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Nematicidal Activity of (*E*,*E*)-2,4-Decadienal and (*E*)-2-Decenal from *Ailanthus altissima* against *Meloidogyne javanica*

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ABSTRACT: Methanol extracts of various plant parts of *Ailanthus altissima* were tested against the root knot nematode *Meloidogyne javanica*. Extracts of bark (ABE), wood (AWE), roots (ARE), and leaves (ALE) from *A. altissima* were investigated against freshly hatched second-stage juveniles (J_2). AWE was the most active extract, with $EC_{50/3d}$ of 58.9 mg/L, while ALE, ARE, and ABE did not show nematicidal activity. The chemical composition of the extracts of *A. altissima* was determined by gas chromatography−mass spectrometry, and (*E*,*E*)-2,4-decadienal, (*E*)-2-undecenal, (*E*)-2-decenal, hexanal, nonanal, and furfural were the most prominent constituents. (*E*,*E*)-2,4-Decadienal, (*E*)-2-decenal, and furfural showed the highest nematicidal activity against *M. javanica*, with $EC_{50/1d}$ = 11.7, 20.43, and 21.79 mg/L, respectively, while the other compounds were inactive at the concentrations tested. The results obtained showed that AWE and its constituents (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal could be considered as potent botanical nematicidal agents.

KEYWORDS: *GC−MS, tree of heaven, unsaturated aldehydes, Meloidogyne javanica, botanical pesticide, reactive carbonyl species, (E,E)-2,4-decadienal, (E)-2-decenal*

■ INTRODUCTION

Crop infestation by root knot nematodes (RKNs; *Meloidogyne* spp.) causes 70 billion U.S. dollars of crop losses annually in fruit and vegetable production.[1] Among potential strategies to control these pests, natural nematicides isolated from plants or microorganisms are successfully used as biocontrol agents to reduce non-target exposure to hazardous pesticides and to face resistance development.[2,3] The control of nematodes on cucumbers, tomatoes, and carrots has been performed primarily by fumigants, such us metam sodium and 1,3-dichloropropene, the means of a biological control using bacteria, such us *Bacillus firmus* and *Bacillus chitinosporus*, or using botanical extracts, such as sesame stalk or oil, neem cake, and crab shell meal. Nematicidal application is performed before planting or during crop growth. The pressure to find viable alternatives to the soil fumigant methyl bromide, withdrawn in 2005 according to the Montreal Protocol on Substances that Deplete the Ozone Layer, has been intensified in recent years.

Plant secondary metabolites that have no apparent role in processes of plant structure play an important role in plant−insect interactions,[4] and therefore, such compounds called allelochemicals have insecticidal, hormonal, and antifeedant activities against pests.[2] Reynolds et al. reported that *Meloidogyne incognita* and other generalist nematodes with a wide host range may rely almost exclusively on general plant clues with the right blend and concentration of semiochemicals.[1] Plants may use some known nematicidal cues, such as 2-undecanone, furfural, benzaldehyde, thymol, limonene, neral, geranial, and carvacrol, for defending themselves against the attacker underground.[5−13] On the other hand, some nematodes can be used by plants for the indirect defense against plant herbivores.[14] Another role of secondary metabolites is that of allelopathy, the inhibition of the growth of one plant by another through the production and release of toxic chemicals into the environment.[14] Unsaturated aldehydes are known to be formed in large amounts in plant tissues in responses to wounding and the subsequent action of the lipoxygenase (LOX) enzyme system involving lipid oxidation.[15] According to Wenke et al., belowground volatiles produced by plants facilitate interactions between roots and soil organisms,[16] while Hildebrand[17] suggested that LOX-mediated products, including aliphatic aldehydes, ketones, and alcohols, are involved in plant defense.

The nematode cuticle is flexible, and the exoskeleton is resilient, allowing locomotion and protection and permitting growth by molting. The cuticle is composed of cross-linked collagens, insoluble proteins called cuticlins, glycoproteins, and lipids. The cuticle collagens are encoded by a large gene family, and mutation of individuals genes can result in a range of defects from abnormal morphology to larval deaths, confirming a crucial and essential role of the cuticle structures. Activated, small-molecular-weight carbonyls are an important class of intermediates known as reactive carbonyl species (RCS). In our search for new naturally occurring compounds, we found that the 2-undecanone constituent of the methanol extract of *Ruta chalepensis* and furfural from *Melia azedarach* exhibited strong nematicidal activity against J_2 larvae of *M. incognita* and *Meloidogyne javanica*.[5,6] These compounds are byproducts of cellular metabolism, including lipid peroxidation and glycation, and are activated by α, β, γ, and δ insaturation and/or β-oxo substitution. Among the damage caused by RCS is DNA damage, proteosome degradation, and cellular and extracellular protein alteration. The latter has been recently linked to skin and collagen deterioration.[18] RCS, especially mono- and dicarbonyl compounds, can react with proteins to form a

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variety of adducts through a Maillard reaction. These compounds are known as advanced glycation end products.

Ailanthus altissima, commonly known as "tree of heaven", is a deciduous tree of the Simaroubaceae family. When the leaves and flowers are crushed, they emit a foul-smelling odor. *A. altissima* is native to northeast and central China and was introduced in Europe as a street tree at the end of the 18th century. *A. altissima* grows rapidly and is capable of reaching heights of 10−15 m, and for this reason, it has become an invasive species capable of colonizing disturbed areas. Characteristics of this plant include the versatility of the reproductive methods, the tolerance to unfavorable conditions, and the potential presence of allelochemicals.[14] The tree of heaven has already been used in traditional medicine in many parts of Asia, including China, while the bark and leaves are being used for their bitter-tonic, astringent, vermifuge, and antitumoral properties.[19] Different phytochemical studies reported the presence in the plant of chemical compounds, such as quassinoids, alkaloids, lipids and fatty acids, volatile and phenolic compounds, flavonoids, and coumarins.[19] Kraus et al. reported that the ailanthone extracted with methanol from *A. altissima* seeds turned out to be a potent antifeedant and insect growth regulator.[20]

In this work, we studied the composition of methanol extracts from various parts of *A. altissima* by gas chromatography−mass spectrometry (GC−MS). We also investigated the nematicidal activity of these extracts and their components against RKN *M. javanica* to find potential botanical alternatives to the currently used synthetic nematicidal agents or model compounds for the development of chemically synthesized derivatives with enhanced activity and environmental compatibility.

MATERIALS AND METHODS

Chemicals. Standards of (*E*,*E*)-2,4-decadienal, (*E*)-2-undecenal, (*E*)-2-decenal, (*E*)-2-octenal, nonanal, hexanal, acetic acid, furfural, 2,3-butanediol, hexanoic acid, 5-hydroxymethylfurfural, heptanal, of purity greater than 98%, Tween 20, and dimethylsulfoxide were obtained from Sigma-Aldrich (Milano, Italy). Methanol and water were high-performance liquid chromatography (HPLC)-grade.

Extraction and Chemical Characterization. *Plant Materials.* Leaves, bark, and wood of *A. altissima* were collected before flowering in April 2011 at Cagliari, Italy, and were dried in the absence of light at room temperature. Then, they were sealed in paper bags, stored at room temperature, and kept in the dark until use. Voucher specimens were deposited at the Department of Pharmaceutical Chemistry and Technology, University of Cagliari, Cagliari, Italy, for species identification.

A. altissima Methanol Extracts. Dried leaves, bark, roots, and wood plant parts (100 g) were ground and extracted with methanol (1:10, w/v) in a sonicator apparatus for 15 min, filtered through a Whatman no. 40 filter paper, and centrifuged for 15 min at 13 000 rpm. The extract was analyzed for component identification by means of GC−MS.

GC−MS Analysis. The chromatographic separation and identification of the main components of methanol extracts of *A. altissima* were performed on a Trace GC ultra gas chromatograph (Thermo Finnigan, San Jose, CA) coupled with a Trace DSQ mass spectrometry detector, a split−splitless injector, and an Xcalibur MS platform. The column was a CP-WAX 57CB from Varian (60 m, 0.25 mm inner diameter, and 0.25 μm film thickness; Varian, Inc., Palo Alto, CA). The injector and transfer line were at 200 °C. The oven temperature was programmed as follows: 50 °C (held for 1 min), then raised to 220 °C (3 °C/min), and isothermally held for 13 min. Helium was the carrier gas at a constant flow rate of 1 mL/min; 1 μL of each sample was injected in the splitless mode (60 s). Mass spectrometry acquisition was carried out using the continuous [electron ionization (EI) positive] scanning mode from 40 to 500 amu. *A. altissima* methanol extract components were identified by (1) comparison of their relative retention times and mass fragmentation to those of authentic standards and (2) computer matching against a NIST98 commercial library, National Institute of Standards and Technology (NIST), Gaithersburg, MD. Quantitative analysis of each component was carried out with the external standard method.

Effect of *A. altissima* Extracts on J_2 Motility. Effects of *A. altissima* wood extract (AWE), *A. altissima* leaves extract (ALE), *A. altissima* bark extract (ABE), and *A. altissima* roots extract (ARE) on *M. javanica* J_2 motility were tested at the test concentration range of 15.6−250 mg/L for EC_{50} calculation. Pure compounds contained in the extracts were tested individually against *M. javanica* at the concentration range of 1−50 mg/L for EC_{50} calculation. The compounds used for the paralysis experiment were (*E*,*E*)-2,4-decadienal, (*E*)-2-decenal, (*E*)-2-undecenal, (*E*)-2-decenal, nonanal, heptanal, hexanal, furfural, and 5-hydroxymethylfurfural. Stock solutions were prepared in methanol to overcome insolubility, whereas Tween 90 in distilled water was used for further dilutions. Final concentrations of methanol in each well never exceeded 1% (v/v) because preliminary experiments showed that this concentration was not toxic to nematodes. Distilled water as well as a mixture of water and Tween (0.3%, v/v) (carrier control) served as controls. In all cases, working solutions were prepared containing double the test concentration and mixed in CellstarR 96-well cell culture plates (Greiner Bio-One) at a ratio of 1:1 (v/v) with suspensions of 15 J_2 added to each well. Multi-well plates were covered to prevent evaporation and maintained in the dark at 28 °C.

Juveniles were obtained by an Italian population of *M. javanica* reared for 2 months on tomato (*Solanum lycopersicum*) in a glasshouse at 25 ± 2 °C. Juveniles were observed with the aid of an inverted microscope (Zeiss, 3951, Germany) at 10× after 1, 24, and 72 h and were ranked into two distinct categories: motile or paralyzed. Moreover, at that point, nematodes were moved to plain water after washing in tap water through a 20 μm pore screen to remove the excess of extracts. Numbers of motile and paralyzed J_2 were assessed by pricking the juvenile body with a needle, and they were counted.

Statistical Analysis. Treatments of paralysis experiments were replicated 5 times, and each experiment was performed twice. The percentages of paralyzed J_2 in the microwell assays were corrected by elimination of the natural death/paralysis in the water control according to the Schneider Orelli formula:[21] corrected percent = [(mortality percent in treatment − mortality percent in control)/(100 − mortality percent in control)] × 100, and they were analyzed by analysis of variation (ANOVA) combined over time. Because ANOVA indicated no significant treatment by time interaction, means were averaged over experiments. Corrected percentages of paralyzed J_2 treated with *A. altissima* extracts or pure compounds were subjected to nonlinear regression analysis using the log−logistic equation proposed by Seefeldt et al.:[22] $Y = C + (D - C)/\{1 + \exp[b(\log(x) - \log(EC_{50}))]\}$, where C is the lower limit, D is the upper limit, b is the slope at EC_{50}, and EC_{50} is the *A. altissima* extract or pure compound concentration required for 50% death/paralysis of nematodes after elimination of the control (natural death/paralysis). In the regression equation, the *A. altissima* extract or pure compound concentration (%, w/v) was the independent variable (x) and the paralyzed J_2 (percentage increase over water control) was the dependent variable (y). The mean value of the five replicates per test concentration and immersion period was used to calculate the EC_{50} value.

Scanning Electronic Microscopy (SEM) Analysis. The physical mechanism that (*E*,*E*)-2,4-decacadienal and furfural used to interact with the external nematode cuticle was observed by SEM in the environmental mode (1−20 Torr). Freshly hatched nematodes were treated for 24 h by immersion in a 100 μL solution containing 100 mg/L test compounds. Thereafter, a topographic visualization using a FEI Quanta 200 microscope (FEI, Hillsboro, OR) was performed.

Table 1. Composition of AWE, ALE, ARE, and ABE Determined by GC−MS Analysis, Listed in Order of Elution[a]

compound	t_R (min)	EIMS(m/z) (amu) (abundance)	molecular weight	AWE (mg/kg)	ALE (mg/kg)	ABE (mg/kg)	ARE (mg/kg)
hexanal	10.88	56 (100%); 72 (63%); 82 (47%)	100.2	179			
nonanal	22.71	57 (100%); 82 (51%); 70 (49%)	142.2	61.1	53	76	58
(*E*)-2-octenal	24.27	70 (100%); 55 (82%); 83 (70%)	126.1	44.7			
acetic acid	25.59	60 (100%); 69 (10%)	60.0	108	271	419	280
furfural	25.84	95 (100%); 94 (91%); 59 (13%)	96.1	1.38			
[*R*-(*R**,*R**)]-2,3-butanediol	28.73	60 (100%); 75 (80%)	90.1	7.36			
[*S*-(*R**,*R**)]-2,3-butanediol	30.20	57 (100%); 75 (60%); 72 (20%)	90.1	14.7			
2-decenal	32.89	70 (100%); 55 (77%); 83 (65%)	154.2	33.2			
2-undecenal	36.99	70 (100%); 83 (73%); 55 (62%)	168.3	37.7			
(*E*,*Z*)-2,4-decadienal	37.52	81 (100%); 83 (28%); 67 (17%)	152.2	68.6			
(*E*,*E*)-2,4-decadienal	39.20	81 (100%); 83 (19%); 67 (18%)	152.2	124			
hexanoic acid	40.55	56 (100%); 73 (79%); 87 (24%)	116.2	48	4.0	11.9	13.3
2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	54.52	97 (100%); 126 (79%); 69 (31%)	144.1	nc[b]	nc	nc	nc
5-hydroxymethylfurfural	61.59	144 (100%); 101 (48%); 73 (28%)	126.1	70	55		

[a]Identification was performed by comparison of mass spectra of commercial standards to the respective data of the NIST library in total ion current (TIC) and the literature. [b]nc = not calculated.

RESULTS AND DISCUSSION

Mass spectrometry coupled to gas chromatography is a useful analytical platform for the chemical characterization of plant extracts because it allows for the identification of a large number of plant metabolites. A major disadvantage of this technique is that analytes used must be derivatized to improve the volatility to the injection port. In this work, *A. altissima* methanol extracts were directly injected in GC−MS neither employing derivatization nor any purification steps. Using a CP-WAX 57CB Carbowax column, we were able to separate polar and medium polar plant secondary metabolites, such as (*E*,*E*)-2,4-decadienal, (*E*)-2-undecenal, (*E*)-2-decenal, (*E*)-2-decenal, hexanal, nonanal, acetic acid, furfural, 2,3-butanediol, hexanoic acid, and 5-hydroxymethylfurfural (Table 1). We also tried to chemically characterize the methanol extract by liquid chromatography−mass spectrometry−time of flight (LCMS−TOF), but aldehydes were not detected because of their volatility and low ionization efficiency during atmospheric pressure ionization.

Among unsaturated aldehydes identified in *A. altissima* extracts, (*E*,*E*)-2,4-decadienal, (*E*)-2-decenal, and furfural were the most active against J_2 with $EC_{50/1d}$ of 11.70 and 20.43 mg/L, respectively, while (*E*)-2-octenal, nonanal, heptanal, and hexanal did not provoke paralysis on J_2 (Table 2). Interestingly, 1 h post-J_2 immersion in test solutions, EC_{50} values were calculated even lower [7.5 and 11.75 mg/L for (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal], but this activity was characterized as nematostatic rather than nematicidal because to some extend J_2 regained their movement later. Moreover, no fumigant activity of plant methanol extracts or pure compounds was detected. The activity of (*E*,*E*)-2,4-decadienal and (*E*)-2-decenal against *M. javanica* is rather high if compared to the nematicidal activity of fosthiazate ($EC_{50/1d}$ = 15.9 mg/L).

Table 2. EC_{50} and R^2 Values of Individual Compounds against *M. javanica* Calculated at 1 h and 1 day after Immersion in Test Solutions[a]

	1 h		1 day	
compound	EC_{50} (mg/L)	R^2	EC_{50} (mg/L)	R^2
(*E*,*E*)-2,4-decadienal	7.53	0.98	11.70	0.97
(*E*)-2-decenal	11.75	0.98	20.43	0.93
(*E*)-2-undecenal	>25		>25	
(*E*)-2-octenal	na[b]		>25	
nonanal	>50		na	
heptanal	na		na	
hexanal	na		na	
furfural	>25		21.79	0.98
5-hydroxy-methylfurfural	>25		>25	
fosthiazate	>25		15.9	0.98

[a]If R^2 values are not presented and the EC_{50} values have not been calculated, they were outside the test concentration range and are estimated higher than the upper concentration level (25 mg/L). [b]na = not active in the range of 25−1000 mg/L.

According to the GC−MS analysis (Figure 1), AWE afforded (*E*,*E*)-2,4-decadienal, (*E*)-2-decenal, hexanal, nonanal, acetic acid, furfural, 2,3-butanediol, 2-decenal, 2-undecenal, hexanoic acid, and 5-hydroxymethylfurfural (Table 1), while ALE, ARE, and ABE afforded nonanal, acetic and hexanoic acids, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one. As a result of the GC−MS analysis, 14 plant metabolites, accounting for 82.6% of the methanol extract, were identified. Without taking into account plant compound bioavailability or the synergetic effect when AWE was tested against *M. javanica*, a clear dose−response relationship was established and significant paralysis of J_2 was evident after 3 days of exposure, with an $EC_{50/3d}$ value calculated at 58.9 mg/L (Table 3). This value is rather low

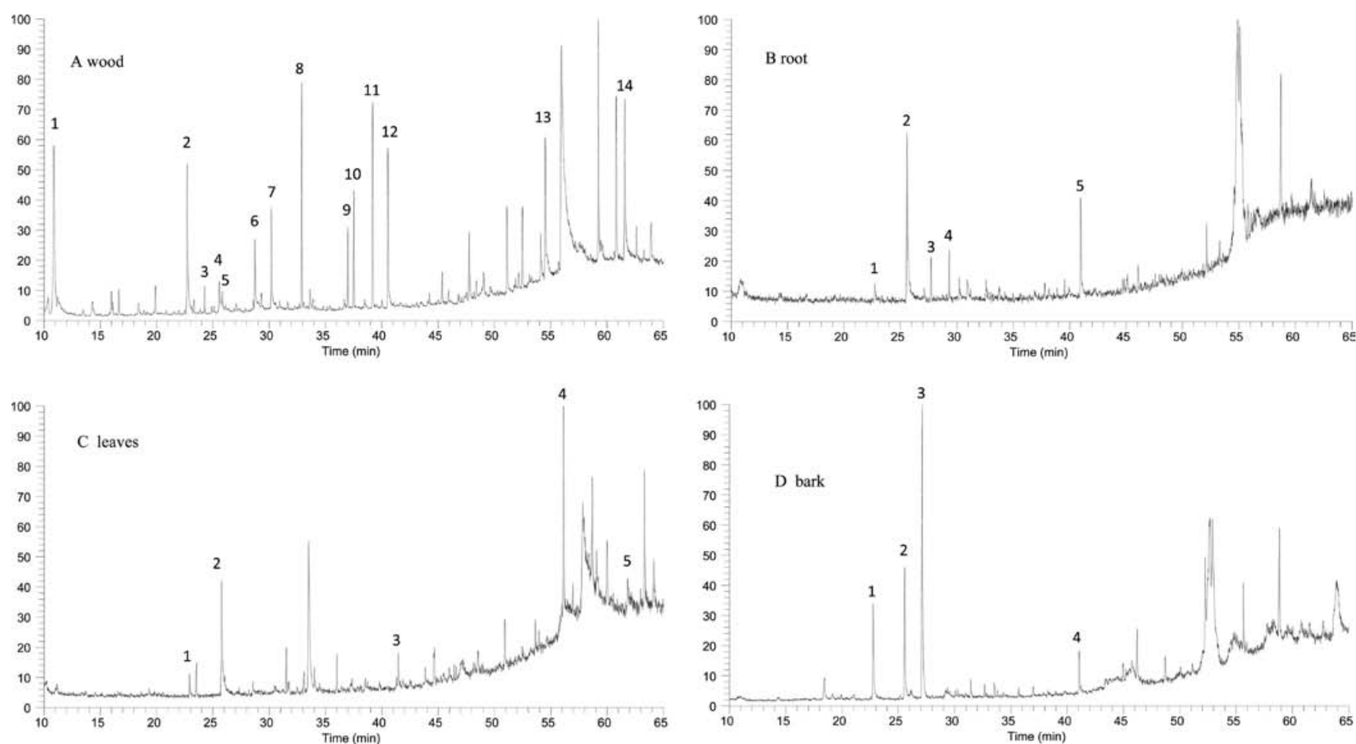

Figure 1. GC−MS chromatograms of *A. altissima* methanol extracts. Peaks A: (1) hexanal, (2) nonanal, (3) (*E*)-2-octenal, (4) acetic acid, (5) furfural, (6) (*E,Z*)-2,3-butanediol, (7) (*E,E*)-2,3-butanediol, (8) (*E*)-2-decenal, (9) (*E*)-2-undecenal, (10) (*E,Z*)-2,4-decedienal, (11) (*E,E*)-2,4-decedienal, (12) hexanoic acid, (13) 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, and (14) 5-hydroxymethylfurfural. Peaks B: (1) nonanal, (2) acetic acid, (3) 2-nonanol, (4) 1-octanol, and (5) hexanoic acid. Peaks C: (1) nonanal, (2) acetic acid, (3) hexanoic acid, (4) 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one, and (5) 5-hydroxymethylfurfural. Peaks D: (1) nonanal, (2) acetic acid, (3) decanal, and (4) hexanoic acid.

Table 3. EC_{50} and R^2 Values of *A. altissima* Extracts (ALE, ABE, AWE, and ARE) against *M. javanica* Calculated after 3 Days of Immersion in Test Solutions[a] and Respective Concentration of Each Extract Provoking 100% Paralysis

	3 days		4 days	
extract	EC_{50} (mg/L)	R^2	100% mortality (mg/L)	100% mortality (mg/L)
AWE	58.9	0.91	500	31.2
ABE	>250		625	312
ALE	>250		>2500	>2500
ARE	>250		>2500	>2500

[a] *A. altissima* methanolic extracts of wood, AWE; leaves, ALE; bark, ABE; and roots, ARE.

considering the activities of *R. chalepensis* methanol extracts against *M. incognita*, exhibiting an EC_{50} value of 1001 mg/L after 1 day of J_2 immersion in test solutions,[5] as well as *Plantago lanceolata*, with an EC_{50} value of 43.7% after 2 days of immersion.[23]

This is the first report of the irreversible nematicidal activity of unsaturated aldehydes as constituents of *A. altissima* against *M. javanica*. According to our results, (*E,E*)-2,4-decadienal and (*E*)-2-decenal were the principal nematicidal constituents of AWE. Interestingly, the other aldehydes or ketones were not found nematicidal against RKN (data not shown). Our results clearly indicate that $\alpha,\beta,\gamma,\delta$-unsaturated C_{10} aldehydes are generally more potent nematicidal than their shortest C chain counterparts versus *M. javanica in vitro* experiments. Kim et al. reported that α,β-unsaturated aldehyde 2-decenal showed the highest nematicidal activity at 200 mg/L against the pine wood nematode (*Bursaphelenchus xylophilus*) if compared to other non-unsaturated C_8−C_{10} aldehydes.[24] On the other hand, Andersen et al. reported that C_9 unsaturated aldehydes, i.e., (*E*)-2-nonenal and (*E,Z*)-nonadienal, showed the strongest antifungal activity against *Alternaria alternata* if compared to shortest chain aldehydes, concluding that the effectiveness is due to their increased propensity to react with thiols and amino groups of the target fungi.[15]

Understanding the mode of action of the α,β- and $\alpha,\beta,\gamma,\delta$-unsaturated C_{10} aldehydes is of practical importance for developing new formulations and delivery systems for nematode control. We observed that nematodes treated with aldehydes and ketones were paralyzed in a straight shape, in a similar way as reported by Kim et al. that treated nematodes with plant essential oils,[24] while Kong et al. reported that pine wood nematode treated with muscle activity blockers levamisole or morantal tartrate were paralyzed in semi-circular and coiling shapes, respectively.[25] Moreover, we have recently reported the circular shape paralysis of J_2 after immersion with the organoposphorous fosthiazate.[5]

Aliphatic aldehydes and to a lesser extent ketones are relatively reactive compounds. The carbonyl carbon is an electrophilic site and reacts with primary amines and thiols, resulting in the formation of substituted imines, called Schiff bases and hemiacetals, respectively. Aldehydes bringing one or two insaturations become even more reactive, being easily the site of nucleophilic attack. Taking into account the reactivity of $\alpha,\beta,\gamma,\delta$-unsaturated aldehydes and environmental scanning electron microscopy (ESEM) experimental photographs of the external nematode cuticle following treatment with (*E,E*)-2,4-decadienal and furfural at 100 mg/L led us to hypothesize the reaction of $\alpha,\beta,\gamma,\delta$-aldehydes with the nematode cuticle

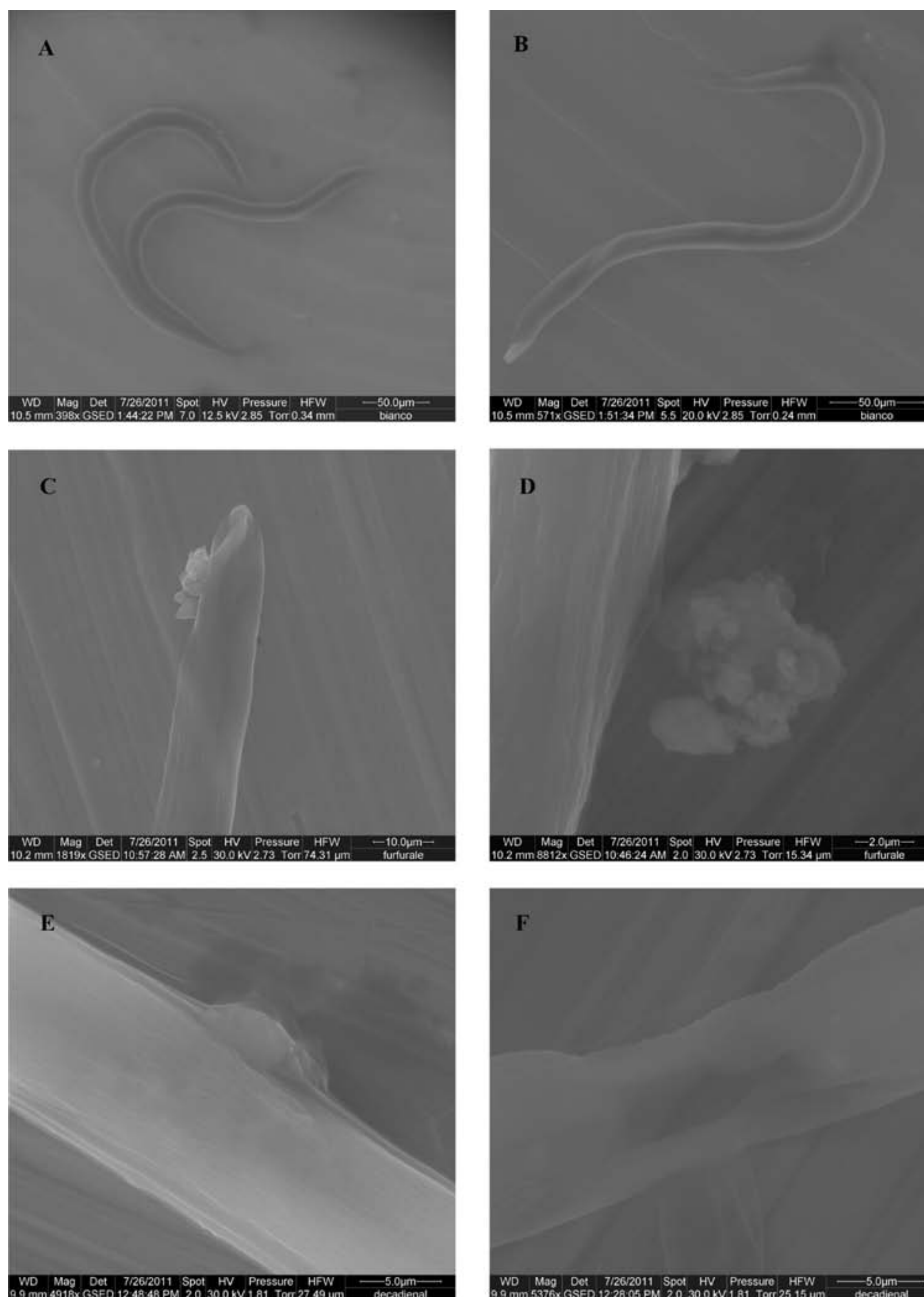

Figure 2. ESEM topographical images from immersion treatment of J_2 with (A and B) water, (C and D) furfural at 100 mg/L, and (E and F) treatment with (*E*,*E*)-2,4-decadienal at 100 mg/L. (D−F) Degradation of the external cuticle of *M. incognita* juveniles is evidenced by the leakage of internal fluids.

through a Michael addition. This reaction consists of a nucleophilic addition of a cuticle amino or thiol group to an *α*,*β*-unsaturated carbonyl. This interaction leads to evident cuticle damage and leakage of the internal fluid nematode material (Figure 2). Similar nematode cuticle damages were reported for *Panagrellus redivivus* caused by a unique fungal structure on the vegetative hyphae of *Coprinus comatus*. The latter was also able to produce potent nematicidal toxins, such as 5-mehylfuran-3-carboxylic acid and 5-hydroxy-3,5-dimethyl-furan-2(5*H*)-one.[26,27] Moreover, Luo et al. observed that the fungus *Stropharia rugosoannulata* produced a severe mechanical damage on the cuticle of nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus* through finger-like projections called acanthocytes.[28]

The exact mechanism underlying the interaction of unsaturated aldehydes with the nematode cuticle is still unclear. Currently, we are investigating the interaction of aldehydes with the nematode cuticle through a proteomic approach to well-understand protein glycation.

Abbreviations Used

GC−MS, gas chromatography−mass spectrometry; AWE, *A. altissima* wood methanolic extract; ALE, *A. altissima* leaves methanolic extract; ABE, *A. altissima* bark methanolic extract; ARE, *A. altissima* roots methanolic extract

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