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ORIGINAL RESEARCH

Age-Dependent Variations of Volatile Emissions and Inhibitory Activity Toward *Botrytis cinerea* and *Fusarium oxysporum* in Tomato Leaves Treated with Chitosan Oligosaccharide

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Abstract We investigated variations in the level and composition of volatiles emitted by tomato leaves at different ages. Our focus also included their antifungal properties and responses to chitosan oligosaccharide. Based on leaf position, the release of volatiles decreased over time. Young leaves produced high levels of C6-aldehyde, which is mainly composed of hexenal, while the volatiles emitted by more mature leaves largely comprised terpenes, particularly β-phellandrene and caryophyllane. In young upper leaves, the main components (up to 86% of the total) were hexenal, β-phellandrene, and caryophyllane. Their levels decreased steadily over time, from 386.3 μg g^{-1} fresh weight (FW) in young leaves to 113.2 μg g^{-1} FW in old tissues. Volatiles emitted from young leaves exhibited the best antifungal activity against spore germination and hyphal growth by *Botrytis cinerea* and *Fusarium oxysporum*. Leaves became more susceptible to oligosaccharide treatment with increasing age. When young tissues were exposed to chitosan, we found declines in both the quantity of volatiles and their ability to inhibit fungal growth. Compared with the control, the amount of volatiles from young tissues was 88.4% lower after such treatment. In contrast, contents of volatiles from old and adult leaves were dramatically increased by chitosan oligosaccharide. Likewise, their inhibitory effect was significantly enhanced. Therefore, our results suggest that these volatiles are responsible for antifungal activity and may play a role in age-related resistance by tomato.

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Volatile organic compounds (VOCs) include alkanes, alkenes, alcohols, aldehydes, ethers, esters, and carboxylic acids. This group of 30,000 compounds is dominated by terpenoids and fatty-acid derivatives that have now been synthesized via a range of physiological processes in many different plant tissues (Pichersky and Gershenzon 2002). Numerous factors can affect the quality and quantity of plant VOCs such as developmental stage, growing conditions, and stress. For example, the release of isoprene and C6-aldehyde from bean leaves depends upon their level of maturity (Kuzma and Fall 1993; Zhuang et al. 1992). Furthermore, Staudt et al. (1997) have shown that patterns for monoterpene emissions from *Pinus pinea* (L.) are influenced by seasonal and diurnal factors. Dramatic changes in the composition of essential oils from *Micromeria fruticosa* are related to leaf position and maturation (Dudai et al. 2001). Various pathogens (Moalemiyan et al. 2007) and bacterial strains (Huang et al. 2003; Cardoza and Tumlinson 2006) also can induce differential volatile emissions from plants.

Low-molecular-weight volatiles with specific functional groups serve a vital role in the plant life cycle, providing a means for interacting with the surrounding environment (Dudareva and Negre 2005). Many plant VOCs and essential oils inhibit microbes or herbivores and function as volatile elicitors in defense responses (Dudareva et al. 2004; Holopainen 2004). Most constituents released from tomato leaves can block fungal growth and may play a key role in plant defenses (Hamilton-Kemp et al. 1992). Several components of tomato VOCs, e.g., (*E*)-2-hexenal and β-phellandrene, can somewhat inhibit *Botrytis cinerea* and *Fusarium oxysporum* (He et al. 2005a; Zhang et al. 2006).

Plant tissues often exhibit varying degrees of resistance depending on their stage of development. The relationship between maturity and disease resistance (age-related resistance, ARR) is well documented in different plant–pathogen systems (Kus et al. 2002; Panter and Jones 2002; Yun and Choi 2003; Rusterucci et al. 2005; Zeier 2005; Kwon et al. 2009). The onset of some ARR forms is associated with flowering, whereas others are correlated with plant age, leaf size, or the synthesis of secondary metabolites or defense proteins (Panter and Jones 2002). In tomatoes, resistance is conferred by *Cf-9B*, a novel form of ARR with homology to other genes; this occurs between early flowering and early fruiting (Parniske et al. 1997; Lauge et al. 1998; Panter et al. 2002). Over time, however, such tomato leaves become more susceptible to disease.

Zhang et al. (2008) have reported that both the production of volatile compounds and their inhibitory activity increase as tomato plants mature. Chitosan oligosaccharide, which is produced by the degradation of the chemical chitin, can enhance the formation of those volatile compounds and decrease plant susceptibility to *B. cinerea* (He et al., 2005b). Obara et al. (2002) also have found that chitosan oligosaccharide can trigger the emission of linalool, MeSA, and β-caryophyllene in rice plants (*Oryza sativa* L.). Therefore, our study objective was to clarify how the levels of volatile emissions and their inhibitory activity toward *B. cinerea* and *F. oxysporum* are changed in tomato leaves over time and in response to treatment with chitosan oligosaccharide.

Materials and Methods

Plant Materials, Growing Conditions, Definitions of Leaf Age, and Fungal Strains

Seeds of tomato (*Lycopersicon esculentum* Mill. cv. Qingyan1) were obtained from the Academy of Agricultural Science in Qingdao, China. For all experiments, plants were reared in growth chambers (37×25 cm) under a 16 h light/8 h dark cycle at 25°C/18°C. Plants exhibiting 15 to 16 leaves at anthesis were sampled. These tissues were numbered in ascending order according to their time of appearance. Leaves 5 to 6 were defined as "old," leaves 9 to 10 as "adult," and leaves 13 to 14 as "young."

Pathogen Preparations

B. cinerea Pers. pv. tomato and *F. oxysporum* f. sp. tomato were kindly donated by Professor Li Tian (The First Institute of Oceanography, State Oceanic Administration, Qingdao 266061, China). The former was propagated on potato dextrose agar at 22°C for 10 days, and suspensions were prepared at a density of 10^5 conidia ml^{-1} of distilled water. Suspensions for the latter were obtained by culturing that pathogen in potato dextrose agar at 28°C for 7 days, then adjusting the concentration of conidia to 10^5 spores ml^{-1} of distilled water.

Chitosan Oligosaccharide Treatment

The properties of chitosan oligosaccharide (Dalian Institute of Chemical Physics, Chinese Academy of Sciences) included an Mr<2,000 and a degree of polymerization (DP) of 2 to 10. The influence of this compound on volatile emissions was studied by exposing plants at anthesis to 5.0 mg ml^{-1} of chitosan oligosaccharide (suspended in distilled water). The control was distilled water only. All treatment solutions were applied with a hand sprayer to run-off. Plants were kept in a chamber at 24±1°C and 70% to 80% relative humidity. After 72 h, leaves representing three different ages were collected. All materials were frozen immediately in liquid nitrogen and stored at −80°C.

Inhibitory Effects of Volatiles on *B. cinerea* and *F. oxysporum*

The inhibitory effects of volatiles on pathogen growth were evaluated as described by Hamilton-Kemp et al. (1992) and Zhang et al. (2008). Single drops of *B. cinerea* or *F. oxysporum* spore suspensions were added to a 1.0-cm^3 block of 1.2% water agar, which was then placed in a 5-cm-diameter glass Petri dish. This dish was put into a 9-cm-diameter Petri dish containing crushed leaf tissue. A larger dish covered this entire system and was sealed with plastic packaging. Samples (0.5 g) of tissue were taken from three crushed leaves from both control and treatment groups, and 2 ml of water was added to produce a slurry. The control was a 9-cm-diameter glass Petri dish that contained only 2 ml of water. The effects of VOCs on spore germination and hyphal growth of *B. cinerea* were determined after 12 h of incubation at 23°C, whereas the influence of volatiles on *F. oxysporum* was evaluated after 12 h of incubation at 28°C. Hyphal lengths in the control and treatment groups were measured under a microscope (net micrometer ocular at ×250 magnification on the agar surface). Each treatment had five replicates. The rate and inhibition of spore germination and the inhibition of hyphal growth were calculated as described by He et al. (2005a). The following formulae were used: (1) Germination Inhibition Rate = [(germination rate of control − germination rate of treatment group)/germination rate of control × 100%]; and (2) Mycelium Growth Inhibition Rate = [(length of mycelia in control group − length of mycelia from treatment group)/length of control mycelia × 100%].

VOC Collection

Leaves of different ages were excised and sprayed with chitosan oligosaccharide. The procedure for VOC collection was based on methods described by Zhang et al. (2008). All leaf samples (5.0 g) were pulverized in liquid nitrogen with a chilled pestle, and the resulting slurry was quickly transferred to a 500-ml Purge-and-Trap apparatus. To this was added 1.0 ml of distilled water and 5.0 μl of camphor as an internal standard (20,000 mg l^{-1} dissolved in ether). Volatiles were trapped at 52°C for 2 h in a 150.0× 3.0 mm glass tube containing 150 mg of Tenax TA resin. The flow rate for nitrogen gas was 150 to 200 ml min^{-1}. Afterward, the trap tube was dried for 0.5 h at room temperature at a nitrogen flow rate of 100 to 150 ml min^{-1}. The trapped VOCs were eluted three times with 3 ml of ether then concentrated to 1.0 ml with nitrogen at a flow rate of 100 ml min^{-1}. Finally, 1-μl samples were subjected to analysis by gas chromatography-mass spectrometry (GC/MS). A blank was prepared without plant material.

GC/MS Analysis

An Agilent HP-6890 GC/MS (Agilent Technologies) with a capillary column (HP-5MS 5% phenyl methyl siloxane; 60 m×0.25 mm×0.25 μm film) was operated under the following conditions: injector temperature, 200°C; detector temperature, 280°C; pressure of helium carrier, 6.82 kpa; flow rate, 1 ml min^{-1}. The column temperature program was set initially at 50°C for 3 min and ramped at 5°C min^{-1} to 200°C and at 10°C min^{-1} to 270°C then held at 270°C for 3 min. MS conditions included an EI of 70 Ev; the mass detector was operated in the scan mode in the range of 40 to 350 amu, at 1.0 s per scan. The pulsed splitless technique was used.

Peaks were identified by comparing them to the blank. Our evaluation was based on chromatographic and mass spectral comparisons with authentic standards or via tentative identification using the National Institute of Standards and Technology mass spectral library and reference data in cases where no standards were available. Amounts of individual volatiles were calculated from peak areas on the ion chromatogram, based on the internal standard of camphor, as follows: volatile ($\mu g\ g^{-1}$) = area of volatile/peak area of internal standard) × 20.

Statistical Analysis

Results were expressed as means±standard deviation ($n \geq 3$). Comparisons among our three leaf ages were made through an analysis of variance, and those means were compared by Tukey's multiple range tests ($P<0.05$). Analysis between the control group and the chitosan oligosaccharide treatment group was performed with a Student's t test, and differences were considered significant at $P \leq 0.05$ or $P \leq 0.01$.

Results

Effects of Age on the Emissions of Volatiles from Tomato Leaves

Analysis of the volatiles emitted from leaves taken from different positions on the same plant revealed that age affected the production and/or release of biologically active volatiles (Fig. 1, Table 1). In all, 20 constituents belonging to four kinds of organic compounds were identified based on their mass spectra, retention index data, and an online library. The total amounts of volatiles over time varied markedly. Young leaves, near the top of the plant, produced 41.5% more volatiles than the adult leaves and 208.5% more than those from old leaves.

This age-related variation was also reflected in the composition of those volatiles, with 20 compounds being released from young leaves, 18 from adult leaves, and 11 from old leaves. The major VOC components emitted from young leaves were C6-aldehydes and, particularly, hexenal, whereas terpenes were the most important group released from adult and old leaves. Regardless of age, considerable amounts of terpenes, including monoterpene, and sesquiterpenoids (mostly β-phellandrene and caryophyllene) were found in our samples. Of all the volatiles, terpenes accounted for 52.6% from young leaves, 58.7% from adult leaves, and 73.6% from old leaves. Only one aromatic compound, methyl salicylate (MeSA), was detected in the volatiles from young and adult leaves. Except for (+)-4-carene, the levels of all volatiles from the different leaf ages decreased with time. For example, the amount of (*E*)-2-hexenal declined from 114.0 μg g^{-1} fresh weight (FW) in young tissue to 17.5 μg g^{-1} FW in the old leaves. For β-phellandrene, levels from young leaves were 73.8% higher than from adult leaves and 193.2% higher than from old tissues. Similarly, the amount of caryophyllene emitted by young leaves was 1.3-fold greater than from adult leaves and 3.0-fold higher than from old leaves. In contrast, the amount of (+)-4-carene was very low (5.85 μg g^{-1} FW) in the volatiles released from young leaves but increased to 13.05 μg g^{-1} FW for old leaves.

Certain compounds were detected only at a single developmental stage, e.g., ocimene and camphene in young leaves. Another seven compounds, including ethylcyclohexane, methyl salicylate, 3-carene, α-farnesene, copaene, germacrene D, and caryophyllene oxide, were not detected in samples from old leaves.

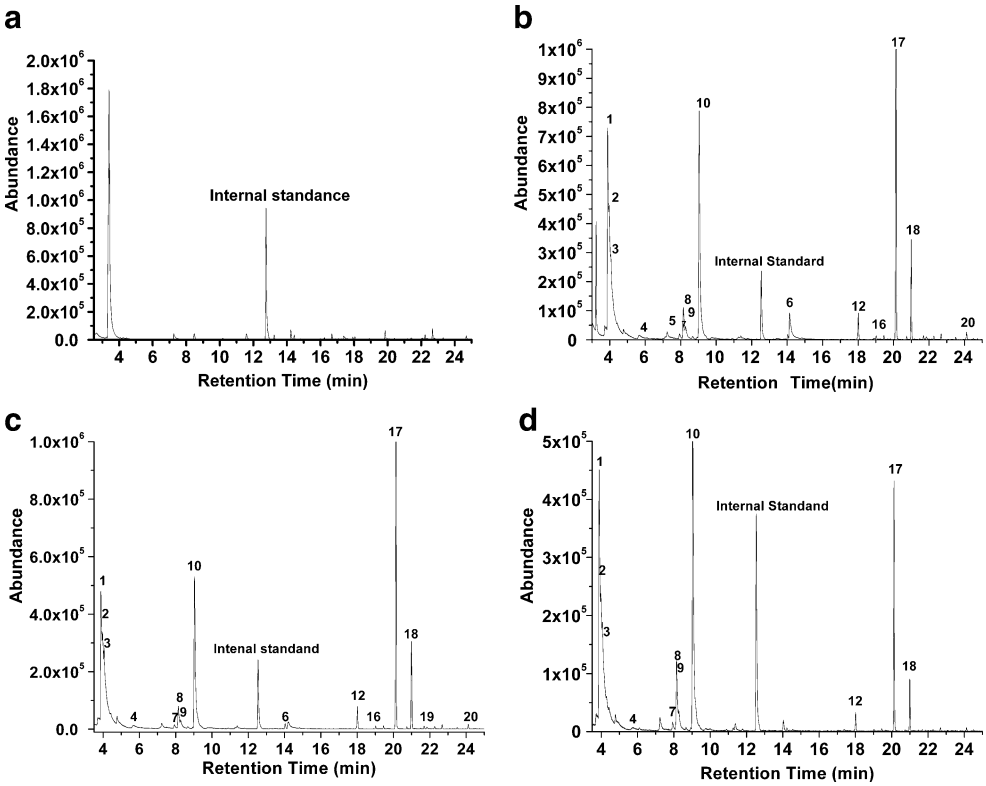

Fig. 1 Typical GC/MS chromatographic profiles of volatiles emitted from different-aged tomato leaves: **a** blank, **b** young, **c** adult, and **d** old. Compounds represented by peaks were identified as: (*E*)-2-hexenal (*1*), 2-hexenal (*2*), (*Z*)-2-hexen-1-ol (*3*), (*E,E*)-2,4-hexadienal (*4*), ethyl-cyclohexane (*5*), methyl salicylate (*6*), β-myrcene (*7*), (+)-2-carene (*8*), α-phellandrene (*9*), β-phellandrene (*10*), (+)-4-carene (*12*), copaene (*16*), caryophyllene (*17*), α-caryophyllene (*18*), germacrene D (*19*), and caryophyllene oxide (*20*)

Chitosan Oligosaccharide Induces Changes in the Emissions of Volatiles from Leaves of Different Ages

Compared with the control, the amounts of volatiles emitted from old and adult leaves were increased significantly, by 85.7% and 47.3%, respectively, after treatment with chitosan oligosaccharide (Fig. 2). However, the levels of volatiles from young leaves were decreased to 88.4% of the control after the same treatment. Old leaves showed a 217.8% increase in oxygenated aliphatic compounds and a 74.2% rise in monoterpenes compared with the control, while aromatic compounds from adult leaves were 356.7% higher than from the control. Young leaves had 28.5% more monoterpene after this treatment.

Application of chitosan led to a 460.0% increase in (*E*)-2-hexenal from old leaves and a 140.0% rise in the adult leaves versus the control, but its level in young leaves was decreased to 28.2% of the control value. (*Z*)-2-Hexen-1-ol was significantly elevated in old leaves but nearly unchanged for the other age groups. Methyl salicylate, the only aromatic compound detected here, was significantly enhanced in adult leaves and was 5.5 times higher than in the control after chitosan oligosaccharide treatment. This same treatment also was associated with the detection of another compound, 1R-α-pinene, at all three stages. For all leaves, α-phellandrene was slightly increased, whereas β-phellandrene and (+)-2-carene were markedly enhanced by chitosan oligosaccharide. α-Farnesene was significantly increased, by 4.9-fold in adult leaves and 5.2-fold in young leaves, compared with the control. Six other compounds—two monoterpenes (1R-α-pinene and camphene) and four sesquiterpenoids (copaene, α-farnesene, caryophyllene oxide, and germacrene D)—also were detected in old leaves following this treatment.

Age and Treatment with Chitosan Oligosaccharide Change the Ability of Emitted Volatiles to Inhibit Spore Germination and Hyphal Growth by *B. cinerea* and *F. oxysporum*

Leaf age affected the degree to which volatiles inhibited fungal growth (Fig. 3a, b). Those volatiles emitted from young leaves had the best antifungal activity against spore germination, with an inhibitory rate of almost 99.0%. For *B. cinerea* and *F. oxysporum*, the rates of hyphae inhibition were 92.9% and 82.0%, respectively. Volatiles emitted from adult leaves also showed good activity against *B. cinerea*, with rates >80% for both spore germination and hyphal development. The antifungal activity of volatiles from adult leaves on spore germination was higher than on hyphal growth for *F. oxysporum*. Compared with young and adult leaves, volatiles emitted by old leaves had much less antifungal activity. No significant inhibition of *Fusarium* spore germination was detected from that age group. However, their volatiles did

Table 1 Effects of leaf age on emissions of volatiles from tomato

Peak	Compounds	Content (μg g^{-1} FW)		
		Young leaves	Adult leaves	Old leaves
Oxygenated aliphatic compounds				
1	(*E*)-2-Hexenal	113.99±12.02 c	63.74±5. 94 b	17.46±3.16 a
2	2-Hexenal	69.14±7.71 c	51.83±5.52 bc	16.78±2.93 a
3	(*Z*)-2-Hexen-1-ol	7.10±2.65 c	7.08±2.09 bc	1.36±0.24 a
4	(*E*,*E*)-2,4-Hexadienal	4.44±0.79 b	2.41±0.45 a	3.05±0.87 ab
5	Ethyl-cyclohexane	1.83±0.28 b	0.31±0.11 a	–
Total		196.50	125.37	38.65
Aromatic compounds				
6	Methyl salicylate	17.81±3.21 b	7.47±1.68 a	–
Total		17.81	7.47	
Monoterpenes				
7	β-Myrcene	2.45±0.52 ab	1.71±0.21 a	4.07±0.97 b
8	(+)-2-Carene	11.23±1.81 b	6.69±1.08 ab	5.93±1.05 a
9	α-Phellandrene	7.15±1.13 b	4.67±0.69 a	5.93±0.92 ab
10	β-Phellandrene	91.44±8.34 c	52.61±5.58 b	31.19±3.23 a
11	Ocimene	0.42±0.13	–	–
12	(+)-4-Carene	5.80±1.24 a	7.32±1.71 b	13.05±2.26 c
13	Camphene	0.94±0.16	–	–
14	3-Carene	2.09±0.46 a	5.47±1.21 b	–
Total		121.52	78.47	60.17
Terpenoids				
15	α-Farnesene	0.99±0.25 a	3.11±0.78 b	–
16	Copaene	1.04±0.16 a	3.27±0.82 b	–
17	Caryophyllene	91.12±9.43 c	71.44±6.99 b	30.00±4.02 a
18	α-Caryophyllene	20.63±3.10 a	22.18±3.15 a	17.80±1.97 a
19	Germacrene D	0.94±0.21 a	3.66±0.98 b	–
20	Caryophyllene oxide	1.83±0.35 a	5.76±1.04 b	–
Total		116.55	109.42	47.80
Total VOC	452.38	320.73	146.62	

Values not followed by the same letter within a column are significantly different at 5%

block hyphal growth, resulting in rates against *B. cinerea* and *F. oxysporum* of 64.7% and 67.0%, respectively.

Treatment with chitosan oligosaccharide caused the inhibitory activity of these volatiles to change (Fig. 3c, d), most notably with old leaves, i.e., increasing to 45.2% and 27.2% for spore germination and 78.7% and 78.4% for hyphal growth in *B. cinerea* and *F. oxysporum*, respectively. An analogous but less pronounced improvement in inhibitory activity was detected from adult leaves that had been sprayed: 19.0% and 10.6% for spore germination and 11.4% and 22.2% for hyphal growth in *B. cinerea* and *F. oxysporum*, respectively. In contrast, inhibition by volatiles from young leaves was slightly diminished after chitosan oligosaccharide treatment.

Discussion

Throughout their life cycle, plants release diverse blends of volatile compounds, not just from flowers and fruits, but also from vegetative tissues (Farmer 2001; Dudareva and Negre 2005). Those from the latter, as part of a defense system, can either directly repel microbes and animals or indirectly protect the plant via tritrophic interactions that attract the natural predators of attacking herbivores (Dudareva and Negre 2005; Hu et al. 2008). Plant volatiles can be influenced by many factors, e.g., stress, growing conditions, elicitors, and developmental stage. In soybeans, levels of C6-aldehydes are high in younger leaves but are decreased markedly as that tissue matures (Zhuang et al. 1992). Dudai et al. (2001) have

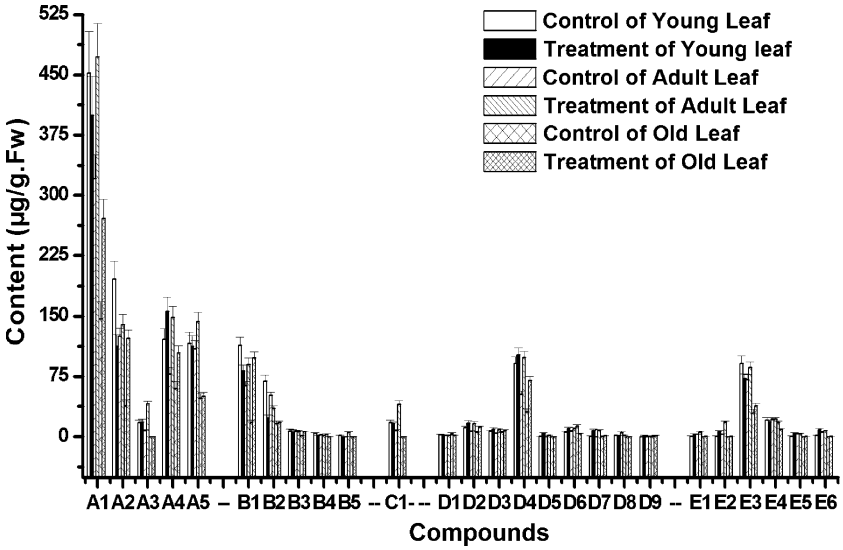

Fig. 2 Effects of chitosan oligosaccharide on quantities of emissions for volatiles at three stages of leaf development, as determined via GC/MS at 72 h post-treatment. *A1* all volatile compounds; *A2–A5* oxygenated aliphatic compounds, aromatic compounds, monoterpene, and sesquiterpenoids; *B1–B5* quantity of (*E*)-2-hexenal, 2-hexenal, (*Z*)-2-hexen-1-ol, (*E*,*E*)-2,4-hexadienal, and ethyl-cyclohexane; *C1* methyl salicylate; *D1–D9* β-myrcene, (+)-2-carene, α-phellandrene, β-phellandrene, ocimene, (+)-4-carene, camphene, 3-carene, and 1R-α-pinene; *E1–E6* copaene, α-farnesene, caryophyllene, α-caryophyllene, germacrene D, and caryophyllene oxide

studied the developmental control of monoterpene content and composition in *M. fruticosa* (L.) Druce and have discovered that the amounts of most compounds increase, while only a few decrease over time. Between emergence and maturation, the level of isoprene emissions rises >100-fold in the leaves of velvet beans (Kuzma and Fall 1993).

Although the composition of volatiles emitted from tomato leaves has been described previously (He et al. 2005b), little

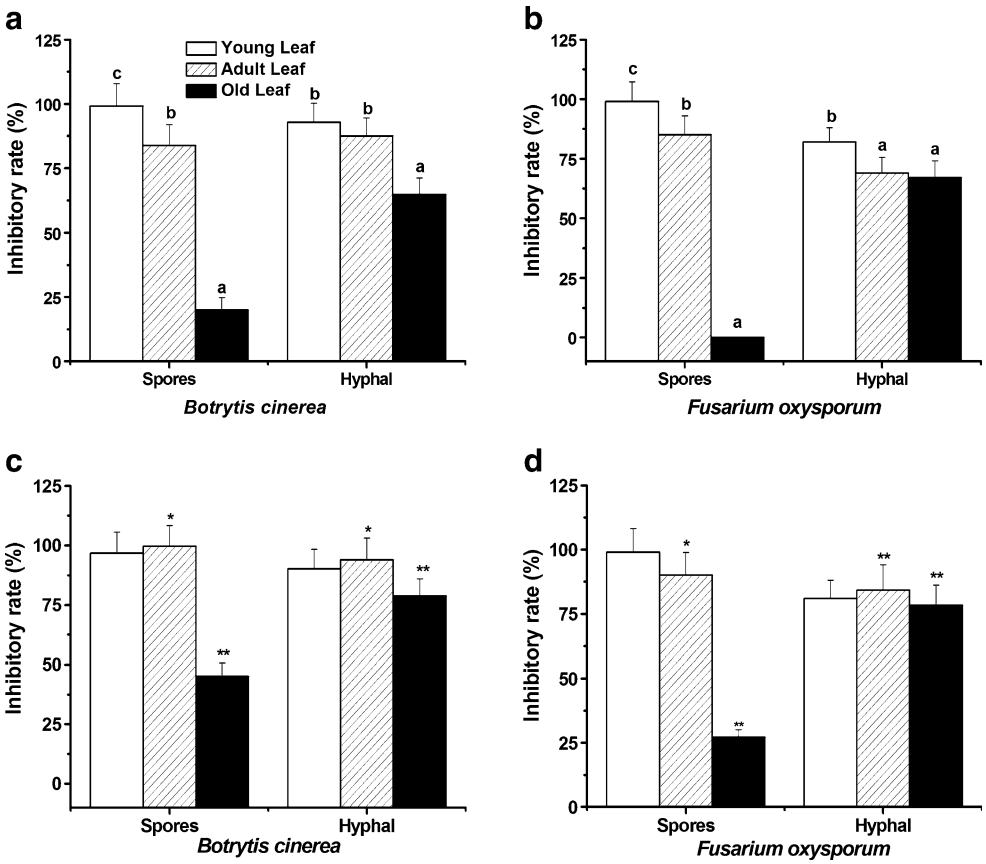

Fig. 3 Effects of volatiles on spore germination and hyphal growth by *B. cinerea* and *F. oxysporum*. **a** Inhibitory rates of volatiles emitted from different-aged leaves against *B. cinerea*; **b** inhibitory rates of volatiles emitted from different-aged leaves against *F. oxysporum*; **c** inhibitory rates of volatiles emitted from plants sprayed with chitosan oligosaccharide (5 mg ml^{-1}) against *B. cinerea*; **d** inhibitory rates of volatiles emitted from plants sprayed with chitosan oligosaccharide against *F. oxysporum*. Values not followed by the same letter are significantly different at 5% among leaf ages; $*P \leq 0.05$ and $**P \leq 0.01$ by *t* test denote significant differences between control and chitosan oligosaccharide treatments

has been known about how leaf age influences that composition or the plant's ability to inhibit pathogens. Here, a quantitatively and qualitatively different blend of volatiles was released by leaves, depending upon their developmental stage. These compounds were oxygenated aliphatic compounds, aromatic compounds, monoterpenes, and sesquiterpenoids. Young leaves produced high levels of volatiles, mainly (*E*)-2-hexenal, 2-hexenal, β-phellandrene, caryophyllene, and α-caryophyllene. As leaves developed, however, those emissions decreased markedly in quantity and quality.

The complex array of chemical responses that plants display during pathogen attack includes the induced emissions of VOCs (Cardoza et al. 2002; Huang et al. 2003; Cardoza and Tumlinson 2006; Park and Paek 2007). These may serve as a direct defense. The most important volatile compounds emitted by tomatoes strongly inhibit radial growth by *B. cinerea* (He et al. 2005a) and *F. oxysporum* (Zhang et al. 2006). Although volatiles emitted from old leaves significantly inhibited hyphal growth here, antifungal activity was greater from the volatiles of younger leaves. Those emitted by the latter were the most effective in suppressing spore germination and hyphal growth by both pathogens, with *B. cinerea* being more affected. We have previously reported that the degree of antifungal activity by certain compounds also depends upon their specific amounts and compositions (He et al. 2005a; Zhang et al. 2006).

Here, we also clarified the influence of leaf age on volatile emissions and their inhibitory role when tomatoes were sprayed with chitosan oligosaccharide. In the treated groups, older leaves generally released more volatile emissions than younger leaves. This response by the latter was attenuated or delayed because of the quantity of the volatiles and their effectiveness against *B. cinerea* and *F. oxysporum*. Whereas contents of volatiles from old and adult leaves were dramatically increased over time (compared with the control), those levels from young leaves were diminished following such treatment. We previously showed that methyl salicylate not only inhibits the growth of pathogenic fungi but also has teratogenic effects; for example, the hyphae of *B. cinerea* grow abnormally when exposed to 10~20 μmol L^{-1} MeSA (He et al. 2005a; Zhang et al. 2006). Here, that airborne signal, which induces resistance in neighboring plants (Shulaev et al. 1997), was slightly decreased by chitosan oligosaccharide in young leaves but was obviously enhanced in adult and old leaves compared with the control (data not shown). This might be a result of higher antifungal activity by those two older leaf groups.

We earlier confirmed that ten components of VOCs emitted from tomato can inhibit *B. cinerea* and *F. oxysporum* to varying degrees (He et al. 2005a; Zhang et al. 2006). Oxygenated aliphatic compounds—(*E*)-2-hexenal and nonenal—have strong antifungal activity against spore germination and fungal growth, while other constituents (MeSA, β-phellandrene, caryophyllene, and α-caryophyllene) may be essential components for such defenses in tomato after chitosan oligosaccharide treatment (He et al. 2005a; Zhang et al. 2006).

Rates of fungal inhibition were highest for volatiles emitted from treated old leaves. Activity was analogous but less pronounced by treated adult leaves but slightly decreased for volatiles from treated young leaves. These marked changes in the amounts and chemical compositions of volatiles were primarily due to developmental stage. Although responses increased with leaf age, we had determined that older tomato leaves became more susceptible to infection by *B. cinerea*. Nevertheless, the relative efficacy of chitosan oligosaccharide application improved with leaf age after such infection. Amounts of volatile emissions were strongly dependent on developmental leaf stage, and fluctuations in antifungal activity paralleled the contents of emissions over time. Therefore, our findings suggest that these VOCs contribute to plant age-related resistance.

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