Research Article

Received: 22 December 2009 Accepted: 3 March 2010 Published online in Wiley Interscience: 19 May 2010

(www.interscience.wiley.com) DOI 10.1002/ps.1967

Herbivore-induced plant volatiles allow detection of *Trichoplusia ni* (Lepidoptera: Noctuidae) infestation on greenhouse tomato plants

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Abstract

BACKGROUND: Monitoring of insect populations is an important component of integrated pest management and typically is based on the presence and number of insects in various development stages. Yet plants respond to insect herbivory and release herbivore-induced plant volatiles (HIPVs), which could be exploited in monitoring systems. The present objective was to investigate whether the information associated with HIPVs has potential to become part of advanced technologies for monitoring pest insect populations.

RESULTS: In a laboratory experiment, it was determined that tomato plants, *Lycopersicon esculentum* Mill cv. clarence, each infested with 20 caterpillars of the cabbage looper, *Trichoplusia ni* (Hübner), emit HIPVs, of which (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene and β-caryophyllene were selected as chemicals indicative of herbivory. Using an ultrafast portable gas chromatograph (zNose™) in a research greenhouse and in a commercial greenhouse, it was possible (i) to reveal differential emissions of these three indicator chemicals from plants with or without herbivory, (ii) to detect herbivory within 6 h of its onset, (iii) to track changes in indicator chemical emissions over time and (iv) to study the effect of environmental and crop-maintenance-related factors on the emission of indicator chemicals.

CONCLUSION: HIPVs appear to be promising as reliable indicators of plant health, but further studies are needed to fully understand the potential of this concept.

Keywords: HIPVs; pest monitoring; greenhouse tomato; *Trichoplusia ni*; zNose™

1 INTRODUCTION

The greenhouse vegetable industry is an important and growing segment of Canadian agriculture. Tomato crops alone account for >50% of all revenues. Monitoring insect populations is an essential component of integrated pest management (IPM). Most current pest monitoring systems rely on counting insect numbers[1] or finding indicators of insect presence or damage.[2] Even modern monitoring systems are based on insect-related indicators.[3–7] They enable growers to estimate insect populations or predict outbreaks. However, all such systems still involve human scouts to determine the location of insects within a field or a greenhouse.[8] Scouts must visually inspect a large number of plants, or plant parts, for the presence of various pests. In fields and greenhouses, visual inspections are challenging, and some pests may be overlooked. In spite of extensive training, the performance of scouts varies with degree of experience and personal attributes.[9] Monitoring plants and their response to herbivory offers an alternative approach to scouting for insects.

Emission of herbivore-induced plant volatiles (HIPVs) is a well-documented response of plants to biotic stressors.[10] Considering the vast knowledge about HIPVs and plant responses to the environment, it is conceivable to integrate these volatile indicators of herbivory into pest monitoring systems. HIPV emission is affected by several factors,[11,12] including light intensity,[13] relative humidity,[14] temperature[15] and photoperiod.[16]

Technological advances have facilitated and accelerated the acquisition and analyses of HIPVs from plants within their growing environment.[17–21] In this work, the zNose™ (Newbury Park, CA), an ultrafast portable gas chromatograph, was used for analyses of volatile emissions from uninfested tomato plants and from

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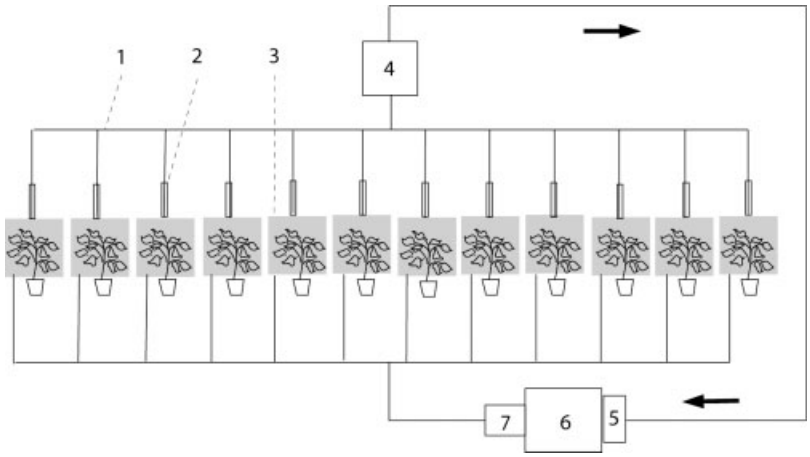

Figure 1. System for collection of plant volatiles: 1 – vinyl tubing; 2 – Porapak Q volatile trap; 3 – Pyrex jar; 4 – vacuum pumps; 5 – activated charcoal; 6 – air purifier filter; 7 – air micromist filter.

those infested with larvae of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), in the laboratory, a research greenhouse and a commercial greenhouse. During the sampling period in the commercial greenhouse, environmental factors were also recorded, and note was taken of greenhouse maintenance practices such as removing shoots or picking fruits.

2 EXPERIMENTAL METHODS

2.1 Experimental insects and plants

Cabbage looper larvae were obtained from a research colony maintained for 50 generations at the University of British Columbia. Tomato plants, *Lycopersicon esculentum* Mill cv. clarence, were obtained from Houweling's Hot House (Delta, BC). Intact 30–40 cm tall tomato plants were stored in a growth chamber at 25 °C ± 1 and a 16 : 8 h light : dark photoperiod until they were used in experiments.

2.2 System for collection of plant volatiles

A closed-loop stripping system was designed (Fig. 1) and placed inside a growth chamber maintained under a 16 : 8 h light : dark photoperiod at 25 °C. Two vacuum pumps (GAST Miniature Diaphragm 15D 1150 series; IDEX Corporation, Benton Harbor, MI) drew purified air at 0.6 L min^{-1} through each of 12 in-parallel-placed glass jars (each 20 × 15 cm wide), and through a glass tube (10 × 0.7 cm ID) filled with 50 mg of Porapak Q (100–120 mesh; Supelco, St Louis, MO) fitted to each jar. Air was purified by drawing it through activated charcoal, an air purifier filter (Pisco FTA 300 MD-B; Bensenville, IL) and an air micromist filter (Pisco UADR 300 AD; Bensenville). All components of the entire system were interconnected with vinyl tubing (2236 PL-4-NT, 4 mm ID; Festo, Mississauga, ON). Organic molecules emanating from the plant in each jar were adsorbed on Porapak Q for 24 h and then desorbed with 2 mL of HPLC-grade pentane into each of 12 separate vials.

2.3 Laboratory experiment

Using the closed-loop stripping system and environmental conditions described above, ten third-instar *T. ni* larvae starved for 3 h were placed on each of six randomly assigned treatment plants, while six control plants were kept without larvae. Volatiles from each plant were collected for 24 h. The procedure was replicated 5 times for a total of 30 treatment and 30 control plants.

2.4 Research greenhouse experiment

After indicator volatiles of larvae-infested plants had been determined, an experiment inside the UBC horticulture greenhouse was conducted. The main objectives were (a) to compare volatile emissions from uninfested and infested plants using the zNose™, (b) to monitor changes in the volatile emissions over time and (c) to explore the possibility of detecting herbivory at an early stage.

In each of two subsequent volatile sampling events, eight-week-old tomato plants were placed inside the greenhouse 48 h prior to volatile collection, thus allowing plants to acclimatize. Of these 50 plants, 40 with no signs of mechanical or insect damage were selected and spaced 1 m apart from each other in a completely randomized design, and 20 plants each were assigned to treatment and control groups. Above each plant, a clear plastic bag (Fisher Precision Ultraclean Bag; Fisher Scientific, Ottawa, ON) was suspended, covering the upper half of the plant canopy. Cross-shaped wooden frames wrapped in aluminum foil were attached to the exterior upper part of bags, thus keeping them in cube-shaped form and minimizing contact with plants and condensation. The lower part of the canopy was covered by a fine mesh screen which allowed ventilation but prevented larvae from leaving the plants in the treatment group. A short tube (5 × 1 cm ID) was attached to the upper part of the bag to form an opening for volatile collection.

To eliminate the effect of plant size on volatile emissions, the amount of all indicator volatiles from treatment and control plants was quantified 1 h prior to placing larvae on treatment plants. All changes in volatile emissions could then be compared with this baseline. The fresh weight of all plants, including feces of *T. ni* larvae, was also recorded as soon as volatile collections were completed. As a negative control, ten empty bags and screens were randomly suspended in various locations among the bags partially covering experimental plants. After establishing the baseline in all groups, 20 third-instar *T. ni* larvae starved for 3 h were placed randomly on all parts of the canopy of treatment plants. After 6, 12 and 24 h, the level of indicator volatiles in all groups was quantified. These time periods were considered as a factor, as opposed to a continuous variable.

2.5 Commercial greenhouse experiment

Plant volatiles were sampled and analysed by the zNose™ at Houweling's Hot House. As part of an unbiased representative

sampling strategy, 30 locations were randomly selected within the greenhouse with Google Earth™ software (Google Inc., Mountain View, CA). The GPS coordinates were then extracted from the software, and the sampling sites (plants) inside the greenhouse were located using a Garmin GPSmap 60CSx (Garmin, Olathe, KS). Each sample plant was then marked with a piece of ribbon to ensure consistent sampling over time. Maintenance practices affecting sample plants, such as removing shoots, picking fruits or opening windows above plants, were recorded at the time of sampling. Temperature, relative humidity, light intensity and airflow were recorded at each sampling site with a Fisher Scientific Enviro-Meter™. Samples were collected once a week for 8 weeks commencing in June 2008.

2.6 Analysis of plant volatiles by GC-MS

Aliquots of Porapak Q extracts were analyzed by a Varian Saturn 2000 Ion Trap GC-MS fitted with a DB-5 column (30 m × 0.25 mm ID; J&W Scientific, Folsom, CA), using the following temperature program: 50 °C for 2 min, 10 °C min^{-1} to 280 °C. Heptyl acetate served as an internal standard. External standards were (*Z*)-3-hexenyl acetate [obtained by esterification of (*Z*)-3-hexen-1-ol (Aldrich 98%) with acetic anhydride and pyridine], (*E*)-β-ocimene (27 : 69, *Z* : *E*; International Flavour and Fragrances, New York, NY) and β-caryophyllene (Sigma).

2.7 Calibration and program properties of the zNose™

The zNose system was tuned prior to its application in each experiment. Each of (*Z*)-3-hexenyl acetate, (*E*)-β-ocimene and β-caryophyllene was diluted in pentane, and 0.1, 0.2, 0.4 and 0.8 µL aliquots containing 20, 40, 80 and 160 ng, respectively, of chemicals were injected in each of five replicates into the zNose™ via the zNose™ 3500 sample injector to establish calibration curves.

In the research greenhouse experiment, the zNose™ inlet, valve and initial column temperature were 200 °C, 165 °C and 40 °C respectively. During analyses, the column temperature was increased at 10 °C s^{-1} to 200 °C. The SAW sensor was kept at 50 °C and the trap at 250 °C. The helium flow during the 10-s sampling period was set at 3.00 mL min^{-1}. The sampling period was set for 10 s at a sample flow of 20 mL min^{-1}, after which the system switched to 20 s of data acquisition. Thereafter, the sensor was heated to 150 °C for 30 s, and the parameters (see above) were reset. In the commercial greenhouse experiment, the SAW sensor was kept at 40 °C. The sampling period was set for 60 s at a sample flow of 20 mL min^{-1}. For standardized sampling, a stainless steel tube (15 × 20 cm) was affixed to the zNose™ inlet, ensuring a consistent distance from the plants during sampling. The tube was cleaned and baked at 250 °C for 2 h prior to each sampling period.

2.8 Statistical analyses

Quantities of indicator volatiles from control and treatment plants 1 h prior to placement of larvae on treatment plants were analyzed by ANOVA (SPSS v.16). Data obtained in the greenhouse experiment were analyzed with generalized estimating equations (GEEs, R v.2.9.0)[22] to determine changes in volatile emissions of treatment and control plants. GEE models are commonly used

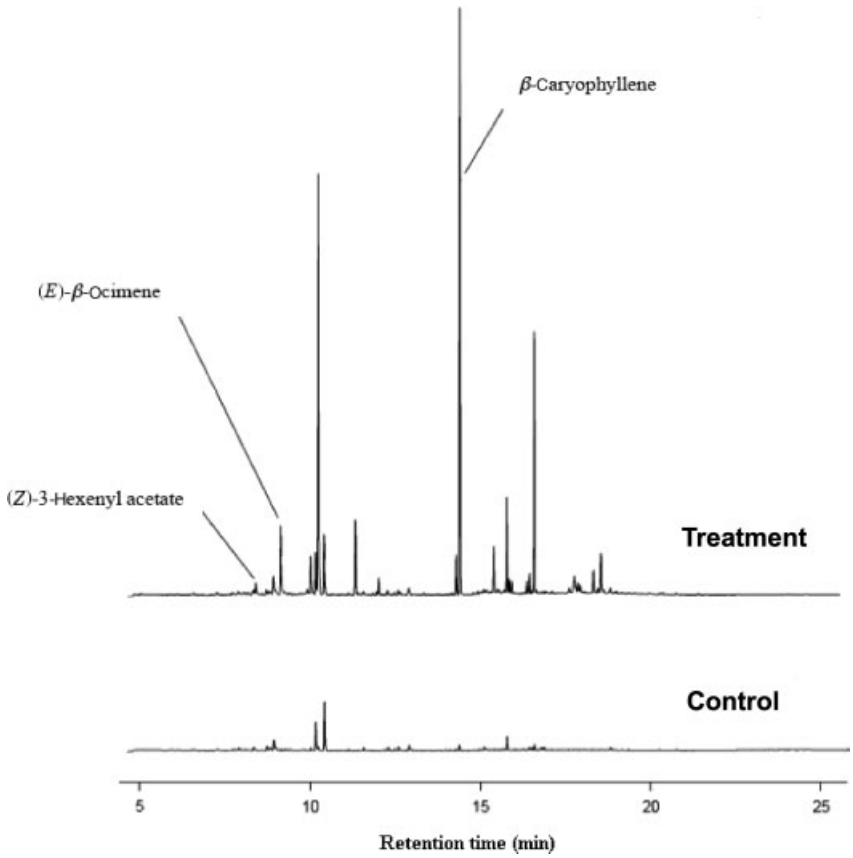

Figure 2. Representative gas chromatograms of headspace volatiles of a tomato plant either fed on by 20 cabbage looper larvae (upper trace) or void of larvae.

for the analysis of longitudinal data. They are robust for missing data, and for data obtained at irregular intervals, as in this study. GEEs make no distributional assumptions on data, and estimates are the same as those produced by regression analyses when the dependent variable is normally distributed and no within-response correlation is assumed.

In this study, time was considered a factor (with three and seven levels in the research greenhouse and commercial greenhouse, respectively), as opposed to a continuous variable. This allowed more flexibility for the model. In the commercial greenhouse experiment, the incidence of herbivores or their damage, maintenance practices (open window, removing shoots or picking fruits), environmental factors (temperature, RH, light intensity and airflow) and time were each considered a factor. The following model was fitted to the data to explain changes in indicator chemicals over time with respect to the treatment the plants received:

$$\Delta Chemical = \beta_0 + \beta_1(treatment_1) + \cdots + \beta_n(treatment_n) + \beta_{n+1}(time_1) + \beta_{n+2}(time_2) + \cdots + error$$

In this equation, β_1 to β_5 represent quantitative changes in indicator chemical level with a unit change in treatment. Linear discriminant analysis (LDA) was used for classification of volatile emission levels in control and treatment groups (using R v.2.9.0).

3 RESULTS

3.1 Laboratory experiment

There were both quantitative and qualitative differences in the emission of volatiles from plants infested with *T. ni* larvae and uninfested control plants (Fig. 2). (*Z*)-3-Hexenyl acetate, (*E*)-β-ocimene and β-caryophyllene were selected as indicators of herbivory. All of them had previously been reported in volatile blends of tomato plants.[23–25] None of the indicator volatiles was detected in empty bags.

3.2 The zNose™ calibration

The calibration curve for each of the indicator volatiles was linear, as follows:

(*Z*)-3-hexenyl acetate

$$y = 214.78x - 805.78,\ r^2 = 0.9984$$

(*E*)-β-ocimene

$$y = 189.39x + 1098.6,\ r^2 = 0.9905$$

β-caryophyllene

$$y = 162.3x + 3055.1,\ r^2 = 0.9703$$

where y = SAW kilocount and x = nanogram.

3.3 Research greenhouse experiment

Prior to placement of larvae on treatment plants, there were no significant quantitative differences in the amount of volatiles emitted from plants in treatment and control groups. Feeding by larvae on treatment plants for 6, 12 and 24 h increased the amount of indicator volatiles, which also changed over time (Fig. 3; Table 1). For example, the emission level of (*Z*)-3-hexenyl acetate increased during the first time period (6–12 h) but decreased during the second (12–24 h) (Fig. 3).

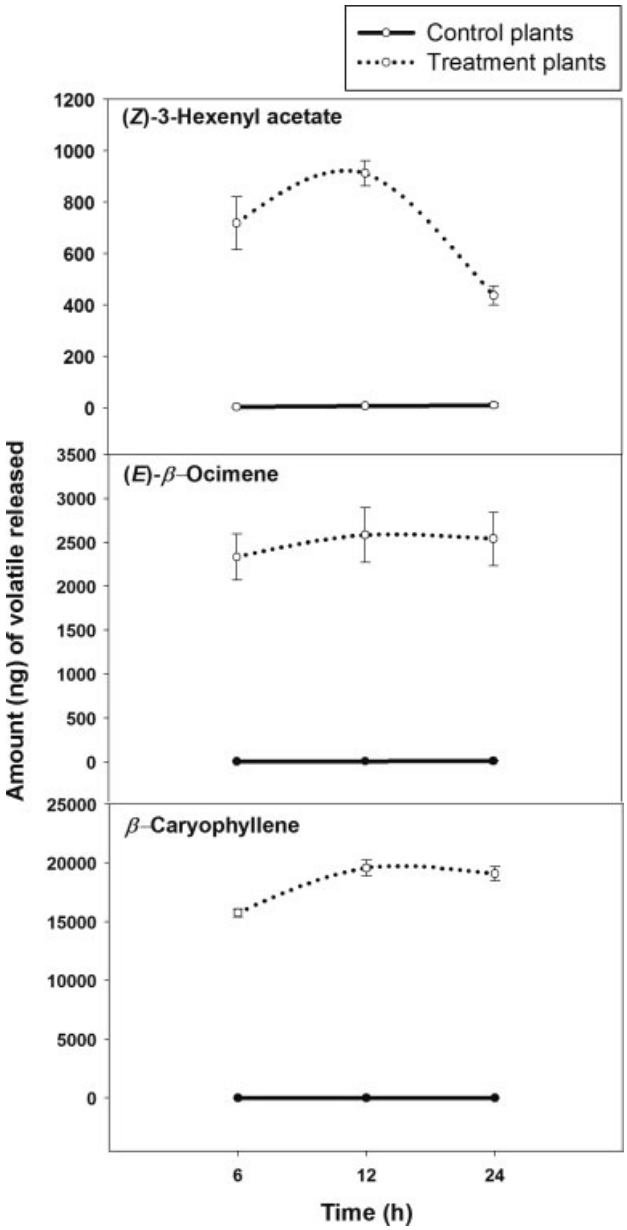

Figure 3. Mean (± SE) amounts (ng) of (*Z*)-hexenyl acetate, (*E*)-β-ocimene and β-caryophyllene emitted over time by tomato plants in the research greenhouse experiment. Plants were not infested (control) or each was infested with 20 third-instar larvae of the cabbage looper. For statistical analyses of data, refer to Table 1.

3.4 Classification of plants as not infested or infested

Based on the emission level of indicator volatiles, plants could readily and unequivocally be diagnosed as not infested or infested with *T. ni* larvae feeding for 6 h (Table 2).

3.5 Commercial greenhouse experiment

Throughout the sampling period, *T. ni* larvae were encountered only on one of the sampling plants (Fig. 4). On other occasions, symptoms of damaged plant foliage were found, but no larvae (Fig. 5). No plant disease was found at any sampling sites.

The level of ~3000–4000 ng of β-caryophyllene emitted from uninfested plants significantly increased to ~10 000 ng when plants had damage symptoms, and to ~60 000 ng when plants

Table 1. Generalized estimating equation (GEE) regression coefficients of indicator chemical emissions over time from tomato plants in response to feeding by cabbage looper larvae

Indicator chemical	Factors	Estimate	Std error	Wald test	*P*
(*Z*)-3-Hexenyl acetate	Intercept	7.28	5.50	1.75	0.19
	Treatment	708.13	7.22	9627.68	<0.001
	Time (6–12 h)	98.76	12.99	57.79	<0.001
	Time (12–24 h)	−136.86	18.37	55.51	<0.001
(*E*)-β-Ocimene	Intercept	−49.46	6.83	52.5	<0.001
	Treatment	2435.27	44.16	3040.8	<0.001
	Time (6–12 h)	126.95	15.41	67.9	<0.001
	Time (12–24 h)	106.19	12.79	18.9	<0.001
β-Caryophyllene	Intercept	−1220.1	139.4	76.6	<0.001
	Treatment	18 191.6	71.2	65 353.5	<0.001
	Time (6–12 h)	1913.7	220.6	75.3	<0.001
	Time (12–24 h)	1665.5	189.2	77.5	<0.001

Table 2. Linear discriminant analysis (LDA) of tomato plant volatiles after 6 h of feeding by cabbage looper larvae

	Not infested Mean	Infested Mean	LDA coefficient	Misclassification rate (%)
(*Z*)-3-Hexenyl acetate	458.25	1176.70	0.01139335	0
(*E*)-β-Ocimene	1173.775	3515.600	0.00427449	0
β-Caryophyllene	1310.175	17 062.375	0.00370752	0

were fed on by *T. ni* larvae. Opening windows above plants did not have a significant effect, but removing shoots and picking fruits did (Table 3).

Emission levels of other indicator chemicals in response to feeding *T. ni* larvae also differed compared with herbivore-free plants, but differences were less pronounced. While the presence of *T. ni* larvae had a significant effect on emission levels of (*Z*)-3-hexenyl acetate, no significant difference was found between plants with just damage symptoms and undamaged plants. Opening windows and environmental factors had no significant effect on the emission level of (*Z*)-3-hexenyl acetate (Table 4).

Emission levels of (*E*)-β-ocimene from plants that showed damage symptoms or that were fed on by *T. ni* larvae were significantly altered compared with intact herbivore-free plants. Environmental factors did not significantly affect emission levels of (*E*)-β-ocimene, but fruit picking did (Table 5). For all indicator chemicals, time was found to have a significant effect, indicating that volatile emission levels change as plants grow.

Table 3. Generalized estimating equation (GEE) regression coefficients of β-caryophyllene emission over time from tomato plants in the commercial greenhouse experiment in response to herbivory by cabbage looper larvae, variation in environmental factors and crop maintenance practices

	Estimate	Std error	Wald test	*P*
Intercept	5.43e+03	1.24e+03	1.92e+01	<0.001
Looper feeding	5.60e+04	1.65e+02	1.16e+05	<0.001
Looper-caused damage symptoms	8.71e+03	1.32e+03	4.36e+03	<0.001
Open window	2.38e+02	6.20e+02	1.50e−01	*0.70149*
Removing shoots	6.02e+02	1.51e+02	1.59e+01	<0.001
Picking fruits	−9.62e+02	1.42e+02	4.61e+01	<0.001
Temperature	−2.62e+01	2.47e+01	1.13e+00	*0.28878*
Relative humidity	−7.08e+01	1.89e+01	1.41e+01	<0.001
Light intensity	9.99e−02	5.27e−01	4.00e−02	*0.84981*
Airflow	3.45e+01	4.58e+01	5.70e−01	*0.45168*
Time (week 1–week 2)	−2.96e+02	1.51e+02	3.87e+00	<0.05
Time (week 2–week 3)	1.68e+03	1.22e+02	1.88e+02	<0.001
Time (week 3–week 4)	1.26e+03	1.58e+02	6.40e+01	<0.001
Time (week 4–week 5)	1.23e+03	1.38e+02	7.83e+01	<0.001
Time (week 5–week 6)	1.29e+03	1.66e+02	6.00e+1	<0.001
Time (week 6–week 7)	1.21e+03	1.52e+02	6.32e+01	<0.001
Time (week 7–week 8)	−1.90e+02	1.74e+02	1.19e+00	*0.27450*

4 DISCUSSION

The data support the conclusion that herbivore-induced volatiles can be utilized for detecting an insect infestation in greenhouses, and that the zNose™ as a portable and ultrafast gas chromatograph is applicable for monitoring both quantitative and qualitative changes in plant emission of HIPVs.

In UBC's horticultural greenhouse, the zNose™ proved capable of (i) revealing differential volatile emissions from plants with or without herbivory, (ii) detecting herbivory at an early stage and (iii) monitoring changes in volatile emissions from infested plants over time. In the commercial greenhouse, *T. ni* larvae were encountered only once during the sampling period. However, their effect was significant for all indicator chemicals. Specifically, the emission of β-caryophyllene increased almost 20-fold in response to the presence of *T. ni* larvae. Damage symptoms had significant effects on emission levels of (*E*)-β-ocimene and β-caryophyllene, but not on those of (*Z*)-3-hexenyl acetate. However, interpreting these results is difficult because the exact time of infestation remains unknown.

Among crop maintenance practices, ventilation was a key concern for all growers. They wanted to know whether indi-

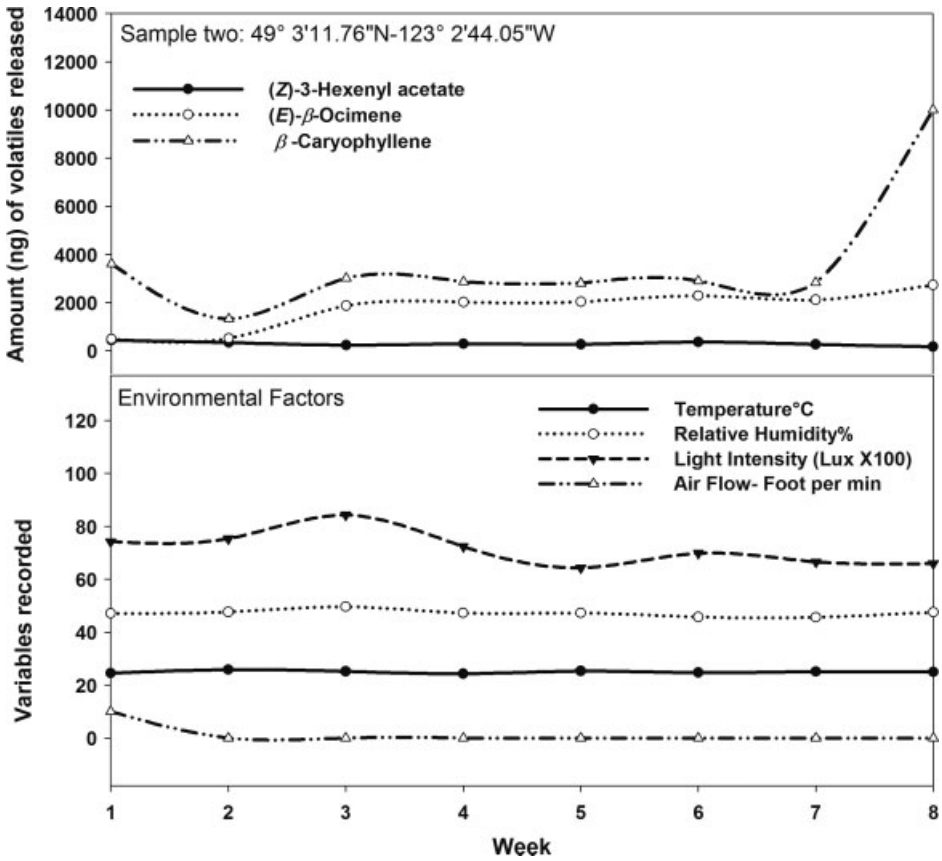

Figure 4. Variation in indicator chemicals and in environmental and crop-maintenance-related factors. Symptoms of plant damage were observed at week 8 on sampling plant site No. 2. For statistical analyses of data, refer to Tables 3 to 5.

Table 4. Generalized estimating equation (GEE) regression coefficients of (*Z*)-3-hexenyl acetate emission over time from tomato plants in the commercial greenhouse experiment in response to herbivory by cabbage looper larvae, variation in environmental factors and crop maintenance practices

	Estimate	Std error	Wald test	*P*
Intercept	408.1501	93.2760	19.15	<0.001
Looper feeding	41.9823	13.8600	9.17	<0.01
Looper-caused damage symptoms	4.8714	8.7314	0.31	*0.5769*
Open window	50.1083	44.1009	1.29	*0.2559*
Removing shoots	658.0727	23.5872	778.38	<0.001
Picking fruits	−304.4295	16.2413	351.34	<0.001
Temperature	−0.7988	1.5553	0.26	*0.6075*
Relative humidity	−1.7296	1.5446	1.25	*0.2628*
Light intensity	0.0229	0.0823	0.08	*0.7811*
Air flow	−2.0955	3.7390	0.31	*0.5752*
Time (week 1-week 2)	−77.3690	14.7965	27.34	<0.001
Time (week 2-eek 3)	−90.6191	14.2321	40.54	<0.001
Time (week 3-week 4)	−39.3291	12.7390	9.53	<0.001
Time (week 4-week 5)	−62.3358	11.1357	31.34	<0.001
Time (week 5-week 6)	21.1675	14.3251	2.18	*0.1395*
Time (week 6-week 7)	−79.9492	11.4569	48.70	<0.001
Time (week 7-week 8)	−158.2364	14.9140	112.57	<0.001

Table 5. Generalized estimating equation (GEE) regression coefficients of (*E*)-β-ocimene emission over time from tomato plants in the commercial greenhouse experiment in response to feeding by cabbage looper larvae, variation in environmental factors and crop maintenance practices

	Estimate	Std error	Wald test	*P*
Intercept	1371.752	784.186	3.06	<0.05
Looper feeding	3524.058	112.122	987.88	<0.001
Looper-caused damage symptoms	986.089	68.117	201.99	<0.001
Open window	−239.640	378.154	0.40	*0.53*
Removing shoots	119.818	83.635	2.05	*0.15*
Picking fruits	−295.688	47.253	39.16	<0.001
Temperature	−12.995	15.076	0.74	*0.39*
Relative humidity	−11.859	11.222	1.12	*0.29*
Light intensity	−0.138	0.215	0.41	*0.52*
Air flow	32.121	31.182	1.06	*0.30*
Time (week 1 – week 2)	86.261	53.595	2.59	*0.11*
Time (week 2 – week 3)	1560.005	30.102	2685.80	<0.001
Time (week 3 – week 4)	1626.192	54.465	891.46	<0.001
Time (week 4 – week 5)	1628.971	51.159	1013.86	<0.001
Time (week 5 – week 6)	1835.454	64.405	812.18	<0.001
Time (week 6 – week 7)	1706.823	65.817	672.51	<0.001
Time (week 7 – week 8)	1371.301	69.834	385.59	<0.001

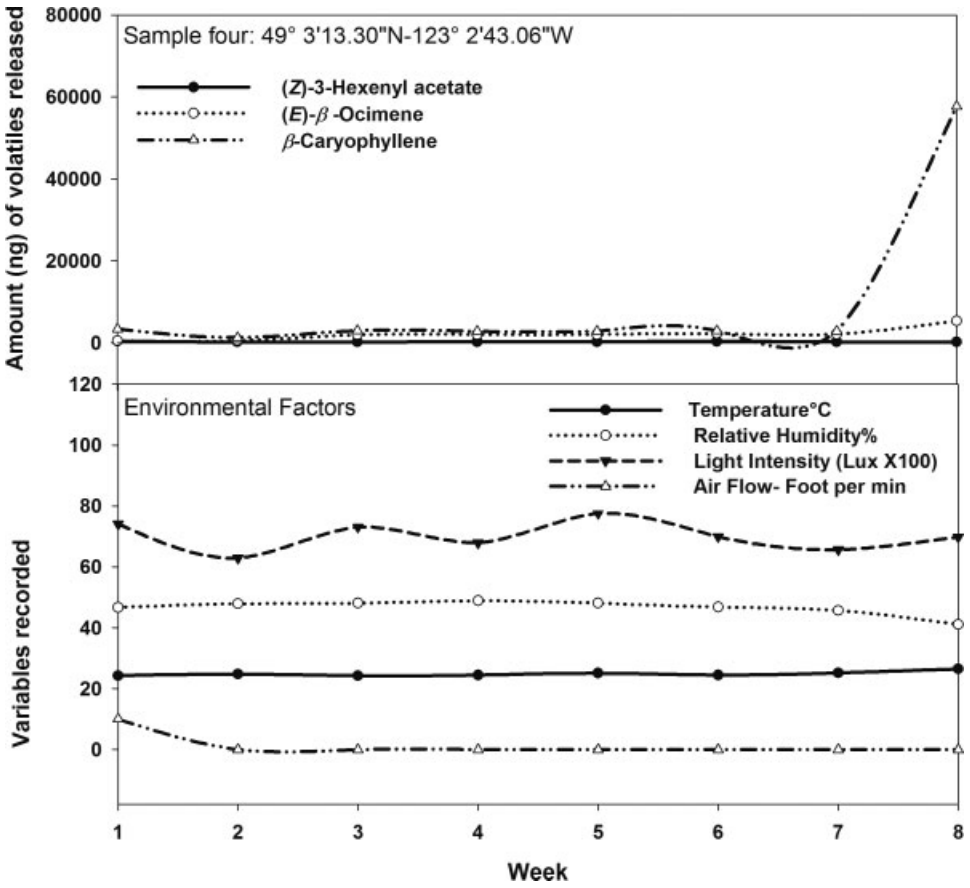

Figure 5. Variation in indicator chemicals and in environmental and crop-maintenance-related factors. Cabbage looper larvae were observed at week 8 on sampling plant site No. 4. For statistical analyses of data, refer to Tables 3 to 5.

cator chemicals would still be detectable when windows are open. The present results clearly indicate that opening windows and screens does not significantly affect emission or detection of volatiles. Removing shoots and picking fruits, on the other hand, were found to significantly alter the emission of almost all indicator chemicals. Some greenhouse practices cause an increase in the emission of indicator volatiles,[26] but the effect of these practices is modest compared with that of herbivory.

Light intensity and airflow did not significantly alter emission levels of any indicator chemical, but relative humidity did alter the emission of β-caryophyllene. Environmental factors affect the release of HIPVs,[11,12] but their impact in the present study was moderate. Time, in contrast, was an effective and significant factor. Temporal variation in the emission levels of volatiles[27] and rhythmic emission of volatiles at different times of day[28–30] have previously been reported. Insect herbivory, however, remains the most significant factor.

The three chemicals selected in the present study as indicators of herbivory have previously been associated with the response of plants to biotic or abiotic stresses. (*Z*)-3-Hexenyl acetate is a green-leaf volatile (GLV) that plants form through the hydroperoxide lyase pathway of oxylipin metabolism when tissue is disrupted.[31] GLVs activate the production of jasmonic acid, which primes the plant's defense against biotic stresses.[32] (*E*)-β-Ocimene as a monoterpene belongs to the most abundant and varied class of HIPVs. *Medicago truncatula* Gaertn. plants undergoing herbivory release (*E*)-β-ocimene from both insect-damaged and undamaged leaves, suggesting that (*E*)-β-ocimene plays an active role in indirect defense against insects.[33] Finally, β-caryophyllene is a well-known HIPV both above ground[34] and below ground.[35]

Whether plant-derived cues are intelligent or reflexive, or whether they have an evolutionary *raison d'être*[36,37] remains controversial, but it is accepted that most plants are capable of responding to changes in their surroundings and of conveying information about their overall health.[38] Plants respond to events as subtle as insects crawling over their foliage,[39] or depositing eggs,[40,41] and they stage a different type of defense according to the offending organisms, which may include microbes, nematodes, arthropods or mammals.[42] Plants certainly respond to insect herbivory (Refs 43 to 45 and this study), and the resulting indicator chemicals in their volatile emissions could be utilized in pest insect monitoring systems.

Key requisites for using plant-derived cues instead of insect presence in insect monitoring systems are (i) the detectability of cues, (ii) the reliability of recordings and (iii) instantaneous results. The zNose™ system appears to meet these criteria, based on the present data obtained in a research greenhouse and a commercial greenhouse. Only 6 h after the onset of herbivory, the zNose™ detected the resulting changes in plant volatiles emission (Fig. 5), reliably discerned between plants with or without herbivory (Table 2) and immediately made data available for assessment. Also, in the commercial greenhouse, in spite of variations in microclimate, the zNose™ produced consistent results.

In conclusion, HIPVs appear to be promising as reliable indicators of plant health. The information they convey could become an integral part of sophisticated pest monitoring systems with advanced technologies such as the zNose™.

ACKNOWLEDGEMENTS

The authors thank Dr Jocelyn Millar for providing helpful feedback on the manuscript. This project was funded in part by an NSERC Industrial Postgraduate Scholarship to SM in conjunction with the BC Greenhouse Growers' Association.

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