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Monitoring Crop Health Status at Greenhouse Scale on the Basis of Volatiles Emitted from the Plants

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This review focuses on the monitoring of crop health status at greenhouse scale, based on the measurement of volatile organic compounds (VOCs) emitted from the plants. The review includes the most important factors that affect the emission of these VOCs from greenhouse crops. Since both, stress factors as well as non-stress factors have an effect on the emission, they are covered separately. The review provides an overview of processes that affect the gas balance of plant VOCs in the greenhouse including the loss processes. These processes are considered as important since they contribute to the time-dynamic concentration profiles of plant-emitted VOCs. In addition, we review the most popular techniques currently in use to measure volatiles emitted from plants with emphasis on greenhouse application. Dynamic sampling in combination with gas chromatography coupled to mass spectrometry is considered as most appropriate method for application at greenhouse scale. It is recommended to evaluate the state-of-the-art in the fields concerned with this method and to explore the development of a new instrument based on the specific needs for application in greenhouse practice. However, to apply such an instrument at greenhouse-scale remains a challenge, mainly due to the high costs associated with it.

Keywords : crop, greenhouse, inspection, monitoring, plant health, volatiles

INTRODUCTION

Greenhouse cultivation offers the advantage of a year-round production which stabilizes and secures the growers' income. On the other hand, it also requires a vast amount of labour throughout the entire year. Labour intensive tasks include harvest and crop maintenance. Also crop inspection, the task to detect emerging factors that may harm the crop, requires a substantial amount of time and requires skilled personnel which in-turn leads to high costs.

The traditional inspections of greenhouse crops are done by greenhouse personnel and rely on visual symptoms on the crop. This working method has its limitations. Namely, visual symptoms are often difficult to notice, or when seen, it may be too late to correct the problem. For example,

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early signs of pathogen infections and herbivore infestations often appear on the abaxial side of leaves or on stem parts that are hidden by the foliage. When these symptoms remain unnoticed, such infections or infestations may disperse rapidly and result in irretrievable crop damage.

These limitations have led to the emergence of a wide range of methods to improve the inspection of greenhouse crops. In an ideal manner, such method would enable the continuous monitoring of individual plants in order to reveal health problems at an early stage. This would enable a grower to take early action, and prevent further crop damage. One approach to monitor the health status of plants is based on the volatile organic compounds (VOCs) emitted from them. This approach is based on previous laboratory-scale studies which established that plants emit different types and amounts of VOCs during their decline in health status (*e.g.* Jansen et al., 2009b).

This review focuses on the monitoring of crop health status at greenhouse scale, based on the measurement of plant-emitted VOCs in greenhouse air. The first section of the review is basically plant-oriented and covers knowledge related to the emission of VOCs from greenhouse crops. The second section of this review is mainly greenhouse-oriented and covers aspect related to factors that affect the gas balance of plant VOCs in the greenhouse including their loss processes. The third section of this paper evaluates this concept from a technical point of view and reviews the most popular techniques currently in use to measure volatiles emitted from plants with emphasis on the application of these techniques in greenhouse practice. The last section of this paper deals with trends and future possibilities and gives an outlook on the possibilities for crop health monitoring based on plant-emitted VOCs.

EMISSION OF VOCs FROM GREENHOUSE CROPS

The first part of this section reviews factors which affect the emission of VOCs from greenhouse crops. The second part is addressing the specificity of stress-induced emissions and explains how plant-emitted volatiles can be used to characterize the stress factors that contribute to plant health problems.

Factors affecting the emission of VOCs from greenhouse crops

An extensive overview of factors affecting VOC emissions from crop and non-crop plants have appeared in the literature (*e.g.* Kesselmeier and Staudt, 1999). In this paper, we explain those factors that affect the emission of plant volatiles from crop plants grown in greenhouses. These factors are divided into two categories. The first category includes factors that have been shown to correlate with plant health. Since plant health is generally associated with responses to stresses, we termed these factors as “stress factors”. The second category includes factors that do not show this correlation. These factors were termed as “non-stress factors”.

The term “stress factor” is extensively used in this review paper. However, this term is subjective and used with various meanings in different situations (see Gaspar et al., 2002). Since the aim of greenhouses is to produce, we define stress factors as those factors that adversely affect crop productivity.

Stress factors affecting the emission of VOCs from greenhouse crops

A greenhouse crop might be challenged with numerous stress factors. However, the number of stress factors that generally occur in greenhouse cultivation is limited, primarily due to monoculture and environmental control. The effects of these generally occurring stress factors in terms of plant volatile emissions are described below. The factors are divided into two types, biotic and abiotic stress factors.

Biotic stress factors are those factors that are caused by biological sources. Two biotic stress factors that generally occur in greenhouse crop production are herbivore infestation and pathogen infection. The herbivore-induced emission of VOCs has been widely studied over the past few decades. Most of these studies were performed at the laboratory scale. In these studies, numerous

plant species were subjected to insect-, mite-, and snail-species to study the plant-response in terms of volatile emissions. Usually, these herbivores were applied on aerial parts of the plants (*e.g.* Wei et al., 2007) but herbivores were also applied on the root zone of plants (*e.g.* Rasmann and Turlings, 2007). In general, these studies reported a significant increase in the types and amounts of VOCs emitted after herbivore infestation.

In contrast to the large amount of studies that report about herbivore-induced emission, only few studies reported about pathogen-induced emissions. These limited amount of studies include the increased emission of VOCs from silver birch (*Betula pendula*) upon a fungal infection (Vuorinen et al., 2007), from tomato plants (*Lycopersicon esculentum*) upon a viral infection (Deng et al., 2004), and from tobacco plants (*Nicotiana tabacum*) upon a bacterial infection (Heiden et al., 2003). In these studies, the aerial parts of the plants were infected. However, also root infections may result in increased emissions of certain VOCs. Preliminary experiments using cucumber plants (*Cucumis sativus*) inoculated with the root pathogen *Pythium aphanidermatum* did indicate such systemic plant response (Jansen et al., 2007).

Abiotic stress factors are those factors which are caused by non-biological, environmental forces. Water deficiency, nutrient deficiency, and air pollution are abiotic stress factors that may occur in greenhouse practice, and have a negative effect on crop health (Peet, 1999). Greenhouse crops might be monitored for the presence of nutrient deficiency based on volatile emissions since several studies have indicated an effect of fertilization rate on volatile emission. For instance, Gouinguené and Turlings (2002) reported that the emission of volatiles was minimal when corn plants (*Zea mays*) were grown under low nutrition, even when results were corrected for plant biomass. Greenhouse crops might also be monitored for water deficiency based on VOCs emitted from drought-stressed plants since several studies demonstrated an increase in the amount and types of plant volatile emitted after drought (*e.g.* Ebel et al., 2006). Emitted substances after drought were characterised by alcohols and aldehydes, probably as a result of the gradual collapse of the cellular structure of the plant leaves during the drying process. Finally, crop might be monitored for air pollution damage by plant-emitted volatiles since several researchers (*e.g.* Wildt et al., 2003), have demonstrated that harmful ozone concentrations induced an increased emission of several VOCs from a number of plant species including sunflower (*Helianthus annuus*) and Scots pine (*Pinus sylvestris*).

Non-stress factors affecting the emission rate of VOCs from greenhouse crops

Temperature and light are well described factors that affect the VOC emission rate from plants. These factors can be stressful for plants during periods of excessive temperature and/or light. However, due to climate control, these excessive temperature- and/or light-conditions are generally avoided in modern greenhouse practice and thus regarded as “non-stress”. The effects of temperature and light in terms of volatile emissions are described below.

Temperature increases the emission rates of most VOCs exponentially up to an optimum by enhancing the biosynthetic enzymatic activities, by raising the VOC vapour pressure and by decreasing the resistance of emission pathway (Niinemets et al., 2004).

Among the studies that have examined the light dependency of plant volatile emission, there have been mixed findings, with evidence that some emissions are mainly temperature controlled (Loreto et al., 2000), while others are also significantly affected by light (*e.g.* Schuh et al., 1997). However, most of the literature suggests that dependencies on temperature are much stronger than those on light. Interestingly, similar chemical classes of VOCs might respond quite different to light. When the concentration of the sesquiterpene α-copaene was examined at greenhouse scale, a clear diurnal emission pattern was evident, with an increase during the day and a decrease at night. However, the concentration of another sesquiterpene (β-caryophyllene) remained constant (Jansen et al., unpublished data). A similar observation was obtained during laboratory-scale studies in which volatiles from tomato plants were analysed (Maes and Debergh, 2003). The

investigators suggested that α-copaene requires light for its biosynthesis and/or emission. In summary, temperature and light might have a strong effect on the emission of certain VOCs. Since temperature and light fluctuate in horticulture practice, these two factors have to be taken in account when correlating the concentration of such volatiles to any plant-health issue.

Besides fluctuations in temperature and light, other "non-stress factors" generally occur in greenhouses that affect the emission of VOCs. Such "non-stress factors" include elevated CO_2 concentrations (Loreto et al., 2006), phenological events such as budding, flowering, and fruit setting (Peñuelas and Llusià, 2001), and activities of greenhouse workers such as pruning and fruit picking (Jansen et al., 2009a).

Specificity of stress-induced emissions

A monitoring system that detects plant health problems at an early stage would enable a grower to take early action. The opportunity to identify the stress factor would be of great value to such system as it would allow to decide on the proper control measure such as the release of natural enemies of white fly (*Bemisia tabaci*) in case the stress factor was identified as "white fly infestation".

To identify a stress factor through the measurement of plant-emitted VOCs requires the emission of highly specific chemical substances upon the onset of stress, or a highly specific time course of the stress-induced change in VOC emissions.

The emission of highly specific substances seems unlikely since it is well established that emission of many of the same substances is induced upon different biotic and abiotic stress factors. For example, most of the substances reported upon pathogen infection of tomato plants were also reported upon herbivore infestation of tomato plants (Deng et al., 2004; Kant et al., 2004). Same substances were also induced when different plant species were challenged with a similar stress factor. For example, herbivore damage of the plant species cucumber, apple, lima bean, corn, potato, tobacco, and cotton all induced an increase in the emission of (*E*)-β-ocimene (Paré and Tumlinson, 1999). Chemical substances which are frequently reported after a stress-induced change in VOC emissions — independent of the stress factor and independent of the plant species — are presented in Fig. 1. This list is certainly not complete. But, to the best of our knowledge, no chemical substance has ever been exclusively ascribed to one particular stress factor. Therefore it is improbable that stress factors can be identified based on plant-emitted VOCs only.

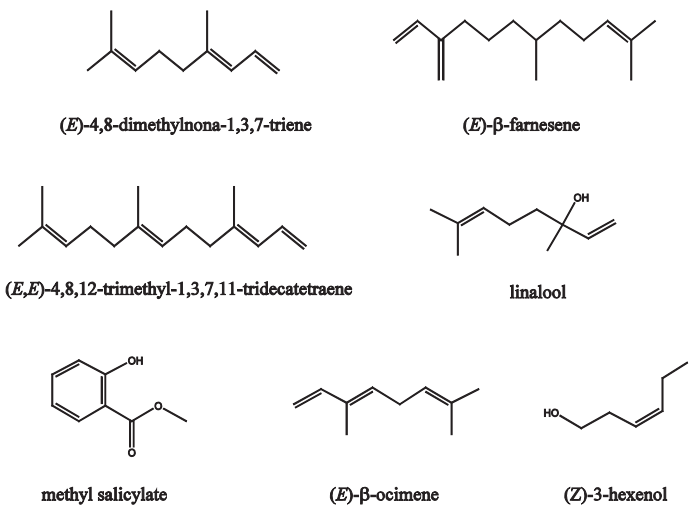

Fig. 1 Chemical substances which are frequently reported after a stress-induced change in VOC emissions from plants.

Several researchers addressed the time course of stress-induced volatile emissions from plants (*e.g.* Kunert et al., 2002). These studies demonstrated that the emission of certain substance can increase directly after the onset of stress followed by rapid returns to low emission rates while increased emissions of other substances were delayed for some hours up to several days after the onset of stress. The time period between the first response and the delayed response in terms of increased volatile emissions might indicate the stress factor exposed to the plant. For instance, this time period was dissimilar for tobacco plants in response to different strains of *Pseudomonas syringae* (Huang et al., 2003). However, to the best of our knowledge, no time course has ever been exclusively ascribed to one particular stress factor. Therefore it is improbable that stress factors can be identified based on the time course of stress-induced volatile emissions only. But, how stress-induced changes in VOC emissions might be used to characterize the stress factor is explained below.

The first way to characterize the stress factor is based upon the chemical substances present in the mixture of the plant-emitted VOCs upon the onset of the stress. These substances are to a large extent related to the plant-structure that emits these VOCs. Previous studies suggested an arbitrary classification of confined sub-structures, and the entire plant as emitting structure.

There are several examples in which the emission from confined plant sub-structures changes upon stress. A first example is the release of VOCs from local plant tissue after damage of involved cell-membranes due to *e.g.* herbivore infestation (Wei et al., 2007) or pathogen infection (Wildt et al., 2003). Damage of these cell-membranes will result in the local emission of several C_6-alcohols and C_6-aldehydes at the site of damage. The emissions of these C_6-compounds originate from the oxidative cleavage of C_{18}-fatty acids in the presence of oxygen and enzymes such as lipoxygenases (Hatanaka, 1993; Fall et al., 1999; Matsui, 2006). These C_6-compounds thus characterize stress factors in which damage of cell-membranes (that contain fatty acids) plays an important role. Also non-stress factors may damage cell-membranes. For instance, shoot removal was responsible for the detection of C_6-compounds at greenhouse-scale.

A second example of plant sub-structures that emit VOCs during stress is the local emission of VOCs from damaged trichomes due to *e.g.* herbivore infestation (Loughrin et al., 1994), or pathogen infection (Jansen et al., 2009b). These trichomes are outgrowths of the plant epidermis and collectively constitute the pubescence of the plant surface. A local damage of these plant sub-structures will result in the local emission of terpenes that are stored in them. These terpenes thus characterize stress factors in which damage of trichomes plays an important role.

Also “non-stress factors” may damage trichomes. For instance, fruit picking resulted in the damage of trichomes and a subsequent increase in the concentration of mono- and sesquiterpenes at greenhouse-scale (Jansen et al., 2009a).

Plants are attacked at different parts in different ways by the multitude of stress factors. As a result, it can be expected that some types of plant sub-structures are involved, while others are not, depending on the stress factor. As a consequence, the chemical substances associated with the particular type of sub-structure might thus be used to characterize the stress factor that harms the plant.

The emission of methyl salicylate can be cited as an example in which the entire shoot can be regarded as emitting structure. The emission of this volatile phytohormone is generally believed to increase, but only after a certain period following the local inoculation of, or local application of herbivores (Röse et al., 1996; Shulaev et al., 1997; Kant et al., 2004). An example of the delayed increase in emission of methyl salicylate from tomato plants after inoculation with the fungal pathogen *Botrytis cinerea* is given in Fig. 2.

Instantaneous damage to plants, *e.g.* the punching of holes within a short time period, did not result in increased emission of methyl salicylate from tomato plants (Deng et al., 2005b). Probably, a stress factor needs to be continuously sustained in order to increase the emission of methyl

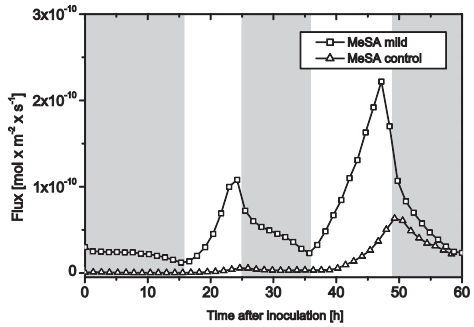

Fig. 2 Typical time courses of the emission flux of methyl salicylate from tomato plants inoculated with the fungus *Botrytis cinerea* and for control plants (Jansen et al., unpublished data). Squares refer to an experiment in which plants developed a mild infection. Triangles refer to a control experiment. Shaded areas represent dark periods. Measurements were performed using gas chromatography coupled to mass spectrometry.

salicylate and/or other stress-associated VOCs. It is also believed that chemical signals derived from the stress factor, *e.g.* derived from herbivore secretions, are required to increase the emission of methyl salicylate and/or other stress-associated VOCs (Arimura et al., 2005). Thus methyl salicylate might be used to characterize stress factors in which continuously sustained damage is, and/or chemical signals are involved.

Besides the chemical substances present in the mixture of the plant-emitted VOCs, also the time course of the stress-induced change in VOCs emissions may characterize the stress factor.

For example, severe *B. cinerea* infections resulted in a large increase in emissions a few hours after inoculation while mild infections resulted in a small increase in emissions several hours after inoculation (Jansen et al., 2009a). The importance of the duration and the intensity of damage as main factor with respect to stress-induced changes in emissions were also elegantly demonstrated using MecWorm, a robotic device designed to reproduce tissue damage caused by herbivore attack (Mithöfer et al., 2005). Besides local emissions, also systemic emissions depend on the duration and intensity of damage. For instance, the emission of systemically emitted volatiles from Brussels sprouts was depending on the duration of caterpillar feeding (Mattiacci et al., 2001).

As mentioned before, the opportunity to identify the stress factor would be of great value to a plant health monitoring system. This section explained how plant-emitted volatiles can be used to characterize the stress factors that contribute to plant health problems. This might be sufficient since the diversity of stress factors that occur per greenhouse is often limited, primarily due to monoculture and environmental control.

FACTORS THAT AFFECT THE GAS BALANCE OF PLANT VOCs IN THE GREENHOUSE

This section covers aspect related to factors that affect the gas balance of plant VOCs in the greenhouse. The crop inside of the greenhouse is probably the most important source of plant volatiles in a greenhouse. However, the gas balance of plant VOCs in the greenhouse might also be affected by the unpredictable transfer of typical plant VOCs from outside to inside of the greenhouse. Greenhouse ventilation is likely to be the most important source in that respect.

Loss processes are regarded as important aspects of the gas balance since they contribute to the time-dynamic concentration profiles of plant-emitted volatiles. On the one hand, a slow loss will cause the accumulation of VOCs in a greenhouse and thus promote the detection. On the other hand, a fast loss of VOCs enables the detection of short time dynamics which might be required since VOC emissions during stress sometimes appear as burst followed by rapid returns to low

emission rates (*e.g.* Beauchamp et al., 2005; Deng et al., 2005a).

The first loss process for plant-emitted VOCs is the removal of these VOCs by greenhouse ventilation. Ventilation involves removing air from inside the greenhouse and replacing it with external air. This ventilation may be natural —caused by wind and temperature forces— or mechanical, accomplished by using fans.

The second loss process considers the degradation due to gas-phase reactions. In lower atmosphere, the major degradation processes for plant VOCs are reactions with hydroxyl radicals (OH), nitrate radicals (NO_3) and ozone (O_3) leading to a number of breakdown products (Atkinson and Arey, 2003). Such oxidative breakdown of compounds not only affects the concentration of VOCs in the air surrounding unstressed plants but also the concentration of VOCs in the air surrounding stressed plants. For example, it was recently demonstrated that exposure of plants to moderately enhanced O_3 levels resulted in the partial degradation of VOCs emitted upon herbivore infestation (Pinto et al., 2007).

The third process leading to removal of VOCs from greenhouse air is the sorption on air-contact surfaces such as the floor of the greenhouse. Many researchers have shown that material surfaces interact with VOCs (*e.g.* Jorgenson, 1999). Most of this work involved relatively simple test chamber experiments where material surfaces were exposed to VOCs and the concentration in the test chamber was monitored (Huang et al., 2006). The material surfaces in a greenhouse are a complex mixture of materials such as glass, steel, plastics and concrete. Therefore it is difficult to estimate the effect of sorption on air-contact surfaces beforehand.

The fourth process to take into account is the solution of VOCs in water bodies occurring on cold greenhouse surfaces due to condensation. This water originates from plant transpiration and the amount mainly depends on greenhouse climate and plant size. The Henry's Law constant is a key parameter to estimate the maximum amount of VOCs that can be solved into water. This Henry's Law constant for chemical air-water partitioning is defined as the ratio of a chemical partial pressure in air to its mole fraction in water at equilibrium. However, care should be taken since Henry's Law assumes no further chemical breakdown of chemical compounds when dissolved in water.

The fifth process for losses of VOCs is absorption on the plant cuticle. Welke et al. (1998) proved that the plant cuticle can adsorb many VOCs and the amount absorbed is correlated with the concentration in air. This adsorption process might be relevant if the absorbed compounds are metabolised and the uptake potential remains.

The sixth possible loss process for VOCs is the uptake of these compounds due to absorption through the stomata. Uptake of VOCs through stomata requires a lower concentration of the compounds in the stomatal cavity then in the greenhouse air. This concentration difference is important since gasses move along the concentration gradient between the inside and the outside of the leaf. The stomatal cavity is covered by water. Therefore, compounds should be solved in this water and thereafter metabolized in plant tissues to maintain the continuous uptake potential. So, uptake through the stomata of a certain VOC depends on the water solubility of this compound and its metabolism. This loss process might thus be particularly relevant for polar VOCs such as alcohols.

TECHNIQUES TO MEASURE THE EMISSION OF VOCs FROM PLANTS AT GREENHOUSE SCALE

Several excellent papers are available that review the techniques currently in use to measure the emission of VOCs from plants (Tholl et al., 2006; Ortega and Helmig, 2008). However, none of these papers describe how these techniques can be applied to monitor crop health at greenhouse scale. This section is intended to fill this knowledge gap.

In general, the measurement of plant emission consists of three steps: (1) collection of the plant-emitted VOCs, (2) separation of the plant-emitted VOCs in the mixture, and (3)

identification, and/or quantification of the separate VOCs. These three steps are explained below.

Collection of the plant-emitted VOCs

In the first step, a fraction of the compounds emitted from the plants is collected. This sampling step is in general combined with the pre-concentration of the VOCs in the air to achieve the detection limits of commonly applied analytical instruments. Several reviews are dealing with the pre-concentration of VOCs in air (*e.g.* Harper, 2000; Dettmer and Engewald, 2002). Therefore, we briefly mention the basic concepts and focus on appropriate methods for pre-concentrating plant-emitted VOCs with emphasis on the application of these methods in greenhouse practice.

Two methods are generally applied to pre-concentrate the VOCs present in air. The first method is based on the dynamic pre-concentration of VOCs. This method is referred to as dynamic because the air is actively pumped through a cartridge packed with a material that traps the compounds of interest. The second method is based on the static pre-concentration of VOCs. In this case, a material is exposed to the air, in which the trapping of VOCs mainly depends on mass diffusion processes. In both cases, the selection of the material is crucial in order to trap the VOCs of interest. There are a huge number of different materials available for the pre-concentration of plant-emitted VOCs in air (supplemented in Tholl et al., 2006). For most materials, *e.g.* the porous polymer Tenax [poly-(2,6-diphenyl-*p*-phenylene oxide] and carbon-based adsorbents, the pre-concentration depends on adsorption. For a few other materials, *e.g.* polydimethylsiloxane, the pre-concentration depends on absorption. The appropriate material —or combination of materials— should meet the following criteria: (1) homogeneous and inert surface to avoid artefact formation, irreversible adsorption, and catalytic effects during sampling and desorption; (2) complete and fast adsorption or absorption of the volatile organic compounds of interest; and (3) low affinity with water.

This inventory is not meant to be a complete list of criteria but rather to demonstrate the range of different aspects to consider. It is therefore obvious that care should be taken in the selection of materials since the pre-concentration step offers the opportunity to reduce the required sensitivity of the detector. It is recommended to investigate available materials in order to improve the efficiency of this step. Derivatization techniques might be employed to improve the properties of these materials in order to increase the efficiency of air sampling (see Deng et al., 2005a).

Separation of the plant-emitted VOCs in the mixture

Before identification and/or quantification of the plant-emitted volatiles, the mixture of compounds is often separated. Gas chromatography (GC) is then the method of choice in most applications. This method is a type of chromatography in which the mobile phase is a carrier gas, usually an inert gas such as helium, and the stationary phase is a layer of a polymer on an inert solid support, inside a glass or metal column. The properties of this column should be selected with care since they have a large effect on the ability to separate plant-emitted volatiles.

Identification and quantification of the plant-emitted VOCs

After separation, a detector is used for the identification and/or quantification of the individual VOCs present in the sample. A key-specification of any detector is their limit of detection (LOD). This LOD is generally defined as the lowest quantity of a substance that can be distinguished from the absence of that substance within a stated confidence limit, *i.e.*, where it can be assured that a certain substance is present. The limit of quantification (LOQ) should be considered if besides detection also quantification of the concentration is required for the task of crop health monitoring. This LOQ is the minimum concentration that can be quantitatively determined with satisfying certainty. The LOQ is normally defined as 10 times the standard deviation for blank samples, and is thus approximately three times higher than the LOD. In this review, we use two units of measure to approximate the LODs per instrument: absolute amounts in nanograms (ng) or picograms (pg), and the concentrations in air defined as nanograms per litre of air (ng L^{-1}) or picograms per litre of air (pg L^{-1}).

There are various types of detectors available on the market to identify and quantify plant-emitted VOCs. The most popular detectors in use are the flame ionization detector and the mass spectrometer. Electronic noses are also widely used to detect plant-emitted VOCs in air (Kunert et al., 2002). More recently, biosensors have emerged as promising tool to identify and quantify low levels of VOCs in ambient air. These four types of instruments are briefly described below.

Flame ionization detector

This technique involves the detection of ions. The response of the detector is determined by the number of carbon atoms hitting the detector per unit time. This makes the detector sensitive to the mass rather than the concentration, which is useful because the response of the detector is not greatly affected by changes in the carrier gas flow rate. Flame ionization detectors (FID) have been commonly used to measure VOCs emitted from plants (*e.g.* Greenberg et al., 1994). It offers a stable response, a wide dynamic concentration range, and a high sensitivity with limits of detection (LOD) in the order of picograms to nanograms (Tholl et al., 2006).

Mass spectrometer

The mass spectrometer (MS) and its applications are extensively covered in a variety of journals and books (*e.g.* McMaster, 2008). Therefore we only briefly mention its operating principle and focus on aspects related to the application of this instrument for the identification and/or quantification of plant-emitted VOCs at greenhouse scale.

Mass spectrometers measure the mass of charged molecules. Often the MS is combined with a chromatographic column (GC). This combination has become the method of choice for quantification and identification of plant-emitted VOCs at laboratory scale. It offers a high selectivity and resolution, good accuracy and precision, a high sensitivity, and a wide dynamic concentration range. Most current GC-MS instruments can achieve LODs in the low femtogram range. However, GC-MS LODs for realistic analytes are often in the picogram to nanogram range.

Conventional GC-MS systems are delicate instruments usually restricted to laboratory use. As a consequence, air samples collected in the greenhouse should be transferred to the laboratory for further analysis. The disadvantage of this transfer is the time delay between sampling and analysis. This time delay is undesirable in case the detection of plant health problems require an immediate act *e.g.* in case of the detection of a highly transmittable disease. Air samples should therefore preferably be analysed on-site. More robust GC-MS systems have therefore appeared on the market and have been applied, for example, to detect air contaminants in field settings (*e.g.* Smith et al., 2005) and to monitor a biogas tower reactor for the presence of potentially toxic VOCs (Matz et al., 1998).

Electronic nose

The term electronic nose (e-nose) first appeared in the literature around the late 1980s. Before this time, these sensors were referred to as gas sensors. Many aspects of electronic noses have been already reviewed in detail (*e.g.* Arshak et al., 2004) and thus we mention only those aspects relevant to the detection of plant-emitted VOCs in greenhouse air.

E-nose instruments are good at addressing the chemical integrity of a sample, which means to determine whether the sample is the same as or different than a certain standard. In general, they are not useful for the identification and quantification of individual components (Gardner and Bartlett, 1999). However, the identification of the volatiles being emitted may not be needed if the comparison and recognition of patterns in the volatile profile are sufficient for crop health monitoring through the analysis of plant-emitted volatiles. Such a profile can be obtained through the use of sensor arrays. This converges with research on volatile based inspection of potato tubers based on e-nose systems which rely on the recognition of fingerprints of volatiles released from them. For instance, a prototype device incorporating three metal oxide sensors was able to discriminate between sound tubers and the same tubers with one *Erwinia carotovora*-infected tuber added (de Lacy Costello et al., 2000). De Lacy Costello et al. (2003) recognized the problems associated with

air humidity and low air temperatures. However, these authors claimed that the system was able to differentiate between sound and infected tubers when operating at 4°C and 85% relative humidity while the sampling time necessary to allow discrimination was reduced to 10 s.

A combination of the marker-compound-approach with the e-nose technique can result in e-nose systems that have the ability to quantify VOC concentration in air as demonstrated for the differentiation of fresh and rancid butter based on volatiles (Hofmann et al., 1997). This development seems to be quite promising. The remaining drawback of e-noses based on sensor arrays is that the threshold of determination of most of these systems is in the low ppm-range. However, this drawback can be overcome by utilization of pre-concentration techniques. Such a combination of a gas-chromatographic system equipped with a pre-concentration unit and e-nose was successfully applied to detect plant emitted volatiles in a small cuvette (Kunert et al., 2002). They reported LODs for relevant VOCs in the low ng levels.

Biosensor technology

A biosensor is a particular type of chemical sensor that uses the recognition properties of biological components in the sensitive layer. Since its inception, biosensors have been expected to play a significant analytical role in medicine, agriculture, food safety, homeland security, environmental and industrial monitoring (Luong et al., 2008). However, despite the large amount of biosensors developed in research laboratories, the commercialization of biosensor technology is still in its infancy.

Nevertheless, steady improvements of well known basic principles have resulted in improved sensitivity, reliability and stability of traditional enzymatic biosensors. Also new affinity sensors such as transmembrane sensors and sensors utilising whole cells or cell networks significantly improve. For example, The Centre for Environmental Biotechnology at the University of Tennessee developed a proof-of-concept for a whole cell bioluminescent bioreporter for the detection of VOCs (Vijayaraghavan et al., 2006). These bioluminescent bioreporters generate visible light in response to specific chemical or physical agents in their environment. Measurements were obtained at vapour phase concentrations of less than one $\mu g\ L^{-1}$. Despite the lag in response and lack of correlation between concentration and bioluminescence it was hypothesized that the bioreporter can produce qualitative as well as quantitative results.

Today even whole animals or certain organs of animals are used in biosensors. For example, Schütz (2001) developed a biosensor to detect volatiles emitted from artificially and herbivore damaged potato plants (*Solanum tuberosum*). This biosensor, based on the intact antennae of the Colorado potato beetle (*Leptinotarsa decemlineata*), was also able to detect volatiles emitted from potato plants infected with *Phytophthora infestans*, the causal agent of the late blight disease (Schütz, 2001). Sensitivity and dynamic range can compete with the performance of GC-MS instruments (LOD $<$ 1 ng L^{-1}) while the response, dead and adaptation time, are shorter by a factor 10.

TRENDS AND FUTURE POSSIBILITIES

So far, most of the research related to plant health monitoring through plant-emitted VOCs is undertaken at the laboratory scale to pinpoint marker VOCs that can be used to indicate certain health problems (*e.g.* Laothawornkitkul et al., 2008). Recently, experimental evidence demonstrated that the detection of plant damage based on plant-emitted VOCs is also feasible at greenhouse scale (Jansen et al., 2009a). A characteristic of their experimental system was the rather small-scale with 60 plants grown at a floor area of 42 m^2. However, commercial greenhouses are much larger in size. For example, at present, the majority of commercial greenhouses in Western European countries, such as the Netherlands, range between 10^3 and 10^4 m^2 (Henten, 2006).

Proper experiments can be done to determine whether plant-emitted volatiles can be detected

in these full-scale greenhouses. However, this will be a time consuming and costly operation since the effects of various greenhouse characteristics must be evaluated. A potential cost reduction can be attained through the use of model-based predictions. For that reason, mass-transfer models are increasingly being used to bridge the gap between experimental measurements and real world applications. Such model approach is considered to be cost saving in translating the results obtained in a small-scale greenhouse into the potential application of plant health monitoring in full-scale greenhouses.

This review indicates the potential of monitoring crop health status at greenhouse scale on the basis of volatiles emitted from the plants. It reflects on how technological developments in the field of analytical chemistry can be used in an agricultural setting. Most of these developments are driven by research in which the detection of trace level amounts of volatile contaminants in food, air, or water is the subject. Approaches to detect these contaminants are based on highly sensitive instruments including flame ionization detectors, mass spectrometers, electronic noses and biosensors. It is recommended to evaluate the status of these instruments and to explore the development of new instruments that meet the specific needs for application in greenhouse practice.

At this moment, we consider gas chromatography coupled to mass spectrometry as the best method for monitoring the health status of crops on the basis of plant-emitted VOCs at high-input greenhouse facilities. This preference is based on its favourable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range, high sensitivity, and the current commercialization of robust GC-MS systems.

A disadvantage associated with GC-MS application is the large and complex data generated by this instrument. As a consequence, experienced and skilled analysts are often required to process these data in order to extract the concentrations of the chemical compounds of interest. However, developments in computer science technology and software will increase the opportunity to automatically process GC-MS data at an affordable price which will promote the efficient application of this instrument outside the laboratory. Another disadvantage related to the application of GC-MS is the relatively high cost of purchase. However, improvements in the field of mass spectrometry will likely results in affordable systems.

In conclusion, plants emit different types and amounts of volatiles during their decline in health status. Probably, it will be difficult if not impossible to identify the stress factors based on VOC emissions on. However, plant-emitted volatiles can be used to characterize the stress factors that contribute to plant health problems. There are instruments available (GC-MS) meeting the required technical specifications to detect these VOCs at greenhouse scale. Only due to the high costs, we are years away from having this kind of instruments in horticultural practice. But, the ongoing expansion and intensification of greenhouse production and the concern among consumers about the potential intake of pesticide residues on fruits and vegetables will support the prospected application of plant health monitoring in a commercial setting.

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