Journal of Elementology ISSN 1644-2296

Sikorska-Zimny K.M., Wędzisz A., Rogowska M. 2017. *Spinosad decay dynamics and mineral content of some elements in chosen vegetables*. J. Elem., 22(2): 737-746. DOI: 10.5601/jelem.2016.21.1.1135

ORIGINAL PAPER

SPINOSAD DECAY DYNAMICS AND MINERAL CONTENT OF SOME ELEMENTS IN CHOSEN VEGETABLES

Kalina M. Sikorska-Zimny[1], Anna Wędzisz[2], Maria Rogowska[3]

[1]Fruit and Vegetables Storage and Processing Department
Research Institute of Horticulture in Skierniewice
[2]Chair of Toxicology and Food Sciences
Medical University of Łódź
[3]State College of Applied Sciences in Skierniewice

Abstract

Spinosad is an insecticide with a completely unique and novel mode of action. It is used in a great variety of crops: fruit, vegetables, and ornamental plants. Its dissipation was studied in three vegetables: cabbage (the cultivar Stonehead), carrot (cv. Perfekcja) and onion (cv. Wolska). The vegetables were sprayed with a dose of 96 g a.i. ha^{-1} of spinosad and collected after 1, 3, 5, and 7 days. The residues were analyzed using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection after purification on a column with methanol. Elements were determined with *flame atomic absorption spectroscopy* (FAAS), and the determinations were made on the 1st and 7th day after spraying. The determined amounts of spinosad were in the range of 0.943-0.072 mg kg^{-1} for carrot and onion, respectively, on the 7th day. Spinosad decomposed completely in cabbage, and the highest level of the pesticide after 7 days remained in carrot, although the shape of all decomposition curves is quite similar. Most elements in onion were on significantly higher levels after sprays than in the control variant, opposite to cabbage, where higher levels of the analyzed elements were determined in the control. Concentrations of the elements determined in carrot were statistically significant for manganese, iron and magnesium. The spinosad decomposition time and the content of elements depend on the species of vegetables. The amount of spinosad on day 7 found in onion and cabbage was equal to the Maximum Residue Level (MRL) available in the literature.

Keywords: spinosad, vegetables, HPLC with UV, column purification, MRL, elements.

dr n. farm. inż. Kalina Sikorska-Zimny, Fruit and Vegetables Storage and Processing Department, Research Institute of Horticulture in Skierniewice, 96-100 Skierniewice, Konstytucji 3 Maja 1/3, e-mail: kalinasikorska@op.pl, kalina.sikorska@inhort.pl

INTRODUCTION

Spinosad is a naturally derived fermentation insecticide (Gao et al 2007). It is a tetracyclic macrolide, which has a mode of action against many insect orders: *Lepidoptera, Thysanoptera* and *Coleoptera* (Leeuwen et al. 2005, Figureoa et al. 2015, Lan et al. 2015) – Figure 1.

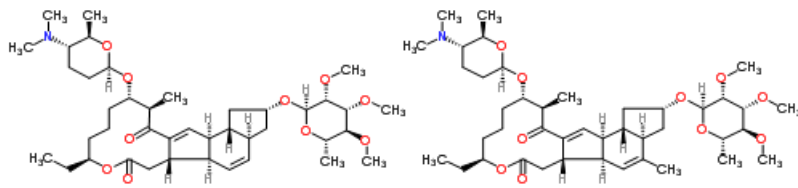

Fig. 1. Spinosad's chemical structure spinosyn A and D
(ChemSpider; Royal Society of Chemistry)

Spinosad is a fermentation product of the actinomycete bacterium *Saccharopolyspora spinosa*. Two spinosyns called spinosyn A and spinosyn D are active ingredients, which differ from each other by a single methyl substitute at position 6 of the polyketide (Zhi-Hua et al. 2006, Somers et al. 2015).

Spinosyns have very short half-lives in soil (aerobic soil metabolism study): for spinosyn A it is 17.3 (silt loam) and 9.4 (sandy loam) days, and for spinosyn D – 14.5 days (loam soil) (Fan et al. 2008). It needs to be added that terrestrial dissipation half-lives of spinosyns are shorter. Research conducted by Dasenaki et al. (2016) shows spinosyns $t_{1/2}$ at 1.2 days in onion. Liu et al. (2013) established $t_{1/2}$ of spinosad in zucchini at 3.5-3.9 days. Spinosyns are known as fast degrading compounds (Liu et al. 2013, Vassilakos, Athanassiou 2015). Photolysis is their major degradation pathway; both spinosyns degrade under ultraviolet light (Adak, Mukherjee 2015). This is a great advantage because it allows a short time before consumption of the fruit or vegetable treated with spinosad (Srinivas et al. 2012, Liu et al. 2013, Vassilakos, Athanassiou 2015, Dasenaki et al. 2016). Spinosad is registered for 150 crops (U.S.) in more than 30 countries (Rathore, DAS 2001, Nollet 2012, USA EPA 2015).

Analytical methods for the determination of spinosyn residues are based on the following techniques: immunoassay, HPLC and UPLC-MS/MS (Rutheford et al. 2000, IR-4 Project 2005, Gao et al. 2007, Lockender et al. 2015). The immunoassay technique may not require clean-up before determination (FAO 2001) and it is based on a Test Kit. High performance liquid chromatography may be applied with +Ion APCI mass spectrometry detection or with UV detection (IR-4 Project 2005). Mass spectrometry allows the determination of single spinosyns such as A and D. UV detection determines spinosad as a sum of two major spinosyns: A and D (Sannino 2007). It is worth adding

that the US authorities have defined spinosad residues as the sum of spinosyns A and D (FAO 2001). The WHO recommends that "the spinosad content (sum of spinosyns A and D) is determined by reversed-phase HPLC, using UV detection at 280 nm and external standardization. Definitive identification is by positive-ion ESI LC-MS, as no other technique is sufficiently specific" (WHO 2008). All residue analysis methods need to consider the sensitivity of spinosad to sunlight and heat. Spinosad starts decomposition at a temperature of 172°C (WHO 2008) and is sensitive to light.

Spinosad residues have been determined in tomatoes, potatoes, almond nuts, and hulls, eggs, and poultry citrus crops, orange processed commodities using HPLC with UV detection, but also with mass spectroscopy, MS (West et al. 2000, Ueno et al. 2006, WHO 2008).

The aim of this study was to evaluate the rate of dissipation of spinosad in three crops and the correlation between spinosad sprays and the content of micro- and macro-elements in the vegetables.

MATERIAL AND METHOD

An analytical method for determination of spinosad residues is presented underneath. It is based on the HPLC method with UV detection and purification on a column. Three species of vegetables have been analyzed: cabbage (cv. Stonehead), carrot (cv. Perfekcja) and onion (cv. Wolska). Experiments were conducted according to EPPO (European and Mediterranean Plant Protection Organization OEPP/EPPO 2004) and in line with generally accepted laboratory methodologies and principles of observation in experiments. All plants were grown in summer 2008, in fields near the town of Skierniewice, on 15m^2 plots, in sandy soil (soil quality class: IVa), with four replications. The vegetables were sprayed with a dose of 96 g a.i. ha^{-1} of liquid spinosad formulation using a slit nozzle Tee-Jet XR 8002 (operating pressure: 3 bar, operating speed: 1.6 km h^{-1}, height boom suspension above plants: 50 cm). The first harvest of onion and carrot was on 18 August and that of cabbage on 9 September (first day after spraying); subsequent harvests took place on 3rd, 5th and 7th day after spraying. Vegetables were harvested (selected randomly from each of the plots in the blocks) according to the BBCH scale: onion: main development phase 4.45 405, carrot: 4, 46; cabbage: 4.45-46. The harvested vegetables were put in plastic bags ad kept frozen at -20°C for 3 months (as appropriate for food storage) – Table 1.

For every determination, five edible parts of vegetable were taken, homogenized and examined in four replicates. For HPLC assays, vegetables were thawed (only edible parts), homogenized, weighed (25 g each sample) and dissolved in 50 ml of methanol. They were shaken for 1 h and filtered through a large-pore filter. A 2 cm^3 aliquot was transferred to a column filled with purified florisil (magnesium silicate). The florisil purification process

Table 1

Atmospheric conditions during the plant growing season

Specification	Day after spraying with spinosad						
	1	2	3	4	5	6	7
Onion; Carrot							
Date	18.08	19.08	20.08	21.08	22.08	23.08	24.08
Min temp. (°C)	11.6	15	16	15.3	12.1	16	10.6
Max temp. (°C)	25	28.3	27.4	24.3	26.7	23.8	21.4
Min temp. of ground (°C)	9.7	11.6	13	12.4	9.7	13.8	7.4
Average relative humidity (%)	67	61	72	65	71	76	78
Hours of solar radiation (h)	11.8	12.6	7.9	10.3	8.5	4.5	4.9
Cabbage							
Date	09.09	10.09	11.09	12.09	13.09	14.09	15.09
Min temp. (°C)	12.4	9.4	12.6	9.4	3	5.4	7.4
Max temp. (°C)	21.4	23.8	20.1	15.9	10.9	11.3	12.8
Min temp. of ground (°C)	9.9	6.4	9.4	8.9	0.3	1.2	4.1
Average relative humidity (%)	77	79	94	79	85	85	83
Hours of solar radiation (h)	9.1	7.8	1.8	0.1	1.1	0.1	0.1

was performed according to the manufacturer's recommendations. 5% v/w of distilled water was added to florisil desiccated at 140°C, for 14 h, and all the florisil was kept in an airproof jar. The column was rinsed with methanol and the eluate was collected in two 25 ml volumetric flasks. Each gathered solution was fortified with technical spinosad. Samples were transferred to an HPLC–UV apparatus. Analyses were carried out on a HPLC Varian 9050; LDC Analytical fluoro Monitor 4100 with UV detector and Star Chromatography Working Station Version 5.2, using a 5 μm Inertsil ODS3 (10×4,6 mm) column at room temperature. The detection wavelength was 250 nm. The flow rate of methanol/acetonitrile: water (95:5) was 1 ml min^{-1}. Five individual edible parts of each plants species were examined daily (in three replicates), together with the control and fortification samples (recovery: 82%, LOD = 0.067 μg ml^{-1}, LOQ = 0.20 μg ml^{-1}).

Determinations of mineral compounds were conducted using flame atomic absorption spectroscopy (FAAS) on an AVANTA instrument – Table 2.

Followed by the mineralization of a sample, the ash was dissolved in nitric acid (1 mol dm^{-3}). The determination procedure was confirmed with standard material (NCSZC73014 Tea), obtaining a recovery rate of 94.35 to 102.73% for iron and manganese, respectively. Data were evaluated by the *t*-student test and the statistical significance was accepted for p values <0.05 according to the control.

Table 2

FAAS Avanta instrument working parameters

Parameters	Air flow 10 $dm^3 min^{-1}$, acetylene flow 2 $dm^3 min^{-1}$					
	mineral compound					
	Cu	Ca	Zn	Fe	Mg	Mn
Wavelength λ (nm)	324.7	422.7	213.7	248.3	285.2	279.0
Max lamp current mA)	3.0	5.0	5.0	7.0	5.0	5.0
Slit (nm)	0.5	0.5	0.5	0.2	0.5	0.2
Recovery (%)	97	102	98	94	98	97
LOQ (μg ml^{-1})	0.1	0.2	0.2	0.1	0.05	0.1
LOD (μg ml^{-1})	0.033	0.08	0.08	0.05	0.025	0.04
R^2	0.998	0.999	0.974	0.994	0.988	0.998

RESULTS AND DISCUSSION

The results of the analyses are shown below (Table 3, Figure 2). On the 1st day after spinosad spraying, the highest amount of the pesticide was in carrot, lower in cabbage and the lowest in onion.

Table 3

Dynamics of spinosad degradation in vegetables (mg kg^{-1})

Vegetable	Average spinosad content ±SD (mg kg^{-1})			
Crop species	1st day after spray	3rd day after spray	5th day after spray	7th day after spray
Onion	0.310±0.115	0.270±0.133	0.167±0.129	0.072±0.044
Carrot	16.543±3.535	6.297±0.860	5.880±1.564	0.943±0.123
Cabbage	13.133±1.850	3.820±0.904	0.810±0.328	0.000±0.000

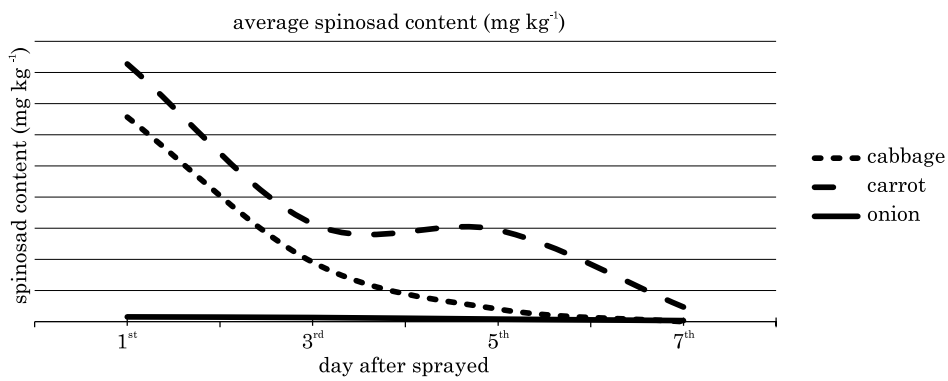

Fig. 2. Spinosad disappearance in vegetables

In onion and carrot, the spinosad degradation process slows down from the 3rd to 5th day after the pesticide's application. The results displayed as percentages show that while 98.2% of spinosad in carrot and 79.8% of spinosad in onion had decomposed within 7 days, spinosad in cabbage had decomposed completely by the 7th day (Tables 4, 5).

Table 4

Spinosad residues in crops (%)

Vegetable	Spinosad residues (%)			
	1st day	3rd day	5th day	7th day
Onion	100.0	87.10	53.87	20.23
Carrot	100.0	38.06	35.54	1.79
Cabbage	100.0	29.09	6.17	0.00

Table 5

Spinosad's half-life period in different crops (h)

Period	Vegetable variety		
	Onion	Carrot	Cabbage
$t_{1/2}$ (h)	125	87	71

Most elements (except calcium) in onion on the 1st day after spraying with spinosad were on significantly higher levels relative to the control. After 7 days, only zinc, manganese, calcium and magnesium were significantly higher compared to the control. The determined concentrations of manganese, iron (only after 7 days) and magnesium were statistically significant in carrot. The determination of the tested elements in cabbage shows significant differences in amounts of cooper, iron, calcium and manganese and magnesium (only after 7 days following the application of spinosad) – Table 6.

The highest amount of spinosad found in carrot and the lowest one determined in onion (after one day) might have been caused by the different structures and the differences between the edible parts of the two vegetables. Cabbage is a multi-layered vegetable. Leaves are succulent, dark green on the outside and covered with waxy coating. Cabbage has smooth-leaf, firm heads. The size of a cabbage head depends on a variety and growth conditions. The edible part (taken for analysis) is a whole cabbage head except external leaves. Carrot is a root vegetable, where edible parts are whole roots with a peel. This part of the plant composed our samples. Onion samples consisted of internal leaves (flattened and grown out of the basal disc) and some external layer of the dry scale (discarded for analytical purpose).

Table 3 shows that the highest amount of spinosad was found in carrot, which might be associated with its slower soil decomposition

All examinations should be repeated several times to account for different weather conditions, especially that all the tested vegetables ripen on different days and months.

Table 6

Content of elements in vegetables (mg kg^{-1} f.w.)

Vegetable	Days after spinosad spraying	Mean content of chosen elements (mg kg^{-1} f.w.) $n = 6$					
		Zn	Mn	Cu	Fe	Ca	Mg
Onion	0 (ctrl)	1.950	0.800	0.120	14.43	272.8	105.4
	1	3.150*	1.180*	0.260*	18.51*	301.8	120.2*
	7	2.190*	1.060*	0.070	9.780*	317.1*	116.9*
Carrot	0 (ctrl)	8.130	0.970	0.140	14.04	334.8	173.2
	1	7.030	0.590*	0.180	15.67	234.7	215.2*
	7	9.460	0.590*	0.120	11.04*	390.0	128.6*
Cabbage	0 (ctrl)	3.800	0.560	0.260	1.660	409.9	116.9
	1	3.400	0.490	0.230*	1.910*	284.9*	101.8
	7	2.820	0.410*	0.330*	1.420*	374.9*	102.0*

* statistically significant

As mentioned before, photolysis is the main pathway of spinosad degradation (FAO 2001) and as this process is absent in soil, that might explain the higher amount of spinosad in carrot, which could continuously absorb spinosad during its growth.

The lowest amount of spinosad was found in onion. This crop (like all the others) was sprayed with spinosad just before harvest, implying that the plant could have developed some of its ligneous shell, which may have prevented spinosad penetration.

Cabbage is not a root crop, its edible part grows above the ground, and it does not develop ligneous leaves, which might clarify the complete spinosad degradation in cabbage. As it has been shown above, all decomposition curves are otherwise quite similar. The results of the study reveal that spinosad decomposes in a similar way in different vegetables.

Of the three crops, the greatest amount of spinosad expressed as a percentage was left in onion. This could suggest that spinosad decomposition was the slowest in onion, although this vegetable had absorbed the lowest amount of spinosad. This might be correlated with the morphological structure of onion, where the ligneous shell prevents the penetration of spinosad inside the plant while, on the other hand, it inhibits the breakdown of spinosad which has already penetrated into the onion.

The spinosad maximum residue level in cabbage (cv.Stonehead, included wrapper leaves) is 0.01mg kg^{-1}, according to supervised trials carried out in the USA (FAO 2001). The Official Journal of the European Union shows the

following maximum residue level of spinosad (as a sum of spinosyn A and D) in examined vegetables (Table 7).

Table 7

Spinosad MRLs (mg kg^{-1})

Crop	MRLs (mg kg^{-1})
Cabbage	2.0
Carrot	0.2 (provisional)
Onion	0.1

Seven days after being sprayed, a high amount of spinosad was observed in carrot, which coincided with its complete absence in cabbage. There was a very low amount of spinosad in onion. After 7 days, the levels of the measured spinosad residues in cabbage and onion were in compliance with the MRL data available in the literature (Table 7). Only the spinosad residue in carrot on day 7 after spraying was not in accordance with the above data

The morphological traits and location of edible parts of the vegetables plants as well as spinosad decay under the influence of sunlight may suggest that the dynamics of spinosad decomposition is dependent on the species of a vegetable. The fastest decomposition of spinosad was in cabbage, where spinosad was probably mostly absorbed by external leaves which are exposed to sunligt; the spinosad decomposition rate was the slowest in carrot, where the root (away from direct sunlight) was analyzed. As seen in Table 3, the highest amount of spinosad was absorbed by carrot and less by onion. This may also be associated with the morphological structure of the vegetables, namely as the decomposition of spinosad is the slowest in soil, roots (e.g. the edible part of carrot) can absorb the pesticide for longer time. Onion develops an external ligneous shell, which prevents spinosad penetration into the vegetable's edible part.

It was noticed that the level of mineral compounds in onion was the lowest in the control, opposite to cabbage, where the control had a higher level of mineral compound than the sprayed variants. This can substantiate the conclusion that morphological traits affect the content of minerals depending on the time of spinosad application. Certainly, the study should be repeated to verify the results. The authors hope that the current research outcome will contribute to further research on the decay of spinosad in field lying at Polish latitudes.

CONCLUSIONS

It was found that the dynamics of decay of spinosad in field conditions varied depending on the species of vegetables. Spinosad decomposes the fastest in cabbage and the slowest in carrots. Most spinosad is absorbed by carrots while onions absorb the smallest amounts of the preparation.

The influence of spinosad on the level of mineral nutrients in vegetables has not been determined unambiguously. A higher mineral content was noticed in the treated onions than in the control ones. Cabbage not treated with spinosad had a higher mineral content than cabbage sprinkled with spinosad.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Adak T., Mukherjee I. 2015. *Investigating role of abiotic factors on spinosad dissipation.* Bull. Environ. Contam. Toxicol., 96(1): 125-9.

Commission Regulation EU No 556/2012. *Amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for spinosad in or on raspberries*. Official Journal of the European Union, 27.06.2012, L 166/67.

Dasenaki M., Bletsou A., Hanafi A., Thomaidis N. 2016. *Liquid chromatography – tandem mass spectrometric methods for the determination of spinosad, thiacloprid and pyridalyl in spring onions and estimation of their pre-harvest interval values.* Food Chem., 213(15): 395-401.

Dow AgroSciences (DAS) LLC. 2001. *Spinosad Technical Bulletin*. http://www.dowagro.com/PublishedLiterature/dh_0064/0901b803800647cc.pdf?filepath=/PublishToInternet/InternetDOWAGRO/usag/pdfs/noreg/010-80032&fromPage=BasicSearch Accessed Jan 2001

European and Mediterranean Plant Protection Organization OEPP/EPPO 2004. *Efficacy evaluation of plant protection products*. Vol. 3. *Insecticides and acaricides.* OEPP/EPPO, Paris (FR)., 3: 250.

Fan S., Segawa R., Kim V., Levine V., Ganapathy C., Wofford P., Hsu J., Lee P. 2008. *Environmental monitoring results of spinosad aerial applications for the Mexican fruit fly eradication in valley center. California* Department of Pesticide Regulation. http://www.cdpr.ca.gov/docs/emon/epests/mexfly/mxff_final.pdf

Figueroa J., Coronado R., Pineda S., Chavarrieta J., Martínez-Castillo A. 2015. *Mortality and food consumption in Spodoptera frugiperda (Lepidoptera: Noctuidae) larvae treated with spinosad alone or in mixtures with a nucleopolyhedrovirus*. Fla. Entomol., 98(3): 1009-1011. DOI: http://dx.doi.org/10.1653/024.098.0340

Food and Agriculture Organization (FAO) of the United Nations. 2001. *Spinosad 203*, www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/2001_eva/14%20Spinosad.pdf

Gao R., Dong J., Zhang W., Chen W. 2007. *Dietary risk assessment of spinosad in China.* Regul. Toxicol. Pharmacol., 49(1): 31-42.

IR-4 Project, Rutgers, The State University of NJ. 2005. *Spinosad.* ir4.rutgers.edu/Other/Analytical_Methods/Prot_Sec-28_&_29/SPINOSAD.doc

Kashyap L., Sharma D.C., Anil. 2015. *Dissipation behaviour of spinosad in polyhouse grown tomato under mid-hill conditions of Himachal Pradesh, India.* Environ. Monit., Assess, 187(3): 75. DOI: 10.1007/s10661-014-4210-y

Lan Z., Zhao C., Guo W., Guan X., Zhang X. 2015. *Optimization of culture medium for maximal production of spinosad using an artificial neural network - genetic algorithm modeling.* J Mol Microbiol Biotechnol, 25(4): 253-261. DOI: 10.1159/000381312

Liu Y., Sun H., Wang S. 2013. *Dissipation and residue of spinosad in zucchini under field conditions*. Bull Environ Contam Toxicol, 91(2): 256-259.

Rathore H., Nollet L. 2012. *Pesticides: evaluation of environmental pollution.* CRC Press. ISBN 9781439836255 - CAT# KE11646 p.19

Rutherford BS., Gardner RC., West SD., Robb CK., Dolder SC. 2000. *Residues of spinosad in meat, milk, and eggs*. J. Agric. Food Chem., 48(9): 4428-4431.

Sannino A. 2007. *Determination of three natural pesticides in processed fruit and vegetables using high-performance liquid chromatography/tandem mass spectrometry*. Rapid Commun. Mass Spectrom., 21(13): 2079-86.

Somers J. , Nguyen J., Lumb C., Batterham P., Perry T. 2015. *In vivo functional analysis of the Drosophila melanogaster nicotinic acetylcholine receptor Dα6 using the insecticide spinosad*. Insect Biochem. Mol. Biol., 64: 116-127. DOI: 10.1016/j.ibmb.2015.01.018

Srinivas P., Banerjee K., Jadhav M., Ghaste M., Lawande K. 2012. *Bioefficacy, dissipation kinetics and safety evaluation of selected insecticides in Allium cepa* L. J Environ Sci Health B, 47(7): 700-709.

Ueno E., Oshima H., Matsumoto H., Saito I., Tamura H. 2006. *Determination of spinosad in vegetables and fruits by high-performance liquid chromatography with UV and mass spectrometric detection after gel permeation chromatography and solid-phase extraction cleanup on a 2-layered column*. J. AOAC Int., 89 (6) : 1641-1649.

United States Environmental Protection Agency 2015. *Memorandum spinosad and spinetoram*. UE-EPA Washington, D.C. 20460. Office Of Chemical Safety And Pollution Prevention. 2015. https://www.regulations.gov/document?D=EPA-HQ-OPP-2013-0727-0012

Van Leeuwen T., Dermauw W., Van de Veire M., Tirry L. 2005. *Systemic use of spinosad to control the two-spotted spider mite (Acari: Tetranychidae) on tomatoes grown in rockwool*. Exp. Appl. Acarol., 37(1-2): 93-105.

Vassilakos T., Athanassiou C. 2015. *Long-term residual efficacy of spinetoram on concrete and steel surfaces for the management of three stored product beetle species*. J. Econ. Entomol., 108(4) online DOI http://dx.doi.org/10.1093/jee/tov088

West SD., Turner LG. 2000. *Determination of spinosad and its metabolites in citrus crops and orange processed commodities by HPLC with UV detection,* J. Agric. Food Chem., 48(2): 366-372.

World Health Organization (WHO) 2008. *Specifications and evaluations for public health pesticides. Spinosad*. March 2008, www.who.int/entity/whopes/quality/Spinosad_eval_only_March_2008.pdf

Zhi-Hua J., Jian-Ping W., Yuan Z., Xiu Ch., Li-Rong Y., Pei-Lin C. 2015. *Improvement of spinosad producing Saccharopolyspora spinosa by rational screening*, J. Zhejiang Univ. Sci. A, 7(Suppl. II): 366-370.