; DuPont
9 Crop Protection Ltd, Stevenage, UK) are acetylcholine esterase inhibitors (Opperman &
10 Chang, 1990), which are reported to control PCN by interfering with host finding abilities
11 of the infective juvenile (Evans *et al.*, 1982), which ultimately reduces the extent of root
12 damage during infection. However, fosthiazate and oxamyl have been reported as being
13 ineffective for the control of *G. pallida* (Whitehead *et al.*, 1994). The challenge for
14 nematicides to achieve effective control of *G. pallida* is commonly ascribed to their short
15 term persistence in soil (Whitehead *et al.*, 1984; Whitehead *et al.*, 1991); Haydock *et al.*,
16 2012). The rapid degradation of fosthiazate and oxamyl combined with the slower hatching
17 of *G. pallida* (Whitehead, 1992; Ryan *et al.*, 2003) means that the peak juvenile hatch can
18 avoid effective nematicide concentrations (Evans, 1993), and potato roots are infected for a
19 longer period. A study of the hatching behaviour of *G. pallida* in relation to persistence of
20 the nematicide oxamyl, for instance, showed that an effective control will require it to
21 extend for greater than 3 weeks (Haydock & Evans, 1998)

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

1 to an inability to utilize lipid stores and death. Previously, we have presented findings on
2 the efficacy of fluensulfone against *G. pallida* under field conditions in Shropshire,
3 England (Norshie *et al.*,2016), where it was shown that a full rate application of
4 fluensulfone (as Nimitz 15G at 27 kg ha⁻¹) at potato planting could be an option for the
5 control of *G. pallida*. During these field experiments, the loss of fluensulfone from the
6 potato beds was also determined. Since persistence influences nematicide efficacy, it was
7 critical to establish whether fluensulfone will persist in soil for a sufficient period to reduce
8 *G. pallida* effectively. The specific objectives were to determine the dissipation rate (*k*)
9 and the time to 50% dissipation (DT₅₀) for the full rate application of fluensulfone in
10 comparison to the field rate application of the nematicide fosthiazate (Nemathorin 10G).

11

12 **Materials and methods**

13

14 EXPERIMENTAL SITES, NEMATICIDE APPLICATION AND GENERAL 15 AGRONOMY

16

17 The experiments used commercial potato growers' fields at Woodcote (UK Ordnance
18 Survey Grid Reference: SJ 76901 15708) in 2010, and at Howle (UK Ordnance Survey
19 Grid Reference: SJ 69485 23830) in 2011. The soils at both sites were sandy clay loam
20 (1.8% organic matter, 6.6 pH, 14.8% moisture content at 5 KPa at Woodcote; 2.2% organic
21 matter, 5.6 pH, 13.9% moisture content at 5 KPa at Howle). The growers cultivated the
22 fields according to standard commercial practice. Both fields had received fosthiazate
23 treatments five years prior to the experiments. Experimental plots measured 3.6 m wide
24 and 6.0 m long, and comprised of four ridges (drills) with the outer two acting as guards.
25 The experiments utilised certified potato (*Solanum tuberosum* ssp. *tuberosum*) seed (Super

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

9

10 SOIL SAMPLING, DETERMINATION OF SOIL TEMPERATURE AND AMOUNT OF 11 PRECIPITATION

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

21

22 SOIL ANALYSIS

23 Technical-grade fluensulfone (> 95% purity, lot number 130291-PF-2) was supplied by
24 ADAMA Agricultural Solutions Ltd, Airport City, Israel. Analytical standard grade

1 fosthiazate (98.6% purity) was purchased from Fluka Analytical UK Ltd. Acetonitrile
2 (99.99% purity) and orthophosphoric acid (85% purity) were purchased from Fisher
3 Scientific Ltd and BDH Laboratory supplies UK Ltd, respectively. Water for all analyses
4 was prepared by Purite Stillplus HP Pack. Fluensulfone and fosthiazate were quantified in
5 the soil samples by HPLC (High Performance Liquid Chromatography) analysis on an
6 Agilent Technologies 1100 series apparatus (Agilent Technologies Ltd, Stockport, UK),
7 which was equipped with an auto-sampler, a binary pump system, multiple wavelength UV
8 detectors, and operated by Agilent ChemStation B.03 software for windows. The
9 chromatographic conditions for analysing fluensulfone were set according to a protocol by
10 ADAMA Agricultural Solutions Ltd. The separation column was a reversible Hypersil
11 Gold column (250 x 4.6 mm, and 5 μm particle size), and was used at 40°C oven
12 temperature. The mobile phase comprised acetonitrile (eluent A) and 0.1%
13 orthophosphoric acid in water (eluent B) mixture, and was set to flow at the gradient
14 shown in Table 1. The injected volume (20 μL) was monitored at 254 nm peak area.
15 Fosthiazate was analysed using the same column. , and a 20 μL aliquot was injected and
16 but monitored at 230 nm peak area (Osborn *et al.*, 2010). The mobile phase was an
17 acetonitrile and water mixture (1/1 v/v), and flowed at 1 mL min^{-1} . Under the above
18 conditions, fluensulfone and fosthiazate eluted at ca. 18.1 min and 5.9 min, respectively.

19

20 CALIBRATION OF HPLC EQUIPMENT

21

22 A stock solution each of fluensulfone and fosthiazate was prepared by dissolving 10 mg
23 chemical in 100 mL solvent (1/1 v/v mixture of acetonitrile and water) to give a dilution of
24 100 $\mu\text{g mL}^{-1}$. Dilutions of fluensulfone and fosthiazate (0.001, 0.01, 0.1, 0.25, 0.50, 1.00,
25 2.5, 5 and 10 $\mu\text{g mL}^{-1}$) were prepared from the stock solutions. Each dilution was injected

1 into the HPLC four times, starting with the lowest. The stock solutions were stored at 4°C
2 until were needed for analysis where fresh working dilutions were prepared. The validities
3 of the methods were determined by correlations between peak areas and concentrations.
4 The correlations in each case were positively linear, and occurred over 0.1, 0.25, 0.50 and
5 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
6 mL for fosthiazate ($a = 14.56b + 0.25$, $r = 0.999$).

7

8 VALIDATION OF EXTRACTION METHODS

9

10 The recovery of fluensulfone from field soil was determined by spiking the soil with either
11 technical grade fluensulfone or Nimitz 15G. The field soil was collected from the plots left
12 untreated at Woodcote and Howle in 2010 and 2011, respectively. The soil was air dried
13 and sieved to < 2 mm. Triplicate 20 g sub-samples were weighed into 100 mL glass
14 shaking bottles, and was either spiked with 1 mL of 50 µg mL⁻¹ of fluensulfone or mixed
15 with 27 mg Nimitz 15G. The samples were allowed to stand for ca. 30 min before a 20 mL
16 acetonitrile/water mixture (1/1, v/v) was added, and the mixture agitated at 300 rpm for 1h
17 on a HS 501 Digital reciprocal shaker (IKA®-Werke GmbH & Co. KG, Staufen,
18 Germany). The samples were then allowed to stand for ca. 1h before 1 mL of the
19 supernatant was removed with a 2 mL syringe (BD Plastics Ltd, UK) and sieved through a
20 0.2 µm pore size Polyvinylidene Difluoride (PVDF) syringe filter (GE Healthcare UK Ltd)
21 into a screw cap 2 mL HPLC glass vial. A similar protocol was used to determine the
22 recovery of fosthiazate from soil, but only analytical grade fosthiazate was used and the
23 samples were shaken for a period of 3h (Osborn *et al.*, 2010). The mean recovered
24 fluensulfone from the Nimitz 15G treated-soil was 85.8% (82.8 – 88.7%), which was lower
25 than 98.2% (92.4 – 103%) for the technical grade fluensulfone spiked-soil. For this reason,

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

4

5 RELEASE OF FLUENSULFONE FROM THE GRANULAR FORMULATION

6 As part of the validation of the method, two tests were conducted to determine the release
7 rates of fluensulfone from the granular formulation. The tests were made in order to
8 optimise the extraction of fluensulfone from the soil samples, and to determine any effects
9 of the formulation on the availability of fluensulfone upon application to the soil. Test 1,
10 which was repeated three times, quantified total fluensulfone in the formulation. With this,
11 27 mg of granules (4.05 mg fluensulfone) were transferred in 100 mL glass bottles and
12 shaken at 300 rpm for 1, 2, 3, 4 and 12 h in 20 mL of acetonitrile without soil. This was
13 repeated three times. At each time point, 1 mL of the solvent was sampled and analysed for
14 fluensulfone. Test 2 (repeated twice) was undertaken to determine a water-induced release
15 of fluensulfone from the formulation. In Test 2, 27 mg of the granules were placed in each
16 of ten plastic tubes (1.0 cm wide × 2.0 cm high) sealed at the bottom with nylon mesh (53-
17 µm aperture). Half of the tubes were placed upright in the wells of a 24-well plate to which
18 1 mL of distilled water was added, submerging the granules. The plate was covered, sealed
19 with parafilm and incubated at 5°C (to minimise potential degradation). The other half was
20 similarly treated and incubated at 20°C. After 1 h of incubation, each tube was carefully
21 lifted, and the entire water was removed by a pipette and transferred to 100 mL conical
22 flasks. The well was rinsed twice, and the water added to the flask. Fresh distilled water was
23 added to the well and the plates incubated as before. The water in the flask was made up to
24 the 100 mL mark (1/100 dilutions), and a 1 mL subsample was filtered through 0.2 µm

1 sieves to analyse for fluensulfone. Sampling and analysis for fluensulfone was repeated at
2 12h and 24h, and then at 3.5, 7, 17, 23, 35 and 41 days after incubation.

3 QUANTIFICATION OF FLUENSULFONE AND FOSTHIAZATE IN SOIL

4

5 Soil samples to be extracted were removed from -20°C storage, and left in plastic trays
6 overnight to thaw. Each sample was then thoroughly mixed, and a 20g sub-sample
7 transferred in a 100 mL glass shaking bottle and agitated in 20 mL of acetonitrile and water
8 (1/1 v/v) at 300 rpm for 3h. The bottle was left to stand for at least 30 minutes when 1mL
9 of the supernatant was sampled, and was either analysed immediately or stored at -20°C
10 for future analysis.

11

12 DATA ANALYSIS

13

14 The measured concentrations for each replicate plot were regressed against time (DAA),
15 and the parameters k and DT_{50} estimated from curves which best fitted the data (Osborn *et*
16 *al.*, 2010). Curve fitting and parameter estimations were carried out using SigmaPlot V.12
17 (Systat Software, Inc. London, UK). The parameters obtained were analysed by one-way
18 ANOVA using Genstat v.14 (VSN International Ltd., Hemel Hempstead, UK). There were
19 a few instances when the 0 DAA samples contained lower concentrations than were
20 detected at 7 DAA. In these instances, the starting concentration for calculating the DT_{50}
21 value was estimated from the fitted curve as recommended by Forum for the Co-ordination
22 of pesticide fate models and their use, FOCUS. (FOCUS 2006).

23

1 **Results**

2 SOIL TEMPERATURE AND PRECIPITATION

3

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

8

9 DISSIPATION OF FLUENSULFONE AND FOSTHIAZATE IN POTATO BEDS

10

11 Figure 2 shows the concentration of fluensulfone and fosthiazate in the 20 cm soil depth of
12 potato beds over 126 days at Woodcote and Howle in 2010 and 2011, respectively. The
13 mean concentration of fluensulfone at 0 DAA at Woodcote was 2.35 mg Kg⁻¹ soil, and was
14 slightly higher by 7 DAA (2.69 mg Kg⁻¹ soil). It then dissipated quite rapidly through 14
15 DAA and 21 DAA to 0.54 mg Kg⁻¹ soil (ca. 63% dissipation) by 28 DAA. Subsequent
16 losses occurred rather slowly, with the concentrations fluctuating between 0.27 and 0.12
17 mg Kg⁻¹ soil. The loss of fluensulfone from the potato beds at Howle followed a similar
18 trend to that observed at Woodcote. The mean concentration at 0 DAA was 1.90 mg kg⁻¹
19 and was slightly higher (2.10 mg Kg⁻¹ soil) by 7 DAA before dropping rapidly through 14,
20 21 and 28 DAA to 0.27 mg Kg⁻¹ soil (ca. 75 % dissipation) by 35 DAA. Further losses
21 appeared rather slowly, with fluctuating concentrations detected up until the final sampling
22 at 126 DAA. Overall, fluensulfone dissipated at similar rates in the two fields (Table 2),
23 and the trends followed a sigmoidal equation (Figure 2). The DT₅₀ varied from 19.6 to 30.0
24 days, with a mean of 24.3 days at Woodcote, and varied from 13.9 to 31.5 days, with a

1 mean of 23.7 days at Howle. Fosthiazate dissipated at different rates in the two fields
2 (Table 2). The trend was exponential at Woodcote (Figure 2), with 34 % dissipation
3 occurring within the first 7 DAA. This was then followed by a period of no significant loss
4 until 42 DAA when 0.23 mg Kg⁻¹ soil remained (ca. 87% dissipation). The trend was
5 exponential sigmoidal at Howle (Figure 2), with no losses until after 7 DAA when the 0
6 DAA concentration (1.80 mg kg⁻¹ soil) dissipated significantly ($P < 0.001$) to 0.52 mg Kg⁻¹
7 soil by 21 DAA (ca. 71% dissipation). The DT₅₀ ranged from 10.4 to 35.2 days, with a
8 mean of 21.3 days at Woodcote, and 16.3 – 21.1 days, with a mean of 18.8 days at Howle.
9 The DT₅₀ values obtained did not differ between the nematicides or the fields (Figure 3).

10

11 RELEASE OF FLUENSULFONE FROM THE GRANULAR FORMULATION

12

13 Fluensulfone extracted by acetonitrile and the release from the granular formulation into
14 water as functions of time are shown in Figure 4. Assuming no degradation had occurred
15 during the test, the extraction with acetonitrile showed that ca. 91% (3.67 mg) of the
16 expected fluensulfone (4.05 mg) was available for extraction by 12h of shaking, and much
17 of this (3.66 mg) was extracted by the 4th hour. Water, on the other hand, induced a
18 gradual, but incomplete, release of fluensulfone from the formulation within 41 days of
19 incubation. The amount and rate of release depended mainly on the temperature and
20 duration of incubation. Except for the samples collected at 1 and 12h (first two points
21 shown on figure 4b), when as much fluensulfone was released thereafter at 5°C as at 20°C
22 incubation temperature, the amount released thereafter at later sampling times was greater
23 at 20°C than at 5°C, figure 4b. The percentage cumulative release, as of 41 days of
24 incubation, was significantly lower (2.93 mg) for material incubated at 5°C than that seen

1 at 20°C (3.53 mg) ($P < 0.001$), and the amounts released at either incubation temperature
2 correlated positively ($r^2 = 0.99$; $P < 0.001$) with the duration of incubation.

3

4 **Discussion**

5 The DT₅₀ for fluensulfone in this study suggests the half-life (persistence) is no
6 longer than 24 days, and was similar to that observed for fosthiazate. This implies that half
7 the concentration of both nematicides may have dissipated prior to peak J2 hatch at
8 between 42 – 56 days (Haydock and Evans, 1998) Conversely, whilst the DT₅₀ may be an
9 important index for environmental persistence it may convey limited, if any, information
10 on the minimum effective dosage for control of *G. pallida* or the length of that control.
11 Indeed, control of *G. pallida* by the full rate fluensulfone treatments was evident in the
12 field experiments (Norshie *et al.*, 2016), which provided the soil for these laboratory
13 analyses. This suggests that soil concentrations were at effective concentrations. Also,
14 preliminary *in vitro* experiments (unpublished data) showed that fluensulfone acted against
15 *G. pallida* hatching of J2 *G. pallida* from two-year-old cysts incubated in the technical-
16 grade at concentrations ranging from 0.00425 to 0.608 mg L⁻¹ and it was deduced from the
17 cumulative hatch curve that there was complete inhibition of J2 emergence by the fifth
18 week of incubation in fluensulfone. Similarly, the motility of J2 *G. pallida* was reduced
19 following incubation in fluensulfone at 0.0078 – 32 mgL⁻¹ for 24 – 72h. The concentration
20 of fluensulfone remaining in the beds beyond 24 DAA ranged from 0.6 to 1.2 mg Kg⁻¹ soil
21 at Woodcote, and from 0.4 to 0.9 mg Kg⁻¹ soil at Howle. Even with the calculated DT₅₀
22 suggesting short persistence, fluensulfone may remain in the soil in sufficient
23 concentrations to control *G. pallida*, and the same can be said of fosthiazate, which had a
24 similar persistence to fluensulfone.

1 The dissipation kinetics for fluensulfone were similar for both fields. This could be
2 explained by the similar soil and growing conditions under which the experiments were
3 done. The release kinetics of fluensulfone from the formulation into water suggest that the
4 active substance may be readily available for entry into water once the granules become
5 hydrated and that the carrier material is unlikely to be a limiting factor to its availability to
6 soil water. Even though the incubation tests are made under laboratory conditions not
7 directly comparable to field situations, gradual release of fluensulfone from the granular
8 formulation in the soil is plausible, and may influence the persistence of fluensulfone by
9 retarding its availability to degradation and/or leaching processes, which are concentration
10 dependent. The lag phase of seven days preceding the dissipation of fluensulfone in both
11 fields could be due to an initial delay in release which could limit availability in the soil
12 during this period. A general property of non-fumigant nematicides is that their availability
13 and subsequent redistribution in the soil are achieved by soil moisture as affected by
14 rainfall or irrigation application (Noling, 2003; Smelt & Leistra 1992; Rich *et al.*,2003).
15 As shown in Figure 1, no precipitation occurred at either site until 7 DAA. Therefore, the
16 release kinetics from the formulation may partly explain the initial delay in dissipation
17 measured in the field plots. Indeed, the loss of fluensulfone from the plots in both fields
18 coincided with the onset of precipitation/rainfall, and the subsequent rapid decline in
19 concentration occurred during the period of highest precipitation. This highlights the likely
20 importance of soil moisture on the overall persistence of fluensulfone. If the laboratory,
21 results could be extrapolated to field situations, then both the amount and frequency of
22 irrigation/rainfall, following application of fluensulfone to potato beds, could affect the
23 persistence in the rhizosphere of the potato and, thus, its ability to protect roots from
24 invasion. Soil samples in this study were only collected from the depth of incorporation
25 (i.e. topmost 15 – 20 cm) and, assuming that there was leaching during the sampling
26 period, it could be that fluensulfone was leached to deeper layers rather than being

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

5 In summary, the short DT₅₀ demonstrated for fluensulfone in this study is an attribute in
6 that it may pose a negligible hazard to the environment. However, its persistence at an
7 effective dose may be long enough to be effective over the peak hatch period of *G. pallida*.

8

9 **Acknowledgements**

10

11 We thank the staff at the Crop and Environment Research Centre at Harper Adams
12 University, and Mr Matthew Rodenhurst, in particular, for his efforts in securing field sites
13 and applying the nematicides during the field experiments. Also, we appreciate the
14 guidance and assistance by Mr Kevin Jones and Mr Amjad Ali during HPLC analyses of
15 the soils. A special mention goes to the late Dr Patrick P. J. Haydock, who initiated the
16 work reported in this paper. The first author was in receipt of a Ph.D. studentship from
17 ADAMA Agricultural Solutions Ltd, Airport City, Golan Street, Israel.

18

19 **References**

20 Evans, K. (1993). New approaches for potato cyst nematode management. *Nematropica* 23,
21 221-231.

22 Evans, S.G. and Wright, D.J., (1982). Effects of the nematicide oxamyl on life cycle stages
23 of *Globodera rostochiensis*. *Annals of applied Biology*, 100, pp.511-519. DOI: 10.1111/j.1744-

24 7348.1982.tb01417.x

- 1 Focus (2006). *Guidance Document on Estimating Persistence and Degradation Kinetics*
2 *from Environmental Fate Studies on Pesticides in EU Registration. Report of the*
3 *FOCUS (FORum for the Co-ordination of pesticide fate models and their USE) Work*
4 *Group on Degradation Kinetics, EC Document Reference SANCO/10058/2005 version*
5 *2.0, June 2006.* <http://focus.jrc.ec.europa.eu/> (accessed 2 March 2012).
- 6 Haydock, P.P.J. & Evans, K (1998). Management of potato cyst nematodes in the UK: an
7 integrated approach? *Outlook of Agriculture* 27, 253-260.
- 8 Haydock, P.P.J., Ambrose, E.L., Wilcox, A. & Deliopoulos, T. (2012). Degradation of the
9 nematicide oxamyl under field and laboratory conditions. *Nematology* 14, 339-352. DOI:
10 10.1163/156854111X597485
- 11 Karpouzias, D.G., Pantelelis, I., Menkissoglu-Spiroudi, U., Golia, E. & Tsiropoulos, N. G.
12 (2007). Leaching of the organophosphorus nematicide fosthiazate. *Chemosphere* 68, 1359-
13 1364. DOI: <https://doi.org/10.1016/j.chemosphere.2007.01.023>
- 14 [Kearn, J., Lilley, C., Urwin, P., O'Connor, V. and Holden-Dye, L., \(2017\). Progressive](#)
15 [metabolic impairment underlies the novel nematicidal action of fluensulfone on the](#)
16 [potato cyst nematode *Globodera pallida*. *Pesticide Biochemistry and Physiology*.](#)
17 [Article in Press. DOI: <https://doi.org/10.1016/j.pestbp.2017.01.009>](#)
- 18 Morris, K.A., Langston, D.B., Dickson, D.W., Davis, R.F., Timper, P. & Noe, J.P. (2015).
19 Efficacy of Fluensulfone in a Tomato–Cucumber Double Cropping System. *Journal of*
20 *Nematology* 47, 310-315.
- 21 Noling, J.W. (2003). Movement and toxicity of nematicides in the plant root zone in *Fact*
22 *Sheet ENY-041 (formerly RF-NG002) 2003.* DOI: <http://edis.ifas.ufl.edu/ng002>. (accessed 5 May
23 2011).

- 1 Norshie, P.M., Grove, I.G. & Back, M.A., (2016). Field evaluation of the nematicide
2 fluensulfone for control of the potato cyst nematode *Globodera pallida*. *Pest*
3 *management science* 72, 2001-2007. DOI: 10.1002/ps.4329.
- 4 Oka, Y. (2014). "Nematicidal activity of fluensulfone against some migratory nematodes
5 under laboratory conditions." *Pest Management Science* 70, 1850-1858. DOI: 10.1002/ps.3730
- 6 Oka, Y., Shuker, S. & Tkachi, N. (2009). Nematicidal efficacy of MCW-2, a new nematicide
7 of the fluoroalkenyl group, against the root-knot nematode, *Meloidogyne javanica*. *Pest*
8 *Management Science* 65, 1082-1089. DOI: 10.1002/ps.1796
- 9 Oka, Y., Shuker, S. & Tkachi, N. (2012). Systemic nematicidal activity of fluensulfone
10 against the root-knot nematode *Meloidogyne incognita* on pepper. *Pest Management*
11 *Science* 68, 268-275. DOI: 10.1002/ps.2256
- 12 Oka, Y., Shuker, S. & Tkachi, N. (2013). Influence of soil environments on nematicidal
13 activity of fluensulfone against *Meloidogyne javanica*. *Pest Management Science* 69,
14 1225-1234. DOI: 10.1002/ps.3487
- 15 Opperman, C.H. & Chang, S. (1990). Plant-parasitic nematode acetylcholinesterase
16 inhibition by carbamate and organophosphate nematicides. *Journal of Nematology* 22,
17 481-488.
- 18 Osborn, R.K., Edwards, S.G., Wilcox, A. & Haydock, P.P.J. (2010). Potential enhancement
19 of degradation of the nematicides aldicarb, oxamyl and fosthiazate in UK agricultural
20 soils through repeated applications. *Pest Management Science* 66, 253-261. DOI:
21 10.1002/ps.1866
- 22 Rich, J.R., Dunn, R.A. & Noling, J.W., (2004). Nematicides: Past and present uses. In: Chen
23 ZX, Chen SY and Dickson DW, (Eds) *Nematology Advances and Perspectives Volume*

- 1 2: *Nematode Management and Utilisation*. Wallingford, UK, CAB International pp.
2 1179-1200.
- 3 Ryan, N.A., Deliopoulos, T., Jones, P. & Haydock, P.P.J. (2003). Effects of a mixed-isolate
4 mycorrhizal inoculum on the potato – Potato cyst nematode interaction. *Annals of*
5 *Applied Biology* 143, 111-119. DOI: 10.1111/j.1744-7348.2003.tb00275.x
- 6 Smelt, J.H. & Leistra, M., (1992). Availability, movement and (accelerated) transformation
7 of soil-applied nematicides. In: Gommers FJ and Maas PW Th (Eds) *Proceedings*
8 *Second International Nematology Congress*. European Society of Nematologists. pp.
9 266-293.
- 10 Whitehead, A.G. (1992). Emergence of juvenile potato cyst-nematodes *Globodera*
11 *rostochiensis* and *Globodera pallida* and the control of *G. pallida*. *Annals of Applied*
12 *Biology* 120, 471-486. DOI: 10.1111/j.1744-7348.1992.tb04907.x
- 13 Whitehead, A.G., Nichols, A.J.F. & Senior, J.C. (1991). Control of potato pale cyst-
14 nematode, *Globodera pallida*, with a granular nematicide and partially resistant
15 potatoes. *Annals of Applied Biology* 118, 623-636. DOI: 10.1111/j.1744-7348.1991.tb05352.x
- 16 Whitehead, A.G., Nichols, A.J.F. & Senior, J.C. (1994). The control of potato pale cyst-
17 nematode (*Globodera pallida*) by chemical and cultural methods in different soils. *TJ.*
18 *Agricultural Science* 123, 207-218. DOI: <https://doi.org/10.1017/S0021859600068477>
- 19 Whitehead, A.G., Tite, D.J., Fraser, J.E. & Nichols, A.J.F. (1984). Differential control of
20 potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, by oxamyl and the
21 yields of resistant and susceptible potatoes in treated and untreated soils. *Annals of*
22 *Applied Biology* 105, 231-244. DOI: 10.1111/j.1744-7348.1984.tb03047.x

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 \pm standard error.

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

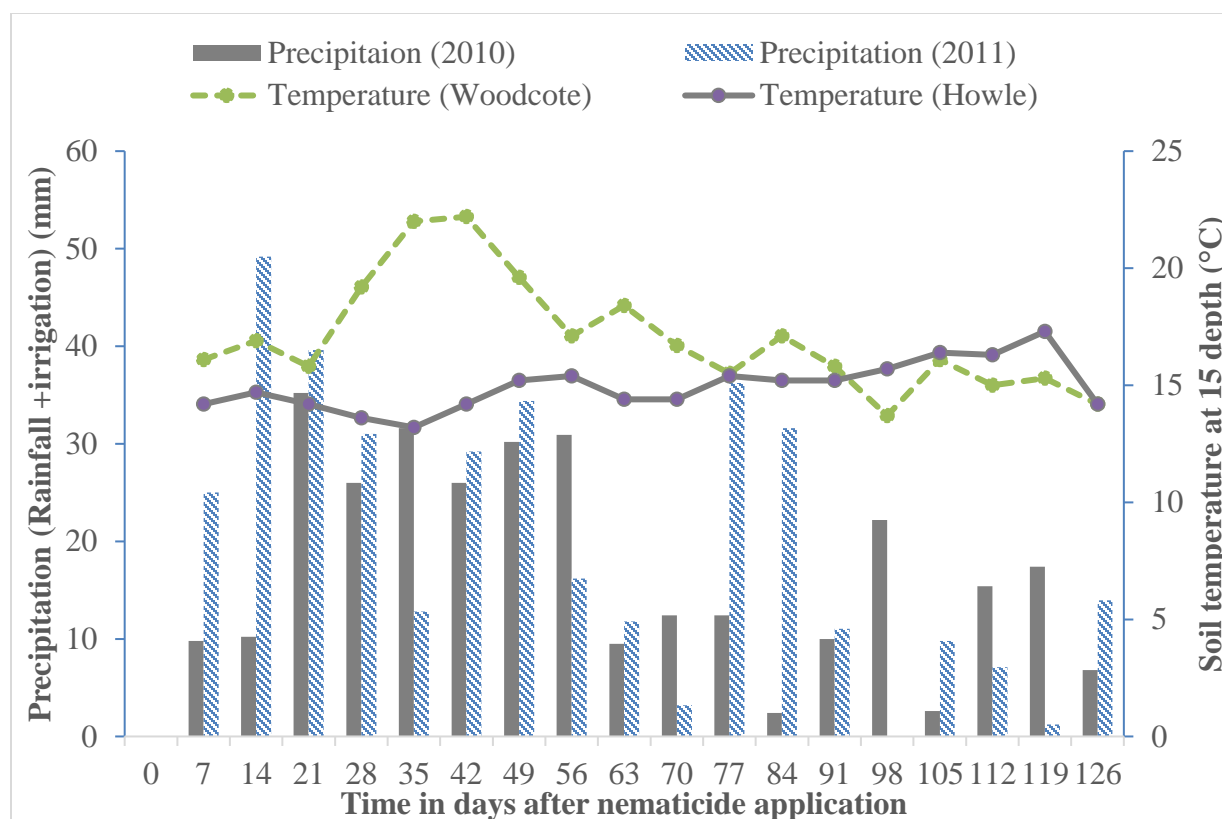


Figure 1. Mean soil temperature (°C) at 15 cm depth of potato beds and total precipitation (rainfall + irrigation) as recorded at Harper Adams University over 126 days of in-field dissipation studies of nematicides fluensulfone and fosthiazate at Woodcote in 2010 and at Howle in 2011.

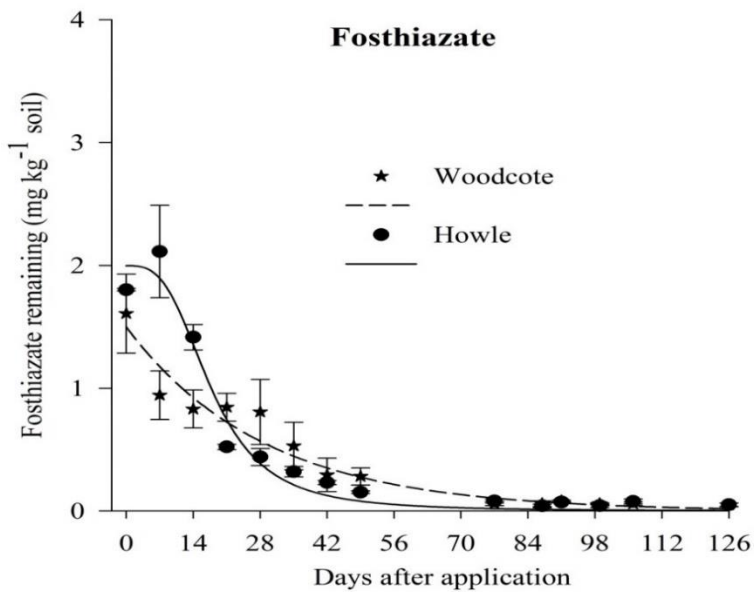
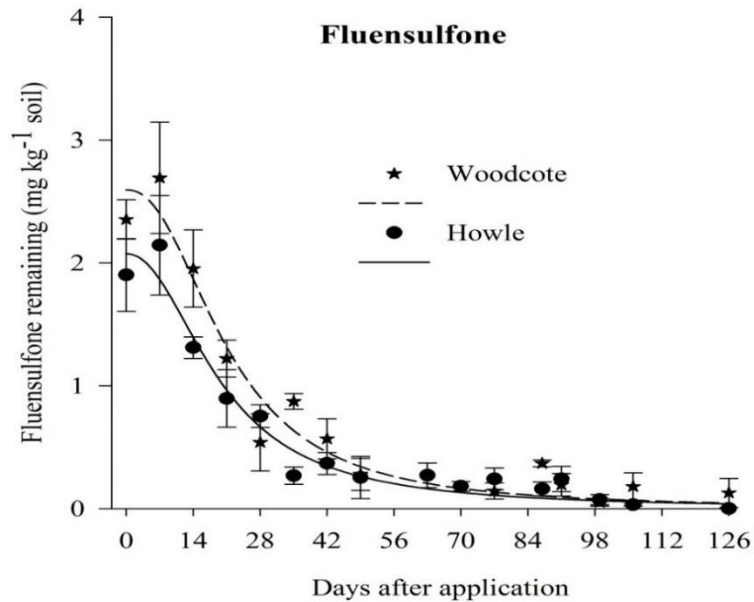


Fig. 2. Nematicides fluensulfone and fosthiazate quantified in the topmost 20 cm soil layer of potato beds following treatments with Nimitz 15G at 27 kg ha⁻¹ and Nemathorin 10G at 30 kg ha⁻¹ at Woodcote in 2010 and at Howle in 2011. The loss of fluensulfone followed the equations $Y = 4.04 / (1 + e^{-(X-10.38)/-16})$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

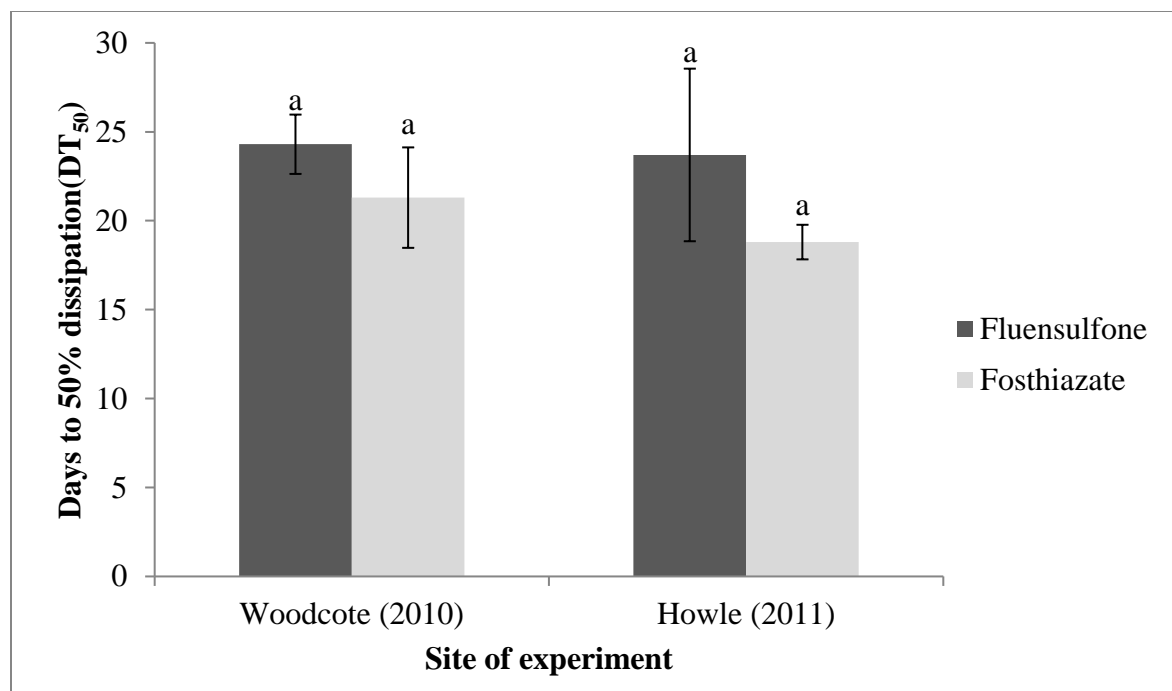
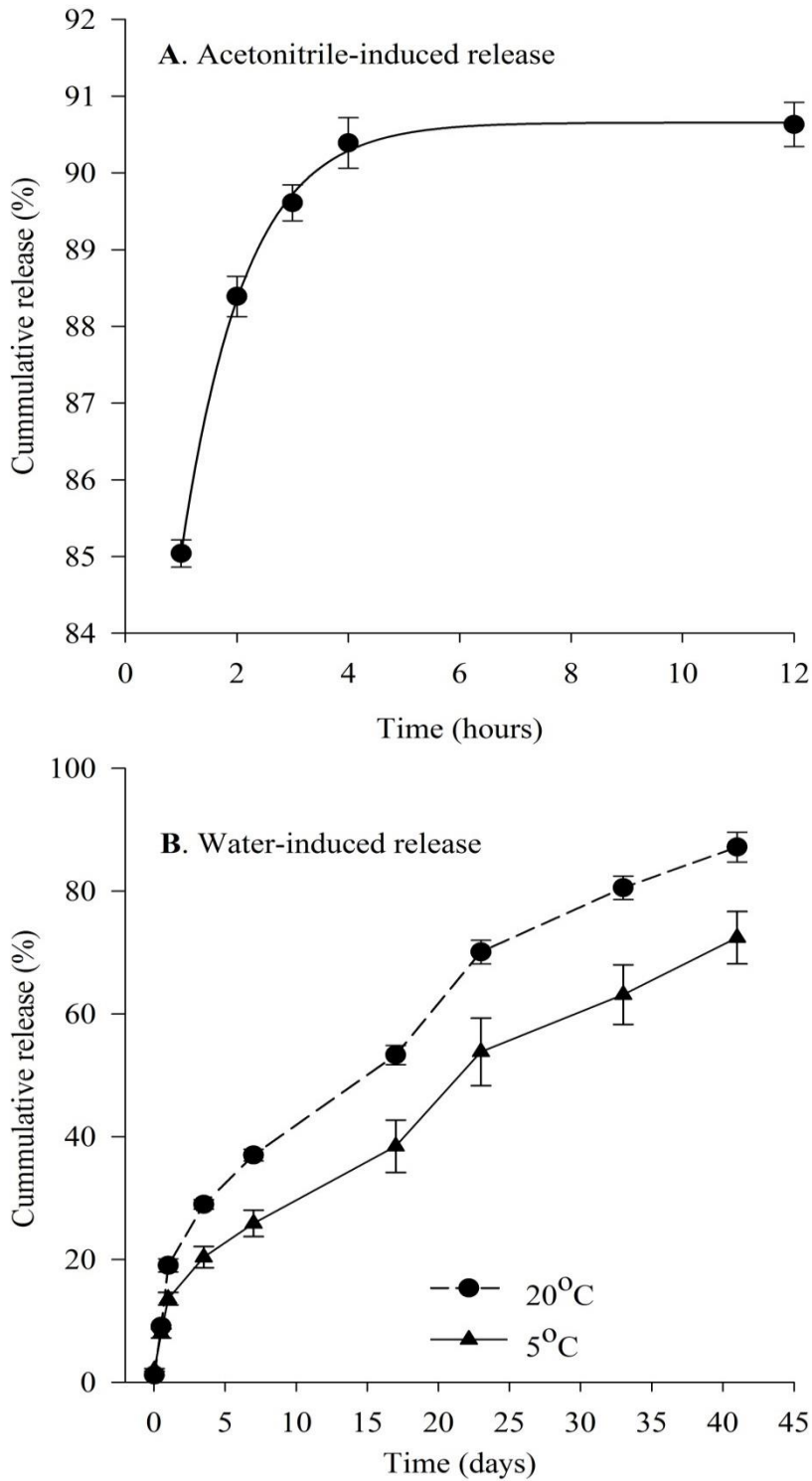


Figure 3. Comparisons of half-lives (DT₅₀) obtained for nematicides fluensulfone and fosthiazate at 20 cm depth of potato beds treated with Nimitz 15G at 27 kg ha⁻¹ and Nemathorin 10G at 30 kg ha⁻¹ at Woodcote in 2010 and at Howle in 2011. Bars represent standard error values (n = 5).



1

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