LIMITS OF VOLATILE CHEMICAL DETECTION OF A PARASITOID WASP, *Microplitis croceipes*, AND AN ELECTRONIC NOSE: A COMPARATIVE STUDY

G. C. Rains, J. K. Tomberlin, M. D'Alessandro, W. J. Lewis

ABSTRACT. *Volatile chemical signals are used by many animals to find food, mates, or hosts. While the keen sense of smell of dogs has been used for centuries, other animals have not been significantly utilized. Recent studies have indicated that many insect species have the ability to learn volatile chemical compounds in association with food or other resources. These insects present a novel approach to volatile chemical detection that could provide a highly sensitive, inexpensive, flexible, and portable sensor. One characteristic of insects that makes them desirable as a potential chemical detector is their ability to detect extremely low levels of chemical compounds. A parasitoid wasp,* Microplitis croceipes, *was used as the model insect for determining the threshold of response for four compounds: 3−octanone, a compound found in many fungal pathogens; myrcene, a volatile constituent released by cotton plants fed on by cotton bollworms; and putriscene and cadaverine, two products of the breakdown of dead animal protein by microorganisms. Eighteen wasps were trained to each of these individual compounds at one dosage and tested at decreasing dosage levels until their responses were negligible. Each dosage was tested with 18 freshly trained wasps. The wasp response to the odor was determined by a searching behavior called antennating. Wasp response was measured by the length of time the wasp antennated when exposed to the odor. The mean wasp response fell below 10 s at approximately 3.1 × 10^{-7}, 2.9 × 10^{-7}, 3.9 × 10^{-6}, and 4.5 × 10^{-7} mol L^{-1} of compound for 3−octanone, myrcene, cadaverine, and putriscene, respectively. For comparative purposes, the detection limits of an electronic nose, the Cyranose 320, was determined for two of the four compounds. The response limits of the wasp for the compounds 3−octanone and myrcene were 74 and 94 times better than the electronic nose, respectively. The response limit of the wasps to putriscene, 3−octanone, and myrcene was approximately 10 times better than to cadaverine.*

Keywords. *Electronic nose, Sensors, Volatile compounds.*

Many animals are known to have a remarkable ability to detect, recognize, and locate target materials based on olfactory, visual, and other cues. However, the capacity of these animals has remained poorly understood and largely unused, other than a long history of using the dog's keen sense of smell for bomb detection and illegal drug discovery as well as for arson and forensics investigations. In addition, the dog's keen olfactory system is being used in non−traditional ways, such as medical diagnoses to recognize cancerous skin melanoma (Pickell et al., 2001).

Emerging information regarding the chemical detection capabilities of insects has revealed the potential for using them as chemical detectors. Although insects have an extremely keen sense of smell, until recently responses to particular volatile chemicals were believed to be innate. Innate responses of insects have been utilized in the development of at least one odor detector using gravid face flies (Raman and Gerhardt, 1997). In this detector, the behavioral responses of six gravid flies in a container were recorded with a microphone.

Lewis and Tumlinson (1988) discovered that a parasitoid wasp, *Microplitis croceipes*, could associatively learn chemical cues from its host and respond to these cues when searching for hosts in varied environments. Female wasps of *M. croceipes* are parasitoids of three highly polyphagous larval hosts, *Heliocoverpa zea*, *Heliothis virescens*, and *Heliothis subflexa* (Lepidoptera: Noctidae). Once a host is found, the wasp oviposits an egg directly into the caterpillar larvae. The wasp larva, after feeding on the caterpillar, emerges and weaves a cocoon. An adult wasp emerges in 7 to 10 days.

This parasitoid has learned to associate specific volatiles with its host and host environment in order to improve its foraging ability in a complex environment (De Moraes et al., 1998; Turlings et al., 1990). As conditions change (e.g., host plant changes from cotton to soybean), the wasp learns to recognize new chemical cues that best indicate a habitat for its host caterpillar and food. Laboratory−reared wasps, allowed to smell a chemical while tasting sugar water or stinging a host, can quickly learn to recognize and respond to

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extremely low concentrations of that compound regardless of its molecular structure (Takasu and Lewis, 1993, 1996; Wäckers and Lewis, 1993; Olson et al., 2003). Because of the wasp's keen sense of smell, the potential for developing volatile chemical detection devices that are highly sensitive, portable, cheap to reproduce, and easy to use is immense. In agriculture, a sensor to detect chemicals that indicate a specific crop disease could be used to determine the spatial and temporal distribution of that disease within a field. Other applications are numerous, including drug discovery, forensics investigations, and land−mine detection.

Dogs trained to detect explosives can respond to chemical levels in the low ppb (Williams et al., 1998). If insects have the same discriminatory ability and sensitivity as dogs, they may be used as an alternative in specific situations that require quick training and a quick response. However, portable electronic nose devices are becoming increasingly available and may provide the discrimination necessary to detect low levels of volatile chemicals. One such electronic nose is the Cyranose 320 (Cyrano Sciences, 2000). This nose is portable, relatively low−cost, and includes some of the latest technology available in electronic nose detection. The sensitivity of electronic nose technology compared to a biological sensor such as the wasp would help determine application limitations of each. The objectives of this study were:

- Determine the threshold level of sensitivity for the wasp to four chemicals that hold promise for applications in forensic investigations and agriculture.
- Compare the sensitivity of the Cyranose 320 electronic nose and trained wasps for two volatile chemical compounds that have agricultural applications, one released by damaged plants (myrcene) and the other a constituent of several fungal pathogens (3−octanone).

Methods and Materials

The Cyranose 320 electronic nose and the parasitoid wasp *M. croceipes* were both trained to detect volatile chemicals and tested to determine their limits of detection for those chemicals. Two volatile compounds, myrcene and 3−octanone, were used to compare the electronic nose's sensitivity to that of the wasps. Myrcene has been found to be part of the volatile chemical mixture released by cotton plants when stressed by bollworm feeding (Turlings et al., 1995; Paré and Tumlinson, 1997). The common fungal volatile 3−octanone has been discovered in the fungus *Sclerotium rolfsii* (Cardoza et al., 2003), a plant fungal pathogen of over 200 plant species (Aycock, 1966), and on *Fusarium sporotrichoides*, a fungus that produces toxins on grains (Schnürer et al., 1999). Further study of the wasp's sensitivity was analyzed by training and testing with two additional chemicals, putriscene and cadaverine. These two chemicals are products of protein breakdown by microorganisms. They can indicate the presence or past presence of dead animal organic material and could therefore be used for location of bodies in forensic investigations.

Wasp Training and Testing

Compounds Examined

Responses of wasps to seven concentrations of either 3−octanone (Aldrich Chemical Company, Inc., Milwaukee, Wisc.), cadaverine (Aldrich Chemical Company, Inc., Milwaukee, Wisc.), or putrescine (Sigma, St. Louis, Mo.) ranging from 10 ng to 10 mg per 20 µL dichloromethane and six concentrations of myrcene (Aldrich Chemical Company, Inc., Milwaukee, Wisc.) ranging from 100 ng to 10 mg per 20 µL dichloromethane were recorded. The control for each compound being examined was 20 µL dichloromethane alone.

Insects

The trained organism used in this comparison is the parasitoid wasp *Microplitis croceipes*, a beneficial insect that is approximately 10 to 12 mm long (fig. 1). Adult wasps live approximately 2 weeks.

In this study, the antennating behavior was used as the behavioral response indicating the presence of the target odor. A wasp rubbing its antennae on the surface and turning its body in circles over the odor source characterizes the antennating behavior. Therefore, wasp testing involves three steps: (1) placing the wasp over the odor with forceps, (2) recording the length of time the wasp exhibits the antennating behavior, and (3) removing the wasp from the odor source.

M. croceipes were reared on *H. zea* larvae using methods described by Lewis and Burton (1970). Wasp colonies were maintained at 28˚C and 60% to 70% RH with 16:8 (L:D) h. *Helicoverpa zea* larvae reared on a pinto bean artificial diet were provided as hosts for *M. croceipes*. Emergent females were not provided honey for 2 days and were then used in the experiments.

Conditioning and Testing

Eighteen wasps (3 per day for 6 days) were conditioned and tested to each concentration of the compounds. Individual wasps were conditioned to a 1 mg per 20 µL dichloromethane concentration placed on a 2.5 cm diameter Whatman No. 1001325 filter paper (Fisher Scientific, Norcross, Ga.).

Prior to conditioning the wasps, the treated filter paper was placed on a petri dish under a ventilation hood for 1 min to allow the solvent to evaporate. Following evaporation, the filter paper and an 8 mm Fisherbrand magnetic octagonal bar (Fisher Scientific, Norcross, Ga.) were placed in a 250 mL glass jar. The jar was immediately covered with an 8 × 8 cm sheet of aluminum foil, sealed with a screw lid, and placed on a Corning magnetic stirrer (Fisher Scientific, Norcross, Ga.), as shown in figure 2. The stirrer was set at a rotation rate

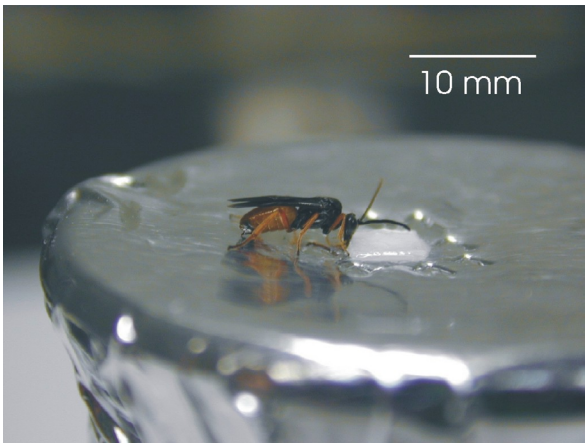

Figure 1. *Microplitis croceipes* feeding on sugar water.

of 770 rpm for 5 min to distribute the volatile inside the jar, after which seven holes (each approximately 1 mm diameter, separated by approximately 2 mm) were placed in a circle near the center of the foil. A droplet (<0.5 mL) of 33% sucrose solution was placed in the center of the ringlet of holes as a food resource for the wasps (fig. 2). Wasps were placed individually and sequentially on the aluminum foil and allowed to feed for 10 s. During this time, the wasps were exposed to the odor diffusing through the holes. Each wasp had three 10 s conditioning periods with approximately 3 min between each session. After the final training session, the wasps were held individually in 5 mL glass vials for 15 min before testing.

The design described above, but excluding the sugar water, was used to test the behavioral response of individual wasps to a concentration of the compound (target) used to condition them. The amount of time the wasps remained within a 1 cm radius of the holes searching (i.e., antennation and circular rotation of the body) was recorded. Total time searching and percentage of wasps examined to respond for $\geq$ 10 s were recorded. Wasps that responded for < 10 s were recorded as a negative response. All glassware was cleaned with distilled water, acetone, and hexane, respectively, prior to being dried for 24 h in an Econotherm oven (model 1025−155, Precision−NAPCO, Winchester, Va.) set at 225˚C.

Concentration of material was calculated in moles per liter (molarity). Vapor pressure of the solvent dichloromethane was 350 mm Hg, and the vapor pressure of myrcene and 3−octanone were 2.01 and 1.50 mm Hg, respectively. Consequently, the solvent evaporation at atmospheric pressure was extremely fast compared to the tested compounds. In addition, conditioned wasps, tested to the solvent, showed no behavioral response, indicating that the solvent was absent the conditioning regime. Therefore, it was reasonable to assume that the solvent was completely evaporated before placing the filter paper in the jar. However, it is not possible to know the precise amount of tested compound that had already evaporated before placing the filter paper in the jar. Assuming that all the material tested on the filter paper diffuses into the air within the jar, the concentration is calculated by:

$$C = \frac{D}{MW \times V} \quad (1)$$

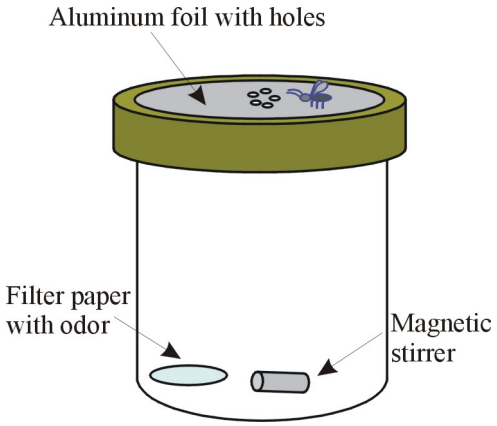

Figure 2. Jar used for training and testing Cyranose 320 and wasps.

where

C = theoretical concentration of chemical in jar (mol L^{-1})
D = dosage in jar (g)
MW = molecular weight of chemical (g mol^{-1})
V = volume of air in jar (0.250 L).

The concentration calculated in equation 1 indicates the highest concentration possible in the jar at the time of the test. The actual concentration (molarity) will be some percentage of C. However, the percentage of C should be consistent between comparisons of the wasp and electronic nose.

Statistical Analysis

Fisher's LSD test was used following an ANOVA ($P < 0.05$) to separate mean responses to each dose (SAS, 2001). A chi−squared test ($P < 0.05$) was used to determine if the percent responses at each concentration were significantly different.

Cyranose 320 Electronic Nose

The Cyranose 320 (fig. 3) is a portable electronic nose that employs 32 chemiresistor polymers for sensing volatile vapors (Cyrano Sciences, 2000). The device comes complete with data pre−processing algorithms, model development algorithms, and cross−validation procedures for training the nose to detect volatile compounds. Each chemiresistor sensor has a unique chemical composition that changes resistance as it absorbs volatile chemicals. The amount of absorption depends on the chemical vapor properties and the chemiresistor's affinity to the vapor. When a sample is "sniffed" by the Cyranose 320, the maximum change in resistance of each chemiresistor is stored and used to identify the sample by comparison to chemical classes that the Cyranose 320 has been trained to recognize. If the chemical does not fit any of the trained classes, it is given an "unknown" classification.

Figure 4 shows a strip chart recording of the response of six chemiresistor sensors to myrcene sampled by the Cyranose 320. There are three steps in sampling a volatile chemical: (1) baseline purge, (2) sample intake, and (3) sam−

Figure 3. Cyranose 320 handheld electronic nose.

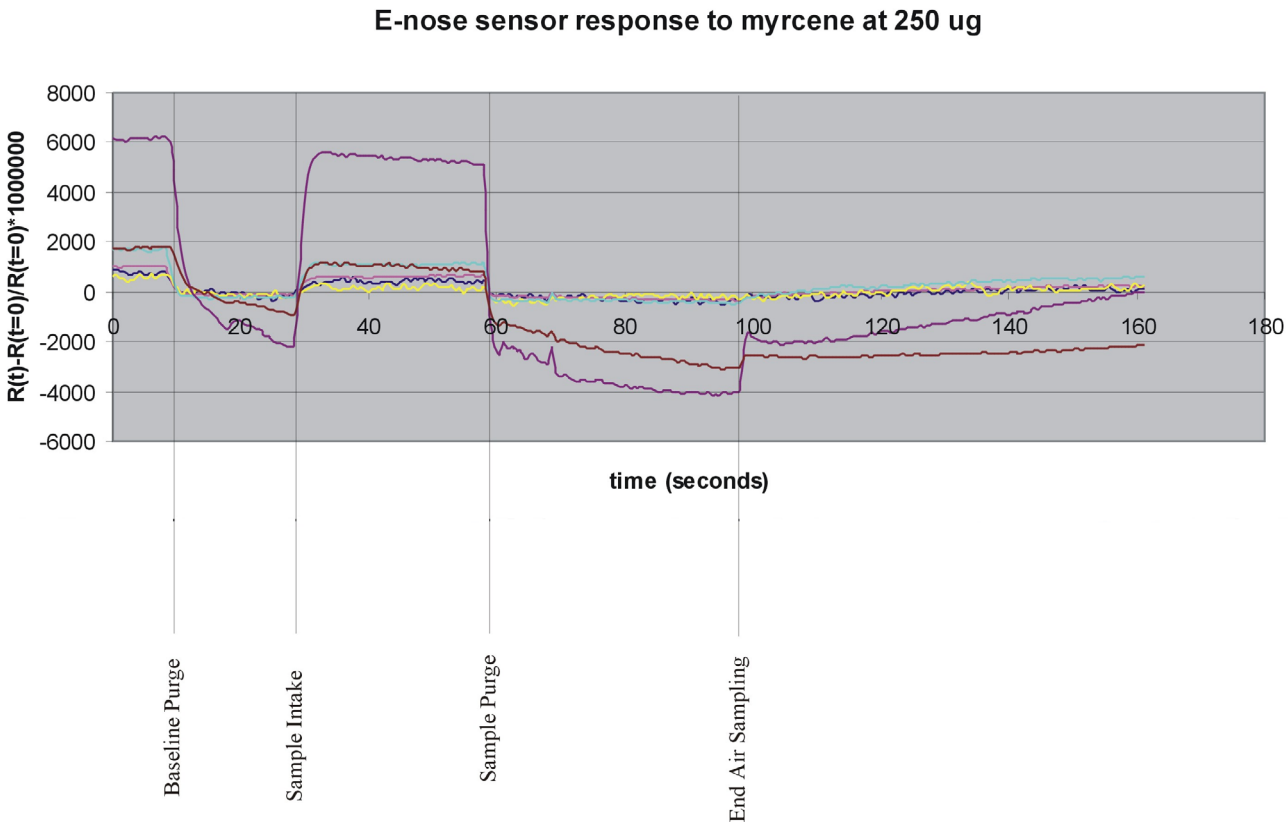

Figure 4. Strip chart recording of Cyranose 320 response to myrcene.

ple purge. The first step (baseline purge) purges the system with clean ambient air so that a baseline value for the sensor resistance can be obtained. The next step (sample intake) pulls an air sample into the Cyranose 320 and over the chemiresistors for analysis. During this period, the sensors are changing resistance in response to the volatile chemical sampled. After the sample intake has been completed, the third step is to purge the sensor chamber to allow the chemiresistors to desorb the volatile chemicals and return to a baseline condition. An internal pump and valve system controls the plumbing of the Cyranose 320.

Electronic Nose Training and Method Development

A "method" is the setting and training data set of the Cyranose 320 used to train and detect volatile chemical compounds. Once the method is set, it cannot be changed without re−training the Cyranose 320. A "class" is an individual volatile compound within a method that the electronic nose is trained to detect.

Method development includes selecting the pump speed; the baseline purge, sample intake, and sample purge times; the sensor temperature; and the pre−processing and pattern recognition (PARC) algorithms. The following parameters were used to establish a method for detecting myrcene and 3−octanone:

- Substrate temperature of the chemiresistors was set 7˚C higher than the ambient temperature expected during operation. The temperature difference helped to stabilize the substrate temperature of the chemiresistors. In this study, substrate temperature was set to 35˚C.
- Pump speed was selected to provide the optimum odor source over the chemiresistors. For headspace applications, pump speed was set low to conserve the potentially small amount of odor to sample. For applications with an infinite source of sample, a high setting was recommended. In this study, sample speed was selected to be low for headspace collection.
- The time to purge and sample an odor was dependent on the amount of odor available for the sample, the time it took to reach a steady−state change in the chemiresistors, and the time required to purge (desorb) the odor from the chemiresistors. Repeated testing of the Cyranose 320 with samples of myrcene and 3−octanone provided the parameters shown in table 1 for the air pump speeds and purge and sample times indicated.
- The lowest concentration of volatile chemicals that the Cyranose 320 would be detecting was usually used for training. In this study, strip chart recordings of the resistance changes of the chemiresistors to several chemical concentrations were used to determine the lowest concentration at which the Cyranose 320 could reliably obtain a steady−state change in the chemiresistors. The lowest reliable response was obtained at 1.0 mg of 3−octanone and myrcene.
- Digital filtering was activated and the data were normalized with the normalization−1 routine provided with the Cyranose software. Digital filtering was recommended in the user's manual unless the raw data were to be analyzed by the user's own algorithms. There were two normalization routines included, but no explanation of the difference between the two rou−

Table 1. Methods settings for identifying different doses of 3−octanone and myrcene with the Cyranose 320.

Flow Settings		
	Time (s)	Pump Speed
Baseline purge	15	Low
Sample draw	30	Low
Sample draw 2	0	Low
Snout removal	0	
1st sample gas purge	0	High
1st air intake purge	10	High
2nd sample gas purge	30	High
2nd air intake purge	0	High
Digital filtering	On	
Substrate heater	On	35°C
Training repeat count	1	
Identifying repeat count	1	
Data Processing		
Active sensors	All 32	
Algorithm	KNN	
Preprocessing	Autoscaling	
Normalization	Normalization−1	
Identification quality	Lower	

tines was given. Changes in normalization did not appear to change the outcome of the training, so normalization−1 was used. Autoscaling was used to standardize the sample response. Autoscaling subtracted the sample mean from each chemiresistor response and divided by the sample standard deviation.

Once parameters were set for the Cyranose 320, it was trained to discriminate between two classes of odors, 3−octanone and myrcene. The Cyranose 320 was trained to a 1 mg per 20 μL dichloromethane concentration of each compound placed on a 2.5 cm diameter Whatman No. 1001325 filter paper (Fisher Scientific, Norcross, Ga.) (1 mg was the lowest concentration on the filter paper for which the Cyranose 320 could be trained). Prior to training, the treated filter paper was placed on a petri dish under a ventilation hood for 1 min to allow the solvent to evaporate. Following evaporation, the filter paper and an 8 mm Fisherbrand magnetic octagonal bar (Fisher Scientific, Norcross, Ga.) were placed in a 250 mL glass jar. The jar was immediately covered with an 8 × 8 cm sheet of aluminum foil, sealed with a screw lid, and placed on a Corning magnetic stirrer (Fisher Scientific, Norcross, Ga.). The stirrer was set at a rotation rate of 770 rpm for 5 min to distribute the volatile inside the jar. A training sample of the chemical was acquired by piercing the aluminum foil, placing the Cyranose 320 stainless steel snout into the jar, and then sampling the air. The Cyranose 320 was trained ten times to 1 mg of each compound.

The 20 trainings (ten to myrcene and ten to 3−octanone) were initially viewed as a principal component analysis (PCA) 3−D plot. The PCA reduced the 32−sensor response vector into a vector that could be plotted in 3−D space. The PCA plot was used to visually ascertain if the volatile chemicals were clearly distinguishable and helped to determine if there were outliers in the training set that need to be addressed. The two chemicals appeared to be clearly separated in the PCA plot.

A PARC (pattern recognition) algorithm was used to create a model to identify 3−octanone and myrcene and to cross−validate the training data set. There were two statistically based PARC algorithms to select from in the Cyranose 320. One was based on a cluster analysis (CA) algorithm, K−nearest neighbor (KNN), and the other was a canonical discriminant analysis (CDA) algorithm. While the Cyranose manual gave some advice on choosing algorithms, the choice was basically determined by which algorithm provided the best cross−validation of the training data set. The KNN PARC algorithm provided the best cross−validation with the training data set and was chosen for chemical identification. KNN is an algorithm in which an unknown is classified according to the majority vote of its nearest neighbors in the training set. Cross−validation involves removing one data point from the training set, re−calculating the model algorithm, and then using the model algorithm to determine if it can correctly identify the data point that was removed. This process is repeated for all data points in the training set. The Cyranose manual recommended accepting a model only when it accurately classified each data point during cross−validation.

The final parameter to select for the method was the identification quality. The Cyranose 320 could be set to choose an odor only when it was highly likely that the odor was the correct odor (higher setting). It would otherwise identify the chemical as unknown. To always choose one of the odors in the method, the Cyranose 320 could also be set to "always choose." There were also two gradations between those settings. The four selections to choose from were higher, medium, lower, and always choose. As the setting was lowered, the probability of choosing an odor incorrectly increased. The "lower" setting was chosen since there were only two chemicals to discriminate and chances of false identification were small due to the controlled setting of the training and testing. Table 1 shows the method settings for detecting the two classes (myrcene and 3−octanone).

Electronic Nose Identification and Testing

The Cyranose 320 was tested to determine its detection limits to the chemical compounds 3−octanone and myrcene. The electronic nose was tested using the same methodology for training. Six samples were taken for each concentration. The first concentration tested was 1 mg of compound on filter paper. The concentration was reduced by 0.25 mg increments until reaching 0.25 mg for each subsequent test. The Cyranose was tested six times at each dosage. The classification of the odor (3−octanone, myrcene, unknown) and rating (number of stars) were recorded. The Cyranose response to the control (dichloromethane) was also tested.

RESULTS

WASP DOSAGE RESPONSE

Tables 2 and 3 illustrate the time and percent response of 18 wasps conditioned to 3−octanone, myrcene, putriscene, and cadaverine. Mean wasp response time ranged from 70 to 0.4 s for all the concentrations and compounds. Using 10 s as the necessary response time to obtain a positive indication of the chemical compound, the wasps were most sensitive to 3−octanone and least sensitive to myrcene and cadaverine. One issue of interest was the response to 3−octanone at the different dosages. Wasps responded more vigorously (higher mean response times) at dosages below the training dosage (1 mg) until reaching 100 ng of chemical. For all four compounds, the mean response time at the 10 mg dosage was lower than the mean response time at the conditioning dosage.

Table 2. Mean response times and significance levels for wasps conditioned and tested to four individual compounds and ANOVA test results.

	Mean Response Time (s) (n = 18)[a]			
	Cadaverine	Putriscene	Myrcene	3−Octanone
ANOVA Pr > F	<0.0001	<0.0001	<0.0001	<0.0001
Control	0.7 d	0.0 e	3.0 d	1.0 c
10 ng	4.8 d	0.4 e	−−	7.3 c
100 ng	7.3 dc	5.4 de	4.1 cd	11.7 cb
1 µg	5.3 d	14.7 dc	6.0 cd	27.7 b
10 mg	18.5 c	18.6 c	12.9 cb	68.7 a
0.1 mg	35.4 b	35.3 b	33.2 a	52.1 a
1 mg[b]	57.4 a	69.7 a	42.6 a	19.7 cb
10 mg	11.8 dc	6.9 dce	16.3 b	4.2 c

[a] Values in columns followed by common letters are not significantly different by Fisher's LSD test, P ≤ 0.05.
[b] Training dosage.

Table 3. Percent of wasps responding for more than 10 s to four individual compounds and chi−squared test results.

	% of Wasps Responding ≥10 s[a]			
	Cadaverine	Putriscene	Myrcene	3−Octanone
Chi−squared Pr > F	<0.0001	<0.0001	<0.0001	<0.0001
Control	6	0	0	0
10 ng	11	0	−−	33
100 ng	22	17	17	83
1 µg	17	39	0	83
10 µg	44	67	17	100
0.1 mg	89	94	100	100
1 mg[b]	94	100	100	83
10 mg	28	17	100	50

[a] $x/n \times 100$, where x = number responding, and n = 18.
[b] Training dosage.

Electronic Nose Dosage Response

Initially, 2 mg and 1 mg were tested by the Cyranose 320. The Cyranose provided very good responses at those levels. Then the dosage was reduced by an order of magnitude to 100 µg. The Cyranose 320 responded to the 100 µg dosage with an unknown identification. Then the dosage response test was changed to 2, 1, 0.75, 0.5, and 0.25 mg per dosage. Table 4 shows the identification output of the Cyranose 320 for the test dosages. Five stars was the highest quality rating and indicated the highest confidence in detecting the odor accurately. One star was the lowest quality rating and represented the lowest level of confidence when detecting the odor. As can be seen, the quality of the detection quickly degraded at about 0.5 mg for 3−octanone and 0.75 mg for myrcene. Unknown classifications indicated that the odor did not fit any of the classes for that method. When both myrcene and 3−octanone were identified, as was the case with the 0.25 mg dosage of 3−octanone in sample 2, the Cyranose 320 identified the sample as a member of both classes. The thresholds of response appeared to be approximately 0.75 mg and 1.0 mg for myrcene and 3−octanone, respectively.

Table 4. Sensor response to different doses of 3−octanone, myrcene, and control (pure solvent). The number of stars corresponds to the E−nose's indication of a high certainty (*****) to a low certainty (*) that the compound was determined correctly; "unknown" indicates that the measurement did not fit either of the two chemicals in its method. Responses are shown for three samples tested with the E−nose.

Chemical	Dose (mg)	Sample	Sensor Response
3−octanone	2	1,2,3	*****, *****, *****
		4,5,6	*****, *****, *****
	1	1,2,3	*****, *****, *****
		4,5,6	*****, *****, *****
	0.75	1,2,3	*****, *****, *****
		4,5,6	*****, *****, *****
	0.5	1,2,3	*****, ***, * (myrcene)
		4,5,6	Unknown, unknown, unknown
	0.25	1,2,3	*, unknown, 3−octanone/myrcene?
		4,5,6	Unknown, 3−octanone/myrcene?, *
Myrcene	2	1,2,3	*****, *****, *****
		4,5,6	*****, *****, *****
	1	1,2,3	*****, *****, *****
		4,5,6	*****, *****, *****
	0.75	1,2,3	***, * , unknown
		4,5,6	Unknown, *****, ***
	0.5	1,2,3	***, ***, unknown
		4,5,6	Unknown, ***, unknown
	0.25	1,2,3	Unknown, unknown, *
		4,5,6	Unknown, ***, unknown
Pure CH_2Cl_2	100 µL	1,2,3	Unknown, unknown, unknown
		4,5,6	Unknown, unknown, unknown

Using equation 1, the detection limits of the Cyranose 320 for 3−octanone and myrcene were 2.3×10^{-5} and 2.9×10^{-5} mol L^{-1}, respectively. Since the chemical on the filter paper did not entirely evaporate into the jar, the actual detection limits were probably lower but could not be determined without quantitative testing of the volatile compounds using GC/MS (gas chromatography/mass spectrometry).

Wasps Versus Electronic Nose

Using equation 1, concentration levels of each dosage within the 250 mL jar were calculated. The wasp detection threshold was determined to be the smallest dosage at which the wasp's mean response time was ≥ 10 s. The Cyranose 320 detection threshold was the smallest dosage at which at least three of the six tests indicated the correct compound with 5−star certainty. The resulting thresholds indicated that the trained wasps were 74 and 94 times more sensitive than the Cyranose 320 to 3−octanone and myrcene, respectively (table 5). The wasps also showed some differential threshold

Table 5. Limits of detection for wasp and Cyranose 320.

Compound	Molecular Weight (g)	Wasp Detection Threshold (mol L^{-1})	As a Fraction of 3−Octanone Threshold for Wasp	Cyranose 320 Detection Threshold (mol L^{-1})	As a Fraction of 3−Octanone Threshold for Wasp
3−octanone	128.21	3.1×10^{-7}	1	2.3×10^{-5}	74.1
Myrcene	136.236	2.9×10^{-7}	0.94	2.9×10^{-5}	93.5
Cadaverine	88.15	3.9×10^{-6}	12.6	[a]	−−
Putriscene	102.179	4.5×10^{-7}	1.45	[a]	−−

[a] Not tested.

levels within the compounds tested. The wasps were approximately 10 times more sensitive to putriscene, 3−octanone, and myrcene than to cadaverine.

Conclusions and Discussion

The Cyranose 320 electronic nose and conditioned parasitoid wasps were tested to determine and compare their limits of response. A method was developed to optimize the detection by the electronic nose. The wasp conditioning and testing methods were developed based on previous studies on learning and conditioning protocols.

When comparing the response thresholds for the two sensors, it was apparent that for the compounds tested, the trained wasps had a much lower response threshold. Response limits of the trained wasps were 74 and 94 times lower than those of the Cyranose 320 for 3−octanone and myrcene, respectively, each a volatile chemical compound associated with pests in cotton and peanuts.

The wasps had similar detection limits for three of the four chemical compounds tested. Different detection thresholds for the wasp to specific chemicals could be the result of the abundance of, or lack of, olfactory receptors sensitive to specific chemicals. Further investigation of the response to specific chemicals at the receptor neurons and/or antennal lobe would be required to ascertain this possibility. Better discrimination and lower threshold levels might have been measured if a better "upstream" behavioral response had been detectable. A human observer looking for antennation may not have noticed subtle behavioral changes that occurred at lower thresholds. Current testing of an electronic device to measure behavioral responses without relying on human interpretation is underway.

Statistically, it would be difficult to discriminate between higher and lower dosages based on wasp response since many mean response times were statistically the same over a wide dosage range. For example, when studying the response to cadaverine, the mean response time to the control was statistically the same as the response to 10 ng, 100 ng, 1 ug and 10 mg. Therefore, it would have been difficult to estimate concentration based on response time. The same difficulty was apparent when examining the percent of wasps that responded for greater than 10 s (table 3). However, if detection of the chemical were all that was required, then wasps would work extremely well. It became apparent as well that the training dosage might have been critical to eliciting a response that was statistically different from the control response.

While it is apparent that a wasp is very sensitive to volatile chemicals, being a biological organism, it requires more maintenance than an electronic device such as the Cyranose 320. The wasp's physiological state must be maintained to a certain level of hunger when training it to food−associated odors. Furthermore, it lives only about two weeks as an adult. On the other hand, wasps are easy and inexpensive to rear in the hundreds, are trained in less than 10 min, and are very easy to work with. It is expected that the sensitivities of wasps and other trained invertebrates may prove to be useful instruments to detecting odors in the medical, forensic, illegal drug, and environmental areas as well as for detection of plant stress through volatiles emitted by plants or more directly emitted by insects and plant pathogens. As mentioned above, developments are underway to refine a portable volatile detection unit using confined conditioned wasps to detect chemicals identified with specific applications (Rains et al., 2000).

The Cyranose 320 also proved to be very easy to operate and train and provided a substantial software package of analytical tools for developing methods for detecting volatile chemicals. Although the detection limits were not as good as those of the trained wasp, the Cyranose was very reliable up to that limit of detection and could be used for accurately detecting odors when properly trained. Further testing of the Cyranose will focus on detecting plant odors and developing a healthy and stressed plant training data set for various crops.

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