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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Grower Summary

Headline

This project demonstrated clear potential to use the new technology of 'electronic noses' for early detection of pests, diseases or plant damage within commercial crops grown under protection. However, growers will need to wait for the equipment to be refined over the next 3-5 years before commercial use is possible and cost effective.

Background and objectives

Early detection of pest attack is vital for effective biological control. Late detection allows pest populations to become established and economic damage can occur before biological agents provide adequate control. Traditionally, early detection has depended on crop workers with the time and knowledge to identity the first stages of attack, but this is increasingly difficult with current staff profiles.

In response to pest attack, plants produce a cocktail of volatile compounds that in nature contribute to their anti-pest defences. Laboratory measurement of these volatiles show that they are characteristic of particular pests, and if such measurements were possible in crops, this would provide a way of early detection of attack. Unfortunately, the equipment used in lab-based measurements is very expensive, bulky, slow, and requires skilled operators to process samples, so is unlikely to be suitable for commercial use.

Electronic noses (e-noses) are a relatively new technology that can detect particular 'fingerprints' in mixtures of volatile chemicals. They are compact, relatively low cost and require no specialist skills to use. E-nose technology is developing rapidly, for example for use in medicine and security. This scoping project investigated the ability of e-noses to detect changes in the 'volatile fingerprint' of crops attacked by pests.

Summary of the project and main conclusions

In tomato, the instrument used was able to distinguish between an undamaged crop, plants which had been mechanically wounded (from pruning for example), plants attacked by caterpillars, and plants infected by powdery mildew. In cucumber, the e-nose could distinguish undamaged plants from plants which had been mechanically wounded and plants attacked by red spider mite.

The project showed the potential for e-noses to detect pest attack, and distinguish different types of attack. The current generation of e-nose instruments is too insensitive for

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immediate application in crops. However, the technology is developing very rapidly, and a new generation of e-noses, more likely to the suitable for commercial use in horticulture, is expected to be available for further assessment within the next 3-5 years.

Financial benefits

There are no immediate financial benefits to be gained from growers from this work, but the long-term objective is to improve the efficiency of biological pest and disease control.

Action points for growers

This was a scoping project seeking to identify a new approach to pest control within IPM and, at this stage there are no immediate action points for growers. However, the project showed for the first time that e-nose technology has the potential in the future to provide early detection for pest and disease attack. While further development of e-nose systems is required before the technology can be applied in a commercial context, growers should keep a ‘watching brief’ on advances in this technology in the future.

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Science Section

Introduction

Most plants normally produce a range of volatile organic compounds (VOCs). These compounds may vary qualitatively and quantitatively according to plant species and status, which is determined by biotic and abiotic factors [1]. When attacked by pests and diseases, plants emit much greater variety of VOC composition than non-attacked plants [1, 2]. Moreover, VOC profiles also have degree of specificity corresponding to types of attackers, which are distinct from those emitted due to artificial damage [3-6]. The changes in plant VOC composition according to type of attackers not only provide a potential possibility to track plant health status, but also provide a possibility to develop plant VOC tracking system that can be useful in horticulture, where effective plant health monitoring is crucial.

Several methods of VOC trapping and analysis have been used [7]. Among those, the gas chromatograph [8] is the traditional and routine method for VOC identification. However, this method involves several procedural steps which are time consuming, especially at the stage of VOC trapping and sample preparation [8]. During the past 5 years, technological advances have led to development of so-called “electronic noses” (e-noses), which provide an easier and quicker alternative to GC-MS for VOC detection. Unlike GC, e-nose does not need complex sampling preparation procedures. The instrument comprises of an array of non-specific, gas sensitive, chemical sensors as artificial odour receptors. The technology based on discotic liquid crystal (DLC) coatings and a unique technique for extracting data relating to individual VOCs in a mixture from both DLC and conducting polymer sensors. Rather than quantification of an individual compound, e-nose is designed to characterise the overall profiles of a VOC mixture into a digital fingerprint. It can be “trained” to distinguish VOC signatures from different sample headspace with appropriate tuning. Such technology has been used in a variety of applications, e.g. food quality measurements [9-11], disease diagnosis [12], and micro-organism identification [13]. A wide range of e-nose applications suggests the power of this technology as rapid, sensitive, specific, non-destructive and easy-to-use instrument.

The objective of the present study was to investigate the potential use of an e-nose system (Bloodhound BH114, Leed, UK) employing a 14 conducting polymer sensor array [13] to discriminate different types of plant VOC head space. The VOCs recorded and reported represent a broad overview of types of VOC species that could be expected from commercially grown protected food crops (cucumber, pepper, and tomato) and a protected

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ornamental (petunia). We focused on discrimination of VOCs from control, artificial damaged, diseased, and herbivore (pest) damaged leaves. The efficiency of the e-nose in discrimination of plant VOCs from tomato and cucumber leaves was assessed by comparing the results with those from gas chromatography mass spectrometry (GC-MS).

Materials and methods

Plant material

Cucumber (cv), pepper (cv Mazurka) and tomato plants (cv Carousel) were grown in the glasshouse with day/night temperature of 27 °C to 18 °C. Supplementary light of 300 µmol m^{-2} s^{-1} from 600W sodium (SON-T) lamps was provided to extend daylength to 16h. Within each type of plant species, fully expanded leaves with similar size from the same leaf level were used in the experiments. Petunia plugs were purchased for a local nursery and grown-on under the same conditions.

Caterpillar and arthropod rearing

Eggs of *Manduca sexta* (Tobacco hornworm) were obtained form a laboratory culture reared on wheat-germ based artificial diet at Department of Biology and Biochemistry, University of Bath. Eggs were placed in a plastic pot and maintained at 25 °C, and 50% relative humidity. After hatching, larvae were reared on artificial diet until reaching 3rd instar stage, when they were used in experiments. A colony of red spider mite (*Tetranychus utricae* Koch.) reared on tomato was obtained from Dr. P. Croft's laboratory at Stockbridge Technology Center, UK. The colony was maintained on cucumber plants in the laboratory at the condition of 25 °Cday/18°C night, 14/10-h light regime. The powdery mildew used in these studies was *Oidium neolycopersici*, which was isolated from natural infections occurring at Lancaster. The leaves with 80-100 % coverage by the fungus were used in the analysis.

Plant treatments

The artificial wounding on cucumber, pepper, and tomato leaves was done by using a plastic hole-punch with fine tip (0.9mm in diameter). Along the main leaf vein, a leaf was punched for 40 times. This was done in a pattern of 7, 6, 4, and 3 punches (in 4 rows) on one side of leaf vein, and in the same pattern on the other side. The punched holes were approximately 1.5 mm apart.

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To challenge tomato leaves by *M. sexta*, third instar larvae were starved for 2 hours before placed on fully expanded tomato leaves (2 larvae per leaf). The larvae were left to feed on the leaves for 5 hours before the analysis. To challenge cucumber leaves with spider mites (*T. urticae* Koch.), 40 spider mites were placed on the adaxial surface of the fully expanded cucumber leaf. The leaves then were placed in the condition of 25 °Cday/18°C night, 14/10-h light regime for 10 days before the analysis. Leaves infected with powdery mildew were inoculated with conidia from stock plants of *Oidium neolycopersici*.

Volatile sampling and analysis

In preliminary analysis made before the start of this project, plants had been enclosed in transparent bags and samples taken for injection in to the e-nose system. In the initial stages of this project it became clear that this approach was not suitable for further use. This was partly because it proved difficult to obtain repeatable samples from the bags because we believe it was difficult to obtain good mixing with the sample bags. However, the more significant issue was that the sensitivity of the e-nose was more limited than expected, and this required the development of a more precise sampling system. The method that was developed was to connect the e-nose (BloodhoundTM ST214, Scensive Teechnologies Limited, Yorkshire, UK) to the leaf cuvette of a Li-6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). This cuvette is commonly used for in situ measurements of photosynthesis and encloses a defined area of the leaf, providing defined conditions of light (1000 µmol m^{-2} s^{-1} PAR), temperature (30°C) and carbon dioxide concentration (380 µl l^{-1}). The air flow rate through the chamber at the rate of was also fixed at 450 µmol s^{-1}. This sampling approach also allowed the air input to be filtered using a carbon filter to remove volatiles present in the ambient air. This had the added advantage of allowing direct determination of the capacity of the e-nose to differentiate the signatures of “ambient” air and plants subject to various treatments.

The measurement was initially conducting using the basic operating mode of the instrument (BloodhoundTM ST214 Version 2.1 Control Software: Scensive Technologies Limited, Yorkshire, UK). In brief, two acquisition settings was used for volatile analysis: 1) 7 s absorption, 0 s pause, 20 s desorption, 5 s flush, 2) 12 s absorption, 0 s pause, 25 s desorption, 5 s flush. However, it was recognised that these settings could be optimised and during the course of the project, acquisition settings were adjusted to improve the sensitivity of e-nose to volatiles from treatments of cucumber plants. The instrument was calibrated with a standard solution of 2% (v/v) butan-2-ol.

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Gas-chromatography mass spectrometry (GC-MS) was used to confirm the sensitivity of e-nose to volatile bouquets from leaf treatments. The methods were the standards used for this type of analysis in our laboratories. In brief, volatiles were trapped onto sampling tubes containing the adsorbent resins Tenax TA and Carbotrap (Supelco Inc., Bellefonte, PA, USA). The sampling air passed through a tube at a rate of 200 ml min-1 for 20 min. The volatiles were analysed by GC-MS. For analysis, samples were desorbed using automated thermal desorption (Perkin Elmer, ATD 400, Norwalk, CT, USA). Tubes were heated for five minutes at 280 °C and focusing the desorbed volatiles on a Tenax TA cold trap at -30 °C for six minutes. The cold trap was then flash-heated to 300 °C and the sample injected onto an Al2O3–KCl PLOT column (50m × 0.32mm ID) via a heated transfer line held at 200 °C. Volatiles were identified by Wieley-nist library, followed by authentic compounds if commercially available.

Statistical analysis

The composite resistance from 14 e-nose sensors in an array was recorded as values of divergence, absorption, desorption, and area. These four explanatory variables from each sensor then were checked for consistency of responses to volatile bouquets. Sensors that gave inconsistent response or did not respond to volatiles were eliminated from the analysis. The further data reduction was done by principle component analysis (PCA) to reduce multicolinearity within the explanatory variables. The mathematic procedure underline the PCA was described [12].After data reduction, the variables from the sensor output which represented the volatiles bouquets from leaf treatments were tested for the difference by multivariate analysis of variance (MANOVA) in SPSS for window, v11.5 (SPSS Inc., Chicago, USA). The requirement to perform MANOVA prior to the discriminant analysis (DA) has been described before [14].

DA was performed using both SPSS software (SPSS Inc., Chicago, USA), and XLSTAT software v2007.6 (Addinsoft, NY, USA). DA provides a means of simplifying the e-nose output in to a form that can be used to differentiate the between the “fingerprints” of different cocktail of VOCs. DA is used here to test the statistical significance of changes in VOC fingerprints, but the same analysis would ultimately form the basis of automated software in future commercial systems designed to identify particular forms of attack.

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Results

- ***Outcomes relative to objectives***

As detailed below, the core objectives of the project to demonstrate the ability of an e-nose to differentiate the volatile "signatures" from healthy (control) plants and that from crops challenged by pests was achieved. In addition, this objective was developed to consider (i) the ability of the e-nose to differentiate pest from attack from mechanical damage, as might result from pruning and (ii) the ability of the e-nose to differentiate pest attack disease in tomato. The second objective, to relate the changes in volatile "signatures" detected by the e-nose to changes in the underlying chemistry of the volatiles quantified by GC-MS has also been achieved (see below).

The objective of carrying-out preliminarily investigations of the ability of the e-nose to pick up differences in VOC signatures in a commercial context was not achieved, since it was clear early in the project that the existing technology currently lacked the sensitivity to be usable in this context. Additional objectives were added to the experimental programme in place of this element. Sensitivity is also at the heart of the discussions with Scensive Technologies Ltd, a recognised world leader in E-nose technologies, which formed the fourth objective. Some initial suggestions for optimisation have been discussed, but it is clear that such discussions will be on-going. The final objective was to further communicate of the results of the study.

- ***Differences in e-nose signatures between hosts.***

The e-nose could distinguish volatile bouquets from undamaged leaves of cucumber, petunia, and tomato plants ($P<0.005$, Wilks'Lambda, MANOVA). The pattern of the difference could be explained via DA, by which the volatile bouquets from three different plant species were significantly discriminated (Fig. 1). The co-ordinates of the group centroids were -2.831,-3.006 (cucumber), -1.692, 3.672 (petunia), and 4.071,-0.546 (tomato). These group centroids were positioned along 2 axes (discriminant function (F) 1 and 2) which were both significant ($P<0.05$, Wilks' Lambda, DA). This means that both F1 and F2 can be used to explain the significant difference found among type of volatile clusters. The positions of the group centroids suggested that cucumber volatile bouquets could discriminate from Petunia volatile bouquets mostly by F2, while cucumber volatiles were separated from tomato volatiles mainly by F1. Petunia and tomato could be distinguished mainly by F1. The results indicated that e-nose could discriminate volatiles emitted from leaves of different plant species.

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- ***Demonstration of the ability of the e-nose to differentiate the volatile "signatures" from healthy (control) plants and that from crops challenged by pests.***

Once the sampling strategy had been revised, it was clear that the e-nose was able to detect volatiles from the range of host plants used, and changes in volatiles in response to attack.

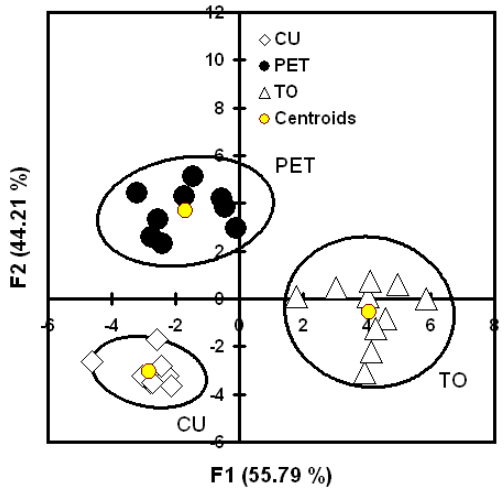

Figure 1 Discrimination analysis of volatiles from control leaves detected by e-nose

In situ measurements of volatiles from petunia (PET), tomato (TO) and cucumber (CU) collected from 2.5 cm^2 of leaf area for 20 min under 30°C and 1000 µmol m^{-2} s^{-1} PAR. Centroids represent the mean variate scores for each group: non-overlapping distribution are significantly different (n = 9).

- ***E-nose detection of changes in VOC emissions following mechanical wounding.***

The fundamental aim of the project was to test the ability of the e-nose to detect changes in VOCs in response to pest attack. However, in many commercial situations, plants are also subject to "mechanical damage" in terms of pruning, harvesting etc. Clearly, if the e-nose could not differentiate between the effects of these management practices and pest attack, it would be of limited value in commercial systems. Thus, although not defined as an objective in the project proposal, additional research was undertaken to assess e-nose signatures in artificially wounded plants.

Undamaged and artificial wounded leaves from pepper, petunia, tomato, and cucumber, leaves were analysed. The analysis of blank cuvette was done to ascertain the ability of e-nose to distinguish plants volatiles from those of background air.

In the case of pepper (Fig 2a), volatiles from the blank cuvette, undamaged and artificial damaged pepper leaves were significantly different ($P<0.001$, Wilk's Lambda, MANOVA). The group centroids for blank cuvette were -7.287, 5.301, undamaged leaves were -3.842, -

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4.708 and artificial damaged leaves were 6.525, 0.881. DA suggested that both variates (F1, and F2) were significant (P<0.003, Wilk's Lambda, DA,) Fig. 2a. This indicated that the separation of sampling groups by MANOVA was based significantly on both axes.

In case of petunia (Fig 2b), volatiles from blank cuvette, undamaged and artificial damaged petunia leaves were significantly different (P<0.01, Wilk's Lambda, MANOVA). DA suggested only F1 was significant (P<0.001, Wilk's Lambda, DA,) Fig. 2b. Therefore the difference of three volatile groups could be described and based only on F1. The group centroids of the three types of volatile bouquet were -15.728, 0.226 (blank cuvette), 4.865, 2.481 (petunia) and 3.873, -2.606 (artificially wounded petunia). Blank cuvette volatiles were very distinct from the other 2 groups of volatiles. However, undamaged and artificial damaged volatiles from petunia leaves were quite similar, even so based on the discriminant score F1, those two types of volatile bouquets were distinguishable.

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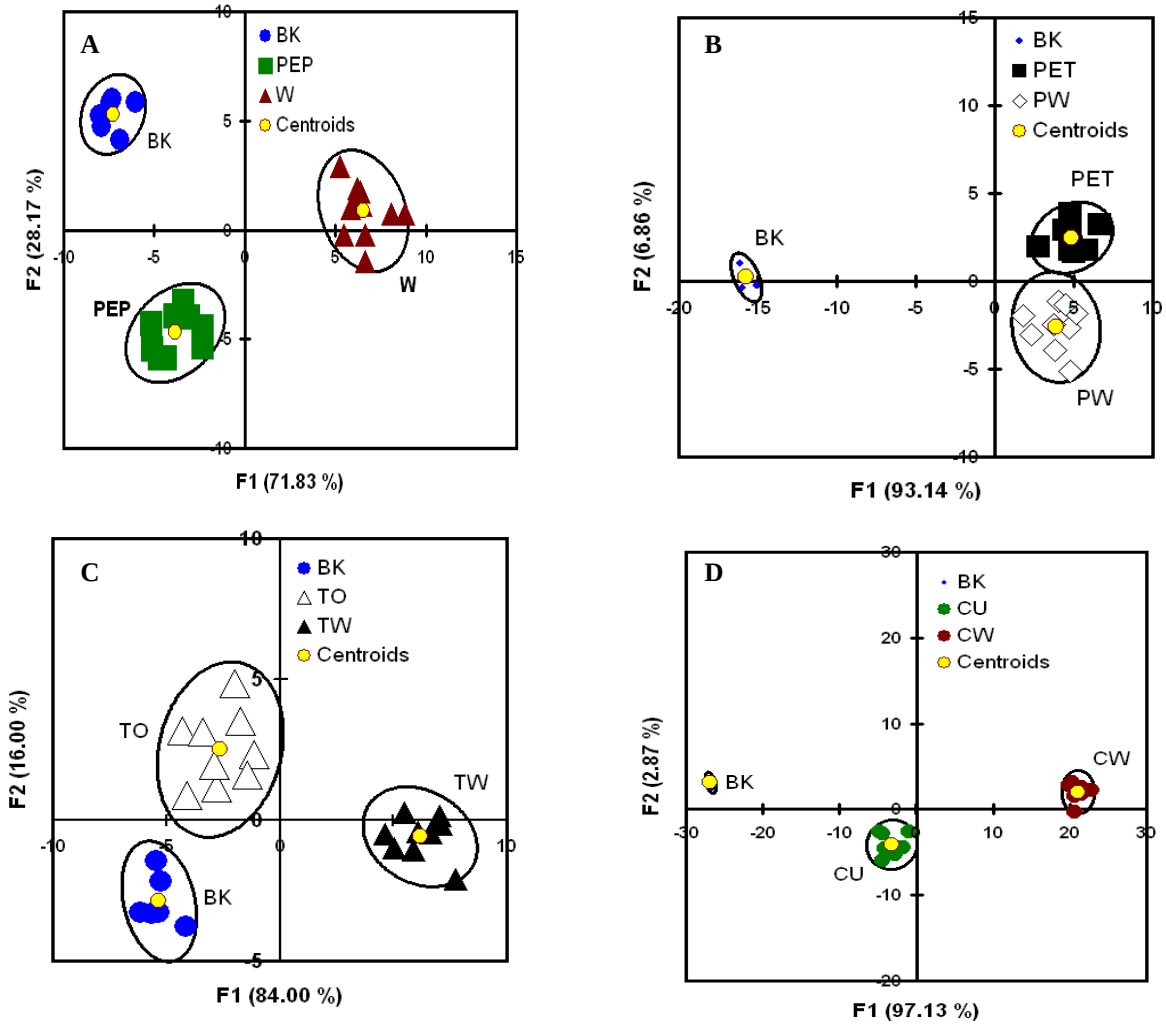

Figure 2 Discrimination analysis of volatiles from control, artificial damaged leaves and blank cuvette detected by e-nose for pepper, petunia, tomato and cucumber

A; volatiles from pepper (PEP), artificial wounded (W) leaves, and blank (BK), B; volatiles from petunia (PET), artificial wounded (PW) leaves, and blank (BK). C; volatiles from control tomato (TO), artificial wounded (TW) and blank (BK) and D; volatiles from control cucumber (CU), artificial wounded (CW), mildew infected (TD). The volatiles were detected from 2.5 cm^2 of leaf area which were intact to whole plants for 20 min under 30°C and 1000 µmol m-2 s-1 PAR ($n \geq 4$). Centroids represent the mean variate scores for each group, $n \geq 5$.

In case of tomato leaves, e-nose measurements of the blank cuvette, undamaged and damaged tomato leaves (Fig 2c) showed significant differences between these three types of volatile ($P<0.001$, Wilk's Lambda, MANOVA). DA suggested only F1 was significant ($P<0.001$, Wilk's Lambda, DA, Fig. 2c). Therefore, discrimination could be done based on F1 alone. Volatile clusters from undamaged tomato leaves lie in between those of blank cuvette and damaged tomato leaves and could be significantly distinguished based on DA (Fig. 2c). The group centroids of three types of volatile bouquets were -5.319,-2.881 (blank cuvette), -2.629, 2.508 (unwounded tomato), 6.174, -0.587 (artificially wounded tomato).

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In the experiment with cucumber leaves (Fig 2d), the e-nose could discriminate volatiles from blank cuvette, artificially damaged cucumber leaves, and undamaged leaves ($P<0.05$, Wilks'Lambda, MANOVA). The co-ordinates of group centroid of blank were -26.821, 3.127, those of undamaged cucumber were -3.193, -4.114, and those of damaged cucumber were 21.073, 2.030 (DA, Fig. 2d). The vertical and horizontal axes (F1, and 2) were significant ($P<0.05$, Wilk's Lampda, DA). The volatiles from artificially damaged cucumber leaves were very distinct from those of the blank cuvette, while volatiles from undamaged leaves was in between volatiles from blank cuvette and damaged leaves. Based on the position of the group centroids, the classification of those 3 groups of volatiles could be described well under F1.

- ***E-nose detection of changes in VOC emissions following mechanical wounding and pest attacks.***

Despite the ability of the e-nose to detect changes in VOCs resulting from artificial wounding, not all pests produced detectable changes in the volatile signature detected by the current e-nose. Thus, the e-nose could not detect changes in petunia or pepper attacked by aphids (data not presented).

Based on the first acquisition setting used in the previous analyses (7 s absorption, 0 s pause, 20 s desorption, 5 s flush), the e-nose could not significantly distinguish volatiles from spider mite infested, artificial wounded, and undamaged control leaves ($P>0.05$ Wilks'Lambda, MANOVA) Fig. 3a. DA suggested that although volatiles from artificial wounded leaves were significantly different from the other two type of volatile mixtures, volatile bouquets from spider mite infested and control leaves were not significantly different.

However, when the acquisition setting was changed by increasing absorption and desorption period (12 s absorption, 0 s pause, 25 s desorption, 5 s flush), the e-nose was then able to discriminate volatiles from artificial damaged, spider mite infested and control undamaged leaves ($P<0.05$ Wilks'Lambda, MANOVA) Fig. 3b. Group centroids of the sampling groups were -4.327, 8.167 (blank cuvette), -4.884,-5.783 (spider mite infested), 11.639, -0.012 (artificial wounded), and -5.313, 3.073 (control undamaged). Thus, the sensitivity of an e-nose can be easily adjusted simply by changing the acquisition settings.

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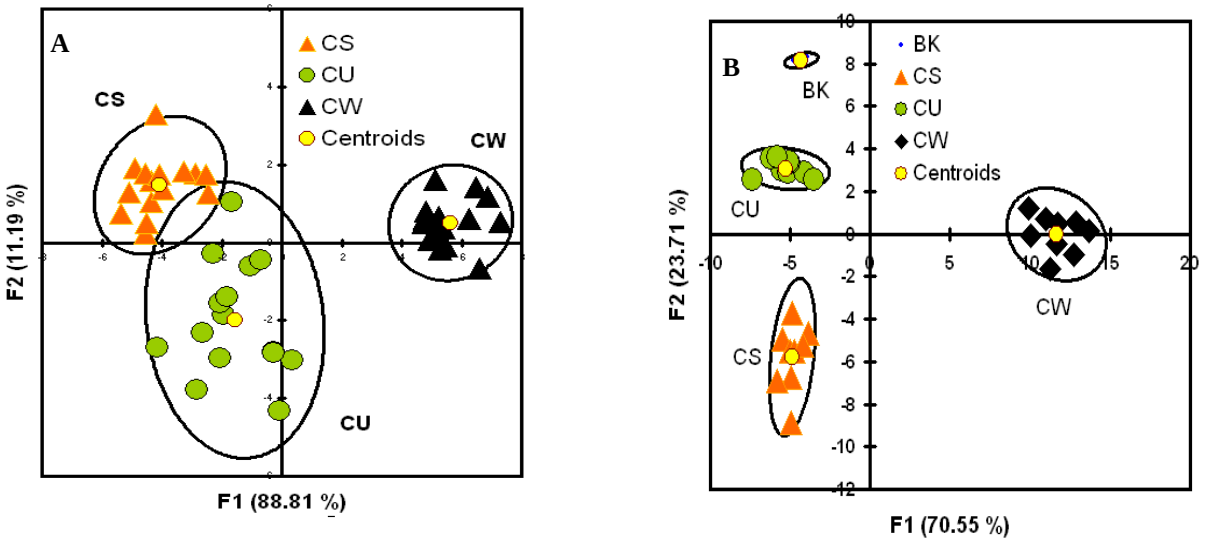

Figure 3 Discrimination analysis of cucumber volatiles from control (CU), artificial damaged (CW), spider mite infested (CS) leaves and blank cuvette detected by e-nose

A; volatiles were detect form e-nose using acquisition setting of 7 s absorption, 0 s pause, 20 s desorption, 5 s flush, B; volatiles were detect form e-nose using acquisition setting of 12 s absorption, 0 s pause, 25 s desorption, 5 s flush. The volatiles were detected from 2.5 cm^2 of leaf area which were intact to whole plants for 20 min under 30°C and 1000 µmol m-2 s-1 PAR ($n \geq 4$). Centroids represent the mean variate scores for each group.

E-nose measurements of volatiles from tomato leaves with powdery mildew, and attacked by caterpillars of tobacco hornworm (*M. sexta*) were done together with undamaged and artificially damaged leaves. The results suggested the significant difference of e-nose response to the difference volatile clusters from control and treated leaves ($P<0.01$, Wilk's Lambda, MANOVA). DA indicated the pattern of the difference based on graphical plot (Fig. 4.). Only F1 was a significant variate ($P<0.01$ Wilk's Lambda, DA), thereby the discrimination of volatiles bouquets from the 4 types of tomato leaves could be described based on F1 alone. The group centroids of the 4 tomato leaf treatments were -9.511, 2.253 (powdery mildew infected), -0.079, -5.088 (tobacco hornworm infested), 4.581, 3.909 (artificially damaged), and 5.481,-2.376 (undamaged). Based on e-nose response, volatiles from powdery mildew infected tomato leaves were the most distinct from the rest of volatiles clusters, i.e. from undamaged, artificially damaged and herbivore (pest) damaged leaves. Although volatiles from herbivore damaged leaves, artificial damaged, and control leaves were positioned close together, volatile clusters resulting from herbivory were more different from the other two. This demonstrated the power of e-nose in detection and discrimination of volatiles from pest, diseased, artificial damaged and control leaves from the same plant species.

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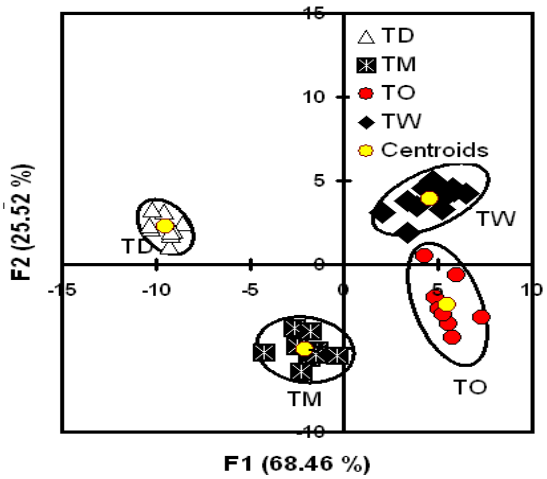

Figure 4 Discrimination analysis of tomato volatiles from control, artifical wounded, pest and disease infested leaves, and blank cuvette detected by e-nose

Volatiles from control (TO), artificial wounded (TW), powdery mildew infected (TD) and tobacco hornworm infested (TM) were analysed by e-nose. The volatiles were detected from 2.5 cm^2 of leaf area which were intact to whole plants for 20 min under 30°C and 1000 µmol m-2 s-1 PAR (n>4). Centroids represent the mean variate scores for each group.

- ***The relationship between the changes in volatile "signatures" detected by the e-nose to changes in the underlying chemistry of the volatiles quantified by GC-MS***

GS-MS analysis of volatile signals in the crops studied revealed highly complex signatures with a range of responses to different forms of attack. Cross referencing the multivariate data from both e-nose and GC-MS is challenging statistically, complex, but initial results show that changes in the e-nose are correlated with genuine chemical changes detected by GC-MS. Data for tomato are shown here to illustrate responses to multiple biotic attacks.

Analysis of volatiles from tomato which was either undamaged, grazed by caterpillars of *Manduca sexta*, or infected with *Oidium neolycopersici* confirmed that the two types of damage had significantly different effects of VOC profiles (Table 1). Of the 18 compounds detected, three (1,6-anhydro-beta-d-glucopyranose, bicyclo-hepten-ol and sabinene) were uniquely associated with mildew: these compounds did not occur in control or grazed plants. Infection, but not grazing, also caused significant increases in butanol methyl acetate and 2-beta pinene. The production of significant concentrations of three compounds (3-hexen-1-ol, beta mycrene and octatriene) was unique to plants grazed by *Manduca* (and similar, but non-significant effects were also seen for 1-hexenol and beta ocimene).

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Other compounds were not present in the VOCs from controls, but were detected at significant concentrations in plants attacked by caterpillar grazing or disease (alpha-humulene, alpha-phellandrene and gamma-terpiene), while ethyl-2-hexene was produced by controls, but not in plants subject to either grazing or mildew. Caryophyllene was detectable in the VOCs from control plants, but significantly increased by both grazing and infection. It has proved very difficult to establish clear correlations between any of these changes in specific compounds and any specific element of the e-nose response. It is clear that the significant changes in the e-nose "signature" are correlated with changes in the chemical composition of the VOC produced by plants under different conditions. With the current technology, this relationship is qualitative rather than quantitative, and the term "volatile signature" remains the best description of the e-nose output. The instrument detects real difference in the overall balance of volatiles that are relate to specific forms of attack or damage, but caution is required in linking this "signature" to specific changes in individual compounds.

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	Total emission (ng/2.5 cm^2)			
	Control plant	Diseased (Mildew)	Pest infested (*M. sexta*)	*P* value
1-hexenol	0.000	0.000	0.107 ± 0.143	0.265
1,6-anhydro-beta-d-glucopyranose	**0.000**	**0.027 ± 0.017**	**0.000**	**0.021***
pentanoic acid, 3-methy-	0.044 ± 0.041	0.000	0.000	0.107
ethyl-2-hexene-1	**0.018 ± 0.006**	**0.000**	**0.000**	**0.001***
2-hexyl-1-hepten-4-ol	0.000	0.000	0.026 ± 0.022	0.079
3-hexen-1-ol	**0.000**	**0.000**	**0.040 ± 0.013**	**0.001***
2-hexenal	0.000	0.001 ± 0.002	0.000	0.422
butanol methyl acetate	**0.008± 0.002**	**0.034 ± 0.011**	**0.000**	**0.001***
beta-ocimene	0.000	0.000	0.042 ± 0.039	0.097
bicyclo-hepten-ol	**0.000**	**0.023 ± 0.016**	**0.000**	**0.039***
beta mycrene	**0.000**	**0.000**	**0.064 ± 0.020**	**0.001***
alpha-humulene	**0.000**	**0.317 ± 0.130**	**0.170 ± 0.076**	**0.012***
alpha-phellandrene	**0.000**	**0.030 ± 0.024**	**0.063 ± 0.024**	**0.022***
gamma-terpiene	**0.000**	**0.011 ± 0.008**	**0.516 ± 0.326**	**0.024***
2-beta-pinene	**0.268 ± 0.085**	**0.902 ± 0.383**	**0.121 ± 0.019**	**0.012***
Sabinene	**0.000**	**0.238 ± 0.028**	**0.000**	**0.000***
Octatriene	**0.000**	**0.000**	**0.020 ± 0.010**	**0.008***
Caryophyllene	**0.069 ± 0.012**	**0.155 ± 0.022**	**0.298 ± 0.107**	**0.012***

Table I. Volatiles emitted by tomato plants

Emitted volatiles were collected from 2.5 cm^2 of leaf area for 20 min under 30°C and 500 µmol m^{-2} s^{-1} PAR. The values indicate the means and STD of each treatment group. The results were evaluated by means of an ANOVA (two-tailed) using the factors of control plant (n=3), mildew plant (n=3), and Manduca plant (n=3). *P*-values denote the significance of the interaction, and those marked with an asterisk (*) denote compounds that significantly depend on the treatments.

Discussion and Conclusions

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This project has confirmed the ability of even an unoptimised e-nose system to detect and differentiate between the VOC signatures produced by a range of factors in several crop plants. The data obtained went further than the original experimental plan in confirming that the e-nose could differentiate between different types of damage: mechanical wounding, pest and attack and mildew infection. Nonetheless, the project also revealed the limits on e-nose technology as it currently stands. These have been considered in detailed with Dr Tim Gibson, of Scensive Technologies Limited, the manufacturers of the e-nose used in this project.

It was clear from the start of the project that the original sampling strategy of enclosing plants within a box or bag to collect volatiles led to very "noisy" e-nose signals. The instrument was able to detect plant volatiles but was also responding to other components of the atmosphere. Discussions with Dr Gibson identified the changing water content of the air within the box as a likely issue. This lead to the use of the leaf cuvette, which allowed direct sampling of volatiles while controlling humidity. This approach was successful, and holds important lessons for the future use of e-noses in horticulture.

The baseline sensitivity of even this unoptimised instrument as sufficient for the effective detection of changes in crop volatiles. It should be noted that the cuvette was not a "sealed" system: a small area of leaf (2.4 cm^2) is enclosed within the cuvette with a flow through the chamber of 200 ml min^{-1}, representing a air change rate >20 changes per minute. Thus, the volatiles produced by the leaf are constantly being diluted but, even so, the signal produced from the e-nose was strong and consistent. Given recent advances in e-nose technology, the latest generation of instruments have sensitivities at least one order of magnitude greater than the instrument used here. Overall, lack of sensitivity should not in itself be a major limiting factor in the application of e-noses in horticulture.

The ability to discriminate between different volatile signatures, rather than simply detect volatiles, requires careful attention to optimizing the e-nose sampling settings. This was evident with cucumber, where optimizing the settings made the difference between no significant separation between treatments, and very clear, highly significant differences. In looking forward to automated systems for commercial use, a key element of development will be to define optimum sampling approaches to ensure robust differentiation of signals that can be automatically "decoded" as "healthy" and "attacked".

Probably the single most important observation of this project is that any commercial use of e-noses in horticulture must solve the problem of "noise", especially due to water vapour. A sensor system that is sensitive to large changes in humidity is not compatible with use in a crop environment, and the cuvette system used here is clearly not compatible with a simple

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automated system for use in horticulture. However, this is a common problem with many applications of e-noses, and changes in e-nose technology show routes forward. Firstly, a new generation of sensors is being developed that has a greatly reduced sensitivity to humidity, maximizing the "signal" of changes in target volatiles against the noise resulting from variation in water vapour. Secondly, automated sampling systems are being developed that "pre-treat" samples to remove water vapour without affecting other volatiles. Such systems are being developed for other challenging applications of e-nose technology, for example in detecting pollutants in the head-space above water or microbes in samples of body fluids. As such technologies mature, they will provide the basis for far more robust systems that should be capable of delivering the type of discrimination seen in this project in a commercial environment, although clearly this will require further research and development.

This project relied on a range of sophisticated data analysis approaches to deal with the highly complex, multivariate data produced by the e-nose. Such analyses are clearly a long way from the type of automated system that will be required in commercial use where, in essence, what is required is a simple "yes/no" output in relation to a particular pest of disease attack. There are multiple routes forward in this respect. Firstly, the sensor used here was not "tuned" to any particular volatile signature. A commercial instrument would be "tuned" by using fewer sensors chosen and optimized for the signature of particular pests and/or diseases. Developments here are partly in the electronics of the sensor but also in the underlying biology i.e. in identifying what elements of a volatile signature should be used for a particular pest or disease. This project begins to identify such targets, for example a sensor optimized to detect 1,6-anhydro-beta-d-glucopyranose, bicyclo-hepten-ol and sabinene would be a good candidate for the specific detection of powdery mildew in tomato. The presence of these compounds would appear on the basis of this project to provide a simple "yes" for the presence of mildew, which could be easily dealt with through automated data analysis software. However, a system "tuned" in this way would need testing in a crop environment to ensure that there were no false positive due, for example, to other pathogens or soil fungi. Future development will require close integration of biology and sensor technology, and this project, by integrating e-nose measurements with GC-MS provides a potential model for such collaborative research, with detailed understanding of the biology of volatiles informing future sensor design.

E-noses for commercial use will be specific for individual pests or pathogens on particular crops. This is self evident, and specificity is a key strength of the technology, but from the grower perspective it will mean individual instruments will be required for each pest or disease of interest. It might be possible to integrate multiple instruments into a unit capable of detecting a suite of major problems of a specific crop, but it seems very unlikely that an

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instrument designed for tomato, could not be used on pepper or cucumber, even for the same pest or pathogen.

Future development will require close integration of biology and sensor technology.

Overall, while the project was a technological success in proving the potential for the use of e-nose technology as an “early warning” of pest and disease attack, it also highlighted the extent of technical development in e-nose systems still required before this potential can be fully exploited in horticulture.

We will continue to discuss the feasibility of further research to deliver an effective e-nose system for use in the UK protected crops industry, but our assessment on the current data is that the initial priority should be in the development of improved e-nose technology.

Technology transfer

The key outcomes of the project were presented to a LU-based session for researchers and representatives of the industry, including the industrial advisers to the project Dr Rob Jacobson, Dr Phil Morley and Mr Neil Bragg. We would be happy to assist in the preparation of a short HDC-News article on the project if this is thought to be desirable. Also, with HDC’s permission, there is scope for wider publicity on the potential use of e-noses for pest and disease attack, in collaboration with the instrument manufacturer.

Glossary

Centroid (or group centroid): a key output of discriminant analysis that represents the “average” e-nose signature of a particular plant or treatment.

Crop volatile: (or VOC) a chemical released by a plant in to the air surrounding it. All plants produce mixtures of volatiles, some of which have been shown to play a defined role in plant physiology or ecology, while the function of others is less well-defined. One defined role is in plant-pest interactions, where volatiles are used by pests to detect suitable hosts, and by predators and parasitoids that attack pests to detect host plants supporting populations of their prey.

DA: (discriminant analysis) is a statistical tool that provides a means of simplifying the e-nose output in to a form that can be used to differentiate the between the “fingerprints” of different cocktail of VOCs.

e-nose: electronic noses are a relatively new technology based on arrays of sensors that each respond to a different group of chemicals, and so produce characteristic multi-variate

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datasets that, with appropriate analyses, can be used to detect particular “fingerprints” in mixtures of volatile chemicals.

GC-MS: gas chromatography-mass spectrometry: an analytical tool for detecting and quantifying chemicals that is used extensively for fundamental research in to plant volatiles

MANOVA: (multivariate analysis of variance,) a statistical tool used to determine the significance of differences between the volatile signatures of different species and treatments

PCA: (principle component analysis) a statistical tool that provides a means of simplifying the e-nose output in to a form that can be used to differentiate the between the “fingerprints” of different cocktail of VOCs.

volatile fingerprint: (or volatile signature): the particular mixture of volatile organic compounds produced by a plant. The signature is characteristic, not only of particular plant species, but also of plants responding to attack by pests and diseases. In this project, the e-nose sensor was used to detect changes in volatile signature.

VOC: volatile organic compound- a generic term for crop volatiles (q.v.)

References

1. Dudareva, N., et al., *Plant volatiles: Recent advances and future perspectives.* Critical Reviews in Plant Sciences, 2006. **25**(5): p. 417-440.
2. Croft, K.P.C., F. Juttner, and A.J. Slusarenko, *Volatile Products of the Lipoxygenase Pathway Evolved from Phaseolus-Vulgaris (L) Leaves Inoculated with Pseudomonas-Syringae Pv-Phaseolicola.* Plant Physiology, 1993. **101**(1): p. 13-24.
3. Costello, B., et al., *Identification of volatiles generated by potato tubers (Solanum tuberosum CV-Maris Piper) infected by Erwinia carotovora, Bacillus polymyxa and Arthrobacter sp.* Plant Pathology, 1999. **48**(3): p. 345-351.
4. Piel, J., et al., *Cellulysin from the plant parasitic fungus Trichoderma viride elicits volatile biosynthesis in higher plants via the octadecanoid signalling cascade.* Febs Letters, 1997. **416**(2): p. 143-148.
5. Dicke, M., *Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods?* Entomologia Experimentalis Et Applicata, 1999. **91**(1): p. 131-142.
6. Cardoza, Y.J., H.T. Alborn, and J.H. Tumlinson, *In vivo volatile emissions from peanut plants induced by simultaneous fungal infection and insect damage.* Journal of Chemical Ecology, 2002. **28**(1): p. 161-174.
7. Tholl, D., et al., *Practical approaches to plant volatile analysis.* Plant Journal, 2006. **45**(4): p. 540-560.

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8. Zhang, H.X., et al., *Quantification of spice mixture compositions by electronic nose: Part II. Comparison with GC and sensory methods.* Journal of Food Science, 2005. **70**(4): p. E259-E264.
9. Pearce, T.C., et al., *Electronic Nose for Monitoring the Flavor of Beers.* Analyst, 1993. **118**(4): p. 371-377.
10. Bourrounet, B., T. Talou, and A. Gaset, *Application of a Multi-Gas-Sensor Device in the Meat Industry for Boar-Taint Detection.* Sensors and Actuators B-Chemical, 1995. **27**(1-3): p. 250-254.
11. Gardener, J.W., Shurmer, H.V., Tan, T.T., *Application of an electronic nose to the discrimination of coffees.* Sensors and Actuators B-Chemical, 1992. **6**: p. 71-75.
12. Fend, R., et al., *Use of an electronic nose to diagnose Mycobacterium bovis infection in badgers and cattle.* Journal of Clinical Microbiology, 2005. **43**(4): p. 1745-1751.
13. Gibson, T.D., et al., *Detection and simultaneous identification of microorganisms from headspace samples using an electronic nose.* Sensors and Actuators B-Chemical, 1997. **44**(1-3): p. 413-422.
14. Field, A., *Discovering statistics using SPSS for windows*. Introducing Statistical methods, ed. D.B. Wright. 2000, London: SAGE Publications Ltd. 496.

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