Identification of insecticide residues with a conducting-polymer electronic nose

Alphus Dan Wilson

Forest Insect and Disease Research, Southern Hardwoods Laboratory, Center for Bottomland Hardwoods Research, Southern Research Station, USDA Forest Service, P.O. Box 227, Stoneville, Mississippi, USA

Author for correspondence: A. Dan Wilson, email: dwilson02@fs.fed.us
Received 1 May 2013; Accepted 28 Jun 2013; Available Online 28 Jun 2013

Abstract

The identification of insecticide residues on crop foliage is needed to make periodic pest management decisions. Electronic-nose (e-nose) methods were developed and tested as a means of acquiring rapid identifications of insecticide residue types at relatively low cost by detection of headspace volatiles released from inert surfaces in vitro. Detection methods were developed for an intrinsically conducting polymer (CP)-type electronic nose gas-sensing device, the Aromascan A32S, to identify and discriminate among eleven insecticides representing eight different classes. A vapor library was developed using diagnostic vapor profile databases (electronic vapor signature patterns) from known insecticides. The A32S e-nose effectively distinguished between eleven different insecticide residues, correctly identifying them at frequencies ranging from 82-99%. The distribution of vapor class components, based on artificial neural net (ANN) training and analysis, indicated the percentage membership of vapor classes shared by insecticide types. Principal component analysis (PCA) indicated the relatedness of insecticides based on sensor array response patterns (vapor profiles) of individual insecticides. Furthermore, PCA generated precise statistical numerical values (quality factors of significance) that also provided some indications of chemical relatedness between insecticides based on pairwise comparisons of headspace volatiles from individual insecticide types. The potential applications of these methods to the detection and identification of insecticide residues on crop surfaces in the field are discussed.

Keywords: Electronic aroma detection; e-nose sensor technologies; Pesticide identification

1. Introduction

Current methods for the detection and identification of pesticide residues on agricultural and landscape plants require time-consuming and expensive chemical analyses [1-4]. This problem causes delays for agricultural land managers in making important crop-management and pest-control decisions involving pesticide applications. The presence of pesticide residues on food crops also is a major health concern, especially on fresh fruits and vegetables because of the broad impacts of health laws regulating the safety of plant-related food and fiber products in commercial markets. The inadequacies of current conventional chemical-analysis methods, such as gas chromatography-mass spectroscopy (GC-MS) for determining the identities of pre-harvest and postharvest crop residues on the surfaces of plant products, has produced a strong need for new more rapid chemical-detection methods to effectively identify pesticide residues on plants in crop fields and in post-harvest storage facilities prior to plant-product introductions into commercial markets.

Many electronic sensor devices have been evaluated for specific capabilities of detecting insecticides in the environment. Most previous insecticide-detection research has focused on organophosphate (OP) insecticides because this broad class of organic compounds is highly toxic to mammals, as powerful cholinesterase inhibitors (nerve toxins), resulting in significant threats to the health of humans and the environmental due to widespread commercial use of OP-insecticides on agricultural lands [2]. Organothiophosphates (P–S, P=S) are related to phosphoryl-type (P=O) organophosphates that include such lethal nerve and chemical warfare agents as VX, Soman and Sarin. Organophosphate residues on agricultural crops, livestock, and poultry products have the potential to migrate into aquifers and contaminate water resources following direct applications to plants and soils or from accidental spills or leaks from storage tanks and waste repositories. Most OP insecticides are non-persistent, but a few OP-contaminants, such as azinphos methyl, have long half-lives and may persist in the environment for long periods of time (up to four years). Insecticides have been detected using a wide range of techniques, including electrochemical [5-8], luminescent [9-11], fluorescent [12-14], optical [15], polymer [16], hydrogels [17], and surface acoustic wave (SAW) electronic-nose sensors [18,19] as well as immunological (antibody) [20-23], enzyme-linked immunosorbent assay (ELISA) [24], enzymatic biosensor [25], microbial biosensors [26] and fiber optic biosensors [27,28].

Electronic chemical-detection methods are ideally suited for repeated, rapid detections needed for making pesticide-management decisions and for monitoring pesticide levels on crops for regulatory safety enforcements. In particular, portable electronic-nose (e-nose) devices are especially useful for these applications due to the capability of rapid detections, sensor recovery, high reproducibility, accurate determinations, and high sensitivity to polar volatile organic compounds (VOCs) typical of most commercial pesticides. This study is part of a series of efficacy studies to assess the relative suitability and capabilities of e-nose devices to detect agricultural pesticide residues in various commercial plant-production settings. The current paper reports on the development and testing of methods for use with an intrinsically conducting polymer (ICP) e-nose technology to potentially identify insecticide residues on plant surfaces in agricultural fields. The objectives of this study were to 1)

Table 1. Chemical classes, formulations and vapor pressure characteristics of insecticides determining chemical interactions with the CP 32-sensor array of the A32S e-nose.

Common name	Trade name	Class[a]	Chemical formula	Formulation[b]	VP[c]	Toxicity[d]
Acephate	Orthene	OP	$C_4H_{10}NO_3PS$	75% WP	2.0×10^{-1}	>60
Carbaryl	Sevin	CB	$C_{12}H_{11}NO_2$	80% WP	2.5	3.8
Cyfluthrin	Baythroid	PY	$C_{22}H_{18}C_{l2}FNO_3$	12.7 % EC	4.4×10^{-3}	>1.7
Diazinon	Spectracide	OP	$C_{12}H_{21}N_2O_3PS$	14% GR	9.7×10^{-2}	>2.5
Fipronil	Combat	PP	$C_{12}H_4Cl_2F_6N_4OS$	2.15% GR	3.7×10^{-4}	0.7
Imidacloprid	Provado	NN	$C_9H_{10}ClN_5O_2$	75% WP	4.0×10^{-7}	3.2
Lindane	Silvanol	OC	$C_6H_6Cl_6$	1.65 EC	4.8×10^{-2}	NH
Malathion	Malatox	OP	$C_{10}H_{19}O_6PS_2$	5% EC	1.6×10^{-2}	>5.2
Methoxyfenozide	Intrepid	DH	$C_{22}H_{28}N_2O_3$	2% F	1.4×10^{-3}	>0.9
Methyl parathion	Penncap	OP	$C_{10}H_{14}NO_5PS$	21.2 % MEF	1.3×10^{-3}	0.12
Spinosad	Tracer	SP	$C_{41}H_{65}NO_{10}$	80% SC	3.2×10^{-5}	>17

[a] Chemical class: CB = carbamate, DH = diacylhydrazine, NN = neonicotinoid, OC = organochlorine, OP = organophosphate, PP = phenylpyrazole, PY = pyrethroid, SP = spinosyn.
[b] Formulation abbreviations: EC = emulsifiable concentrate, F = flowable, GR = granular, MEF = microencapsulated flowable; WP = wettable powder, SC = suspension concentrate.
[c] Vapor pressure (VP) expressed in millipascals (mPa) at 20 °C.
[d] Toxicity by inhalation LC_{50} (ppm for 15 min by rats); NH = considered nonhazardous by inhalation.

determine the capability and effectiveness of ICP e-nose technology to discriminate between different insecticide residues in vitro (as the first testing phase before analysis of residues on plant surfaces in the field), based on analysis of headspace volatiles, 2) assess the tentative potential usefulness of these methods, followed by subsequent field tests on plant surfaces, for making crop-management decisions involving the application and use of insecticides for pest-control applications and 3) a minor objective to determine whether these e-nose methods provide some indications of relatedness between individual insecticide types, based on vapor characteristics and interactions with the sensor array. Some brief preliminary results of this study were published previously [29,30].

2. Experimental Details

Eleven insecticides from several different chemical classes, having different modes of actions against various insect pests, were selected for this study. The insecticides, analyzed and compared using an e-nose type electronic aroma detection (EAD) technology [31], included acephate (Acep), carbaryl (Carb), cyfluthrin (Cyfl), diazinon (Diaz), fipronil (Fipr), imidacloprid (Imid), lindane (Lind), malathion (Mala), methoxyfenozide (Mefo), methyl parathion (Mpth), and spinosad (Spin). The formulations, chemical classes, modes of action, and volatility of each insecticide are presented in Table 1. All insecticides were obtained in formulations that were commercially available from manufacturers, including Monsanto Co. (Acep, Diaz), Bayer AG (Carb, Cyfl), BASF Corp. (Fipr), Makhteshim Agan of North America, Inc. (Imid), Drexel Chemical Co. (Lind), PBI/Gordon Corp. (Mala), Dow AgroSciences LLC (Mefo, Spin), and Cheminova Agro (Mpth), rather than from technical grade preparations, to facilitate practical efficacy testing of formulations actually used in insect-control applications for agronomic crop production.

2.1. Sample preparation and prerun procedures

Small aliquots (5-10 µl) of each insecticide type were analyzed separately by placing them into 14.8 cm^3 uncapped glass vials inserted into a 500 ml Pyrex glass sampling bottle no. 1395 (Corning Inc., Corning, NY) fitted with reference air, sampling, and exhaust ports on a polypropylene bottle cap. Reference air entered the sampling bottle through a 3 mm polypropylene tube extending to just above the bottom of the sampling bottle. The sampling bottle was held in the sampling chamber within the instrument at a constant air temperature of 25 °C and purged with moisture-conditioned reference air for 2 min prior to building headspace. The sampling bottle was sealed and volatiles from each insecticide analyte were allowed to build headspace and equilibrate for 30 min prior to each run. Concentrations of individual insecticides in the sampling bottle, following the building of headspace volatiles, ranged from approximately 5-15 ppm, near or below inhalation LD_{50} toxicity levels in rats for most insecticides tested. Prerun tests were performed as needed to determine sample air relative humidity compared with that of reference air. Reference air was set at 4% relative humidity at 25 °C. The sampling bottle cap and exhaust port were opened between runs to purge the previous sample with conditioned reference air. A reference library (recognition file) for pesticide types was constructed using neural net training by defining vapor classes using reference databases of known pesticides. This recognition file was used to identify unknown samples.

2.2. Instrument configuration and run parameters

Electronic-nose analyses of insecticides were conducted with an Aromascan A32S (Osmetech Inc., Wobum, MA) intrinsically conducting polymer (ICP) e-nose instrument with 32 sensors in the sensor array consisting of polypyrrole, polyanaline, and polythiophene sensor types with 15 volts across sensor paths. Eight sensors, (including sensors 11, 12, 21-23, 26, 31 and 32) that did not respond or did not contribute to the discrimination of pesticide volatiles, were turned off. The response sensitivities of individual sensors, measured as percent changes in electrical resistance response across sensor paths relative to base resistance (%ΔR/Rbase), varied with the type of polymer used in the sensor matrix coating, the type of proprietary ring substitutions used to modify its conductive properties, and the type of metal ions used to dope the matrix to improve and modulate sensor response. Detailed analyses indicating relative analyte sensitivities for individual sensors in the array to various analyte types (representative of different chemical classes) were reported previously [31]. The block temperature of the sensor array was maintained at a constant 30 °C. Reference air was preconditioned by passing room air sequentially through a carbon filter, silica gel beads, inline filter, and Hepa filter to remove organic compounds, moisture, particulates, and microbes, respectively, prior to humidity control and introduction into the sampling bottle. The flow rate of sampled air at the sampling port was maintained at ~702 cm^3/min using a calibrated ADM 3000 flow meter (Agilent Technologies, Wilmington, DE). Sensor surfaces were purged between runs using a 2% isopropanol wash solution. The instrument was interfaced with a personal computer via an RS232 cable and controlled with Aromascan Version 3.51 software. The instrument plumbing (reference air flow route through the instrument) was altered from conventional architecture and specifically configured for static sampling of the headspace by allowing air flow, maintained at 605 cm^3/min flow rate, to be released out of the external vent port of the instrument during analytical runs, and closing the exhaust port on the sampling bottle so that headspace volatiles were taken by vacuum (suction) from a homogeneous static air mass within the sampling bottle.

2.3. Data acquisition parameters and run schedules

Data from the sensor array were collected at 1 s intervals using a 0.2 detection threshold (y-units), a 15–20 y-max graph scale, and with a pattern average of five data samples taken per run during data acquisition. A uniform run schedule was used consisting of reference air 20 s, sampling time 90 s, and wash 20 s, followed by 90 s of reference air for a total run time of 220 s. A 2 min reference air purge was completed between runs after each sample was removed from the sampling bottle.

2.4. Construction of reference libraries and validation

A vapor signature reference library was constructed from eleven known reference insecticides included in this study. All database files were linked to specific (designated) vapor classes defining each sample type or category. All databases were constructed from sensor-array output data collected during a 20 s interval, 85-105 s into the run cycle, immediately prior to the closing of the reference air valve to the sensor array. The following recognition network options (neural net training parameters) were used for each training session: training threshold = 0.60, recognition threshold = 0.60, number of elements allowed in error = 5, learning rate = 0.10, momentum = 0.60, error goal = 0.010 (P = 0.01), hidden nodes = 5, maximum iterations (epochs) = 10,000, using normalized input data, not actual intensity data. Some parameters were modified for improvement of recognition accuracy. A typical training required 2–35 min, depending on the size of the database applied, using an IBM-compatible personal computer with a minimum of 64 mb of RAM and 350 MHz run speed. Neural net trainings were validated by examining training results that compare individual database files for compatibility or by similarity matches to each specific vapor class by test-assigned vapor class distributions among related vapor classes included in each library. The specific detailed analytical methods used for identification of unknowns, data processing, and statistical determinations followed the procedures and specifications indicated by Wilson et al. [31].

2.5. Principal component analysis

Detailed comparisons of relatedness of vapor classes (insecticide types) were determined using principal component analysis (PCA) algorithms provided by the Aromascan 3.51 software. Three-dimensional PCA was used to distinguish between insecticide headspace volatiles released from eleven insecticide types. The mapping parameters for three-dimensional PCA were: iterations = 30, units in Eigen values (%), and use of normalized input data. The degree of relatedness between insecticide vapor classes was determined using three-dimensional principal component analysis (PCA) of headspace volatiles. PCA allowed the determination of the vapor relatedness between individual insecticides via pairwise comparisons that generated a quality factor (statistical significance value) for each vapor comparison and a vapor map showing a 3-dimensional plot of the relatedness of insecticide vapors. The relatedness of vapor profiles between insecticides provided some indications of similarity in vapor elements and chemical characteristics as well as interactions with the sensor array.

3. Results and Discussion

The insecticides tested in this study, similar to other pesticide types, consisted of stronger polar functional groups that tended to produce more intense sensor responses than were observed previously for headspace volatiles derived from microbial biotic sources [31], consisting mostly of primary and secondary metabolic products of oxidative and fermentative respiration. As a consequence, the sensor array was more sensitive to insecticide residues that could be detected at lower concentrations in the 5-10 ppm range. Sometimes the high polarity of certain pesticides resulted in stronger binding of headspace volatiles to individual sensors that caused temporary sensor inactivation or overloaded responses that were not rapidly attenuated because the analyte could not be easily removed from the surface of some sensors during the wash cycle between analytical runs. In extreme cases, some sensors could be permanently inactivated by an analyte that was allowed to build to concentrations that were too high for the sensor array. In such cases, several factors had to be considered prior to analysis including the type of insecticide being analyzed, the concentration (application rate) and total amount of insecticide residues previously applied to crop surfaces, the quantity of sample (e.g. leaf surface containing

the pesticide residue) placed in sampling bottle for analysis, and the time allowed for the sample to build headspace volatiles of the pesticide analyte.

Certain polar insecticides and other pesticides previously have been determined (prior to this study) to be associated with the inactivation of individual CP-sensors included sensors 11, 12, 21-23, and 26) in the A32S e-nose instrument. Analyses of highly-polar insecticide types should be done carefully to avoid overloading the sensor array, leading to potentially permanent sensor damage and inactivation of certain sensor types. The optimum sample size for individual chemical classes of pesticides should be determined prior to data analyses, starting with a small sample pesticide volume of 1-10 µl at ≤1 ppm placed on an inert surface (small glass vial), and testing increasing sample sizes until the minimum effective detection concentration is reached. The A32S e-nose has a detection limit that varies depending on chemical analyte types, but generally ranges below 1 ppm for most pesticide compounds (including insecticides) detectable by the majority of sensors in the sensor array. Concentrations of pesticide residue samples well above (more than 10× greater than the minimum detection concentration) may be avoided by diluting the residue sample with an appropriate solvent if necessary prior to analysis.

3.1. Identification of insecticide analytes

The Aromascan A32S electronic nose provided consistent correct identifications for 10 of the 11 insecticide residue types analyzed based on differences in sensor-array responses to headspace volatiles (Table 2). Only methoxyfenozide (Intrepid) could not be consistently identified at a high level of confidence. Individual sensor responses to each insecticide varied widely within the 2 to 8 sensor-intensity range with good precision as indicated by low standard deviations (SD) of mean values. Sensors 24, 25, and 30 had no responses to some of the insecticides. In particular, sensor 24 could only detect acephate and lindane among the eleven insecticides, whereas sensors 25 and 30 were unable to detect the same five insecticides including fipronil, imidacloprid, methoxyfenozide, methyl parathion, and spinosad.

The instrument correctly identified individual insecticide residues at frequencies ranging from 82-99% among all insecticide residue analytes tested with the exception of methoxyfenozide (Intrepid) of the diacylhydrazine chemical class which was correctly identified in only 73% of samples analyzed. Some samples of methoxyfenozide could not be identified and were classified as unknown due to unexplained variations in vapor signature patterns. For methoxyfenozide samples that were unidentified, the ANN algorithm could not assign the vapor profile to a majority vapor class present in the reference library. Some significant vapor elements in the Intrepid profile were assigned to vapor classes of other insecticides. This resulted in a vapor class distribution that was allocated more widely among other vapor classes. However, none of the insecticide identifications were determined to be incorrect or ambiguous, defined as determinations that resulted in an insecticide residue type being assigned to a different majority vapor class from separate analyses of sample replications.

3.2. Discrimination between insecticide residues

The discrimination of vapor profiles between insecticide residue types was further evaluated following neural net training validation by determining the precise breakdown of vapor class distributions of principal vapor components present in headspace volatiles among the eleven insecticides as summarized in Table 3. Vapor class distributions indicate (on a percentage bases) the proportion of vapor components, present in the headspace volatiles of each insecticide type, that are in common with principal vapor elements of volatiles from other insecticide types present in the reference library. Thus, the degree of overlap among principal vapor elements from volatiles of each insecticide type provides some indication of relatedness between insecticide-residues volatiles based on the chemical nature of volatile principal components present in each residue type. All of the insecticide types identified correctly among the eleven types had a majority proportion of the vapor profile that was assigned to the principal vapor element characteristic of each individual insecticide type.

The range of vapor class distributions attributed to an individual principal vapor element characteristic of each insecticide type ranged from 64.7% in methoxyfenozide (Intrepid) samples to 92.3% in acephate (Orthene) residue samples. Methoxyfenozide residues had a relatively large proportion of secondary vapor elements in common with fipronil (37.5%), diazinon (18.0%), and methyl parathion (16.8%). Consequently, methoxyfenozide residues were determined to have an unknown vapor profile and were not identified. The proportion of secondary vapor elements attributed to vapor classes besides the principal vapor elements ranged from <1% for acephate and spinosad to highs of >25% for fipronil and methoxyfenozide and 50.4% for methyl parathion in common with methoxyfenozide.

The number of principal and secondary vapor elements present in the vapor profiles of individual insecticide residues ranged from four in cyfluthrin to seven in about half of the insecticide residue types with the average number of total vapor elements in common between insecticide residues being approximately 6 to 7 shared vapor elements. Most of the secondary vapor elements in common between insecticide residues contributed to ≤ 11% of the vapor class distribution for most insecticide types with some few exceptions for diazinon (19.8% of vapor secondary elements in common with lindane), fipronil (26.6% and 24.8% of secondary elements in common with methoxyfenozide and spinosad, respectively), lindane (14.2% secondary elements in common with spinosad), and malathion (13.7% and 13.9% of secondary elements in common with lindane and methy parathion).

3.3. Principal component analysis

An analysis of eleven insecticide residues using PCA by pairwise comparisons of headspace volatiles (in all possible combinations) provided greater details of possible chemical relatedness and differences between individual insecticide types based on sensor response patterns (vapor profiles). The results of relatedness between headspace volatiles of insecticide residues from different chemical classes were measured using a statistical algorithm called quality factor (QF) statistical analysis that determines pairwise distances between vapor profiles of insecticide types using Euclidean distance units of measurement. The greater the QF value determined from pairwise comparisons of volatiles, the greater

Table 2. Sensor outputs from the A32S electronic nose sensor array based on conductive polymer analyses of headspace volatiles released from eleven insecticide residues in vitro.

	Sensor number[a]											
Insecticide	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**13**	**14**
Acephate	3.60±0.04	3.17±0.04	3.62±0.04	3.14±0.01	3.15±0.01	3.13±0.01	3.18±0.02	3.31±0.02	2.98±0.03	2.61±0.04	5.62±0.04	5.53±0.04
Carbaryl	4.33±0.04	3.93±0.03	4.45±0.03	2.53±0.01	2.50±0.01	2.53±0.01	4.66±0.01	4.75±0.02	4.06±0.01	3.63±0.01	3.93±0.02	3.65±0.02
Cyfluthrin	4.55±0.04	4.13±0.03	4.66±0.04	3.64±0.01	3.63±0.01	3.61±0.01	4.32±0.02	4.48±0.02	3.58±0.02	2.97±0.01	3.89±0.01	3.80±0.01
Diazinon	4.51±0.01	4.14±0.01	4.71±0.01	3.22±0.02	3.13±0.02	3.22±0.02	4.72±0.01	4.77±0.01	3.93±0.01	3.26±0.03	4.37±0.04	4.35±0.06
Fipronil	4.85±0.02	4.39±0.02	5.03±0.02	2.84±0.01	2.89±0.02	2.85±0.02	5.47±0.03	5.41±0.02	4.47±0.02	4.32±0.03	3.92±0.01	3.62±0.01
Imidacloprid	4.75±0.02	4.30±0.03	4.96±0.02	2.71±0.01	2.69±0.01	2.74±0.01	5.76±0.02	5.53±0.02	4.86±0.02	4.95±0.03	3.78±0.01	3.26±0.01
Lindane	4.35±0.06	4.04±0.05	4.72±0.07	3.35±0.02	3.25±0.02	3.33±0.03	4.93±0.02	4.69±0.01	5.19±0.02	5.05±0.01	5.33±0.10	5.09±0.14
Malathion	4.25±0.04	3.82±0.03	4.37±0.02	2.66±0.01	2.67±0.02	2.64±0.01	4.63±0.03	4.75±0.03	4.29±0.04	3.74±0.02	3.68±0.02	3.47±0.02
Methoxyfenozide	5.16±0.01	4.66±0.01	5.33±0.01	2.71±0.01	2.71±0.01	2.74±0.02	5.59±0.02	5.63±0.01	4.67±0.02	4.35±0.02	3.70±0.02	3.36±0.02
Methyl parathion	5.07±0.02	4.57±0.02	5.27±0.02	2.68±0.02	2.68±0.01	2.67±0.02	5.65±0.01	5.58±0.01	4.65±0.02	4.51±0.03	3.75±0.01	3.34±0.02
Spinosad	4.27±0.03	3.92±0.03	4.63±0.02	2.52±0.01	2.44±0.01	2.47±0.01	5.09±0.00	5.00±0.01	5.45±0.00	5.64±0.03	6.42±0.01	6.43±0.02
	Sensor number[a]											
Insecticide	**15**	**16**	**17**	**18**	**19**	**20**	**24**	**25**	**27**	**28**	**29**	**30**
Acephate	5.31±0.04	5.48±0.04	4.49±0.07	4.73±0.06	4.34±0.06	5.59±0.11	3.58±0.03	3.74±0.03	4.56±0.01	4.85±0.02	4.99±0.02	5.31±0.10
Carbaryl	3.99±0.01	3.75±0.02	5.00±0.02	5.12±0.01	4.80±0.01	4.30±0.06	NR	4.95±0.02	6.28±0.01	6.10±0.01	5.84±0.01	4.93±0.02
Cyfluthrin	3.71±0.01	3.88±0.01	4.37±0.04	4.65±0.03	4.40±0.02	4.38±0.04	NR	4.97±0.02	5.26±0.01	5.45±0.02	5.85±0.02	5.82±0.10
Diazinon	4.22±0.01	4.16±0.04	4.04±0.03	4.25±0.02	4.01±0.03	3.35±0.05	NR	5.25±0.03	5.98±0.01	5.97±0.02	5.95±0.03	4.52±0.05
Fipronil	3.99±0.01	3.82±0.02	5.53±0.03	5.63±0.02	5.33±0.02	4.28±0.07	NR	NR	7.43±0.04	7.12±0.07	6.84±0.08	NR
Imidacloprid	4.06±0.01	3.68±0.01	5.50±0.02	5.54±0.01	5.28±0.01	4.47±0.05	NR	NR	7.67±0.03	7.06±0.05	6.44±0.03	NR
Lindane	5.85±0.09	5.57±0.11	2.78±0.10	3.05±0.09	2.87±0.09	3.73±0.10	3.46±0.04	4.29±0.04	4.27±0.06	4.06±0.03	4.28±0.01	2.49±0.16
Malathion	3.61±0.01	3.59±0.02	4.32±0.06	4.49±0.05	4.20±0.06	4.65±0.07	NR	5.68±0.07	6.17±0.03	6.37±0.04	6.48±0.04	5.48±0.04
Methoxyfenozide	3.83±0.01	3.56±0.02	5.41±0.02	5.51±0.02	5.23±0.01	3.88±0.06	NR	NR	7.59±0.05	7.33±0.08	7.07±0.07	NR
Methyl parathion	3.90±0.01	3.57±0.01	5.29±0.02	5.38±0.03	5.12±0.02	3.92±0.06	NR	NR	7.81±0.06	7.46±0.09	7.14±0.07	NR
Spinosad	6.50±0.03	6.40±0.02	3.61±0.04	3.76±0.04	3.56±0.03	4.28±0.05	NR	NR	6.00±0.01	5.98±0.05	5.66±0.08	NR

[a] Each sensor in the sensor array was coated with a different intrinsically conducting polymer composed of either polypyrrole, polyanaline, or polythiophene derivatives that were modified by proprietary ring-substitutions with different functional groups to impart unique conductive properties (resistance responses to VOCs). All conducting polymers were doped with specific metal ions to improve and modulate polymer conductivity and sensor sensitivity. Values for each sensor are normalized data (transformed from raw data of sensor intensities) expressed as mean ± SD. NR indicates no sensor response was produced or recorded for this insecticide.

Table 3. Distribution of electronic-nose vapor class membership components among eleven insecticide analyte types based on ANN training-algorithm and database validations.

Insecticide	Vapor class distribution (%)[a]										
	Insecticide analytes (abbreviations)[b]										
	Acep	**Carb**	**Cyfl**	**Diaz**	**Fipr**	**Imid**	**Lind**	**Mala**	**Mefo**	**Mpth**	**Spin**
Acephate	**92.3**	4.3	–	0.8	8.9	2.0	2.3	2.5	–	–	–
Carbaryl	2.0	**86.5**	–	7.3	–	8.1	–	2.3	9.9	–	–
Cyfluthrin	–	–	**86.4**	10.1	4.1	–	–	6.8	–	–	–
Diazinon	3.0	10.6	2.3	**90.6**	–	–	19.8	–	8.4	–	–
Fipronil	12.2	–	2.5	–	**87.6**	–	–	–	26.6	–	24.8
Imidacloprid	2.0	6.8	–	–	2.0	**89.3**	–	1.5	2.8	–	3.3
Lindane	1.8	–	–	3.3	–	1.5	**91.3**	1.3	–	9.6	14.2
Malathion	–	5.1	7.1	–	–	4.8	13.7	**87.8**	–	13.9	–
Methoxyfenozide	–	5.7	–	18.0	37.5	5.3	–	–	**64.7**	16.8	8.5
Methyl parathion	–	5.7	1.8	–	–	–	7.7	3.4	50.4	**71.9**	–
Spinosad	–	–	2.0	0.8	10.6	9.1	7.3	–	3.8	–	**87.7**

[a] Mean percent vapor class membership distributions indicated for each insecticide analyte type; values are read from left to right (by row), not top to bottom (by column). Insecticide analyte abbreviations correspond to insecticide types indicated in column 1. Values in bold indicate the major principal component vapor class elements that are representative and unique to each insecticide type.
[b] Insecticide abbreviations: acephate (Acep), carbaryl (Carb), cyfluthrin (Cyfl), diazinon (Diaz), fipronil (Fipr), imidacloprid (Imid), lindane (Lind), malathion (Mala), methoxyfenozide (Mefo), methyl parathion (Mpth), spinosad (Spin).

the difference (or distance) between the vapor signature profiles of the two vapors being compared. In terms of statistical determinations, a QF value of 2.0 is roughly equivalent to a statistical difference at P = 0.10 level of significance. The relatedness among the eleven insecticide types varied greatly based on Euclidean distance as indicated in Table 4. QF values ranged from 0.1 to >70, indicating a very wide range of chemical relatedness between individual insecticides. Among the eleven insecticides compared, a QF of 0.1 indicated a very similar vapor profile and close chemical relationship between diazinon and malathion, both organophosphates, based on PCA. However, other pairwise comparisons also indicated very similar vapor profiles between fipronil and imidacloprid, fipronil and methoxyfenozide, and imidacloprid and methoxyfenozide, even though each of these insecticides within each pair are from different chemical classes. Fipronil and imidacloprid share chlorinated phenyl or pyridyl aromatic groups and structurally-related imidazole or pyrazole groups which could account for the relatedness of vapor signatures. A close vapor profile also was found between methoxyfenozide and methyl parathion insecticides from different chemical classes. Nevertheless, methyl parathion and methoxyfenozide contain phenyl and multiple methyl groups in common within their chemical structure that may contribute to the similarities in vapor signature patterns.

Other PCA data indicated very large differences in vapor signature patterns between carbaryl and fipronil, carbaryl and spinosad, cyfluthrin and fipronil, cyfluthrin and methyl parathion, diazinon and fipronil, diazinon and imidacloprid, imidacloprid and malathion, and malathion and methoxyfenozide, in which the insecticides of each pair were from different chemical classes. By comparison, very large differences were determined between acephate and malathion, both organophosphates, but with quite different chemical structures. Acephate contains an acetamide group, a single sulfanyl group, and a shorter carbon chain compared to malathion.

The relatedness between vapor profiles of eleven pesticide residues based on 3-dimensional PCA, was graphed in the form of an vapor map (Figure 1). The percentages of the total variance for this analysis, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 1 = 75.2%; PC 2 = 23.4%; and PC 3 < 0.5%, representing the x-, y-, and z-axis of the vapor map, respectively. A high proportion (98.6%) of the variation was explained by the first two principal components (PC 1 and PC 2). Almost all of the data points for individual samples of each insecticide residue are closely clustered for most insecticides on the vapor map except for lindane and cyfluthrin. One fairly wide outlying data point for lindane occurred close to acephate from a different chemical class. Cyfluthrin data points had a moderately wide distribution pattern closest to malathion, but significantly unrelated. The close clustering of data points for individual insecticides indicates very good precision between

Table 4. Pairwise-comparisons of relatedness between vapor profiles of eleven insecticide types based on 3-dimensional PCA of headspace volatiles.

Vapor class	Vapor class	QF value[a]	Vapor class	Vapor class	QF value[a]
Acephate	Carbaryl	56.8**	Diazinon	Imidacloprid	>900****
	Cyfluthrin	11.3**		Lindane	17.3**
	Diazinon	72.0**		Malathion	<0.1
	Fipronil	101.6***		Methoxyfenozide	37.3**
	Imidacloprid	126.2***		Methyl parathion	>900****
	Lindane	123.6***		Spinosad	25.4**
	Malathion	>900****	Fipronil	Imidacloprid	<0.1
	Methoxyfenozide	78.4**		Lindane	367.6***
	Methyl parathion	48.2**		Malathion	409.7****
	Spinosad	80.7**		Methoxyfenozide	3.4*
Carbaryl	Cyfluthrin	7.6*		Methyl parathion	<0.1
	Diazinon	13.3**		Spinosad	58.1**
	Fipronil	>900****	Imidacloprid	Lindane	116.0***
	Imidacloprid	186.7***		Malathion	>900****
	Lindane	48.9**		Methoxyfenozide	<0.1
	Malathion	50.4**		Methyl parathion	2.2*
	Methoxyfenozide	95.9**		Spinosad	55.1**
	Methyl parathion	31.0**	Lindane	Malathion	812.5****
	Spinosad	>900****		Methoxyfenozide	71.5**
Cyfluthrin	Diazinon	3.4*		Methyl parathion	151.8***
	Fipronil	>900****		Spinosad	52.4**
	Imidacloprid	202.8***	Malathion	Methoxyfenozide	>900****
	Lindane	10.5**		Methyl parathion	399.4***
	Malathion	33.4**		Spinosad	633.6****
	Methoxyfenozide	233.4***	Methoxyfenozide	Methyl parathion	1.2
	Methyl parathion	>900****		Spinosad	28.1**
	Spinosad	14.1**	Methyl parathion	Spinosad	45.8**
Diazinon	Fipronil	>900****			

[a] Quality factor significant difference levels between vapor classes: * = P < 0.05; ** = P < 0.01; *** = P < 0.001; **** = P < 0.0001. The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 1 = 75.2%; PC 2 = 23.4%. Statistical analyses for pairwise-comparisons were performed for all possible combinations of insecticide types.

e-nose analytical runs which is typical of CP data with good sample analytes and use of the static air sampling method (analyzing headspace that is not diluted by sampling air) rather than dynamic stripping in which the analyte is continuously swept by sampling air that dilutes analyte concentrations as a result of perturbations in carrier-gas mixing with analyte headspace. The high precision of analytical runs also provided higher levels of significant differences in sample (analyte) types. High levels of significant differences between analyte types are not always readily obvious from data point distributions on vapor maps due to 3-dimensional mapping perspectives that are not fully discernible on 2-dimensional (flat) graphs. The differences are more readily observed using analytical software that allows the rotation of 3-dimensional vapor maps to view different perspectives for indications of data-point separations. Thus, statistical values provide a more accurate indication of analyte differences than visual differences observed from a vapor map.

The inability to identify some samples of methoxyfenozide may have been due to chemical decomposition of some sample residues resulting in variations in vapor signatures patterns derived from collective sensor array responses to headspace volatiles. Other possible reasons why methoxyfenozide could not be consistently identified may include insufficient volatiles for analysis, the lack of sufficient principal components in adequate quantities to make up a representative vapor signature profile for this particular insecticide, or the presence of particular VOCs to which the A32S e-nose sensors were not sufficiently sensitive, thus unable to generate a distinctive pattern of sensory outputs. The A32S instrument sensor array generally has detection limits that are below 1 ppm for most strongly polar compounds such as the insecticides tested here. Thus, the concentrations of volatiles released from insecticide residues on crop surfaces usually are well above the detection limits of the instrument even many days after pesticide application. As a consequence, any possible challenges to the capability of the instrument to identify and discriminate insecticide residues are more likely to be due to the occurrence or presence of pesticide mixtures from multiple applications of different pesticides rather than instrument-detection limitations. The presence of plant volatiles should be relative constantly (of one type) due to the common practice of monocultural farming.

Generally, e-noses are set to a level of specificity (run parameters) during neural net training that preclude false positives and result in unknown determinations for samples that cannot be recognized or that have vapor profiles that are missing from the reference vapor library. Diagnostic specificity for discriminations can be improved even further by building e-nose methods and libraries that are specific (application-specific libraries) to particular sample types so that false positive determination are exceedingly rare. Also, the collection of known sample types from the sampling area (crop field) to create vapor reference libraries, representative of the

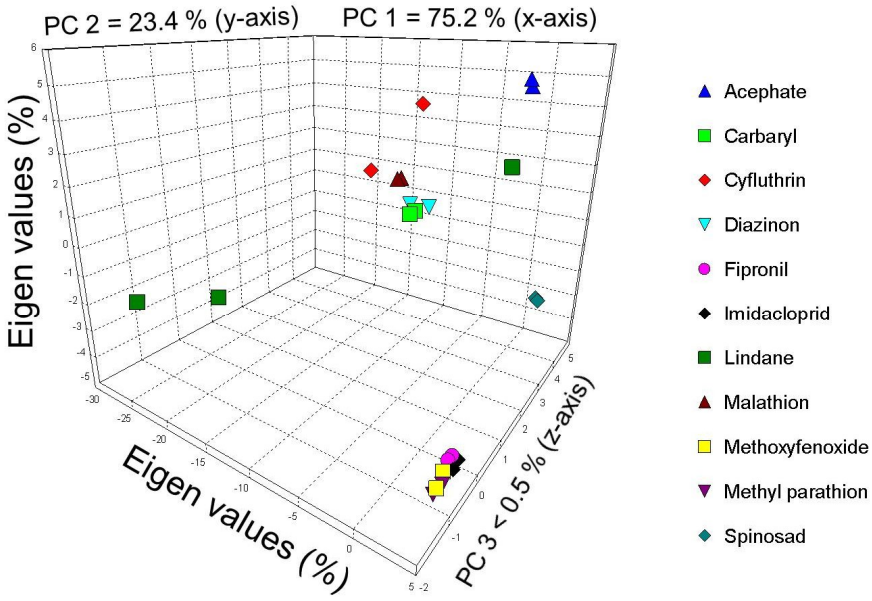

Figure 1. Vapor map showing the chemical relatedness of eleven insecticide residue types using principal component analysis (PCA). The percentages of the total variance, accounting for the variability explained by each orthogonal principal component (PC), are as follows: PC 1 = 75.2%, PC 2 = 23.4%, and PC 3 < 0.5%.

region from which future unknown insecticide residue samples will be collected, reduces the likelihood that geographical variations in individual sample types will not affect determination of insecticide residue sample unknowns. Another way to improve on the e-nose for the detection of a specific chemical group (such as particular pesticide types) is to modify the types of sensors present in the sensor array to include those that are more sensitive and helpful in the identification (discrimination) of chemicals in the particular chemical class of interest. Some e-nose instruments have a large library of sensor types available that can be substituted to improve the overall sensitivity and effectiveness of chemical discriminations by the sensor array. Thus, e-nose instrument sensitivity is probably determined more by the selection of sensors in the array (based on the expected chemical-detection application) rather than the device design (sensors and transducers) of the instrument.

Some limited indications of chemical relatedness of insecticide residue types from the same and different chemical classes were apparent for some pairwise comparisons using PCA. For example, a strong chemical relatedness, based on vapor profiles, was found between the organophosphate insecticides diazinon (Spectracide) and malathion (Malatox), but not between other paired combinations among the four organophosphates tested. The presence of differences in chemical structure and functional groups, among individual insecticides within the organophosphate class, had significant effects on the resulting vapor signature patterns as a result of differential interactions and responses of individual sensors within the sensor array to these molecular differences between different organophosphates. The differential responses of the sensor array to molecular differences also accounted for the low statistical differences between pairs of insecticides from different functional groups. In this case, the apparent chemical relatedness may reflect similarities in largely nonfunctional side (R-) groups rather than the main toxophores that often functionally define insecticide chemical classes.

A few previous studies have indicated the potential feasibility of using e-nose instruments to detect certain types of insecticides. For example, Déjous et al. [19] utilized a surface acoustic wave (SAW) e-nose to detect organophosphate in ambient air. A new experimental e-nose was used more recently to detect organophosphate insecticides on vegetables [32]. Other literature on the e-nose detection of pesticides on fruits, crops, and other plant surfaces are limited [4]. Thus, research on the detection of pesticides using e-nose devices particularly on field crops has just begun.

4. Conclusions

The current study has provided evidence to indicate that the CP A32S e-nose has the capability to discriminate between insecticide residue types (in vitro) from several chemical classes including organophosphates, carbamates, pyrethroids, phenylpyrazoles, neonicotinoids, organochlorines, diacylhydrazines, and spinosyn. Vapor data profiles from e-nose analyses of insecticide residue types resulted in different vapor signature patterns and provided some limited indications of chemical relatedness between insecticide residue types. Thus, e-nose data should theoretically provide a means for discriminating insecticide residue types on plant foliage, once plant volatiles are added to the vapor signatures of insecticide residues taken from crop surfaces. Testing of the e-nose capabilities to detect insecticide residues on crop foliage in the field will be the logical next phase of this study. The capability of identifying insecticide residues on crops will be quite useful for making crop management decisions relating to insect-pest control.

The identification of insecticide residues on foliar surfaces will require the building of application-specific reference libraries of each insecticide on specific types of leaf surfaces (plant or crop species) in order to account for the added plant volatiles that vary with plant species [33]. The reference library must contain all of the possible combinations

of plant species and insecticide types that may be encountered among collected leaf samples (containing insecticide residues) from crop fields to be sampled. The analysis of leaf-insecticide residue combinations not present in the reference database will likely result in unsuccessful (indeterminant) identifications due to the requirement for a precise match of unknown signature patterns with known vapor profiles in the reference library. Ambiguous determinations and false-positive identifications are rare with sample types or combinations not found within the reference database used for analysis [31]. Insecticide residues on leaf surfaces were not analyzed in this study due to the large permutations of possible combinations of headspace volatiles that are possible with multiple crop and insecticide types that are possible in modern crop fields. Nevertheless, the task is easier when application-specific reference databases are created (such as one for each crop species) that includes all of the possible insecticides to be sprayed on each individual crop during the growing season. This method eliminates error and confusion due to cross-species sampling and encountering plant-pesticide volatile combinations not contained in the reference database. However, this method does not eliminate the problem of sampling crops fields with multiple applications of different insecticides in which case reference databases must contain the combination of volatiles from multiple possible combinations of insecticide types in addition to the plant volatiles. More complex sampling of pesticide residues on crops generally requires the use of conventional chemical analysis equipment such as gas chromatographs and mass spectrometers.

Pesticide residue-monitoring methods for insecticides and other pesticides on agricultural products currently are costly, time-consuming, and have limitations based on conventional sampling and analytical technique requirements. Thus, there is a strong demand for the development of quick, simple and reliable methods for the detection and identification of organic-based agricultural insecticide residues on plant surfaces. The advantage of electronic-nose devices over conventional analytical-chemistry instruments, typically used in laboratory chemical analyses, is that e-noses identify the source of headspace volatiles without having to identify individual chemical compounds present in the headspace analyte mixture. Utilization of portable e-nose devices in agricultural crop fields provides a mean to obtaining real-time information of insecticide residues on crops allowing immediate pest-control and crop-management decisions. This study has demonstrated that an ICP e-nose has the capability of identifying and discriminating insecticide residue types based on headspace volatiles released from inert surfaces in vitro.

The time that insecticide residues remain on plant surfaces following application can affect e-nose analysis as a result of chemical vaporization, solar or temperature-induced degradation, leaching or weathering due to dilution by precipitation, or removal in runoff water. Thus, e-nose detection of insecticide residues can be attenuated over time if intense weather conditions remove significant amounts of residues to levels below instrument-detection limits. The maximum duration of residue detection following pesticide application varies not only with weathering conditions, but also the chemical class, volatility or vapor pressure, and initial concentration of the insecticide as well as the sensitivity of the e-nose sensor array to specific analyte residues. Successful insecticide residue detection and identification usually depends on the quality of the sample selected for analysis. Thus, collection of multiple samples from each crop field for analysis provides greater confidence in interpretations of analytical results.

Acknowledgments

The author thanks Charisse Oberle for e-nose instrument operations and analytical runs, constructions of vapor databases, and proofreading the manuscript.

References

1. A. D. Wilson, M. Baietto, Sensors 9 (2009) 5099.
2. S. O. Obare, C. De, W. Guo, T. L. Hay, T. A. Samuels, C. P. Adams, N. O. Masika, D. H. Murray, G. A. Anderson, K. Campbell, K. Fletcher, Sensors 10 (2010) 7018.
3. A. D. Wilson, Proc. Technol. 1 (2012) 453.
4. A. D. Wilson, Sensors 13 (2013) 2295.
5. G. L. Liu, Y. Lin, Anal. Chem. 77 (1995) 5894.
6. G. Palleschi, M. Bernabei, C. Cremisini, N. Mascini, Sens. Actuat. B 7 (1992) 513.
7. D.S. Beaudoin, S.O. Obare, Tetrahedron Lett. 49 (2008) 6054.
8. C. De, T. A. Samuels, T. L. Hay, G. A. Anderson, K. Campbell, K. Fletcher, D. H. Murray, S. O. Obare, Tetrahedron Lett. 51 (2010) 1754.
9. A. L. Jenkins, O. M. Uy, G. M. Murray, Anal. Commun. 34 (1997) 221.
10. A. L. Jenkins, O. M. Uy, G. M. Murray, Anal. Commun. 71 (1999) 373.
11. K. A. Van Houten, D. C. Heath, R. S. Pilato, J. Am. Chem. Soc. 120 (1998) 12359.
12. S. W. Zhang, T. M. Swager, J. Am. Chem. Soc. 125 (2003) 3420.
13. T. J. Dale, J. Rebek, J. Am. Chem. Soc. 128 (2006) 4500.
14. S. Paliwal, M. Wales, T. Good, J. Grimsley, J. Wild, A. Simonian, Anal. Chim. Acta 596 (2007) 9.
15. C. M. Rudzinski, A. M. Young, D. G. Nocera, J. Am. Chem. Soc. 124 (2002) 1723.
16. A. L. Jenkins, R. Yin, J. L. Jensen, Analyst 126 (2001) 798.
17. S. Yamaguchi, L. Yoshimura, T. Kohira, S. Tamaru, I. Hamachi, J. Am. Chem. Soc. 127 (2005) 11835.
18. M. S. Nieuwenhuizen, J. L. N. Harteveld, Sens. Actuat. B 40 (1997) 167.
19. C. Déjous, D. Rebiére, J. Pistré, C. Tiret, R. Planade, Sens. Actuat. B 24 (1995) 58.
20. J. Ngehngwainbi, P. H. Foley, S. S. Kuan, G. G. Guilbault, J. Am. Chem. Soc. 108 (1986) 5444.
21. A. Y. Kolosova, J. H. Park, S. A. Eremin, S. J. Kang, D. Chung, J. Agric. Food. Chem. 51 (2003) 1107.
22. A. Y. Kolosova, J. H. Park, S. A. Eremin, S. J. Park, S. J. Kang, H. S. Lee, D. H. Chung, Anal. Chim. Acta 511 (2004) 323.
23. S. J. Tang, M. Zhang, C. G. Cheng, Y. T. Lu, J. Immuno. Immunochem. 29 (2008) 356.
24. E. K. Lee, T. J. Kim, W. C. Park, T. W. Chung, Y. T. Lee, Anal. Chim. Acta 530 (2005) 143.
25. M. Trojanowicz, Electroanalysis 14 (2002) 1311.
26. Y. Lei, P. Mulchandani, J. Wang, W. Chen, A. Mulchandani, Environ. Sci. Technol. 39 (2005) 8853.
27. K. R. Rogers, C. J. Cao, J. J. Valdes, A. T. Elderfrawi, M. E. Eldefrai, Appl. Toxicol. 16 (1991) 810.
28. W. Lan, G. Chen, F. Cui, F. Tan, R. Liu, M, Yushupujiang, Sensors 12 (2012) 8477.
29. A. D. Wilson, C. S. Oberle, Phytopathology S94 (2004) 110.
30. A. D. Wilson, Phytopathology 102 (2012) S4.136.
31. A. D. Wilson, D. G. Lester, C. S. Oberle, Phytopathology 94 (2004) 419.

32. S. L. Tan, In: Electronics, Robotics, and Automotive Mechanics Conference, Cuernavaca, Mexico (2010) pp. 592-596.
33. A. D. Wilson, D. G. Lester, C. S. Oberle, For. Ecol. Manage. 209 (2005) 207.

Cite this article as:

Alphus Dan Wilson: **Identification of insecticide residues with a conducting-polymer electronic nose.** *Chemical Sensors* 2014, **4:** 3