Residue decline behaviour of the natural insecticide spinosad on apples

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Abstract
Spinosad is a common insecticide not only in organic but also conventional farming. It is a natural derived product from the fermentation of bacteria with a broad ranging control spectrum. The present study, carried out in the orchards of Research Centre Laimburg (Italy), aims to determine the residue decline behaviour of spinosad on apples and to determine different application strategies that results in a residue level not exceeding 0.01 mg/kg. This limit is often requested by European retailers and organic associations. The results show that 1 and 2 treatments, at application rates of 216 g/ha and the last treatment at least applied 20 days before harvest, ensures residue quantities below 0.01 mg/kg.

Keywords: spinosad, insecticide, apples, residue level

Introduction

Spinosad is a naturally derived insecticide produced through the fermentation of the bacteria *Saccharopolyspora spinosa* which occurs in nature and is not genetically engineered.
It is comprised of two complex multi-ring molecules each with a different sugar attached to the central ring structure. These two molecules are very similar in composition and are referred to as spinosyn A and spinosyn D. Commercial formulations typically consist of a mixture of spinosyn A (*CAS Registry No. 131929-60-7*) and spinosyn D (*CAS Registry No 131929-63-0*) in a ratio of approximately 85:15 (*Mertz, F. P. and Yao, R. C , 1990; Thompson, G. D. et al., 1997, 2002; Crouse, G. D. and Sparks, T. C., 1998; Dow, 2001*)
Spinosad control spectrum is quite broad ranging from Lepidoptera, Diptera, Thysanoptera, Hymenoptera, Siphonaptera, and Coleoptera (*Thompson, G.D., et al.. 2000*; *Dow, 2001*). Formulated products containing spinosad are registered worldwide in many countries on a wide range of crops and on farm animals for the control of external parasites.

Spinosad is listed in Annex I of Directive 91/414/EEC (replaced by Reg. (EC) No 1107/2009) since 2007 (*Official Journal of the European Union. 15th February 2007*).

Spinosad has been admitted since many years in the organic standards in different countries (e.g. USA, Switzerland). Due the unsatisfactory situation of insect regulation in organic farming in Southern EU Countries, in 2005 Italy proposed the introduction of spinosad in Annex 2B of the former European regulation for organic agriculture 2092/91/EC. In 2008 spinosad was admitted with the restriction of certification body in a new product category called microbial derivate products (*Official Journal of the European Union. 7th May 2008*).

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The EU- MRL (Maximum Residue Level) according to Regulation (EC) No. 396/2005 *(Official Journal of the European Union, 16 March 2005)* for spinosad (sum of spinosyn A and spinosyn D expressed as spinosad) on apple is currently set at 1 mg/kg. The EU-MRL must be respected both in conventional and organic production. For the spinosad uses approved in the EU the label PHI (minimal Pre Harvest Interval) is 7 days.
The Dow AgroSciences residue data supporting the use of spinosad on apples in the EU demonstrate that applying on apples the maximum label rate (216 g a.i/ha) four time in the season, the spinosad residue level with 7 days of PHI is lower than 30% of EU MRL, as requested by several EU Supermarkets and retailers.
Nevertheless spinosad is not very popular among different organic grower associations in the northern Europe and it is not accepted by some food retailer selling organic products which identify it as a conventional agrochemical because it is part of the screening programme in multimethod analyses for pestizide residues. For this reason some Supermarkets request to achieve 0.01 mg/kg (limit of quantification) in organic fruits.

The aim of the paper was to calculate the probability to achieve residue levels on apples below 0.01 mg/kg as a function of different application factors that correlate with residues (number of treatments, dates between application and harvest, etc). Such information would be useful to provide clear indications to the growers who wish to comply with this more restrictive requirement.

Material and Methods

In 2009, the variety Golden Delicious, cultivated in an apple orchard of the Research Centre Laimburg (Vadena BZ), was treated three times at a distance of three days with the product Laser[5] (Active ingredient spinosad 480 g/L) at a dosage of 20 ml/hl (144 g a.i./ha). The sample taking for the analysis of residues has been carried out 6, 26 and 29 days after the last treatment, the dates are shown in table 1.

Table1: Dates of treatment and sample taking for the analysis of spinosad on fruits in 2009 (Harvest date: 15.09.09)

Dates of treatment			**Dates of sampling**		
13.08.09	17.08.09	20.08.09	26.08.09	15.09.09	18.09.09

As in 2009, also in 2010 the trial was carried out on the variety Golden Delicious, cultivated in an apple orchard of the Research Centre Laimburg (Vadena BZ). One single treatment with the product Laser at a dosage of 30 ml/hl (216 g a.i./ha) was applied. This single treatment has been replicated in 4 different parcels.
Thereupon 4 dates for analysis (indicated in table 2) were fixed, in order to elevate a curve of degradation of the residues, both on foliage and on fruits.

Table 2: Dates of sample taking for the analysis of spinosad on fruits in 2010 (Harvest date: 15.09.09)

Date of treatment	**Dates of sampling**			
03.08.2010	10.08.10	18.08.10	24.08.10	17.09.10

In 2011 the trial was carried out on 7 different varieties. For each variety, one parcel with about 70 trees was treated one time; a second parcel was treated two times with Laser at

a dosage of 30 ml/hl (216 g a.i./ha). The varieties observed, the dates of treatment and sample taking for the analysis of residues are shown in table 3.
The sample taking has been carried out 10 / 16 / 21 / 31 / 41 / and 46 days after the last treatment.

Table 3: Variety, date of treatment and date of sampling taking for the experiment in 2011 (Harvest date: 3rd sampling)

Variety	Dates of treatment*		Dates of sampling					
	1st treatment	2nd treatment after 5 days	1st	2nd	3rd	4th	5th	6th
Red del.	10.08.11	15.08.11	26.08	31.08	05.09	15.09	25.09	30.09
Golden Del.	10.08.11	15.08.11	26.08	31.08	05.09	15.09	25.09	30.09
Braeburn	16.08.11	21.08.11	01.09.	06.09	11.09.	21.09	30.09	05.10
Kanzi	20.08.11	25.08.11	05.09	10.09.	15.09	25.09	05.10	15.10
Granny Smith	2.09.11	7.09.11	17.09	23.09	28.09	08.10	18.10	23.10
Fuji	2.09.11	7.09.11	17.09	23.09	28.09	08.10	18.10	23.10
Pink lady	23.09.11	28.09.11	08.10	13.10	18.10	28.10	08.11	13.11

*the second treatment has been applied only to the second parcel of every variety, while the first treatment was applied to all parcels

Both years, the experimental parcels have been delimitated by trees and rows, in order to avoid drift. The application was carried out with a radial parcel sprayer from Waibl (nozzles Teejet blue – pressure 7 bar) and a water volume of 500 l per meter crown height (equivalent to 1500 L/ha).

The sampling was carried as follows: 20 fruits were harvested randomly from different trees in each plot. In 2010 and 2011 samples were conserved at 1°C until the extraction and analysis. In 2009 the samples were immediately extracted and the extraction samples were kept at -20°C until the analysis

Sampling preparation, extraction, purification and spinosad residue level determination by LC-MS-MS were carried out following the UNI EN 15662:2009 method

Results

The residue data of spinosad recorded (expressed in mg/kg) have been split in different classes on the basis of residue level, setting the value "*true*" = 1 when the residue value fall in a specific class and the value "*false*" = 0 when the value doesn't fall in the same class.
In this way the residue data have been transformed in *qualitative* or *categorical* data

Classes:

1. Residue ≥ EU MRL
2. Residue ≥ 50% EU MRL
3. Residue ≥ 30% EU MRL
4. 30% EU MRL < Residue ≥ 0.01 mg/kg (= LoQ: limit of quantification)
5. Residue < 0.01 (= LoQ: limit of quantification)

The categorical data can be analyzed by *Binary Logistic Multiple Regression* (Logistic Regression is also called logistic model or logit model) which is used for prediction of the probability of occurrence of an event by fitting data to a logistic function. It is a generalized linear model used for binomial regression. Like other forms of regression analysis, it makes use of one or more predictor variables that may be either numerical or categorical
In this case numerical predictor variable are active substance rate, number of treatments, days between application and harvest and categorical predictor variables are varieties.

Equation of Probability

$$P = 1/\ 1 + e^{-z}$$

$$z = a + b_1x_1 + b_2x_2 + b_3x_3 + \ldots\ldots\ldots\ldots\ldots + b_nx_n$$

Where:
a *= constant*
$x_1, x_2, x_3, \ldots\ldots x_n$ *= numerical or categorical factors resulted significant*

In figure 1 are showed the 112 spinosad residue data recorded in 2009, 2010 and 2011 experimental trials

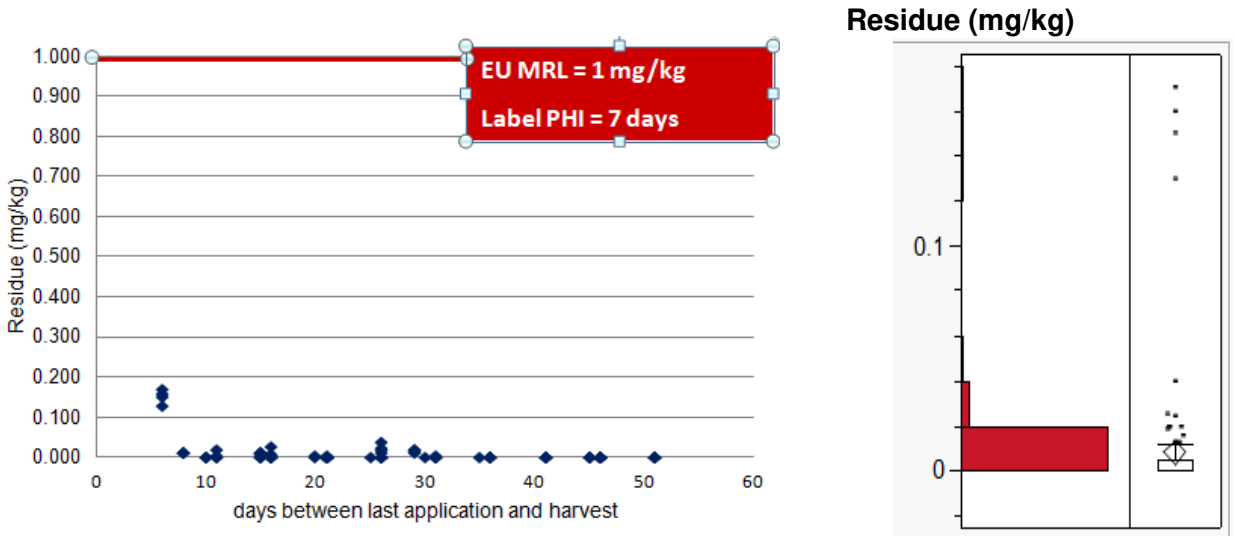

Figure 1: Spinosad residue level (mg/kg) at different days between last application and harvest

All the residue data recorded are lower than 30% MRL (0.3 mg/kg) and most of them (80%) are lower than 0.01 mg/kg with an interval of days between last application and harvest starting from 10 days (figure 2)

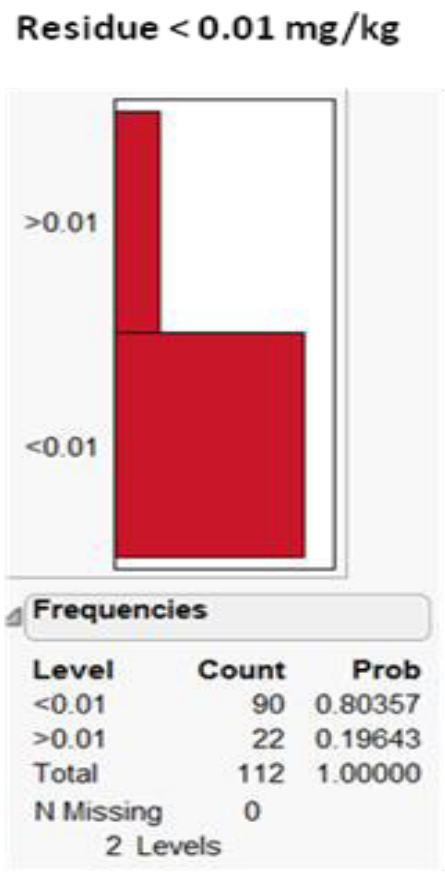

Figure 2: Spinosad residue distribution into the category "Residue lower than 0.01 mg/kg*"*

Carrying out the Binary Logistic Multiple Regression with the 112 spinosad residue data the model obtained considering the factors correlated with residue level, number of treatments (*Prob (ChiSq) = 0.0001*) and days between last application and harvest (*Prob (ChiSq) = 0.0001*), resulted significant: *Prob (ChiSq) = 0.0001 (Prob (ChiSq) < 0.05 is significant)* and R^2*=0.81 (*R^2*>0.2 is good in logistic Regression)*.
The model has also an outstanding predictive capacity (=accuracy): AUC=0.98. (*Accuracy can be expressed as the model's ability to correctly classify 0, or the ability to correctly classify 1 in the holdout dataset. AUC= 0.5 model has no predictive ability; 0.7≤AUC≤0.8 acceptable; 0.8≤AUC≤0.9 excellent; AUC ≥ outstanding*).

The equation of probability is

$P (<0.01) = 1 / 1 + e^{-z}$

Where
***z** = -22.66 +8.56 x*
n. treatment (1) + 14.69 x
n. treatment (2) + 0.93 x days between last application and harvest.

The probability to achieve a residue level of spinosad higher or lower than 0.01 mg/kg are represented in figure 3.

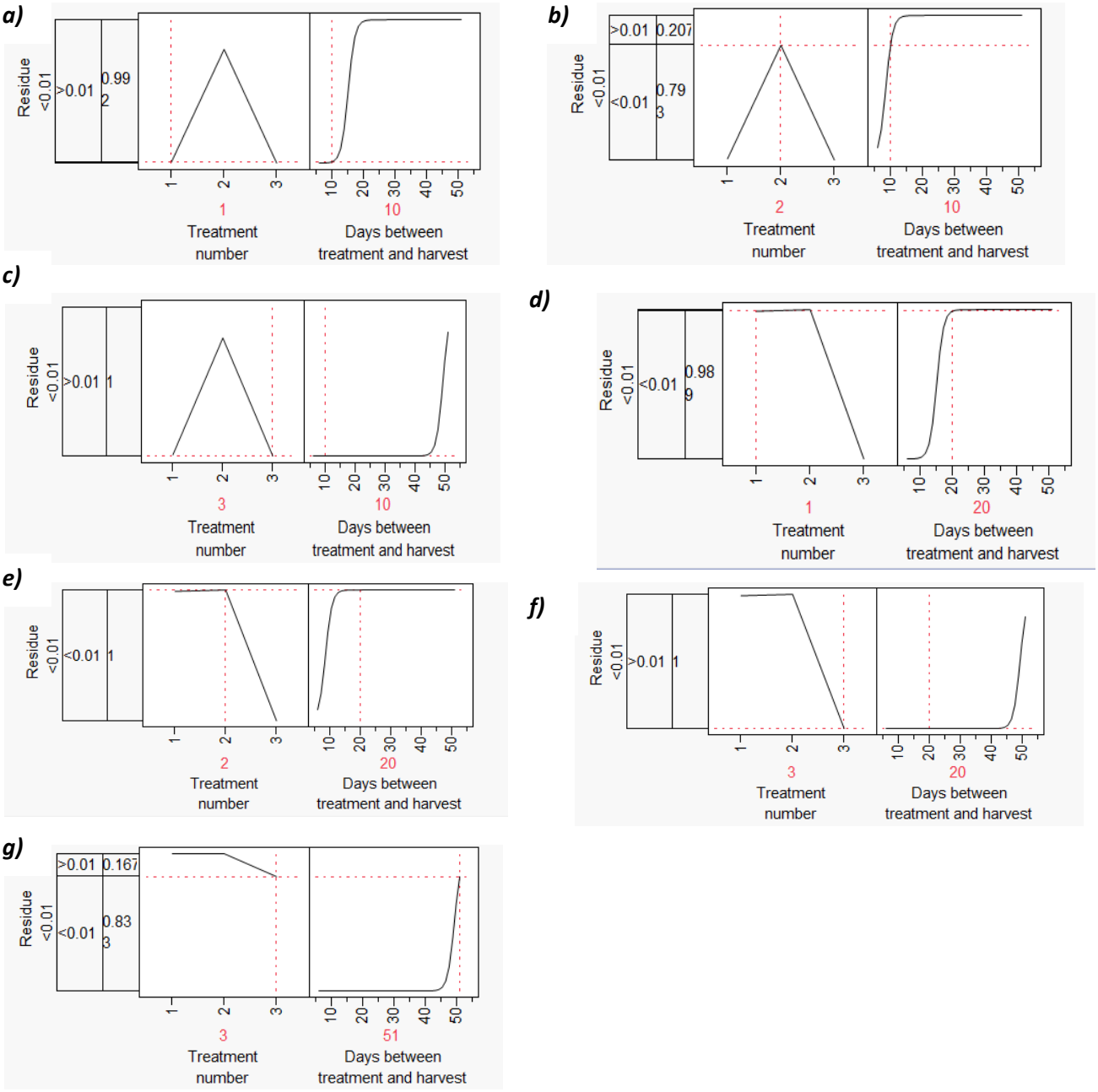

Figure 3: Probability to achieve a residue level of spinosad higher or lower than 0.01 mg/kg calculated by Binary Logistic Multiple Regression Model

In apples harvested 10 days after the last application the probability to achieve a spinosad residue level higher than 0.01 mg/kg is between 80 and 100% independently by the number of treatments (figure 3*a*, 3*b* and 3*c*).
When the days between last application and harvest increase to 20, the probability to find a spinosad residue level in apples lower than 0.01 mg/kg is around 100% with 1 or 2 spinosad treatments (figure 3*d* and 3*e*), whereas with 3 spinosad treatments there is 100% probability to have a residue level higher than 0.01mg/kg (figure 3*f*).
With 3 spinosad applications it is necessary to increase the number of days between last application and harvest to 51 days to achieve the probability of 83% to obtain a residue level lower than 0.01 mg/kg (figure 3*g*).

Conclusions

The experimental trials carried out in the apple orchard of Research Centre Laimburg (Vadena, BZ) to evaluate the residue level of spinosad after the application of 1, 2 or 3 treatments at maximum label rate of 216 g/ha (30 ml/ha of commercial product Laser) on different apple varieties showed that 80% of the total residue data (112 data) are lower than limit of quantification of 0.01 mg/kg.

The statistical analysis (Binary Logistic Multiple Regression) applied to spinosad residue data allow to built a model with an outstanding predictive capacity (AUC=0.98) , which show a very high probability (98-100%) to find out residue of spinosad lower than 0.01 mg/kg (limit of quantification) in apple when 1 or 2 treatments of spinosad are applied and the time interval between last application and harvest is 20 days.

The growers who produce organic apples can take in account this indication on spinosad application in order to be able to supply those EU retailers who may have very restrictive requirements in term of residue in organic fruits.

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