Detection of Diseased Plants by Analysis of Volatile Organic Compound Emission

R.M.C. Jansen,1,4 J. Wildt,2 I.F. Kappers,3 H.J. Bouwmeester,3 J.W. Hofstee,1 and E.J. van Henten1,4

1Wageningen University, Farm Technology Group, 6700 AA, Wageningen, The Netherlands; email: Roel.Jansen@wur.nl
2Institute Plant Sciences (IBG-2), Research Center Julich, D-52435, Julich, Germany; email: J.Wildt@fz-juelich.de
3Wageningen University, Laboratory of Plant Physiology, 6700 AA, Wageningen, The Netherlands; email: Harro.Bouwmeester@wur.nl
4Wageningen UR Greenhouse Horticulture, Wageningen, The Netherlands; email: Eldert.vanHenten@wur.nl

Abstract

This review focuses on the detection of diseased plants by analysis of volatile organic compound (VOC) emissions. It includes an overview of studies that report on the impact of infectious and noninfectious diseases on these emissions and discusses the specificity of disease-induced emissions. The review also provides an overview of processes that affect the gas balance of plant volatiles, including their loss processes. These processes are considered as important because they contribute to the time-dynamic concentration profiles of plant-emitted volatiles. In addition, we describe the most popular techniques currently in use to measure volatiles emitted from plants, with emphasis on agricultural application. Dynamic sampling coupled with gas chromatography and followed by an appropriate detector is considered as the most appropriate method for application in agriculture. 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 agricultural practice. However, to apply such an instrument in agriculture remains a challenge, mainly due to high costs.

Keywords

volatiles, monitoring, plant health, crop, inspection
INTRODUCTION

Humans relied mainly on gathering and hunting for life support until some ten thousand years ago, when agriculture appeared as we know it today. Agriculture has been defined as a system of food, feed, fuel, and fiber production involving a variety of domesticated (i.e., genetically modified) plants and animals together with a sustained input of human effort for cultivating the soil and for tending and harvesting the crops (20). The advent of agricultural production had a great impact on the livelihood of humankind and, for that reason, it sometimes is referred to as the Agricultural Revolution (9).

Agricultural production and productivity increased with time, allowing for the support of a growing world population. Today, nearly 6 billion people rely on agricultural production systems—including farming, livestock production, forestry, and fishery—for their livelihoods yet large differences in productivity and, consequently, the availability of food still exist. Key success factors for the increase in productivity of agricultural production in Western societies have been identified as (a) plant breeding, (b) improved plant nutrition and availability of plant fertilizers, (c) water management and irrigation, (d) increased knowledge of plant production, (e) availability of agrochemicals for pest and disease control, (f) availability of labor-supporting or labor-replacing mechanization and technology (5, 69). Developing countries have implemented similar approaches to achieve a so-called Green Revolution (29).

Yet, there is an urgent need to speed up this (r)evolution because in 2050 the world population is likely to increase to 8–10 billion (56), all relying on agriculture for food, fuel, feed, and fibers. Beyond that, the global society has developed a strong dependency on fossil oil as a resource for fuel and a wide range of chemicals. Thus, the source of oil is being rapidly depleted, and alternative resources need to be explored (73). Additionally, modern agriculture has almost become an industrial production process, putting severe pressure on the limited available resources, such as water, energy, and fertilizers. Agriculture also has strong impacts on the environment and society through the emission of excess amounts of nutrients and agrochemicals. Therefore, there is a growing demand for sustainable ways of agricultural production.

Focusing on disease control, this paper explores opportunities to improve the sustainability of agricultural production in an interdisciplinary fashion, covering aspects of plant pathology, plant physiology, and sensor technology. Throughout this review, the following definition for plant pathology is used: “Plant pathology is the study of the microorganisms and of the environmental factors that cause diseases in plants; of the mechanisms by which these factors induce disease in plants; and of the methods of preventing or controlling disease and reducing the damage it causes” (1).

Plant diseases have troubled agricultural production ever since its advent (1, 96). They result in loss of crop and consequently loss of income; they may cause loss of product quality and in some cases diseases may cause severe pathological effects on humans. Countermeasures included the adoption of new cultivation practices, plant breeding, biotechnology and genetic engineering, biological control, and last but not least, chemical control (96). Chemicals have been used effectively to prevent or cure diseases. However, despite widespread use of chemicals amounting to three billion kg per year at a value of 40 billion U.S. dollars per year, pests and diseases still cause crop losses on the order of 30% to 40% (1, 67). Additionally, widespread use of agrochemicals has many undesired side effects. These include public health effects (acute poisoning, cancer and other chronic effects, pesticide residues in food), domestic animal poisoning, destruction of beneficial natural predators and parasites, resistance in pests and diseases, honeybee and wild bee poisonings and reduced pollination, crop and crop product losses, ground and surface water contamination, fishery losses, losses of wild birds and mammals, and damage to microbes and invertebrates (67). A reduction in the use of agrochemicals in agricultural production is therefore urgently needed.
One way to reduce the use of agrochemicals is to apply the chemical at the right time, at the right place, and in the right amount instead of full field application. Essentially, such an approach is at the core of what is commonly called precision agriculture or precision horticulture. Precision agriculture is defined as “The application of technologies and principles to manage spatial and temporal variability associated with all aspects of agricultural production for the purpose of improving crop performance and environmental quality” (66). In this approach, the site specific application of inputs like fertilizers and water, but also the site specific control of weeds, pests, and diseases, is used to optimize the use of inputs (23, 98).

Precision agriculture is a technology-based approach that includes three main items: (a) sensors, (b) data interpretation and decision making, and (c) actuation or implementation of an action (81). The availability of suitable sensors still seems to be the main bottleneck.

When it comes to sensing the presence of diseases in plants, it is interesting to take note of the following quotation, “Since it is not known whether plants feel pain or discomfort, and since, in any case, plants do not speak or otherwise communicate to us, it is difficult to pinpoint exactly when a plant is diseased” (1). Yet already in 1978, horticultural engineers coined the term “speaking plant approach,” indicating an approach in which crop management would be based on measurements of the status of the individual plants (87). In arable farming, similar observations were done: “If the crop is the best sensor of its own environment then sensing systems that can tap into what the crop is ‘saying’ may provide information on crop condition necessary to direct spatially variable inputs” (52, 81).

Plants have been shown to emit volatile organic compounds (VOCs) when they are affected by disease (8, 78). Although these emissions may not be similar to speech, they may be informative enough for local effective disease control if these signals can be properly interpreted. To be able to interpret these signals, knowledge of plant physiology, biochemistry, and atmospheric chemistry is of crucial importance. Effective disease control furthermore requires selection of the most appropriate technique to measure these signals.

This paper aims to give an overview on the detection of diseased plants by analysis of VOC emission. We provide an overview of studies that examined the impact of plant disease on the emission of VOCs. We then discuss the specificity of disease-induced emissions and provide an overview of processes that affect the gas balance of plant VOCs, as well as an overview of techniques to measure the emissions of VOCs from plants. Finally, we describe current trends and future issues in the field of disease detection in plants by analysis of VOCs.

EMISSIONS OF VOLATILE ORGANIC COMPOUNDS FROM DISEASED CROP PLANTS

To detect diseased plants by analysis of VOC emissions, it is important to know these emissions. This section summarizes and orders the literature on the effect of disease on plant VOC emissions by grouping diseases. Here, plant diseases are grouped by the type of pathogen that causes the disease and classified as infectious, or biotic, plant diseases and noninfectious, or abiotic, plant diseases.

Emissions as a Result of Infectious Plant Diseases

VOC emission is changed by infectious plant diseases. These types of diseases are mainly caused by fungi, prokaryotes (bacteria and mollicutes), parasitic plants, viruses and viroids, nematodes, and protozoa (1).

Studies on fungi-induced VOCs include those on peanut (Arachis hypogaea) upon Sclerotium rolfsii infection (8), on silver birch (Betula pendula) upon Marssonina betulae infection (93), on oil palm (Elaeis guineensis) upon Ganoderma boninense infection (57), and on willow (Salix spp.) upon Melampsora epitea infection (86). Fungi-induced VOCs were also found in tomato (Lycopersicon esculentum) upon Erysiphe
Botrytis cinerea infection of tomato. This pathogen-plant interaction was used as a model system to study whether diseased plants can be detected in greenhouses by analysis of their volatile organic compound emission (38, 40, 84). Photo: Rudi Aerts.

Other studies in which fungi were involved include those on potato (Solanum tuberosum) upon Phytophthora infestans infection (50) and on field mustard (Brassica rapa) upon infection with the fungal pathogen Alternaria brassicae (18). In the above mentioned studies, the infection of aerial parts of the plants was investigated. However, root infections may also result in increased emission of certain VOCs. Preliminary experiments using cucumber plants (Cucumis sativus) inoculated with the root pathogen Pythium aphanidermatum indicated such systemic plant response (41).

Studies in which prokaryotes were involved show altered emission of apple (Malus domestica) and pear plants (Pyrus communis L.) upon infection with Erwinia amylovora, the causal agent of fire blight (80). Also, grapevine (Vitis vinifera) grafted on rootstock Vitis berlandieri × Vitis riparia inoculated with two tumorigenic strains of Agrobacterium vitis showed altered VOC emission (7). Other studies in which prokaryotes were involved describe the emission from tobacco plants (Nicotiana tabacum) upon bacterial infection with Pseudomonas syringae (30, 34).

Not many studies report on the effect of parasitic plants on plant emission. But such an effect can be expected given that recent progress indicates that plant volatiles can act as neighbor detection signals (46). The few studies in which viruses and viroids were involved include the increased emission of VOCs from tobacco plants upon infection with tobacco mosaic virus (TMV) (78) and the increased emission from tomato plants upon infection with TMV (14). Only a few studies report on the effect of nematodes on plant emission. One of them includes the emission of tomato plants infected with the root-knot nematode Meloidogyne incognita (83).

Emission as a Result of Noninfectious Plant Diseases

VOC emissions are also changed by noninfectious plant diseases. These types of disease are mainly caused by too low or too high temperature, lack or excess of soil moisture, lack or excess of light, lack of oxygen, air pollution, nutrient deficiencies, mineral toxicities, soil acidity or alkalinity, and improper use of agrochemicals (1).

Many studies report on the effect of temperature on plant VOC emission. These show that temperature increases the emission rates of most VOCs exponentially up to a maximum. Reasons for the increase are enhancement of the biosynthetic enzyme activities, increases of the
VOCs’ vapor pressure, and decreases of the resistance of the emission pathway (65). Also, lack or excess of soil moisture has an effect on plant volatile emission. For instance, several studies have demonstrated an increase in the amount and types of emitted VOCs after drought (19, 77). Emitted substances after drought include alcohols and aldehydes, probably as a result of the gradual collapse of the cellular structure of the plant leaves during the drying process.

Lack or excess of light has almost certainly an effect on plant VOC emission. For instance, gradual light-dark transitions result in gradual changes in VOC emission, and sudden light transitions result in strong VOC bursts (25). Also, an effect of oxygen deficiency on plant VOC emission can be expected because anoxic conditions of roots elicit a plethora of physiological stress responses, including the enhanced emission of ethanol, methanol, and acetaldehyde (10).

The effect of air pollutants on plant VOC emission is well described. For instance, several studies 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) (95). The most common air pollutant that causes diseases of field crops is ozone, but sulfur oxides may also damage plants. In greenhouses, air pollution is also generated by soft plastics (phthalates) and off-gas products from heating equipment.

Nutrient deficiencies might have an effect on plant VOC emission given that several studies 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) (95). The most common air pollutant that causes diseases of field crops is ozone, but sulfur oxides may also damage plants. In greenhouses, air pollution is also generated by soft plastics (phthalates) and off-gas products from heating equipment.

Whether or not mineral toxicities affect plant VOC emission is—to the best of our knowledge—unknown. However, mineral toxicity occurs when a plant mineral is in excess, and this excess is likely to have an effect on plant molecular composition and thus plant emission. Soil acidity or alkalinity is expected to have an effect on plant emission given that several studies have confirmed the effect of salt stress on plant volatile emission (53). Finally, agrochemicals are expected to have an impact on plant emission. For instance, spraying with the herbicide Paraquat had an impact on volatile emission from Arabidopsis thaliana plants (89).

SPECIFICITY OF DISEASE-INDUCED EMISSION

A monitoring system that detects diseased plants at an early stage would enable a grower to take early action. Identification of the causal agent would improve such a system, as this allows the grower to decide on the proper control measure, such as spraying prophylaxis against gray mold disease in case the agent was identified as Botrytis cinerea. To identify the agent through the measurement of plant-emitted VOCs, the emission of specific chemical substances, a specific blend upon the onset of disease, or a specific time course of the disease-induced VOC emission is required.

Is Emission of Volatile Organic Compounds Unspecific?

The emission of specific VOCs seems unlikely because it is well established that emission of many of the same VOCs is induced upon different infectious and noninfectious diseases. For example, most of the VOCs reported upon fungal infection of tomato plants were also reported upon ozone treatment of tomato plants (39). Emission of the same VOCs was also induced when different plant species were challenged with a similar infection. For example, TMV infection in tobacco as well as in tomato induced an increase in the emission of methyl salicylate (14, 78). Chemical substances that are frequently reported after a disease-induced change in VOC emission— independent of the disease and independent of the plant species—include (Z)-3-hexenol, methyl salicylate, (E)-β-ocimene, linalool, (E)-β-farnesene, (E)-4,8-dimethyl-1,3,7-triene (DMNT), and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). This list is
not complete. To the best of our knowledge, no chemical substance has ever been exclusively ascribed to one particular disease.

Several researchers addressed the time course of disease-induced VOC emission from plants (34, 40). These studies demonstrated that the emission of certain substances can increase directly after the onset of disease followed by a rapid return to low emission rates, whereas increased emission of other substances may be delayed for hours up to several days after the onset of disease. The time period between the first response and the delayed response in terms of increased VOC emission might be indicative of the disease to which the plant is exposed. For instance, this time period differed for tobacco plants in response to different strains of *Pseudomonas syringae* (34). However, it is unlikely that plant diseases can be identified based on the time course of disease-induced volatile emission only.

Nevertheless, disease-induced changes in VOC emission might be used to characterize the plant disease. The first way to characterize the disease is based upon the chemical substances present in the blend of disease-induced VOCs. These substances are to a large extent related to the plant structure from which VOCs are emitted. Previous studies suggested an arbitrary classification of confined substructures, and the entire plant as the emitting structure. There are several examples in which the emission from confined plant substructures changes upon infection. A first example is the release of VOCs from local plant tissue after damage of involved cell membranes because of pathogen infection (95). Damage of these cell membranes results in the local emission of several lipoxygenase (LOX) products at the site of damage (Figure 2). These LOX products originate from oxidative cleavage of fatty acids in the presence of oxygen and enzymes such as noninfected and *Botrytis cinerea*–infected tomato plants. (1) Nonglandular trichome; (2) stomate; (3) stomatal cavity; (4) glandular trichome; (5) trichome-induced emission; (6) system-induced emission; (7) trichome damage–induced emission; (8) *B. cinerea* infection; (9) cell membrane damage–induced emission; (10) stem trichome–induced emission; (11) stem trichome damage–induced emission. Dissimilar colors represent dissimilar VOC blends. Question marks indicate plant parts for which *B. cinerea*–induced emission is unknown. This figure is redrawn from Reference 42.
as lipoxygenases (21, 28, 58). These LOX products thus characterize diseases in which damage of cell membranes (that contain fatty acids) plays an important role. A second example of plant substructures that emit VOCs during stress is the local emission of stored VOCs from damaged glandular trichomes due to pathogen infection (Figure 2). These trichomes are minute structures on the plant surface, characterized by a multicellular stalk and a small glandular vesicle at the tip. Local damage of these trichomes will result in the local emission of stored terpenes or other secondary metabolites. These types of emission thus characterize plant diseases in which damage of glandular trichomes plays an important role. The blend of VOCs per trichome depends on its position on the plant (Figure 2). For instance, in the case of tomato, the portion of \( \beta \)-caryophyllene in stem trichomes was much larger then in leaf trichomes (74). This opens up possibilities to discriminate between stem trichome damage and leaf trichome damage based on trichome-associated VOCs.

Different plant diseases attack different plant parts in different ways. As a result, it can be expected that some types of plant substructures are involved, and others are not, depending on the type of disease. As a consequence, the chemical substances associated with the particular type of substructure might thus be used to characterize the disease that harms the plant, but not to differentiate between different diseases that attack the same part of the plant.

The emission of methyl salicylate and methyl jasmonate, however, can be cited as an example in which the entire shoot can be regarded as an emitting structure. In the case of tomato, systemic emission of methyl salicylate is thought to occur via stomata (Figure 2). The systemic emission of VOCs from diseased plants is generally believed to increase, but only after a certain period following the local inoculation or local application of pathogens (44, 71). 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 (13). Probably, a stressor needs to be continuously sustained in order to increase the emission of methyl salicylate and/or other stress-associated VOCs. It is also believed that chemical signals derived from the stressor, e.g., derived from the infectious agent, are required to increase the emission of methyl salicylate and/or other stress-associated VOCs (2). Thus, methyl salicylate might be used to characterize stressors in which continuously sustained damage and/or chemical signals are involved.

In addition to the chemical substances present in the VOC blend, the time course of the disease-induced VOCs may also characterize the disease. For example, severe \( B. \) cinerea infections resulted in a large increase in emission a few hours after inoculation, whereas mild infections resulted in a small increase in emission several hours after inoculation (40). The importance of the duration and the intensity of damage as a factor in stress-induced changes in emission was also elegantly demonstrated using MecWorm, a robotic device designed to reproduce tissue damage caused by herbivore attack (63). In addition to local emission, systemic emission also depends on the duration and intensity of the damage. For instance, the emission of systemically emitted volatiles from Brussels sprouts was dependent on the duration of caterpillar feeding (59).

As mentioned before, the opportunity to identify the disease would be of great value to a disease detection system. This section explained that emission of VOCs is often unspecific, but this section also explained how plant-emitted volatiles can be used to characterize the disease. This might be sufficient because the diversity of diseases that occur simultaneously is often limited, primarily due to monoculture and in the case of greenhouses, environmental control.

**Is Emission of Volatile Organic Compounds Specific?**

In this section, we argue that although a certain plant species may emit similar VOCs upon induction by different diseases and, furthermore, different plant species may emit the same VOCs
after being challenged with a similar disease, the total VOC blend emitted may be specific for a certain plant-pathogen interaction.

A first argument that supports the presence of a specific VOC blend upon a certain plant-pathogen interaction comes from molecular studies. Increasing molecular evidence exists that different biotic interactions elicit specific signaling cascades in plants. Three major defense signaling pathways have been recognized as important for plant-biotic interactions: the jasmonic acid, the salicylic acid, and the ethylene pathway (47). These signaling pathways individually lead to three different defense responses, such as the production of a certain volatile or volatiles. However, there is extensive crosstalk between these different signal transduction pathways, leading to antagonistic and synergistic interactions. For instance, ample evidence shows that jasmonic acid and salicylic acid can act antagonistically (97). This crosstalk provides plants with an intricate mechanism to fine-tune their defense against different attackers (48). Generally, salicylic acid–dependent defenses are activated more strongly in response to biotrophic pathogens and jasmonic acid– and ethylene-dependent defenses are activated to a higher extent in response to necrotrophic pathogens and herbivorous insects (47). For instance, in maize, the emission of herbivore-induced VOCs was reduced by approximately 50% when plants were also infected with a necrotic fungal pathogen (72). A possible underlying mechanism is that fungal infection likely induces the salicylic acid–based signal transduction pathway, which would reduce signaling through the herbivore-triggered jasmonic acid–pathway because of negative crosstalk (47, 72).

Another argument that supports the presence of a specific VOC blend upon a certain plant-pathogen interaction comes from plant-herbivore studies. Upon insect herbivory, the plant’s endogenous chemistry and metabolite profile are altered (35, 70, 92), as is the VOC blend that is emitted (31, 90). Many carnivorous natural enemies of herbivorous insects and mites use the herbivore-induced volatile information released by the plant to locate their prey. In this speaking plant approach, plants release distress signals to attract only certain parasitoids (16, 17). For pathogens, it is more difficult to demonstrate the relevance of such a specificity for a third party. Nevertheless, it could be that in the case of pathogen infection, specificity in VOC emission occurs as this may have an evolutionary advantage either in a direct way, such as causing toxicity towards the invading pathogen, or in an indirect way as a warning mechanism to the neighbors of the infected plant.

An example of specificity in the case of arthropods is the work of De Boer et al. (11), who show that carnivorous predatory mites preferred the blend from leaves infested with their natural prey, the spider mites, over the VOC blend of nonprey caterpillar-infested leaves even though gas chromatography–mass spectrometry (GC-MS) analysis revealed the presence of many similar compounds in both blends. This shows that predatory mites are capable of distinguishing subtle differences. It could be that differences in such signals are caused by differences in the concentration or in ratios of individual compounds in the total blend or that predatory mites have receptors for compounds that are not conspicuously different in GC-MS analysis.

However, using GC-MS combined with multivariate statistical methods, differences between VOC blends can be visualized. Potato plants exposed to different stressors, representing a pathogen (*Phytophthora infestans*) and four types of herbivores [mites (*Tetranychus urticae*), thrips (*Frankliniella occidentalis*), aphids (*Myzus persicae*), caterpillars (*Spodoptera exigua*), released different VOC blends (Figure 3; I.F. Kappers, unpublished data). Although all induced blends contained the same major compounds, such as methyl salicylate, numerous LOX products, DMNT, TMTT, (E)-β-ocimene, and α-farnesene, each blend was quite characteristic for the applied organism, which can be visualized by principal component analysis (PCA) (Figure 3). The application of jasmonic acid or salicylic acid...
Figure 3
Principal component analysis of the volatile blends of potato plants upon infection with a pathogen (Phytophthora infestans, closed triangles), infestation with cell-feeding spider mites (Tetranychus urticae, closed circles), thrips (Frankliniella occidentalis, open triangles), phloem-feeding aphids (Myzus persicae, stars), leaf-consuming caterpillars (Spodoptera exigua, open hearts), or treated with 0.5 mM jasmonic acid (closed squares) or salicylic acid (open squares), and nontreated plants (open circles). The first two principal components explain 46% and 25%, respectively, of the variation found.

Acid led to a volatile blend that had high similarity with that of herbivore infestation or pathogen infection, respectively (Figure 3). The separate clustering in PCA shows that there are qualitative differences between the VOC blends induced by the different treatments, which suggests that it is possible to discriminate between different plant diseases based on plant VOC emission.

FACTORS THAT AFFECT THE GAS BALANCE OF PLANT VOCs
This section discusses factors that affect the gas balance of plant VOCs in the vicinity of crops.

The crop is probably the most important source of plant VOCs. However, the gas phase concentration of plant VOCs is also affected by the transfer of such VOCs from the outside to the inside of the crop environment. In the case of greenhouses, ventilation is likely to be the most important source. In the case of field crops, wind is the most important transport mechanism.

Loss processes of VOCs are regarded as important aspects of the gas balance because they contribute to the time-dynamic concentration profiles of plant-emitted volatiles (37). On the one hand, a slow loss will cause the
accumulation of VOCs 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 because VOC emission during stress sometimes appears as a burst followed by a rapid return to a low emission rate (6, 12).

The first loss process for plant-emitted VOCs is the removal of these VOCs by air transport. Air transport may be natural, e.g., via wind, or mechanical, e.g., via fans in a greenhouse.

The second loss process is the degradation of VOCs due to gas-phase reactions. In the lower atmosphere, the major degradation processes for plant VOCs are reactions with hydroxyl radicals (OH), nitrate radicals (NO₃), and ozone (O₃), leading to a number of breakdown products (4). Indeed, it was demonstrated that exposure of plants to moderately enhanced O₃ levels resulted in the partial degradation of VOCs emitted upon herbivore infestation (68).

The third process leading to removal of VOCs from air is the sorption on air-contact surfaces. VOCs transported to soil or, in the case of greenhouses, glass and concrete are removed from the gas phase by deposition on such a surface, and many researchers have shown that material surfaces interact with VOCs (for example, see Reference 43). 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 (33). 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 be taken into account as an important loss process is the solution of VOCs in water bodies such as raindrops or condensate. Henry’s Law constant is a key parameter to estimate the maximum amount of VOCs that can be dissolved into water and 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 because Henry’s Law assumes no further chemical breakdown of chemical compounds when dissolved in water.

The fifth process for losses of VOCs is uptake by the plant itself. These losses can occur by adsorption on the cuticle (94) and uptake through the stomata (76). Uptake of VOCs through stomata requires a lower concentration of the compounds in the stomatal cavity than in the surrounding air. This concentration difference is important because gases move along the concentration gradient between the inside and the outside of the leaf. The stomatal cavity is covered by water. Therefore, VOCs that can be dissolved in this water and thereafter metabolized in plant tissues can maintain a continuous uptake potential. This loss process might thus be particularly relevant for polar VOCs such as alcohols.

**TECHNIQUES TO MEASURE VOLATILE ORGANIC COMPOUND EMISSION FROM PLANTS**

Several excellent papers are available that review the techniques currently in use to measure the emission of VOCs from plants (64, 85). However, none of these papers describe how these techniques can be used for detection of diseased plants by analysis of VOC emission. This section is intended to fill this knowledge gap.

In general, the measurement of plant VOC emission consists of three steps: (a) collection of the plant-emitted VOCs, (b) separation of the plant-emitted VOC blend, and (c) identification and/or quantification of the separate VOCs. These three steps are explained below.

**Collection of the Plant-Emitted Volatile Organic Compounds**

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 present in the air to achieve the detection limits of commonly applied analytical instruments. Several reviews are
dealing with the preconcentration of VOCs in air (15, 27). Therefore, we briefly mention the basic concepts and focus on appropriate methods for preconcentrating plant-emitted VOCs with emphasis on the application of these methods in agricultural practice.

Two methods are generally applied to preconcentrate the VOCs present in air. The first method is based on the dynamic preconcentration 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 preconcentration 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 preconcentration of plant-emitted VOCs in air (see Reference 85). For most materials, e.g., the porous polymer Tenax [poly-(2,6-diphenyl-p-phenylene oxide) and carbon-based adsorbents, the preconcentration depends on adsorption. For a few other materials, such as polydimethylsiloxane, the preconcentration depends on absorption. The appropriate material, or combination of materials, should meet the following criteria: (a) homogeneous and inert surface to avoid artifact formation, irreversible adsorption, and catalytic effects during sampling and desorption; (b) complete and fast adsorption or absorption of the VOCs of interest; and (c) 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 because the preconcentration step offers the opportunity to minimize 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 (12).

**Separation of the Plant-Emitted VOC Blend**

The VOC blend is often separated before identification and/or quantification of the individual substances. Gas chromatography (GC) is 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 because 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 its 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. The limit of quantification (LOQ) should be considered if detection and quantification of the concentration are required for the task of crop health monitoring. LODs and LOQs are given in two different units: absolute amounts in nanograms (ng) or picograms (pg), or with respect to the concentrations in air. The latter are given in nanograms per liter of air (ng L\(^{-1}\)) or picograms per liter of air (pg L\(^{-1}\)). Also the parts per notation are used to express LOQs and LODs, particularly at the parts-per-billion (ppb) and parts-per-trillion (ppt) level.

Various types of detectors are available on the market to identify and quantify plant-emitted VOCs. The most popular detectors in use are the flame ionization detector (FID) and the mass spectrometer (MS). Electronic noses (E-noses) are also widely used to detect plant-emitted VOCs in air (49). More recently, biosensors have emerged as promising tools to identify and quantify low levels of VOCs in ambient air.
The FID technique involves the detection of ions. It has been commonly used to measure VOCs emitted from plants (for example, see Reference 26). FIDs offer a stable response, a wide dynamic concentration range, and a high sensitivity with LODs on the order of pg to ng (85).

The MS and its applications are extensively covered in a variety of journals and books (for example, see Reference 61). 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. MS measure the mass of charged molecules or charged molecule fractions. They offer a high selectivity and resolution, good accuracy and precision, a high sensitivity, and a wide dynamic concentration range. Current MS instruments can theoretically achieve LODs in the low femtogram range. However, in practice MS LODs are often in the pg to ng range.

Conventional MS systems are delicate instruments usually restricted to laboratory use. As a consequence, air samples should be transferred to the laboratory for 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 requires immediate action, e.g., in case of the detection of a highly transmittable disease. Air samples should therefore preferably be analyzed onsite. More robust GC-MS systems are available and have been used to detect ambient VOCs (for example, see References 79, 82), to monitor a biogas tower reactor for the presence of potentially toxic VOCs (60), and even to analyze VOC emission from a forest (45).

The term E-nose first appeared in the literature in the late 1980s. Before this time, these sensors were referred to as gas sensors. Many aspects of E-noses have been reviewed in detail (for example, see Reference 3) and thus we mention only those aspects that are relevant to the detection of plant-emitted VOCs. E-nose instruments are good at addressing the chemical integrity of a sample, which is to determine whether the sample is the same as or different from a certain standard. In general, they are not useful for the identification and quantification of individual components (22). 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 detection of a diseased plant. Such a profile can be obtained through the use of sensor arrays. For instance, a prototype device incorporating three metal oxide sensors was able to discriminate between healthy leaves and unhealthy leaves of cucumber, pepper, and tomato (51). A drawback of E-noses based on sensor arrays is that the LOD of most of these systems is in the μg L⁻¹ range. This drawback could be overcome by utilization of preconcentration techniques and chromatographic columns. Then, LODs at the low ng L⁻¹ range can be achieved (54). Such combinations of preconcentration, gas chromatography, and E-noses were successfully used to detect herbivore-induced volatiles from intact tomato and pepper plants (49, 62).

A biosensor is a particular type of chemical sensor that uses the highly sensitive recognition properties of biological components such as an enzyme, antibody, nucleic acid, microorganism or cell. Since its inception, biosensors were predicted to play a significant analytical role in agriculture (88). However, despite the large amount of biosensors developed in research laboratories, the commercialization of biosensor technology is still in its infancy (55). 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 utilizing whole cells or cell networks, have significantly improved. For example, a proof-of-concept for a whole-cell bioluminescent bioreporter for the detection of VOCs has been developed (91). These bioluminescent bioreporters generate visible light in response to specific chemical or physical agents in their environment. LODs of less than one μg L⁻¹ have been reported for such systems.
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, a biosensor based on the intact antennae of the Colorado potato beetle (*Leptinotarsa decemlineata*) was developed to detect volatiles emitted from artificially and herbivore-damaged potato plants (75). This sensor was also able to detect volatiles emitted from potato plants infected with *Phytophthora infestans*, the causal agent of the late blight disease (75). Sensitivity and dynamic range can compete with the performance of GC-MS instruments (LOD < 1 ng L⁻¹), and the response, dead time, and adaptation time are shorter by a factor of 10.

**TRENDS AND FUTURE POSSIBILITIES**

So far, most of the research related to disease detection through plant-emitted VOCs is undertaken at the laboratory scale to pinpoint marker VOCs that can be used to indicate certain plant health problems. Recently, it was demonstrated that the detection of plant damage based on plant-emitted VOCs is also feasible at the greenhouse scale (38). A characteristic of the experimental system used in the later study was the rather small scale with 60 plants grown at a floor area of 42 m². Commercial greenhouses are much larger in size. For example, at present, the majority of commercial greenhouses in Western European countries, such as the Netherlands, have areas between 10⁴ and 10⁵ m² (32). Experiments can be done to determine whether plant-emitted VOCs can be detected in these full-scale greenhouses. However, this approach will be a time consuming and costly operation because the effects of various greenhouse characteristics must be evaluated. A potential cost reduction of the necessary research 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.

This review indicates the potential of disease detection in plants by analysis of VOC emission. 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 GC-FIDs, GC-MS, E-noses, and biosensors. We recommend the evaluation of the status of these instruments and the exploration of the development of new instruments that may meet the specific needs for application in agriculture.

At this moment, we consider dynamic sampling in combination with GC followed by an appropriate detector as the best instrument for detection of plant disease by analysis of VOCs. The most suitable detector should have a favorable combination of high selectivity and resolution, good accuracy and precision, wide dynamic concentration range, and high sensitivity. Such instruments will probably produce large and complex datasets. Experienced analysts are often required to process this data in order to determine the concentrations of the chemical compounds of interest. This manual processing is time consuming, labor intensive, and may be subject to errors due to fatigue. However, developments in computer technology and software have increased the opportunity to automatically process these data within a reasonable time (36).

In conclusion, diseased plants emit different types and amounts of volatiles. It will be a challenge to identify the disease based on VOC emission only. But, plant VOCs can be used to characterize the disease. In addition, instruments are available that meet the required technical specifications to detect these VOCs in an agricultural setting. The high costs of instruments still prevent using such instruments in practice, but the ongoing expansion and...
intensification of agricultural production and the concern among consumers about the potential intake of pesticide residues on fruits and vegetables will support the prospected application of disease detection by analysis of plant volatiles in a commercial setting.

**DISCLOSURE STATEMENT**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

**ACKNOWLEDGMENTS**

Our laboratories have been financially supported by the European Science Foundation Scientific Program “Volatile organic compounds in the Biosphere-Atmosphere System” (VOCBAS grants 814 and 974). We thank the Royal Netherlands Society of Plant Pathology (KNPV) for the financial support to write this paper. We apologize to all our colleagues whose work could not be reviewed here because of space limitations.

**LITERATURE CITED**


87:265–72
Contents

Not As They Seem
George Bruening ................................................................. 1

Norman Borlaug: The Man I Worked With and Knew
Sanjaya Rajaram ................................................................. 17

Chris Lamb: A Visionary Leader in Plant Science
Richard A. Dixon ................................................................. 31

A Coevolutionary Framework for Managing Disease-Suppressive Soils
Linda L. Kinkel, Matthew G. Bakker, and Daniel C. Schlatter .......... 47

A Successful Bacterial Coup d’État: How Rhodococcus fascians Redirects Plant Development
Elisabeth Stes, Olivier M. Vandeputte, Mondher El Jaziri, Marcelle Holsters, and Danny Vereecke ............... 69

Application of High-Throughput DNA Sequencing in Phytopathology
David J. Studholme, Rachel H. Glover, and Neil Boonham ............. 87

Aspergillus flavus
Saori Amaike and Nancy P. Keller ........................................... 107

Cuticle Surface Coat of Plant-Parasitic Nematodes
Keith G. Davies and Rosane H.C. Curtis ................................... 135

Detection of Diseased Plants by Analysis of Volatile Organic Compound Emission

Diverse Targets of Phytoplasma Effectors: From Plant Development to Defense Against Insects
Akiko Sugio, Allyson M. MacLean, Heather N. Kingdom, Victoria M. Grieve, R. Manimekalai, and Saskia A. Hogenbout .......... 175

Diversity of Puccinia striiformis on Cereals and Grasses
Mogens S. Hovmøller, Chris K. Sørensen, Stephanie Walter, and Annemarie F. Justesen ................................................ 197
Emerging Virus Diseases Transmitted by Whiteflies
Jesús Navas-Castillo, Elvira Fiallo-Olivé, and Sonia Sánchez-Campos .................. 219

Evolution and Population Genetics of Exotic and Re-Emerging Pathogens: Novel Tools and Approaches
Niklaus J. Grünwald and Erica M. Goss .................................................. 249

Evolution of Plant Pathogenesis in Pseudomonas syringae: A Genomics Perspective
Heath E. O’Brien, Shalabb Thakur, and David S. Guttman .................. 269

Hidden Fungi, Emergent Properties: Endophytes and Microbiomes
Andrea Porras-Alfaro and Paul Bayman ........................................ 291

Hormone Crosstalk in Plant Disease and Defense: More Than Just JASMONATE-SALICYLATE Antagonism
Alexandre Robert-Seilaniantz, Murray Grant, and Jonathan D.G. Jones ........... 317

Plant-Parasite Coevolution: Bridging the Gap between Genetics and Ecology
James K.M. Brown and Aurélien Tellier ........................................ 345

Reactive Oxygen Species in Phytopathogenic Fungi: Signaling, Development, and Disease
Jens Heller and Paul Tudzynski .................................................. 369

Revision of the Nomenclature of the Differential Host-Pathogen Interactions of Venturia inaequalis and Malus

RNA-RNA Recombination in Plant Virus Replication and Evolution
Joanna Sztuba-Solinska, Anna Urbanowicz, Marek Figlerowicz, and Jozef J. Bujarski ........................................ 415

The Clavibacter michiganensis Subspecies: Molecular Investigation of Gram-Positive Bacterial Plant Pathogens
Rudolf Eichenlaub and Karl-Heinz Gartemann ................................ 445

The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production
Ravi P. Singh, David P. Hodson, Julio Huerta-Espino, Yue Jin, Sridhar Bhavani, Peter Njau, Sybil Herrera-Foessel, Pawan K. Singh, Sukhwinder Singh, and Velu Govindan ........................................ 465

The Pathogen-Actin Connection: A Platform for Defense Signaling in Plants
Brad Day, Jessica L. Henty, Katie J. Porter, and Christopher J. Staiger ................. 483
Understanding and Exploiting Late Blight Resistance in the Age of Effectors  

Water Relations in the Interaction of Foliar Bacterial Pathogens with Plants  
*Gwyn A. Beattie*  533

What Can Plant Autophagy Do for an Innate Immune Response?  
*Andrew P. Hayward and S.P. Dinesh-Kumar*  557

Errata

An online log of corrections to *Annual Review of Phytopathology* articles may be found at [http://phyto.annualreviews.org/](http://phyto.annualreviews.org/)