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LC/DAD/ESI/MS Method for the Determination of Imidacloprid, Thiacloprid, and Spinosad in Olives and Olive Oil after Field Treatment

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ABSTRACT: The behavior in the field and the transfer from olives to olive oil during the technological process of imidacloprid, thiacloprid, and spinosad were studied. The extraction method used was effective in extracting the analytes of interest, and no interfering peaks were detected in the chromatogram. The residue levels found in olives after treatment were 0.14, 0.04, and 0.30 mg/kg for imidacloprid, thiacloprid, and spinosad, respectively, far below the maximum residue levels (MRLs) set for these insecticides in EU. At the preharvest interval (PHI), no residue was detected for imidacloprid and thiacloprid, while spinosad showed a residue level of 0.04 mg/kg. The study of the effect of the technological process on pesticide transfer in olive oil showed that these insecticides tend to remain in the olive cake. The LC/DAD/ESI/MS method showed good performance with adequate recoveries ranging from 80 to 119% and good method limits of quantitation (LOQs) and of determination (LODs). No matrix effect was detected.

KEYWORDS: LC/DAD/ESI/MS, olives, olive oil, pesticide residues, decline curve

INTRODUCTION

Olives are subjected to various diseases in the field caused by insect pests, fungi, and weeds, which could determine a 30% reduction of production.[1] The most dangerous parasite of olives in most of the countries around the Mediterranean Sea is represented by the olive fly (*Bactrocera oleae* (Gmelin), 1788, formerly *Dacus oleae*). The larvae are monophagous and feed exclusively on olive fruits, while adults feed on nectar, honey dew, and other opportunistic sources of liquid or semiliquid food.[1] This parasite causes the quantitative and qualitative falling of the olive oil.[2] Olive safety in the field is obtained by using a large number of conventional insecticides of different families, and numerous methods have been developed to detect pesticide residues in olive and olive oil.[3−9] The increasing public concern about human health risks derived from pesticide use has led to a change in crop protection strategies with particular attention to food quality and safety. For this reason, alternative methods for controlling fruit pests with low toxicological impact and good efficiency in the field have been established. Nowadays, the olive fruit fly is mostly fought using new generation pesticides, botanical pesticides, and semiochemicals (sex pheromones and food attractants) in integrated pest management (IPM) strategies.[10−13] Imidacloprid, thiacloprid, and spinosad are new generation pesticides with good agriculture and toxicological characteristics (Figure 1). Imidacloprid and thiacloprid belong to the nicotinyl family with locally systemic and translaminar characteristics.[14] These pesticides act as agonists of the nicotinic acetylcholine receptor (nAChR) in the central nervous system, thus disturbing the synaptic signal transmissions penetrate the leaf tissues, and form a reservoir of active ingredient (a.i.) within the leaf.[15−17] Imidacloprid and thiacloprid are classified as Group 4 Insecticides[18] and are included in Annex I of Directive 91/414/EEC;[19] they are considered low risk pesticides for human health and against nontarget organs (birds, fish, plants, etc.) and thus can be used in IPM programs. Imidacloprid and thiacloprid have a broad spectrum of activity against several orders of insects (i.e., Hemiptera, Homoptera, Diptera, and Coleoptera) owing to both ingestion and contact activity.[15] Moreover, nicotinoids showed a low ability to develop resistance and a high efficacy in the field, which allowed one to use a low concentration with an overall lower environmental impact.[20] Nicotinoids are applied to a huge number of crops, peaches, pears, courgettes, celeries, apricots,[21] cucumber, lettuce, pepper, tomato,[4] eggplant, grape, grapefruit,[22,23] soil,[24] water,[25] and residues that can be found in transformed products such as honey.[26] Spinosad is a macrocyclic bacterial lactone of the family of spinosyn and is derived from the aerobic fermentation of the actinomycete soil bacterium *Saccharopolyspora spinosa* in aqueous growth media (containing e.g., corn solids, soya bean flour, and cottonseed flour) extraction and recrystallization of technical spinosad.[27−29] Spinosad is a mixture (85:15) of spinosyn A and spinosyn D; because of its microbial origin, spinosad can be considered a biopesticide, and it is useful for the management of many insect pests, including caterpillars, leaf miners, thrips, flies, drywood termites, and some beetles, in various vegetables, field crops, and fruits.[30,31] Spinosad has a unique mechanism of action (MOA) involving the disruption of nicotinic acetylcholine receptors and GABA-gated ion channels of insect nervous systems and has contact activity on all life stages of insects, including eggs, larvae, and adults.[29] Eggs must be sprayed directly, but larvae and adults can be effectively dosed through contact with treated surfaces. Spinosad is most effective when ingested, generally showing a greater selectivity toward target insects and a lesser activity against many beneficial predators as well as mammals and other aquatic and avian animals.[32,33] Foliar applications are not highly systemic, although

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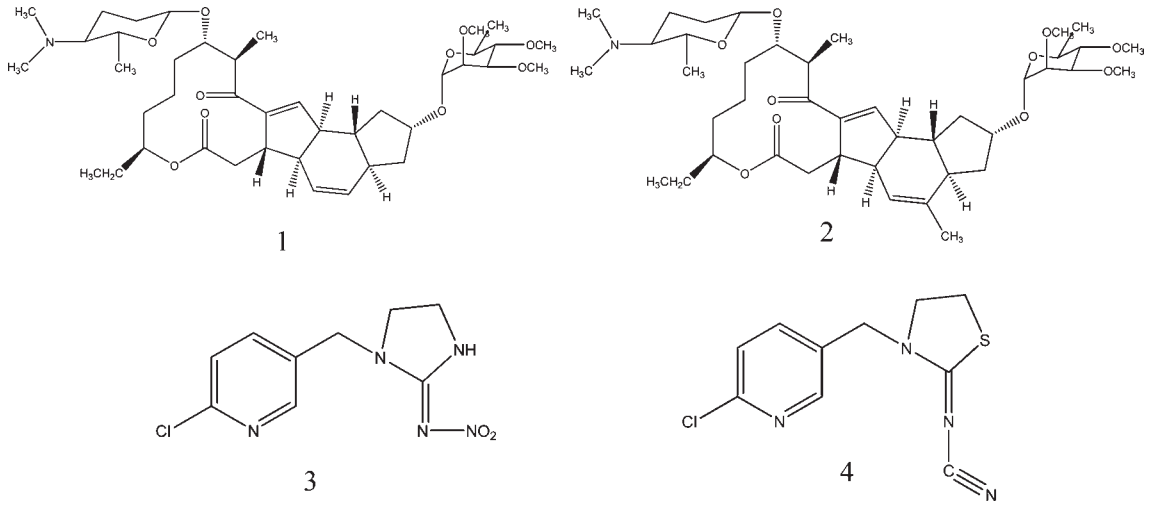

Figure 1. Chemical structures of spynosin A (1), spynosin D (2), imidacloprid (3), and thiacloprid (4).

trans-laminar activity is evident in certain vegetable crops and ornamental plants. It was approved in 1997 by United States Environmental Protection Agency (U.S. EPA) and subsequently in conventional farming in 14 EU and many (>50) non-EU countries.[34] The registration of spinosad on olive fruit has been authorized in Cyprus and Greece from 2002 and in California from 2004; in Italy, it was authorized only in 2008. Spinosad has a safer toxicological profile than that of other botanical pesticides such as rotenone and pyrethrins. On the basis of field studies, it has been established that spinosad is rapidly degraded in soil and can therefore be defined as a non-persistent substance in soil. Moreover, it does not move in the soil, and so there is no danger of it leaching into groundwater.[35] Analysis of spinosad has been previously carried out in cottonseed and cottonseed processed commodities;[41] in soil, sediment, and water;[42] in leafy vegetables, peppers, and tomatoes;[43] in meat, milk, cream, and eggs;[44] in citrus crops and citrus processed commodities;[45] in cabbage, strawberry, green perilla,[46] alfalfa hay, wheat hay, wheat straw, sorghum fodder, and corn stover;[47] and in processed commodities such as fruit and vegetable purées.[48] Its use has led to, especially in California, an increase in insect resistance since it was the only insecticide used on the olive fruit fly.[49] Several methods can be found in literature dealing with the analysis of the different nicotinoids and of spinosad in various crops, and almost all use LC-DAD[22,23] and LC-MS methods.[21,22,25,26,36,37,39,40] These methods involve the analysis of water or high water content matrixes, only a few papers can be found regarding olive or wax matrixes. Fatty matrixes usually require extensive sample extraction and purification steps to partially or totally remove the lipidic components, coextracted with the target compound, even with the advent of advanced hyphenated techniques based on mass spectrometry.[50] The extraction step is mandatory to eliminate or diminish interferences and keep the chromatographic system in good working conditions.[38] Gilbert et al.[9] reported a multiresidue method for the determination of imidacloprid and thiachloprid residues in olives, while Benincasa et al.[51] reported the determination of spinosad and its metabolites in olive oil by LC-DAD detection. No paper was found relating the decline curves of the selected pesticides on olives in the field and on the effect of the technological process on the residue transfer to the oil. Moreover, no data was found on the analysis of nicotinoids in olive oil and of spinosad in olives. IPM strategies suggest the use of pesticides with different side action; thus, residues of different pesticides can be found on the same crop and its processed products.

The aim of this research was to develop a simple and easy to use method for the extraction and determination of imidacloprid, thiacloprid, and spinosad in olive and in olive oil after field treatment. The effect of the technological process and the decline curve in field experiments were reported. Moreover, the HPLC/DAD/ESI/MS method was validated under the EC SANCO/10684/2009 directive, and data were reported.

MATERIALS AND METHODS

Fruit Material and Chemical Analysis. *Field Trials.* Field trials were carried out in an olive grove planted in 1994 (cv. Semidana) with a plant density of 5 × 4 m located at Villasor (Cagliari, Italy). A random-block design with four replications was used, and each block contained 30 trees located randomly in the grove. Treatments were carried out with an F-320 portable motorized sprayer (Fox Motori, Reggio Emilia, Italy). The commercial formulation, used at the doses recommended by the manufacturer, were Warrant SL (imidacloprid 17.1%; Cheminova, Rome, Italy; 200 g/L of water and 1000 L/ha), Calypso SC (thiacloprid 48%; Bayer, Milan, Italy; 96 g/ha), and Success 120 SC (spinosad 120 g/L; 12 mL/10 L of water and 1.8 L/ha). Because treatments with spinosad involve the use of very low amounts of solution per ha, in order to evaluate the decline curves, a second treatment with a.i. concentration 10 times higher than that suggested by the manufacturer was performed using a manual sprayer and spraying a single spot on a selected area on each tree. Sampling for residue control was carried out at 0, 3, 7, and 14 days after the last treatment for imidacloprid and thiacloprid, and at 0, 2, 4, 7, and 10 days for spinosad (Table 1). Random 6 kg samples were collected from each block. For spinosad treatment with the manual sprayer, five olives from each plant were collected.

Chemicals. Acetonitrile, acetone, dichloromethane, *n*-hexane, and ethyl acetate were ultraresidue solvents of analytical grade, purchased from Merck (Darmstadt, Germany). High purity water was distilled and filtered through a Milli-Q apparatus (Millipore, Bedford, MA). Liquid chromatography solvents were filtered with 0.45 μm Teflon membranes before use.

Imidacloprid and thiacloprid (purity ≥95%) were analytical standards supplied by Dr. Ehrenstorfer (Augsburg, Germany), while the spinosad standard (mixture of spinosyn A and spinosyn D, 85−15%) (purity ≥99.9%) was kindly supplied by Dow AgroSciences (Milan,

Table 1. Analytical Method Limits of Quantification (LOQ) and Determination (LOD) of the Studied Pesticides in Olives (mg/kg) and Olive Oil (mg/L) with Correlation Coefficients and CV%

		imidacloprid	thiacloprid	spinosyn A	spinosyn D
MW		256	253	732	746
MRL	olive (mg/kg)	1.00	4.00	1.00 (sum of)	
	olive oil (mg/L)			0.01 (sum of)	
PHI	olive (days)	28	14	7	
LOD	olive (mg/kg)	0.005	0.005	0.001	0.004
	olive oil (mg/L)	0.005	0.008	0.001	0.004
LOQ	olive (mg/kg)	0.01	0.01	0.01	0.01
	olive oil (mg/L)	0.01	0.01	0.01	0.01
linearity range	(mg/kg)	0.01−3.36	0.01−4.04	0.01−3.01	0.01−0.53
$R^2 \pm$ CV%	olive	0.9993 ± 12	0.9992 ± 4	0.9991 ± 15	0.9994 ± 16
	olive oil	0.9997 ± 8	0.9996 ± 6	0.9998 ± 7	0.9989 ± 8
oil yield (%)		15 ± 3	16 ± 1	16 ± 2	

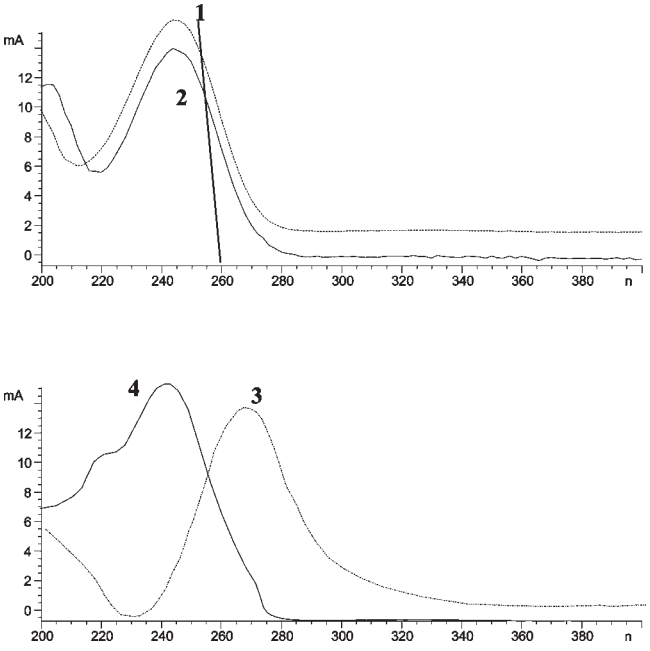

Figure 2. LC-DAD spectra of spynosin A (1), spynosin D (2), imidacloprid (3), and thiacloprid (4).

Italy). Stock standard solutions of imidacloprid, thiacloprid, and spinosad were prepared by dissolving 11.2 mg, 10.1 mg, and 11.8 mg of analytical standard, respectively, in 10 mL of acetonitrile, and the solutions were stored in glass screw-capped flasks at −20 °C. Working standard solutions of a.i.'s were prepared daily by appropriate dilutions of the stock solutions with mobile phase and stored at 4 °C until use. Matrix matched standards were prepared at the same concentrations as that of the calibration solutions by adding the appropriate amounts of stock solution to untreated matrix extracts (control). Several dilutions were prepared to check the linearity response of the detector and to obtain the instrument method detection (LOD) and quantitation (LOQ) limits for the two pesticides.

Liquid Chromatography Diode Array Detector and Mass Spectrometry. An HPLC system (Shimadzu, Milan, Italy) equipped with a SPD11 Avp DAD detector, a SIL 11 AD vp autosampler, and a LC 10 AD binary pump coupled on line with a MS2010 mass spectrometer (Shimadzu, Milan, Italy) was used. Ultraviolet (UV) and MS data were

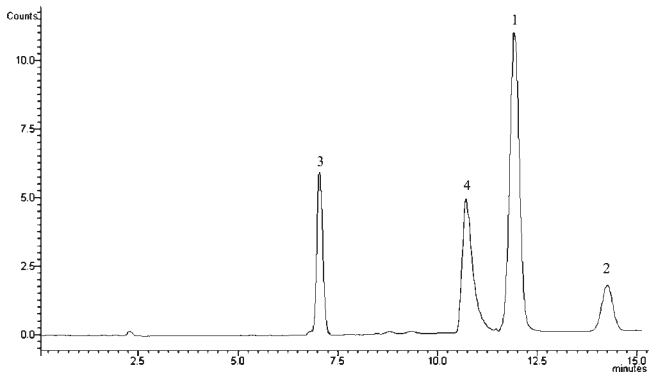

Figure 3. LC/ESI/MS SIM chromatogram at 0.1 mg/kg in olive matrix extract of spynosin A (1), spynosin D (2), imidacloprid (3), and thiacloprid (4).

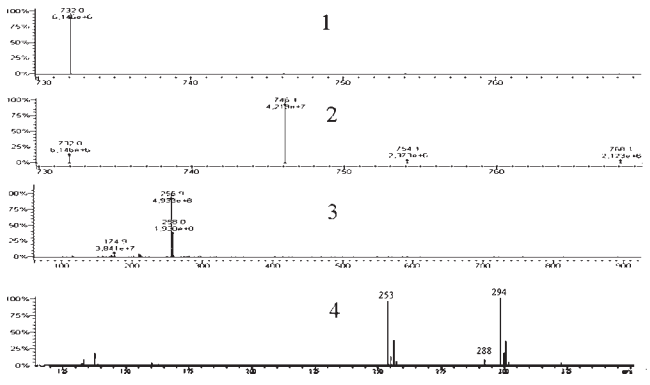

Figure 4. LC/ESI/MS SIM SPECTRA of spynosin A (1), spynosin D (2), imidacloprid (3), and thiacloprid (4).

acquired and processed using the Shimadzu LCMS solution software. Isocratic elution was carried out using the following eluents: A, 99.9% water−0.1% trifluoro acetic acid (TFA), and B, acetonitrile−water (50:50, v/v), for 15 min. The column used was a Waters Symmetry C18, 3.5 μm, 2.1 × 150 mm I.D. (Milford, MA). The injection volume was 20 μL, and the flow rate was 0.4 mL/min. The monitoring wavelengths for UV analysis were 270 and 242 for imidacloprid and

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Table 2. Recoveries in Olives (mg/kg) and Olive Oil (mg/L) at Four Different Levels of Fortification

	recoveries % (n = 4) ± CV %						
	fortification level	imidacloprid	thiacloprid	fortification level	spynosin A	fortification level	spynosin D
olive	0.04	105 ± 13	97 ± 9	0.04	112 ± 5	0.01	93 ± 2
olive oil		110 ± 1	87 ± 19		80 ± 3		92 ± 8
olive	0.20	115 ± 4	99 ± 2	0.10	92 ± 4	0.02	102 ± 5
olive oil		109 ± 5	106 ± 1		96 ± 4		100 ± 3
olive	0.40	119 ± 4	101 ± 3	0.50	105 ± 7	0.09	100 ± 6
olive oil		110 ± 5	85 ± 3		114 ± 3		112 ± 10
olive	1.01	107 ± 3	96 ± 8	1.00	115 ± 5	0.18	101 ± 3
olive oil		110 ± 3	86 ± 2		90 ± 3		103 ± 5

Table 3. Validation Parameters for the Four Pesticides in Olives (mg/kg) and Olive Oil (mg/L) at Four Different Levels of Fortification

matrix	fortification level	imidacloprid	thiacloprid	fortification level	spinosad (sum of spynosin A and D)
		Repeatability (n = 6) CV %			
olive	0.04	14.5	10.6	0.05	10.2
olive oil		12.5	2.4		5.0
olive	0.20	14.1	15.0	0.12	5.9
olive oil		10.1	15.0		5.0
olive	0.40	12.9	13.8	0.59	13.0
olive oil		10.4	14.1		6.0
olive	1.01	9.8	12.1	1.18	13.0
olive oil		8.2	19.8		5.0
		Intermediate precision (n = 36) CV %			
olive	0.04	8.1	14.3	0.05	11.8
olive oil		14.1	14.2		15.7
olive	0.20	17.8	6.4	0.12	12.8
olive oil		10.4	15.9		12.9
olive	0.40	12.7	14.1	0.59	12.8
olive oil		15.8	17.1		19.8
olive	1.01	11.1	11.4	1.18	18.7
olive oil		15.3	9.7		11.5

thiacloprid, respectively, corresponding to the max absorbance in the UV spectrum, and 260 nm for spinosyn A and D (Figure 2). The ESI-MS interface was operated in the positive mode: ESI source probe 250 °C, CDL 250 °C, block at 230 °C, flow gas (N_2) at 4.5 mL/min, and probe voltage at 1.5 kV. Quantitative analysis was performed by selected ion monitoring (SIM) at m/z 256, 253, 732, and 746 for imidacloprid, thiacloprid, spinosyn A, and spinosyn D, respectively. Quantitative data were calculated by comparing peak heights from extracted ion current profiles with those obtained from matrix matched standards. For confirmation purposes, DAD chromatogram spectra and specific MS fragmentation patterns were used for distinguishing the analytes from the matrix interferences thus allowing for greater evidence in compound identification (Figures 2and 4).

Sample Preparation and Extraction. Samples of olives were harvested at ripening and immediately carried to the laboratory for the analysis. Samples were chopped and homogenized by a semiindustrial blender (Malavasi, Bologna, Italy). A modified QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) was used.[9,52] Twenty-five grams of olive sample was weighed in a 250 mL flask and extracted with 25 mL of acetonitrile, 5 g of $MgSO_4$, and 3 g of NaCl. The flask was shaken for 1 min with a vortex apparatus and then agitated for 10 min with a flask shaker (Stuart Scientific CO LTD, Great Britain). The tube was centrifuged at 3500 rpm, and 1 mL of the supernatant was dried under a gentle nitrogen stream. The residue was taken up with 1 mL of HPLC water and injected without cleanup in the HPLC/DAD/ESI/MS system. Olive samples were then processed to olive oil using a stainless steel semiindustrial apparatus with the following procedure: the olives were cleaned from leaves, stems, dirt, rock, and sands. The olives were crushed with a hammer mill, and the paste was subjected to malaxation for 45 min; then, the oil was separated from the water and the feed using a dual phase centrifugal decanter. Oil yields were calculated and are reported in Table 1.

One gram of homogenized olive oil sample was weighed in a 40 mL screw-capped flask, and 5 mL of acetonitrile was added together with 2 g of $MgSO_4$ and 3 g of NaCl. The tubes were shaken with a Vortex apparatus (1 min) and then agitated for 10 min in a rotary stirrer (Falk, Milan, Italy), The tube was centrifuged at 3500 rpm, and 1 mL of the supernatant was dried under a gentle nitrogen stream. The residues were taken up with 1 mL of HPLC water and injected without cleanup in the HPLC/DAD/ESI/MS system.

Table 4. Matrix Effect (% ± CV) of the Active Ingredients in Olive and Olive Oil

	imidacloprid			thiacloprid	
fortification level	olive	olive oil	fortification level	olive	olive oil
0.04	97 ± 10	115 ± 1	0.04	117 ± 11	117 ± 1
0.22	95 ± 12	135 ± 7	0.20	95 ± 12	93 ± 7
0.45	100 ± 6	103 ± 35	0.40	113 ± 6	106 ± 11
1.12	99 ± 9	111 ± 10	1.01	106 ± 9	109 ± 10
	spynosin A			spynosin D	
fortification level	olive	olive oil	fortification level	olive	olive oil
0.05	76 ± 12	109 ± 42	0.01	103 ± 3	117 ± 15
0.10	97 ± 13	80 ± 17	0.02	100 ± 12	83 ± 6
0.50	99 ± 6	94 ± 10	0.07	97 ± 13	101 ± 5
1.00	87 ± 14	102 ± 11	0.18	94 ± 15	85 ± 10

Method Validation. The experimental method was validated by determining the relative standard deviation (RSD) of repeatability, intermediate precision, recovery, and linearity. Repeatability (r) involved the repeated analysis of six samples for olive and olive oil each day, while intermediate precision (IP) was calculated analyzing six samples/day, for each typology, in six different days. Method precision was expressed as relative standard deviation. Method accuracy was determined by the mean of percentage recoveries of the initial analyte concentrations in spiked whole olives and olive oil matrix. Untreated olive and olive oil samples were spiked prior to extraction by adding an appropriate volume of stock standard solution to reach 0.04, 0.20, 0.40, and 1.01 mg/kg for imidacloprid and thiacloprid and 0.05, 0.12, 0.59, and 1.18 mg/kg for spinosad (sum of spynosin A and D) and were processed according to the above-described procedure. The matrix effect was evaluated as the influence of all components of the matrix (olive and olive oil) on the detector response of the studied pesticides versus the analytical standards diluted with the eluent mixture.

Statistical Analysis. This method was validated under the EURACHEM Guide (1998) and CITAC/EURACHEM Guide (2002) recommendations.[54,55] Analysis of variance (ANOVA) was carried out with the software STATISTICA, using Turkey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Analytical Method. The experimental design allowed for the study of the decline curves of the selected pesticides after field treatment to determine the amount of pesticide at harvest on olives and evaluate the transfer of pesticide residues in the olive oil media during the technological extraction process. The chromatographic method allowed a good separation of the four pesticides (imidacloprid, 7.05 min; thiacloprid, 10.90 min; spynosin A, 11.90; and spynosin D, 14.37 min; Figure 3). No interfering peaks were detected in the chromatographic range of interest, and no cleanup was necessary. Pesticides were identified by matching peak retention time (tR) with analytical standards analyzed under the same experimental conditions. The instrumental method limits of quantitation (LOQs) and of determination (LODs) were calculated as 10-fold and 3-fold of the signal-to-noise ratio, respectively, and are reported in Table 1. In any case, the sensitivity of the proposed method was adequate to guarantee a correct identification of the pesticides according to the MRL fixed by the Italian and EU legislation (Table 1). Five point standard calibration curves were prepared for each pesticide. The correlation coefficient (R^2) obtained ranged from 0.9989 (spinosyn D in olive matrix) to 0.9998 (spynosyn A in olive oil matrix), showing a good linearity; the CV % max was detected for spinosyn D in olive (16%; Table 1). Accuracy data were provided by recovery experiments of spiked analytes in olive and olive oil matrix at four concentration levels with 4 replicates for each pesticide. Good recoveries were achieved for all pesticides studied (Table 2) according to the EC SANCO/10684/2009 values.[53] Recoveries ranged for imidacloprid from 105 to 119% in olives and from 109 to 110% in olive oil. Thiacloprid recoveries ranged from 96 to 101% in olives and from 85 to 106% in olive oil, while spinosyn A ranged from 92 to 115% in olive and from 80 to 114% in olive oil, and spinosyn D ranged from 93 to 102% in olives and from 92 to 112% in olive oil. The coefficient of variability ranged from 1 to 19% in the most unfavorable case (Table 2). The obtained values confirmed that the proposed extraction method is suitable for the determination of the residues of the studied pesticides in olive and olive oil matrixes. Repeatability (r) was valued for $n = 6$ and intermediate precision (IP) for $n = 36$ for olive and olive oil samples. Good results were obtained for almost all tests (CV ≤ 20) according to EC SANCO/10684/2009.[53] The maximum variation coefficients (CV) were for imidacloprid, 14.5% in repeatability and 17.8% in intermediate precision, for thiacloprid, 19.8% in repeatability and 17.1% in intermediate precision, and for spinosad (sum of synosin A and D), 13.0% in repeatability and 19.8% in intermediate precision (Table 3). The matrix effect, evaluated at four different levels of concentration, did not show any influence of all components of the matrix (olive and olive oil) on the detector response on the studied pesticides (Table 4). Data ranged from 76% to 135% in the most unfavorable case, remaining mostly in the range 95% to 117%, with a CV_{max} of 42.

Table 5. Residues (mg/kg) of Imidacloprid, Thiacloprid, and Spinosad in Olives after Field Treatment ($n = 4$)

			spinosad[a]
days after treatment	imidacloprid	thiacloprid	sum of spynosin A and D
−0	n.d.	n.d.	n.d.
0	0.140 ± 0.03	0.04 ± 0.01	0.30 ± 0.02
3	0.096 ± 0.02	0.02 ± 0.00	0.15 ± 0.01
7	0.083 ± 0.01	0.01 ± 0.00	0.04 ± 0.01
10			n.d.
14	0.051 ± 0.01	n.d.	
28	n.d.		

[a] Data reported belonged to the experiment carried out at 10 times the dose recommended by the manufacturer.

Residue Analysis. Insecticide residues on olives were, immediately after treatment, extremely low for all insecticides used and well below the MRL set for these compounds by EU (Table 5). Imidacloprid residues decreased with a $t_{1/2}$ of 10 days, being 0.14 mg/kg after treatment and, after two weeks, were 0.051 mg/kg in olives. At PHI, imidacloprid residues were below the LOD set in this experiment. Thiacloprid showed residues well under the MRL and near the LOQ just after treatment. The PHI for this insecticide is 14 days, but after one week of

Table 6. Effect of the Technological Process on the Transfer of Imidacloprid, Thiacloprid, and Spinosad from Olives to Olive Oil ($n = 4$)

matrix	imidacloprid	thiacloprid	spinosad
olive (mg/kg)	1.75 ± 0.12	0.56 ± 0.03	0.36 ± 0.04
olive oil (mg/L)	0.07 ± 0.01	0.05 ± 0.02	n.d.

experiment, the residues were 0.01 mg/kg, and thereafter, they were not detectable. Spinosad treatments involved the use of a very low amount of a.i. in the field; this fact led to residue levels after treatment well under the MRL and near or below the analytical LOD for this insecticide. The data obtained at the doses recommended by the manufacturer do not allow for the definition of a decline curve for this compound. For this reason, a second experiment according to Benincasa et al.[50] was carried out at doses 10 times higher than those recommended by the manufacturer using a handle manual sprayer and collecting only the olives certainly treated. With these operating conditions, spinosad showed a residue level after treatment of 0.30 mg/kg which decreases to 0.04 mg/kg after seven days with a $t_{1/2}$ of one day. All pesticides at PHI showed no significant residue levels; therefore, in order to evaluate the influence of the olive oil processing technology on pesticide residue behavior, olives were harvested immediately after treatments carried out at doses 10 times higher than those recommended by the manufacturer. One liter of oil needs on average 5 to 7 kg of olives depending on the season and on the variety; therefore, we should expect an increase of the pesticide residues from five to seven times. The sample of olives used in this experiment showed an oil yield between 15 and 16% (Table 1), with a theoretical concentration factor between 6 and 7 times. Before processing, imidacloprid residues were 1.75 mg/kg, and after the process, they were 0.07 mg/L; thiacloprid in olives was 0.56 mg/kg and in olive oil, 0.05 mg/L; spinosad was 0.36 mg/kg, and their residues in olive oil were negligible (Table 6). The data obtained showed that the insecticides used in this experiment were not transferred in the oil media or were transferred only in very low amounts. Similar experiments with organophosphorus pesticides showed a different behavior with on average 50% of the pesticide transferred to the oil.[3]

In this article, a simple and rapid method for the determination of imidacloprid, thiacloprid, and spinosad in olives and olive oil was developed and validated. The method showed good validation parameters and good recovery values. Residue data showed that the impact on the environment and on food intake was low or negible. Thanks to the QuEChERS extraction method and the high selectivity of the LC/DAD/ESI/MS method, no cleanup was needed. The lack of residue data of the studied insecticides in olives and olive oil do not allow the making of any comparison on the persistence of different cultivar or countries. The proposed method represents an important tool for the analysis of nicotinoid and spinosad residues in olive and olive oil matrixes; moreover, it can be modified easily for the analysis of pesticide residues on other wax-based fruits.

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