JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Persistence of Two Neem Formulations on Peach Leaves and Fruit: Effect of the Distribution

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Persistence of azadirachtins (A+B) and of the other limonoids (nimbin, salannin, deacetylnimbin, and deacetylsalannin) on peach leaves and fruits was studied using a commercial formulation (form. C) compared with an experimental formulation (form. E) prepared with coformulations allowed in organic culture. Field experiments were carried out using three concentrations: 1×, 5×, and 10× the dose recommended by the manufacturer. The EU maximum residue level (MRL) in fruits and vegetables for azadirachtin A is 1 mg/kg with a preharvest interval (PHI) of 3 days. At the recommended dose, azadirachtin A residue on fruits was not detectable (LOQ < 0.8 μg/kg). After field treatment at the 5× concentration, azadirachtoids were found with 22% in the epicuticular waxes and the remaining 78% on the fruit surface. No residues were found in the fruit pulp. The experimental formulation (E) produced lower residues on leaves and fruit compared with the commercial formulation (C), although formulation E showed greater stability. This is probably due to the amount of the active ingredients that diffuse into the epicuticular wax layer thus enhancing photostability of azadirachtoids.

KEYWORDS: Epicuticular waxes; azadirachtin A; azadirachtin B; deacetylnimbin; deacetylsalannin; nimbin; salannin; peaches; leaves; residues

INTRODUCTION

The cuticle covering all above-ground primary plant organs is a two-dimensional polymer membrane on the plant surface. It is composed of the depolymerizable biopolymer cutin (*1*), the nondepolymerizable polymer cutan (*2*) and associated cuticular lipids also called epicuticular waxes (*3*). Chemical compositions of waxes can show high heterogeneity consisting of long-chain, aliphatic molecules with different functionalities such as alkanes, alcohols, aldehydes, and acids. At room temperature waxes are solid, partially crystalline aggregates (*4*). Epicuticular waxes found on the plant cuticle surface can reflect UV radiation.

Since the permeability of the cuticle to water and to organic compounds increases upon wax extraction by factors between 10 and 1000, the cuticular transport barrier is largely formed by these cuticular waxes (*5*).

In agriculture, plant cuticles often represent the major barrier when chemicals are sprayed on to leaf surfaces (*6*−*8*). Exposure of plants to high temperatures and irradiation increases leaf and fruit surface temperature, and this leads to higher mobilities of water and xenobiotics through the cuticle (*9*, *10*). Moreover, pesticide photodegradation is qualitatively and quantitatively influenced by the presence of amorphous waxes extracted from different fruits (*11*−*14*).

In pesticide formulations surfactants are considered to affect either uptake of pesticide molecules across the cuticle to plant tissues or photodegradation profiles on plant surfaces.

Neem (*Azadirachta indica* A. Juss., Meliaceae) extracts are widely used in organic farming, but have low persistence, limiting their efficacy. Neem extracts consist of a complex group of insecticidally active tetraterpene limonoid compounds among which azadirachtin A is the most abundant and most insecticidally active compound.

The aim of this work was to investigate the distribution of azadirachtin A and related limonoids on the peach surface, epicuticular wax layer, and fruit pulp after field application. After application pesticides can dissipate through photodegradation, evaporation, rainfall elution and growth dilution processes (*15*). On the other hand distribution of the active ingredients into the fruit epicuticular waxes can protect the pesticide from a rapid photodegradation enhancing persistence and thus efficacy.

For these reasons we compared the persistence of azadirachtins (A+B), nimbin, salannin, deacetylnimbin, and deacetylsalannin on surface, epicuticular waxes, and pulp of peach fruits.

MATERIAL AND METHODS

Chemicals. Acetonitrile and methanol were of HPLC grade (Baker, Milan, Italy); sodium acetate and formic acid 99% (Sigma Aldrich,

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10.1021/jf803697h CCC: $40.75
Published on Web 02/23/2009

Table 1. Residues (μg/cm^2 $\pm$ SD) of Azadirachtoids on Peaches after Field Treatment with Two Different Formulations (Form. C = Oikos; Form. E = Experimental) at 125 g a.i./ha (5×)

		form. C				form. E			
time (days)	a.i.	fruit surface	epicuticular waxes	pulp	fruit	fruit surface	epicuticular waxes	pulp	fruit
0	AZA-A	0.284 ± 0.020	0.078 ± 0.012	nd[a]	0.362 ± 0.039	0.124 ± 0.017	0.049 ± 0.007	nd	0.173 ± 0.016
	AZA-B	0.102 ± 0.006	0.031 ± 0.002	nd	0.133 ± 0.006	0.089 ± 0.004	0.016 ± 0.002	nd	0.105 ± 0.006
	DSAL	0.106 ± 0.009	0.041 ± 0.010	nd	0.147 ± 0.011	0.066 ± 0.015	0.019 ± 0.010	nd	0.085 ± 0.008
	DNIM	0.084 ± 0.019	0.054 ± 0.008	nd	0.138 ± 0.023	0.064 ± 0.016	0.021 ± 0.008	nd	0.085 ± 0.016
	NIM	0.290 ± 0.033	0.077 ± 0.012	nd	0.367 ± 0.024	0.110 ± 0.029	0.044 ± 0.010	nd	0.154 ± 0.035
	SAL	0.448 ± 0.045	0.093 ± 0.021	nd	0.541 ± 0.041	0.205 ± 0.042	0.055 ± 0.006	nd	0.260 ± 0.046
	total	1.314	0.369		1.683	0.658	0.204		0.862
1	AZA-A	0.124 ± 0.002	0.035 ± 0.005	nd	0.159 ± 0.012	0.074 ± 0.002	0.027 ± 0.006	nd	0.101 ± 0.013
	AZA-B	nd	0.027 ± 0.001	nd	0.027 ± 0.001	0.053 ± 0.010	0.016 ± 0.007	nd	0.069 ± 0.004
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	0.028 ± 0.005	nd	0.028 ± 0.005	nd	0.019 ± 0.004	nd	0.019 ± 0.004
	NIM	nd	0.062 ± 0.008	nd	0.062 ± 0.008	nd	0.038 ± 0.006	nd	0.038 ± 0.006
	SAL	nd	0.058 ± 0.002	nd	0.058 ± 0.002	nd	0.032 ± 0.001	nd	0.032 ± 0.001
	total	0.124	0.210		0.334	0.127	0.132		0.259
3	AZA-A	nd	0.024 ± 0.009	nd	0.024 ± 0.009	0.061 ± 0.007	0.030 ± 0.004	nd	0.091 ± 0.004
	AZA-B	nd	0.017 ± 0.002	nd	0.017 ± 0.002	0.014 ± 0.005	0.011 ± 0.002	nd	0.025 ± 0.009
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	0.021 ± 0.003	nd	0.021 ± 0.003	nd	0.008 ± 0.002	nd	0.008 ± 0.002
	NIM	nd	0.055 ± 0.005	nd	0.055 ± 0.005	nd	0.034 ± 0.007	nd	0.034 ± 0.007
	SAL	nd	0.033 ± 0.003	nd	0.033 ± 0.003	nd	0.029 ± 0.003	nd	0.029 ± 0.003
	total		0.150		0.150	0.075	0.118		0.118
7	AZA-A	nd	0.014 ± 0.001	nd	0.014 ± 0.001	nd	0.028 ± 0.009	nd	0.028 ± 0.009
	AZA-B	nd	0.017 ± 0.001	nd	0.017 ± 0.001	nd	0.011 ± 0.002	nd	0.011 ± 0.002
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	nd	nd	nd	nd	nd	nd	nd
	NIM	nd	0.009 ± 0.006	nd	0.009 ± 0.006	nd	0.008 ± 0.002	nd	0.008 ± 0.002
	SAL	nd	0.020 ± 0.001	nd	0.020 ± 0.001	nd	0.019 ± 0.001	nd	0.019 ± 0.001
	total		0.060		0.060		0.073		0.073
10	AZA-A	nd	0.018 ± 0.001	nd	0.018 ± 0.001	nd	0.028 ± 0.006	nd	0.028 ± 0.006
	AZA-B	nd	0.011 ± 0.001	nd	0.011 ± 0.001	nd	0.009 ± 0.002	nd	0.009 ± 0.002
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	nd	nd	nd	nd	nd	nd	nd
	NIM	nd	nd	nd	nd	nd	nd	nd	nd
	SAL	nd	nd	nd	nd	nd	nd	nd	nd
	total		0.035		0.035		0.041		0.041

[a] nd = not detectable.

Steinheim, Germany); chloroform was of GC grade (J.T. Baker, Milan, Italy). Water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy) before use. Standards of azadirachtin A (AZA-A), azadirachtin B (AZA-B), deacetylnimbin (DNIM), deacetylsalannin (DSAL), nimbin (NIM), and salannin (SAL) were previously isolated in our laboratory with a purity greater than 95% using a vacuum liquid chromatography method (*16*). Both the commercial formulation OIKOS 25 Plus (AZA-A + AZA-B, 25 g/L), and the neem seed extract powder (AZA-A 1.50%, AZA-B 0.54%, deacetylnimbin 0.47%, deacetylsalannin 0.52%, nimbin 1.44, and salannin 3.07%), used to prepare a laboratory formulation (1.1% of AZA-A + AZA-B) using adjuvants allowed in organic farming (Tween 80 and propylene glycol), were kindly provided by SIPCAM (Milan, Italy).

Apparatus and Chromatography. *LC/MSMS Analysis.* A Varian tandem mass spectrometer (Palo Alto, CA) consisting of a ProStar 410 autosampler, two ProStar 210 pumps, and a 1200 L triple quadrupole mass spectrometer equipped with an electrospray ionization source was used. Varian MS workstation version 6.7 software was used for data acquisition and processing. The chromatographic separation was performed on a Waters XTerra RP-18 column (4.6 mm × 250 mm, i.d. 5 μm). The mobile phase consisted of (A) acetonitrile and (B) bidistilled water containing 0.1% formic acid and 0.01% of sodium acetate. The solvent gradient started at 65% A and 35% B, reaching 90% A at 10 min, and kept in these conditions up to 15 min. The mobile phase, previously degassed with high-purity helium, was pumped at a flow rate of 0.4 mL/min, and the injection volume was 10 μL. The electrospray ionization mass spectrometer was operated in the positive ion mode. The electrospray capillary potential was set to 65 V while the shield was at 725 V. Nitrogen at 49 mTorr was used as a drying gas for solvent evaporation. The atmospheric pressure ionization (API) housing and drying gas temperatures were kept at 54 and 375 °C. Sodium adducts of the parent compounds were subjected to collision induced dissociation using argon at 3.80 mTorr as the collision gas using multiple reaction monitoring (MRM) data acquisitions for the transitions of precursor ions as previously reported (*16*). The scan time was 1 s, and the detector multiplier voltage was set to 2000 V.

Standard and Working Solutions. Six stock standard solutions of AZA-A, AZA-B, DNIM, DSAL, NIM, and SAL (1000 mg/L) were prepared in methanol by weighing 0.01 g of the pure analyte into a 10 mL volumetric flask and diluting to volume. For the LC/MSMS analysis an intermediary mixed standard solution was prepared daily by diluting the stock solutions with the mobile phase as listed above. Standard working solutions were prepared by diluting the mixed standard solution with the extract obtained from the untreated matrix of leaf or fruit of peaches. All standard solutions were stored in the dark at −20 °C until usage.

Efficiency. *(A) Standard Curves and Linearity.* A six-point standard curve for each azadirachtoid was prepared. Standard solutions were prepared in triplicate containing all six azadirachtoids at 1, 10, 50, 100, 500, 1000 μg/kg. Calibration curves were created by plotting the concentration of each compound against the standard peak area of the monitored transition. Simple linear regression analysis was performed to calculate the slope and intercept. The correlation coefficient (r) for each azadirachtoid was also determined.

(B) Repeatability. To evaluate precision, repeatability of both the instrument and the analytical procedure proposed was determined. Intermediate precision was calculated by performing six injections of the same standards on each of six consecutive days.

Extraction of Azadirachtoids from Leaves. Hand-cut leaves (~5 g) were accurately weighed in a 40 mL screw-capped glass tube,

Table 2. Residues (μg/cm^2 $\pm$ SD) of Azadirachtoids on Peaches after Field Treatment with Two Different Formulations (Form. C = Oikos; Form. E = Experimental) at 250 g a.i./ha (10×)

		form. C				form. E			
time (days)	a.i.	fruit surface	epicuticular waxes	pulp	fruit	fruit surface	epicuticular waxes	pulp	fruit
0	AZA-A	0.432 ± 0.052	0.147 ± 0.046	nd[a]	0.579 ± 0.031	0.248 ± 0.013	0.083 ± 0.018	nd	0.331 ± 0.021
	AZA-B	0.172 ± 0.026	0.050 ± 0.013	nd	0.222 ± 0.027	0.128 ± 0.007	0.038 ± 0.009	nd	0.166 ± 0.015
	DSAL	0.126 ± 0.028	0.057 ± 0.010	nd	0.183 ± 0.034	0.067 ± 0.009	0.042 ± 0.014	nd	0.109 ± 0.014
	DNIM	0.147 ± 0.018	0.065 ± 0.003	nd	0.212 ± 0.018	0.146 ± 0.006	0.034 ± 0.001	nd	0.180 ± 0.004
	NIM	0.432 ± 0.086	0.154 ± 0.038	nd	0.586 ± 0.096	0.132 ± 0.014	0.085 ± 0.012	nd	0.217 ± 0.013
	SAL	0.746 ± 0.064	0.260 ± 0.053	nd	1.006 ± 0.070	0.552 ± 0.033	0.110 ± 0.038	nd	0.662 ± 0.070
	total	2.055	0.733		2.788	1.273	0.392		1.665
1	AZA-A	0.258 ± 0.007	0.024 ± 0.002	nd	0.282 ± 0.008	0.151 ± 0.016	0.043 ± 0.008	nd	0.194 ± 0.024
	AZA-B	0.064 ± 0.013	0.038 ± 0.001	nd	0.102 ± 0.012	0.024 ± 0.003	0.038 ± 0.009	nd	0.062 ± 0.007
	DSAL	nd	0.033 ± 0.002	nd	0.033 ± 0.002	nd	0.035 ± 0.006	nd	0.035 ± 0.006
	DNIM	0.049 ± 0.004	0.037 ± 0.001	nd	0.086 ± 0.003	0.028 ± 0.002	0.029 ± 0.010	nd	0.057 ± 0.007
	NIM	0.059 ± 0.019	0.107 ± 0.003	nd	0.166 ± 0.018	0.045 ± 0.009	0.067 ± 0.006	nd	0.112 ± 0.005
	SAL	0.043 ± 0.002	0.085 ± 0.003	nd	0.128 ± 0.005	0.051 ± 0.004	0.032 ± 0.006	nd	0.083 ± 0.006
	total	0.473	0.324		0.797	0.299	0.244		0.543
3	AZA-A	nd	0.024 ± 0.009	nd	0.024 ± 0.009	0.068 ± 0.015	0.038 ± 0.005	nd	0.186 ± 0.021
	AZA-B	nd	0.030 ± 0.002	nd	0.030 ± 0.002	0.016 ± 0.005	0.037 ± 0.007	nd	0.053 ± 0.012
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	0.021 ± 0.003	nd	0.021 ± 0.003	nd	0.010 ± 0.002	nd	0.010 ± 0.002
	NIM	nd	0.055 ± 0.005	nd	0.055 ± 0.005	nd	0.050 ± 0.007	nd	0.050 ± 0.007
	SAL	nd	0.033 ± 0.003	nd	0.033 ± 0.003	nd	0.030 ± 0.004	nd	0.030 ± 0.004
	total		0.150		0.150	0.084	0.165		0.329
7	AZA-A	nd	0.020 ± 0.003	nd	0.020 ± 0.003	nd	0.029 ± 0.009	nd	0.029 ± 0.009
	AZA-B	nd	0.026 ± 0.008	nd	0.026 ± 0.008	nd	0.036 ± 0.008	nd	0.036 ± 0.008
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	0.016 ± 0.003	nd	0.016 ± 0.003	nd	0.008 ± 0.006	nd	0.008 ± 0.006
	NIM	nd	0.031 ± 0.003	nd	0.031 ± 0.003	nd	0.034 ± 0.003	nd	0.034 ± 0.003
	SAL	nd	0.046 ± 0.003	nd	0.046 ± 0.003	nd	0.031 ± 0.005	nd	0.031 ± 0.005
	total		0.139		0.139		0.138		0.138
10	AZA-A	nd	0.015 ± 0.002	nd	0.015 ± 0.002	nd	0.014 ± 0.001	nd	0.014 ± 0.001
	AZA-B	nd	0.017 ± 0.003	nd	0.017 ± 0.003	nd	0.035 ± 0.001	nd	0.035 ± 0.001
	DSAL	nd	nd	nd	nd	nd	nd	nd	nd
	DNIM	nd	nd	nd	nd	nd	nd	nd	nd
	NIM	nd	nd	nd	nd	nd	nd	nd	nd
	SAL	nd	nd	nd	nd	nd	nd	nd	nd
	total		0.045		0.045		0.041		0.049

[a] nd = not detectable.

to which 2 g of NaCl and 20 mL of acetonitrile were added. The tube was agitated for 15 min in a rotary shaker at 9 rpm (FALC Instrumentals, Bergamo, Italy) at room temperature, and 1 mL of the mixture was directly submitted to the chromatographic analysis in the MRM mode.

Extraction of Azadirachtoids from Peaches. *(A) Surface*. Fruit samples of about 1 kg were dipped in water (200 mL) and sonicated for 1 min. The water volume was higher than the solubility of AZA-A in water (50 mg/L) in order to be sure to extract all the pesticide. The aqueous phase was directly submitted to the LC/MS analysis.

(B) Waxes. The extraction of the active ingredient from epicuticular waxes was carried out as described by McDonald (*17*). The same fruits were singularly dipped in chloroform (100 mL) and sonicated for 1 min. One milliliter of the mixture was evaporate to dryness under a gentle nitrogen stream. The residue was dissolved with 100 μL of the mobile phase (35:65, water:acetonitrile, v/v) and submitted to the chromatographic analysis.

(C) Fruits. The same fruits were chopped and the extraction was carried out according to Sarais et al. (*16*).

Recovery Assays. A 50 μL aliquot of pesticide solution at the desired standard concentration was added to each 5 g sample of untreated peaches and leaves. One hundred microliters of leaves extracted was diluted to 10 mL. The fortification levels were 5, 50, 200, and 500 μg/kg. The samples were allowed to settle for 30 min prior to extraction. They were later processed according to the above extraction procedure. Four replicates for each level were analyzed by HPLC/ESI-MS/MS analysis.

Field Trials. Field trails were carried out at a peach orchard (Spring belle cv) located in San Sperate, Italy. The experiment was set up in a randomized block design with 4 replicates of 3 plants per treatment. Treatments were carried out in August 2007 and consisted of (A) the commercial formulation (C) OIKOS 25 PLUS (SIPCAM) containing AZA-A + AZA-B at 2.5% applied at 1× (25 g a.i/ha), 5× (125 g a.i./ha), and 10× (250 g a.i./ha) the concentration recommended by the manufacturer; (B) an experimental formulation prepared in laboratory (E) from the neem seed extract powder at 1.1% of AZA-A + AZA-B, further applied at 25 g a.i/ha (1×), 125 g a.i./ha (5×), and 250 g a.i./ha (10×); (C) control (Tween 80 and propylene glycol). Plants were wetted to the drip point using an AM-190 portable motor sprayer (Oleo-Mac, Reggio Emilia, Italy).

Ripened peaches, 3 kg per block, were harvested from the field for the residue analysis of AZA-A and AZA-B, deacetylnimbin, deacetylsalannin, nimbin, and salannin levels at time 0 (after treatment to dry plant), 1, 3, 7 and 10 days.

For surface determination leaves and fruit were wrapped with aluminum foil and then weighed. The surface was calculated from the weight of a square centimeter.

Because of the low stability of limonoids when exposed to UV light, all samples were collected in dark plastic bags and analyzed immediately after harvest.

RESULTS AND DISCUSSION

Analytical Procedure. An HPLC/ESI-MS/MS method for the separation and quantitation of major azadirachtoids in fruit and leaf extracts was used as previously reported (*16*). Recovery and repeatability data for peach fruits were previously reported (*16*). In leaves, epicuticular waxes and fruit surface recoveries for all compounds tested were >82.9%. The highest and the

Table 3. Residues (μg/cm^2 ± SD) of Azadirachtoids on Leaves after Field Treatment with Two Different Formulations (Form. C = Oikos; Form. E = Experimental) at Two Different Concentrations (5× = 125 g a.i./ha; 10× = 250 g a.i./ha)

			time (days)				
concn	active ingred	form.	0	1	3	7	10
5×	AZA-A	C	0.215 ± 0.034	0.090 ± 0.031	0.028 ± 0.008	nd[a]	nd
		E	0.104 ± 0.016	0.050 ± 0.010	0.046 ± 0.008	0.020 ± 0.005	0.014 ± 0.005
	AZA-B	C	0.075 ± 0.011	0.064 ± 0.002	0.026 ± 0.009	nd	nd
		E	0.038 ± 0.005	0.024 ± 0.002	0.013 ± 0.001	0.011 ± 0.003	nd
	DSAL	C	0.052 ± 0.010	nd	nd	nd	nd
		E	0.023 ± 0.005	nd	nd	nd	nd
	DNIM	C	0.057 ± 0.017	nd	nd	nd	nd
		E	0.023 ± 0.015	nd	nd	nd	nd
	NIM	C	0.183 ± 0.044	nd	nd	nd	nd
		E	0.094 ± 0.012	nd	nd	nd	nd
	SAL	C	0.424 ± 0.081	nd	nd	nd	nd
		E	0.200 ± 0.015	nd	nd	nd	nd
	total	C	1.006	0.154	0.054	nd	nd
		E	0.482	0.074	0.059	0.031	0.014
10×	AZA-A	C	0.604 ± 0.123	0.363 ± 0.079	0.135 ± 0.045	0.010 ± 0.002	nd
		E	0.277 ± 0.075	0.249 ± 0.022	0.171 ± 0.011	0.097 ± 0.015	0.041 ± 0.005
	AZA-B	C	0.187 ± 0.032	0.096 ± 0.015	0.031 ± 0.006	0.011 ± 0.004	nd
		E	0.093 ± 0.023	0.035 ± 0.002	0.020 ± 0.003	0.019 ± 0.002	0.018 ± 0.001
	DSAL	C	0.142 ± 0.050	nd	nd	nd	nd
		E	0.074 ± 0.016	nd	nd	nd	nd
	DNIM	C	0.102 ± 0.010	nd	nd	nd	nd
		E	0.093 ± 0.011	nd	nd	nd	nd
	NIM	C	0.368 ± 0.078	nd	nd	nd	nd
		E	0.184 ± 0.025	nd	nd	nd	nd
	SAL	C	0.747 ± 0.084	nd	nd	nd	nd
		E	0.579 ± 0.115	nd	nd	nd	nd
	total	C	2.150	0.459	0.166	0.021	nd
		E	1.300	0.284	0.191	0.116	0.059

[a] nd = not detectable.

lowest coefficients of variation for leaves were 7.1 and 2.0 for interday and 9.0 and 2.2 for intraday experiments, respectively. For azadirachtin A and B the limits of detection (LOD) and quantitation (LOQ) were 0.4 and 0.8 μg/kg respectively. For DSAL, NIM, and SAL LODs and LOQs were 0.8 and 4.0 μg/kg, while for DNI they were 8.0 and 24.0 μg/kg respectively. Good linearity was achieved for all of the compounds tested with correlation coefficients between 0.995 and 0.998.

Residues on Fruits. Treatments at three concentrations (1×, 5×, 10×) of two different formulations (C and E) were carried out. In order to compare the degradation of azadirachtoids on fruits and leaves, the deposit levels of azadirachtoids on the surface and in the epicuticular layer were expressed as μg/cm^2. The calculated surface/weight ratio was of 90.1 and 1.1 cm^2/g for leaves and fruits respectively.

As previously reported for field experiments on strawberry (*18*), at the concentration recommended by the manufacturer (1× = 25 g a.i./ha) fruit levels of azadirachtoids were below the limit of quantification (LOQ).

In our experiments at 5× concentration, for both formulations, azadirachtoids were mostly distributed on the fruit surface (78%) while a small amount (22%) penetrated the epicuticular wax layer. Moreover, azadirachtoids were not able to penetrate the cuticle, thus no detectable residues were found in the fruit pulp (**Table 1**). The fruit surface residue from the commercial formulation (C) was double that of the experimental one. Just after treatment at the 5× concentration (**Table 1**) the sum of the residue levels of azadirachtoids on fruit was 1.683 μg/cm^2 for formulation C, and 0.862 μg/cm^2 for formulation E.

For formulation C, fruit surface residues were different than those in the epicuticular wax layer. One day after treatment, residues of azadirachtin A were detectable on the fruit surface, while at the preharvest interval (3 days) no residues were detectable. On the other hand, azadirachtoid residues were more persistent in the epicuticular wax. In fact active ingredients disappeared gradually: at 1 day DSAL, at 7 days DNIM and at 10 days NIM and SAL. At 10 days after treatment, residues of AZA-A and AZA-B were 20 and 35% of the initial deposit respectively. In the experiment with formulation E, at 3 days after the treatment, residues of AZA-A and AZA-B were still detectable on the fruit surface, but not at 7 days after treatment. AZA-A and AZA-B residues, in the epicuticular wax has more persistent compared to those from formulation C. Levels of AZA-A halved after 1 day after the treatment remaining steady for the following nine days. The half-life (**Table 4**), calculated based on pseudo first order kinetics, for azadirachtin A in the fruit surface was not calculable for formulation C while it was 3.20 days for formulation E. In the epicuticular waxes layer the rate of degradation of the azadirachtin A was lower than that of residues on the fruit surface (5.26 vs 21.18 days for formulation C and E, respectively). Azadirachtin B showed similar trends.

The dissipation rate for AZA-A and AZA-B for the entire fruit (**Table 4**) was between the values for the surface and for the epicuticular waxes; azadirachtin A and B showed a half-life of 2.38 and 3.73 days and 3.88 and 2.80 days for the formulation C and E respectively.

For the experiments conducted at the 10× concentration we observed the same trend (**Table 2**).

Residues on the Leaves. In the experiment at 5× concentration (**Table 3**), residues on leaves were comparable to the levels observed on fruits. One day after treatment only the residues of AZA-A and AZA-B were detectable on foliage. Data reported in **Table 3** indicated that formulation E was more photostable than formulation C; in fact, AZA-A was detectable 3 days after the treatment for formulation C with a half-life of 1.05 days

Table 4. Half-Life Time (Days) and Coefficient of Correlation (r) of Azadirachtoids on Leaves and Peaches of Two Formulations (Form. C = Oikos; Form. E = Experimental) at Two Concentrations (5× = 125 g a.i./ha; 10× = 250 g a.i./ha)

				$t_{1/2}$	
matrix	form.	concn		azadirachtin A	azadirachtin B
leaf	C	5×		1.05 ($r = 0.994$)	1.87 ($r = 0.901$)
	E	5×		3.83 ($r = 0.965$)	3.74 ($r = 0.923$)
	C	10×		1.19 ($r = 0.998$)	1.71 ($r = 0.979$)
	E	10×		3.74 ($r = 0.991$)	3.48 ($r = 0.901$)
peach	C	5×	surface	nc[a]	nc
			waxes	5.26 ($r = 0.827$)	7.52 ($r = 0.937$)
			fruit	2.38 ($r = 0.843$)	3.88 ($r = 0.774$)
	E	5×	surface	3.20 ($r = 0.902$)	1.11 ($r = 0.998$)
			waxes	21.18 ($r = 0.852$)	12.47 ($r = 0.916$)
			fruit	3.73 ($r = 0.955$)	2.80 ($r = 0.956$)
	C	10×	surface	nc	nc
			waxes	4.52 ($r = 0.718$)	7.44 ($r = 0.965$)
			fruit	2.00 ($r = 0.860$)	3.06 ($r = 0.889$)
	E	10×	surface	1.68 ($r = 0.998$)	1.11 ($r = 0.863$)
			waxes	4.80 ($r = 0.941$)	19.26 ($r = 0.843$)
			fruit	2.22 ($r = 0.996$)	4.57 ($r = 0.894$)

[a] nc = not calculated.

while it was detectable for 10 days after treatment for formulation E, with a half-life time of 3.83 days. Half-lives for AZA-B were 1.87 and 3.74 days for formulations C and E, respectively.

Conclusions. Residues of azadirachtoids differed between the two formulations. Azadirachtoids were more persistent with the experimental formulation compared to the commercial formulation although the initial deposit from formulation E was lower compared to formulation C. Adjuvants in formulation C increased the adhesiveness of active ingredients to the leaves and fruit surface while surfactants in formulation E improved the penetration into the epicuticular wax layer possibly protecting the azadirachtoids from photodegradation. The experimental formulation could be improved through the addition of surfactants that enhance azadirachtoid adhesion on leaves and the fruit surface.

The maximum residue limit (MRL) for azadirachtin A is 1 mg/kg with a preharvest interval of three days (*19*). After treatment at 10× concentration the residue level of azadirachtin A on fruits and leaves was below the MRL. Taking into account levels of AZA-A in this experiment the legal limit could be lowered.

The study of residue dissipation on leaves allows for the evaluation of persistence of azadirachtoids and thus a better understanding of the efficacy of these active ingredients.

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Received for review November 27, 2008. Revised manuscript received January 28, 2009. Accepted January 29, 2009.

JF803697H