Crop Protection 27 (2008) 1295–1302

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Tracking the sex pheromone of codling moth against a background of host volatiles with an electronic nose

R. Martín Negri, D.L. Bernik *

Instituto de Química Física de Materiales, Ambiente y Energía (INQUIMAE), Departamento de Química Inorgánica, Analítica y Química Física (DQIAQF), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2. (C1428EHA), Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 29 November 2007
Accepted 29 February 2008

Keywords:
Mating disruption
Electronic noses
Codling moth
Pest control
Sensors
Apple
Pheromone

ABSTRACT

Mating disruption is now widely used in apple growing regions where control of codling moth is difficult due to insecticide resistance. This strategy comprises the slow release of pheromone from controlled delivery dispensers placed on the crop field with the aim to attract and/or confuse males impeding mating. The success of the technique strongly depends on maintaining an adequate pheromone concentration in the air within the crop. At present, monitoring of the sexual confusion efficiency is done by hand using lures and traps and carrying out a weekly evaluation of the trapped insects, which involves human resources and consumes considerable time and effort.

This article describes the use of an electronic nose capable of detecting and discriminating low amounts of codling moth pheromone (codlemone) immersed in a background odour of plants and fruit volatiles. Laboratory results obtained indicate the feasibility of using a trained electronic nose for tracking in real-time aerial concentration levels of codlemone against a background of common apple host volatiles. Continuous field monitoring can potentially be achieved by combining the electronic nose with automated robotic navigation and remote transference of data.

1. Introduction

This study is focused on the pheromone used for mating disruption treatment of codling moth *Cydia pomonella* (Carpocapsa), one of the most damaging pest insects in apple orchards worldwide (Dorn et al., 1999). Briefly, female codling moths release a sex pheromone (codlemone: E8, E10-dodecadienol; DeSimone et al., 1997) enabling male moths to locate them by following the plume. When the orchard environment is permeated with an adequate vapour concentration of synthetic codlemone delivered from controlled release dispensers hung in tree's canopies male moths are unable to detect individual pheromone trails, resulting in mating disruption (Barnes et al., 1992). The use of this technique has been progressing as complimentary pest control treatments in orchards and crop fields (Judd and Gardiner, 2005; Cork et al., 2008).

A crucial point for a successful mating disruption pest control is to obtain and keep an adequate atmospheric pheromone concentration throughout the crop environment. At first glance, the net amount of pheromone released per unit time can be estimated from the number of dispensers placed in the orchard and the kinetic of pheromone release from each dispenser. However, release kinetic pulses not always follow a zero order, i.e. the amount of pheromone released with time is not constant. In addition, pheromone concentrations are changed by meteorological conditions and should be closely monitored particularly at the field borders, where lower aerial concentrations and less homogenous dispersal of synthetic pheromone are to be expected (Pfeiffer et al., 1993). In recent years, fruit growers and crop consultants have expressed concerns about the seasonal release performance of commercial codling moth mating disruption dispenser products (Tomaszewska et al., 2005). Therefore, a fast and reliable methodology to detect changes in pheromone concentrations in the air would be desirable so that management decisions such as reinforcing with more dispensers and/or spraying prophylactic pesticide doses can be made rapidly.

At present, aerial pheromone concentrations are indirectly evaluated by checking moth catches in traps with conventional monitoring lures. Occasionally, pheromones are detected by field-electroantennography (EAG) and air sampling techniques. The EAG method was a very important development in the study of insects' electrophysiology and also served (as we will recall later) as sensor in robotic navigation. However, several authors reported that predictive value of electrophysiological screening is limited because receptor cells in antennae are not exclusively sensitive to the sex pheromone (Park et al., 2002; Yujie and Xiao Xi, 2004).

* Corresponding author. Tel.: +54 11 4576 3358; fax: +54 11 45763 3341.
E-mail address: dbernik@qi.fcen.uba.ar (D.L. Bernik).

0261-2194/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cropro.2008.02.008

Table 1
Empirical formula, molar mass, density and vapour pressure of the compounds used in this work

Compound	Empirical formula	Mr (g/mol)	Density (g/cm^3)	Vapour pressure (mmHg at 25 °C)
n-Hexanol	$C_6H_{14}O$	102,18	0.816	0.947
n-Hexylaldehyde	$C_6H_{12}O$	100,16	0.801	10.9
n-Hexyl acetate	$C_8H_{16}O_2$	144,22	0.878	1.39
n-Butyl acetate	$C_6H_{12}O_2$	116,16	0.886	11.5
(E,E)-8,10-dodecadienol	$C_{12}H_{22}O$	182,31	0.862	8.86×10^{-4}

Data obtained from ChemSpider chemical database and the United States National Library of Medicine (NIH).

An evolution of the EAG is the single sensillum recording technique (SSR) which has been reported as a more sensitive and specific method of detecting individual pheromone components (Van der Pers and Minks, 1993; Schöning et al., 2000). Nevertheless, both EAG and SSR techniques rely on obtaining of fresh biological tissue from each moth species and the assembling of a portable device, which makes these methodologies difficult to apply for periods of more than an hour without renewal of the biomaterial and also need the supervision of trained technicians for the evaluation of the experimental data obtained.

Electronic noses are devices that comprise an array of non-specific gas chemical sensors coupled with multivariate data analysis methods used for discriminating multicomponent systems through pattern recognition. The sensor's array generates a set of electrical signals when exposed to an odour, which constitutes a pattern (fingerprint) associated with the odour. Therefore, the aim is to discriminate between odours by comparison of the respective fingerprints. When trained to recognize a particular odour, the e-nose can be remotely run in an automated way with the additional advantage of remote transfer of data (Purenne et al., 2007; Pan and Yang, 2007).

In this work aimed to show the feasibility of the use of an electronic nose to monitor low codlemone levels in the air of apple orchards with the presence of other potential masking agents.

2. Materials and methods

2.1. Materials

n-Butyl acetate, *n*-hexyl acetate, *n*-hexylaldehyde (hexanal) and *n*-hexanol were purchased from Sigma–Aldrich Argentina. All solvents were of analytical grade and used as received. Pure codling moth pheromone (codlemone: E8, E10-dodecadienol) was provided by Basf-Argentina. Table 1 shows the physical properties relevant to the present work for these compounds.

2.2. Preparation of samples

Two types of experiment were performed in this work: (1) experiments in closed vials where concentrations can be calculated and (2) experiments in open air conditions, for which concentrations could only be estimated.

The experiments (Figs. 1 and 2) were done in closed 12 cm^3 vials by injecting 1 μl of each volatile separately through the Teflon septa. The aliquots were completely evaporated before measurement. The final aerial concentration were 6.4×10^{-4} M for butyl acetate, 5.1×10^{-4} M for hexyl acetate, 6.7×10^{-4} M for hexylaldehyde and 6.7×10^{-4} M for hexanol.

In the case of codlemone (Fig. 2), 2.4 mg were placed in the vial with a small spatula and then the vial was sealed. Codlemone did not evaporate completely during the course of measurements and the maximum concentration that can be achieved in the closed vial is the equilibrium concentration allowed by its vapour pressure value at 25 °C (8.86×10^{-4} mmHg, see Table 1). Thus, codlemone concentration in air phase was equal or lower than 5.0×10^{-8} M.

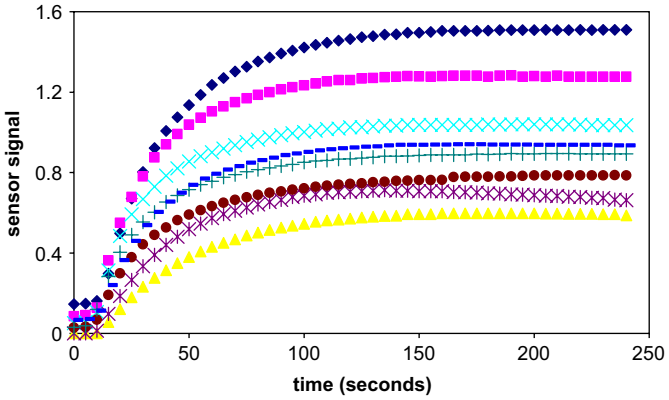

Fig. 1. A representative e-nose measurement. Each curve represents the signal of one individual sensor of the array. The vapours were aspirated since time zero and the signals increase up to reach a plateau. The set of signals at the plateau is the information used for the chemometric data analysis.

Different concentrations of volatiles were placed in the closed vials at concentrations ranging within 5.0×10^{-4} and 2.0×10^{-3} M (Fig. 3). Similarly, varying amounts of codlemone were weighted between 0.2 and 1 mg. Sensor signals were normalized to be used as input data for principal component analysis (PCA) (see Section 2.4).

Two microlitre aliquots of a Golden aroma sample were placed alone (first group of measurements) or together with 0.2–1 mg samples of codlemone (second group of measurements) in 100 cm^3 sealed vials and left to evaporate before measurement (Fig. 4).

2.3. Electronic nose measurements

An electronic nose device developed in our laboratory at the University of Buenos Aires was used in the present work. It consisted of an array of eight polycrystalline tin dioxide-based semiconductor gas sensors placed in a sample chamber with an aspiration pump and a regulating valve. The electrical conductivity of each individual sensor changes in the presence of a target odour. Each sensor is doped with different *ad-hoc* impurities, which modulate the electrical responses, resulting in different sensitivities. The whole set of sensors' signals is referred to as a "fingerprint". We used the electronic nose methodology in previous works to obtain and discriminate the fingerprints of complex mixtures under different conditions (O'Connell et al., 2001; Monge et al., 2004a, b; Branca et al., 2003; Lovino et al., 2005; Diz et al., 2006).

The gas phase present in the headspace of the sealed vials was extracted by means of a controlled flux aspiration pump with

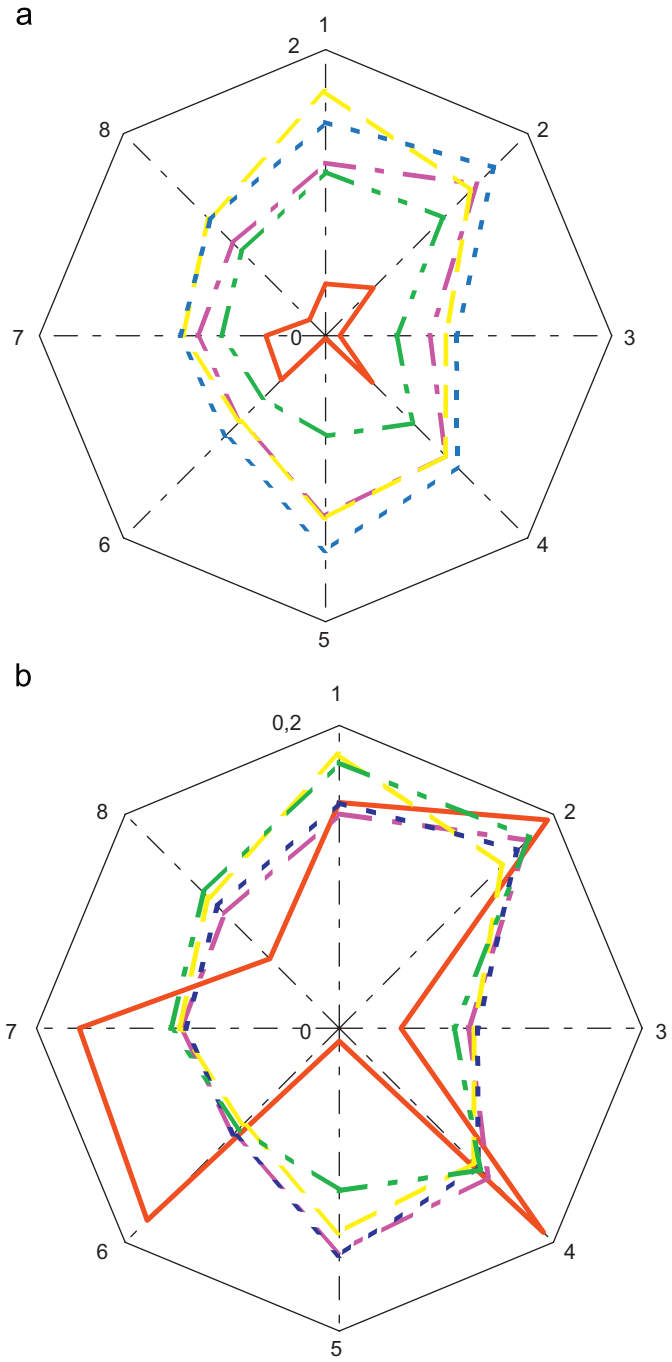

Fig. 2. Radar plots obtained for the e-nose signals of codlemone and four vapours of different volatiles present in apple and pear orchards. Each vertex is associated to the signal taken at 100 s of one sensor of the e-nose. Figs. 2a and b are rough and normalized data, respectively (see Section 2.2). Normalized radar plots are the so-called fingerprints of each compound. —: codlemone, – – –: hexylaldehyde, — — —: butyl acetate, - - -: hexanol, – - –: hexyl acetate.

a syringe injected trough a Teflon septa. Each measurement consisted of records of the sensors' signals during 2–4 min depending on the sample. The data were analysed after subtraction of a baseline achieved by recording the sensors' response to commercial air working at the same controlled flux. The values of the sensors' signals used to make the discrimination analysis were the maximum values at the top or at the plateau level of the curve (Monge et al., 2004b).

Each e-nose measurement provided a set of vectors $\{S_i\}(1<i\leqslant N)$ of the sensors' signals. Then, these vectors can be normalized to obtain a new set $\{S_i'\}$, where $S_i' = S_i/(\Sigma_k S_k^2)^{1/2}$. The parameter $(\Sigma_k S_k^2)^{1/2}$ is the so-called normalization factor, an indicator of the intensity of the odour detected by the e-nose. Thus, the set of data can be either normalized or not depending on the type of information is required.

2.4. Data analysis

2.4.1. PCA

The e-nose methodology uses multivariate data analysis to discriminate between groups of signals and hence to discriminate between samples. PCA is an unsupervised method that is useful for data discrimination and is the most popular for e-nose data analysis. This extraction method consists of a projection of the N-dimensional data set (in this case N is the number of sensors) in a new base of the same dimension N but defined by the eigenvectors of the covariance or the correlation matrix of the data set. The components (projections) of the original data vectors on this new base are the so-called principal components. One set of principal components $\{PC_1,\ldots,PC_N\}$ is obtained for each data set $\{S_1,\ldots,S_N\}$. The important point is that when analysing the new data set $\{PC_1, PC_2,\ldots,PC_N\}$ a large percentage of the total data variance is accumulated in a few of the principal components. For example, in most of the studies associated to e-noses 95% of the total variance is accrued in the three first principal components, this representing a substantial reduction of the dimension and complexity of the problem. In those cases, the data points can be qualitatively discriminated by observing how they group in a two-dimensional (2D) or three-dimensional (3D) plot (*PCA map*) of the principal components.

2.4.2. Cluster analysis (CA)

CA is another unsupervised method that can be implemented using different algorithms (Johnson and Wichern, 2002). In the present work, CA was performed using the partition around medoids (PAM) algorithm. The number of desired classification clusters to which the input data are to be assigned by the algorithm must be fixed in advance. The outputs of PAM indicate not only of which data input the clusters are composed but also provides the so-called silhouette of each cluster. A wide silhouette means a better assignment than a narrow silhouette. As criteria for the goodness of the classification a parameter named average silhouette width (referred as ASW) must be larger than 0.25. The higher the ASW, the better the fit (Struyf et al., 1997).

3. Results

The first step in the experimental work was to record the signals obtained with the electronic nose in the presence of each one of the most abundant volatiles composing the Golden Delicious aroma. Butylacetate is one of the principal components of the volatile fraction of apple essential oils being present in a fraction between 11% and 37% by weight, depending on the apple type. It is also present in the volatile fraction of pears and pear's leaves. Hexylacetate is the second most important ester component in the volatile fraction of apple and the most important component in some pear species. Hexanol is one of the two most abundant alcohol components in the volatile fraction of apples and pears. Although aldehydes are not majority components of apple and pear volatile fractions, hexylaldehyde was chosen to study the e-nose response to another functional group because this compound is abundant in the volatile fraction emitted from leaves (Young, 2002).

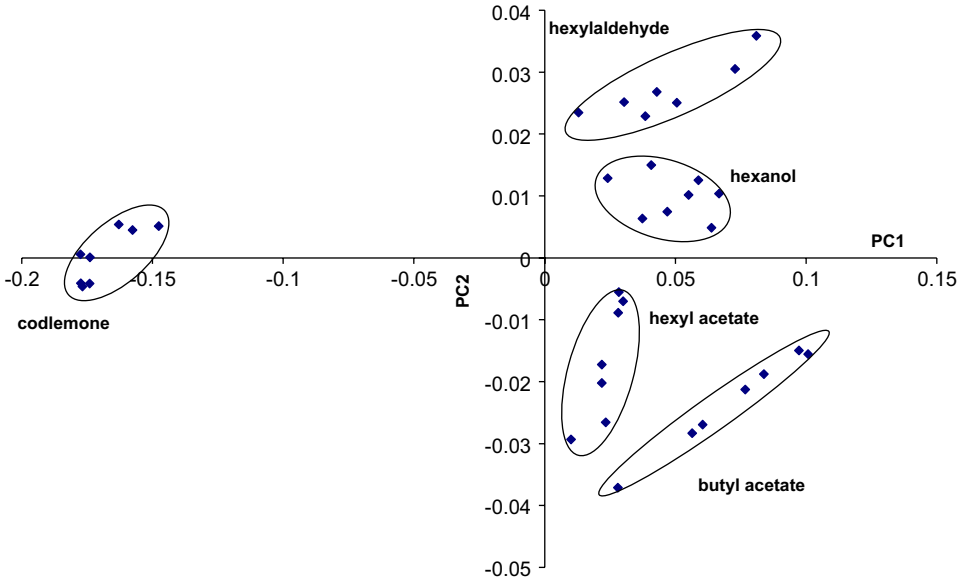

Fig. 3. PCA map for codlemone and the volatile compounds. Each point in this map corresponds to a different concentration in air of the indicated compounds (volatiles between 10^{-3} and 10^{-4} M, codlemone $<5\times10^{-8}$ M). Normalized signals were used as inputs for PCA.

Fig. 1 shows an example of the type of signals recorded with the e-nose during one measurement of butyl acetate 6.4×10^{-4} M in air. The sample is aspirated from a sealed vial. A fast rise in the signal is observed and the signals are recorded for a duration of a few minutes. Each curve represents the signal of one individual sensor of the array obtained after subtraction of a baseline recorded with pure commercial air for that sensor.

Fig. 2 shows the original (part a) and normalized (part b) signals obtained at 100 s drawn as radar plots for the codlemone and the four different volatiles present in apple orchards chosen in this work. Each vertex of the radar corresponds to one sensor signal of the e-nose. The differences in radar area in part (a) are due to the different concentrations and intensities of signals detected by the e-nose and give an idea of the sample concentration. In this regard, it is noticeable that the codlemone radar is very small (red line). By its nature, a trail-marking pheromone has a very low vapour pressure because it must remain where it is deposited in order to be effective (Peterson, 1998) and in fact codlemone does not evaporate during the course of measurements. The concentration in vapour phase was estimated to be lower than 5.0×10^{-8} M (see Table 1 and Section 2.2). The small area of the codlemone radar in comparison with those recorded for the other volatiles accounts for that concentration difference in the air phase.

In part (b), the data were normalized to obtain the so-called fingerprints of each compound. Visual inspection of the normalized radar plots must be considered as the first step towards discrimination by comparing the fingerprint of each sample. The radar plots showed that the codlemone fingerprint is very different to those of the volatiles. This suggests that codlemone can be clearly discriminated from the other compounds using our e-nose, which constitutes a relevant early result of the present work.

Concerning the discrimination between volatiles it must be pointed out that a visual inspection of radar plots is useful and straightforward when fingerprints are clearly different, but can lead to wrong conclusions when small differences are observed, as it is precisely the case of the volatiles' radar plots. Hence, radar plots are useful for observing differences between dissimilar samples and to control reproducibility, but multivariate data analysis methods like PCA or CA must be used to discriminate samples when differences are less obvious.

Fig. 3 shows the results of the PCA representing the so-called PCA map (PC_2 vs. PC_1) for the different samples. Each point in this PCA map corresponds to one normalized measurement with the e-nose. The points are grouped according to their position in the PCA map. Five groups corresponding to each of the five compounds tested in this work are clearly distinguished in Fig. 3: codlemone, the two acetates, hexylaldehyde and hexanol. Within each group, the points represent different concentrations in the air phase (see Section 2.2). Two main results arise from this graph; one is that the e-nose is able to easily discriminate each type of volatile and the other is that codlemone is well separated from any of the other volatiles measured.

On the basis of the excellent discrimination of codlemone, the next step was to explore the discrimination of samples of assembled Golden aroma in air with and without the pheromone. An artificial mixture of the Golden Delicious aroma was prepared using the proportions butylacetate:hexylacetate:-hexanol 11.8:9.7:15.6 according to Saevels et al. (2004). Two microlitres of the mixture, which had a strong apple aroma, were placed in a septum-sealed flask of 100 cm^3 and allowed to evaporate completely. The final concentration of each component in the final mixture was in the order of 10^{-5}–10^{-4} M in air. When codlemone was studied, less than 1 mg was placed in the flask, the sample was sealed with a Teflon septum and thereafter 2 μm of Golden aroma were added and the mixture allowed to equilibrate for 10 min before measurement. As mentioned before, the pheromone microdrop does not evaporate and was present all the time during measurements. Again, the aerial codlemone concentration cannot be larger than 5×10^{-8} M.

Fig. 4a shows an excellent result regarding the ability of the e-nose to discriminate codlemone from Golden aroma, as can be seen from the different shapes of the respective fingerprints (radar plots). Sensor signals were examined by PCA analysis and the results are shown in Fig. 4b. The PCA map shows the separation into two groups consisting of Golden aroma with and without pheromone clearly. This result is of high relevance because the presence of the pheromone is detected even when

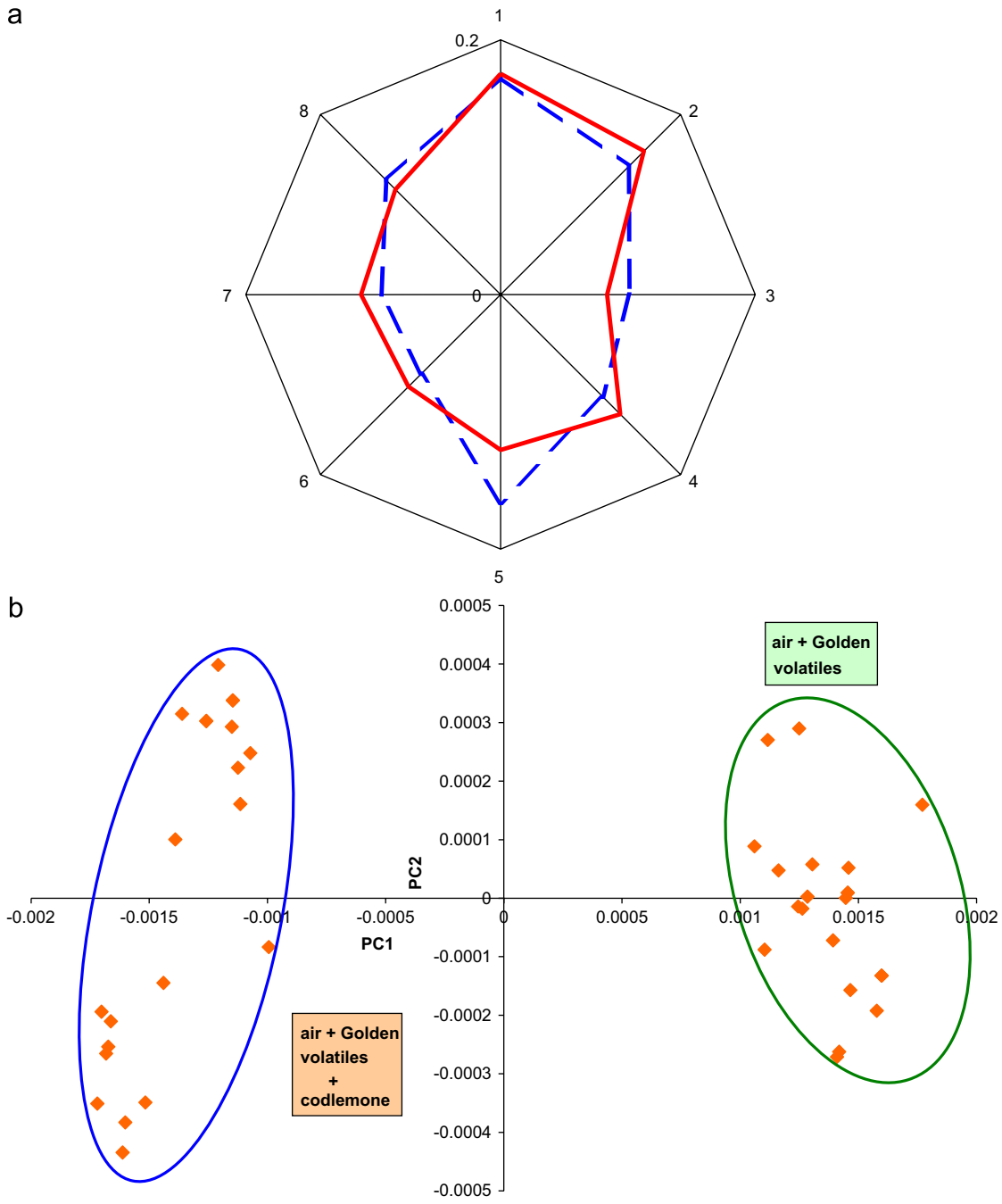

Fig. 4. (a) e-Nose radar plot of normalized signals of Golden aroma (continuous line) and a mixture of Golden aroma and codlemone (dashed line). (b) PCA map of Golden in air and the mixture Golden+pheromone in air.

Golden aroma is present in a relatively high concentration (four orders of magnitude greater).

The above results were obtained for samples placed in closed vials. But a real situation in an orchard is far from this; not only is the atmosphere open but very often a strong and turbulent air convection is caused by wind. In an attempt to reproduce this situation, the first approach was to measure Golden aroma and pheromone in the open environment of the laboratory under different conditions. A diagram showing the spatial disposition of volatile and pheromone sources is depicted in Fig. 5. Firstly, four Petri dishes containing 10 μm of Golden aroma were placed in the corners of a laboratory room (60 m^3) and total evaporation allowed. Thereafter, a measurement with the e-nose was performed during 20 min placing the e-nose on the room midpoint and recording the data at approximately 1.6 m of height, moving the e-nose blower pipe to describe a circle.

Secondly, codlemone was measured after carefully cleaning the room air. The former experiment was repeated, but adding 1 mg of codlemone on each Petri dish (the location of the dishes remained the same). That is, each dish contained 10 μm of Golden aroma (which evaporated almost immediately) and 1 mg of codlemone. The volatiles and codlemone were allowed to permeate in the room for about 5 min and then the odour was recorded for 20 min with similar movements of the blower pipe as in the first experiment. In both experiments, a baseline was recorded with the natural clean room air just before the first experiment with the Golden aroma.

These two experiments provided the first set of data information. Additionally, we tested the reproducibility and reliability of

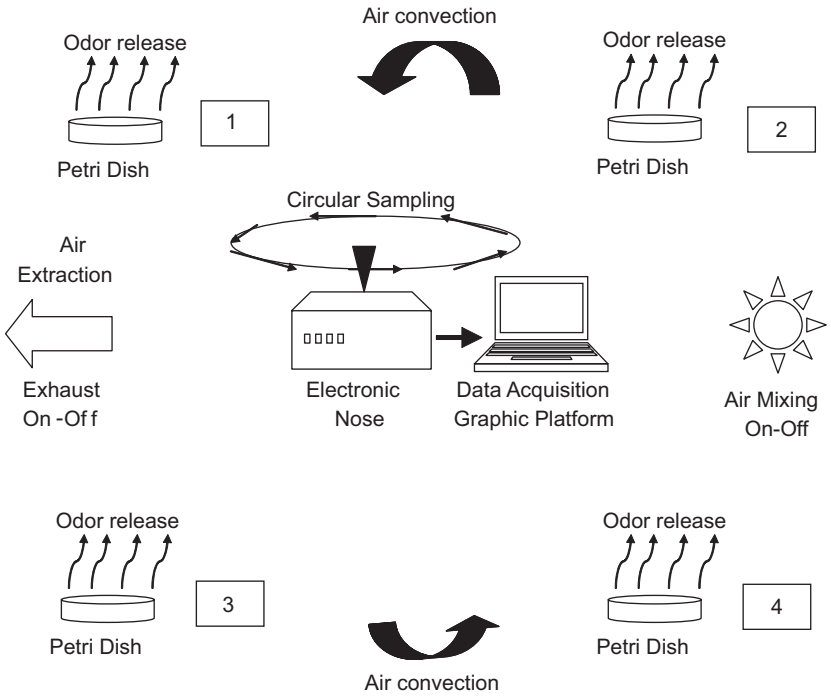

Fig. 5. Experimental set-up for the detection of codlemone in the presence of Golden aroma in a room. Four Petry dishes were placed at the corners of the laboratory room (60 m^3) and the e-nose was placed at the room midpoint. Measurements were performed for 20 min by moving the e-nose blower pipe to describe a circle. See the text for more details.

the discrimination by measuring a so-called "test sample" of codlemone in a room previously filled with Golden aroma. The differences respecting the other experiments were that in this case codlemone was introduced in a room already containing the Golden aroma and in a different location. For doing this, the lab air was cleaned opening windows and using an air extractor and a van. After that, a new baseline was recorded to check that cleaning of the air was already achieved and thereafter the room was filled with Golden aroma in a manner similar to that described previously. The codlemone "test sample" was then introduced in a different location with respect to the original geometry (the four room corners). A new measurement of 20 min duration was recorded and, after subtracting the corresponding second baseline the data were used for PCA and CA.

The results obtained with PCA are shown in Fig. 6a where discrimination of the samples between those with and without codlemone is very clear. This was corroborated by CA, using the obtained pairs {PC_1, PC_2} as inputs for CA and targeting for two-groups clusterization. As results of this CA the "test sample" was grouped in the same cluster than the original codlemones. This means that the test sample was recognized as a "containing-pheromone" sample.

Several additional analyses were performed to check the quality of the classification. In all cases, the numerical results (provided by the parameter ASW in CA) were excellent. For instance, in order to analyse the perturbation of the "test sample" a PCA was performed using the measurements of the Golden aroma background with and without codlemone, but without the test sample. In this case, the classification was almost perfect: PCA contained 99.7% of the variance in the data in the two first components PC_1–PC2 and CA rendered ASW = 0.91 for grouping data into two clusters. When including the test sample the results did not vary much: PCA had 99.3% of data variance in PC_1–PC_2 and ASW = 0.80 for a two-groups clusterization in CA. In both cases (with and without the test sample), 100% of the samples were correctly grouped, that is, the two clusters were composed of samples with codlemone (cluster A) and without codlemone (cluster B) exclusively.

Finally, a very relevant experiment was performed concerning the influence of air turbulences like those produced by wind in orchards. The experimental procedure was repeated but with artificially creating air convection. The room air extractor and a fan were turned on before beginning the measurements, with the aim of creating strong convection. Another "test sample" was then measured. PCA and CA yielded again positive results for codlemone discrimination. The discrimination was still very good in this experiment (see Fig. 6b). The differences between the measurements with and without convection (Figs. 6a and b, respectively) were that now the 99% of the variance in PCA was obtained with three principal components (PC_1, PC_2 and PC_3) and that the clusterization in CA was not as good as in the experiment without air convection. For example, in the analysis without the test sample, the whole set of PC_1–PC_2 contained 95% of the total data variance while 99.3% was contained with PC_1–PC_2–PC_3 and the respective CA rendered ASW = 0.73 (compared with ASW = 0.91 obtained without air convection). When including the test sample PC_1–PC_2 contained 94.2% of the variance while 99.2% was comprised in PC_1–PC_2–PC_3. A value of ASW = 0.71 was calculated in this case which again is slightly lower than the one obtained without air convection (ASW = 0.80). Besides, the points in the PCA map are regularly grouped in the experiments without convection (Fig. 6a) whereas in this latter case the points were in lines (Fig. 6b) showing the influence of air movement. Nevertheless, the main result is that the discrimination of the test sample is very good even in the presence of air convection.

4. Discussion

These results have shown that the electronic nose is able to detect low levels of codlemone in the presence of concentrations

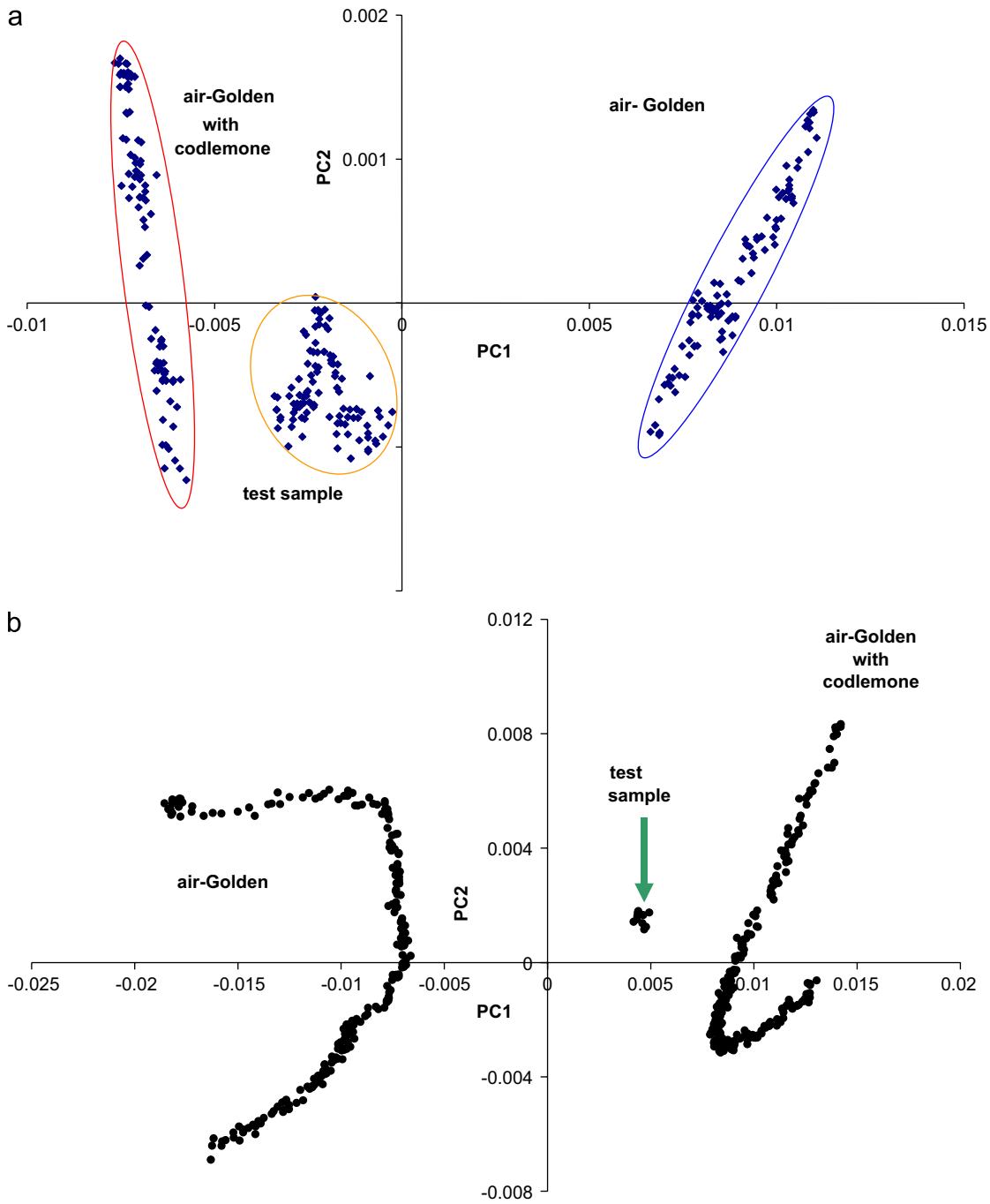

Fig. 6. Results of the experiments using the set up of Fig. 5: (a) without air convection, (b) in the presence of air convection. See the text for the details of the experimental procedure.

four orders greater than a background odour of plant and fruit volatiles. The potential usefulness of the e-nose helping to optimize mating disruption strategy will rely on how the sensors can be placed and measurements automated in an orchard to give real-time readings of pheromone concentrations in the air. This could be particularly important because it would permit continuous and remote monitoring along weeks, whereas the use of alternative possible strategies for measuring pheromone concentrations in an orchard such as EAG and SSR techniques requires a regular replacement of the biological material. Other techniques to quantify pheromone concentrations involve the use of adsorption materials (solid-phase microextraction in the orchard and the transfer of these materials to a laboratory so that can be analysed using chromatographic techniques.

The success of the electronic nose methodology in open field campaigns has been already demonstrated (Bourgeois et al., 2003; Nicolas and Romain, 2004). We have also used our electronic nose in open field trials to detect changes in the signals emitted by plants (*Festuca arundinacea–Lotus corniculatus* pastures) in response to different fertilization treatments and cut frequency and the corresponding influence on its insect communities (Gil et al., 2004).

One of the most studied strategies based on gradient tracking is founded on the male silkworm moth algorithm developed to

track a female moth pheromone plume. In fact, wireless sensor networks mounted on mobile robots has been designed exploiting this control algorithm (Marques et al., 2002; Russell et al., 1995). We propose to place an automated e-nose capable of catching codlemone plume and able to report pheromone concentration in the air. Our strategy is to radially explore the space around dispensers in order to find the odour plume and to test the three-dimensional (3D) codlemone pattern and dispenser's performance. This can be accomplished by assembling a robotic arm with a small electronic nose and performing radial movements. These robotic e-noses will be placed in strategic points at the orchards borders and in places where the farmer usually finds that pest infestation is usual. The key point will be the proper training of the electronic nose device and the programming of the sequence of measurements to be done in order to get reliable data. The results presented in this work provide an excellent precedent for the application of e-noses in pheromone control for pest management.

Acknowledgements

Drs. Bernik and Negri are members of the *Consejo Nacional de Investigaciones Científicas y Tecnológicas* (CONICET), Argentina. This work was supported by the University of Buenos Aires (Programación UBACyT 2004–2007, Proyecto X267) and CONICET (PIP 6382).

References

Barnes, M.M., Millar, J.G., Kirsch, P.A., Hawks, D.C., 1992. Codling moth (Lepidoptera: Tortricidae) control by dissemination of synthetic female sex pheromone. J. Econ. Entomol., 1274–1277.

Bourgeois, W., Romain, A.C., Nicolas, J., Stuetz, R., 2003. The use of sensor arrays for environmental monitoring. Interests and limitations. J. Environ. Monit. 5, 852–858.

Branca, A., Simonian, P., Ferrante, M., Novas, E., Negri, R.M., 2003. Electronic nose based discrimination of a perfumery compound in a fragrance. Sensor. Actuator. B 92, 222–227.

Cork, A., De Souza, K., Hall, D.R., Jones, O.T., Casagrande, E., Krishnaiah, K., Syed, Z., 2008. Development of PVC-resin-controlled release formulation for pheromones and use in mating disruption of yellow rice stem borer, *Scirpophaga incertulas*. Crop Prot. 27, 248–255.

DeSimone, L.A., Howes, B.L., Barlow, P.M., Ebbinghaus, D., Losel, P.M., Lindemann, M., Scherkenbeck, J., Zebitz, C.P.W., 1997. Detection of major and minor sex pheromone components by the male codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). J. Ins. Physiol. 44, 49–58.

Diz, V., Cassanello, M., Negri, R.M., 2006. Detection and discrimination of phenol and primary alcohols in water using electronic noses. Environ. Sci. Technol. 40, 6058–6063.

Dorn, S., Schumacher, P., Abivardi, C., Meyhöfer, R., 1999. Global and regional pest insects and their antagonists in orchards: spatial dynamics. Agric. Ecosyst. Environ. 73, 111–118.

Gil, A., Suárez, S.A., de la Fuente, E.B., Lenardis A.E., Ghersa, C.M., 2004. Señales químicas emitidas por comunidades vegetales y su relación con los insectos: uso de nariz electrónica. In: Proceedings of the II Reunión Binacional de Ecología, Mendoza, Argentina, p. 469.

Johnson, R.A., Wichern, D.W., 2002. Applied Multivariate Statistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

Judd, G.J.R., Gardiner, M.G.T., 2005. Towards eradication of codling moth in British Columbia by complimentary actions of mating disruption, tree banding and sterile insect technique: five-year study in organic orchards. Crop Prot. 24, 718–733.

Lovino, M., Cardinal, M.F., Zubiri, D.B.V., Bernik, D.L., 2005. Electronic nose screening of ethanol release during sol–gel encapsulation. A novel non-invasive method to test silica polymerisation. Biosens. Bioelectron. 21, 857–862.

Marques, L., Nunes, U., Almeida, A.T., 2002. Olfaction-based mobile robot navigation. Thin Solid Films 418, 51–58.

Monge, M.E., Bulone, D., Giacomazza, D., Bernik, D.L., Negri, R.M., 2004a. Detection of flavor release from pectin gels using electronic noses. Sensor. Actuator. B 101, 28–38.

Monge, M.E., Bulone, D., Giacomazza, D., Negri, R.M., Bernik, D.L., 2004b. Electronic nose screening of limonene release from multicomponent essential oils encapsulated in pectin gels. Comb. Chem. High T. Scr. 7, 337–344.

Nicolas, J., Romain, A.C., 2004. Establishing the limit of detection and the resolution limits of odorous sources in the environment for an array of metal oxide gas sensors. Sensor. Actuator. B 99, 384–392.

O'Connell, M., Valdora, G., Peltzer, G., Negri, R.M., 2001. A practical approach for fish freshness determinations using a portable electronic nose. Sensor. Actuator. B 80, 149–154.

Pan, L., Yang, S.X., 2007. A new intelligent electronic nose system for measuring and analysing livestock and poultry farm odours. Environ. Monit. Assess. 135, 399–408.

Park, K.C., Ochieng, S.A., Zhu, J., Baker, T., 2002. Odor discrimination using insect electroantennogram responses from an insect antennal array. Chem. Senses 27, 343–352.

Peterson, J.E., 1998. Method and composition for termite control. United States Patent 5756114.

Pfeiffer, D.G., Kaakeh, W., Killian, J.C., Lachance, M.W., Kirsch, P., 1993. Mating disruption for control of damage by codling moth in Virginia apple orchards. Entomol. Exp. Appl. 67, 57–64.

Purenne, P., Pagé, T., Guy, C., 2007. Odor monitoring at the city of Montreal waste water treatment plant. Water Practice 1, 1–14.

Russell, R., Thiel, D., Deveza, R., Mackay-Sim, A., 1995. A robotic system to locate hazardous chemical leaks. IEEE Int. Conf. Robotic. Autom., 556–561.

Saevels, S., Lammertyn, J., Berna, A.Z., Veraverbeke, E.A., Di Natale, C., Nicolaï, B.M., 2004. An electronic nose and a mass spectrometry-based electronic nose for assessing apple quality during shelf life. Postharvest Biol. Technol. 31, 9–19.

Schöning, M.J., Schroth, P., Schütz, S., 2000. The use of insect chemoreceptors for the assembly of biosensors based on semiconductor field-effect transistors. Electroanalysis 12, 645–652.

Struyf, A., Hubert, M., Rousseeuw, P.J., 1997. Integrating robust clustering techniques in S-PLUS. Comput. Stat. Data An. 26, 17–37.

Tomaszewska, E., Hebert, V.R., Brunner, J.F., Jones, V.P., Doerr, M., Hilton, R., 2005. Evaluation of pheromone release from commercial mating disruption dispensers. J. Agric. Food Chem. 53, 2399–2405.

Van der Pers, J.N.C., Minks, A.K., 1993. Pheromone monitoring in the field using single sensillum recording. Entomol. Exp. Appl. 68, 237–245.

Young, N.Y., 2002. Electroantennogram responses of *Nilaparvata lugens* (Homoptera: Delphacidae) to plant volatile compounds. J. Econ. Entomol. 95, 269–277.

Yujie, L., Xiao Xi, Z., 2004. Electro-antennograms response of male *Helicoverpa armigera* to synthetic sex pheromone components. J. Henan Agric. Univ. 38, 49–53.