Eur J Plant Pathol (2012) 132:91–100
DOI 10.1007/s10658-011-9851-x

Green-odour compounds have antifungal activity against the rice blast fungus *Magnaporthe oryzae*

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Accepted: 12 August 2011 / Published online: 7 September 2011

Abstract Four green-odour compounds—*trans*-2-hexenal, *cis*-3-hexenol, *n*-hexanal, and *cis*-3-hexenal—were applied (0.85 μg ml^{-1} as vapour) to rice plants in laboratory conditions to observe their biological activity against the phytopathogenic fungus *Maganporthe oryzae*, which causes rice blast disease worldwide. Two compounds, *trans*-2-hexenal and *cis*-3-hexenal, showed remarkable disease suppression efficacy (99.7% and 100% suppression, respectively), while *n*-hexanal had moderate (86.5%) and *cis*-3-hexenol had weak (20.8%) disease-suppressing effects. Pre-application and post-application of *trans*-2-hexenal or *cis*-3-hexenal had slight effects on blast incidence, suggesting that these compounds had direct effects to suppress *M. oryzae* infection. In fact, *trans*-2-hexenal and *cis*-3-hexenal exhibited a growth suppression effect on *M. oryzae*. Interestingly, these two compounds inhibited appressorium formation at lower concentrations than the growth suppression. Studies on the hypersensitive response (HR)-like reaction and plant β-1,3-glucanase activity in rice plant confirmed that induced resistance was not the major factor involved in the disease suppression mechanism. Results of this study conclusively showed that *trans*-2-hexenal and *cis*-3-hexenal possess potent inhibitory activities against the growth and the appressorium formation of *M. oryzae* and could be used as antifungal agents to significantly reduce *M. oryzae* infections in rice.

Keywords *cis*-3-hexenal · Green-leaf-derived C6-aroma compounds · Leaf aldehyde · *Magnaporthe grisea* · *Pyricularia oryzae* · *trans*-2-hexenal

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Introduction

Rice blast, caused by *Maganporthe oryzae* B. Couch (anamorph=*Pyricularia oryzae* Cavara) [previously known as *Magnaporthe grisea* (Hebert) Barr] (Couch and Kohn 2002), is one of the most important diseases that affect rice cultivation worldwide (Baker et al. 1997; Talbot 2003). Once in the plant, the fungus develops bulbous secondary hyphae and quickly colonizes the host tissue (Bourett and Howard 1990; Heath et al. 1990; Talbot et al. 1993). Damage to the rice crops result from either leaf blast, which kills or debilitates seedlings, or neck or panicle blast,

which destroys the rice grain during the seed-setting stage (Ou 1985).

In recent years, many techniques have been developed to control the fungus. However, blast disease is still a major threat to global rice production (Skamnioti and Gurr 2009). Therefore, alternative control methods are urgently required for the management of rice blast. It is known that some chemical fungicides can be used to control *M. oryzae* (Kurahashi et al. 1999; Amadioha 2000). A combination of high inoculum pressure, humid conditions that favour pathogen growth and frequent pesticide applications have resulted in the emergence of resistant pathogen strains (Yamada et al. 2004; Takagaki et al. 2004). There are concerns over the increasing loss of efficacy of conventional fungicides due to pathogen resistance, general unacceptability of fungicide usage, and environmental risks.

Natural products from plants have great potential as novel fungicide sources for controlling pathogenic fungi. The use of biologically based compounds in plant extracts may be an alternative to currently used fungicides to control phytopathogenic fungi and bacteria, because they virtually constitute a rich source of bioactive chemicals, such as phenols, flavonoids, quinons, tannins, alkaloids, saponins, and sterols (Isman 2000; Burt 2004). Since these extracts can be active against fungal and bacterial pathogens, are biodegradable to non-toxic products, and are potentially suitable for use in integrated pest management programs, they could become a new class of safer disease control agents. Some phytochemicals of plant origin (e.g., neem seed water extract, azadirchtin, carbvone, and pyrerethroids) have been formulated as botanical pesticides and are used successfully in integrated pest management programs (Satti et al. 2010).

The fresh scent emitted by green leaves has been known by the name "green odor" (Hatanaka et al. 1978). The green-odour compounds (GOCs) are of plant origin; are formed by a biosynthetic reaction from the chloroplast membrane through linolenic acid (Hatanaka 1996); and are known to have various physiological actions as plant-to-plant messengers in allelopathy, insect-attracting pheromone-like substances, bacteriocides, and phytontids (Hatanaka 1999).

Available information on the influence of GOCs on plant diseases often appears to be sporadic and inconclusive. It has been known that oil extracts of seeds of *Azadirachita indica* (neem) significantly reduce the in vitro radial growth of the rice blast fungus and the development and spread of the rice blast disease in the greenhouse (Amadioha 2000). However, there is no information available on which GOC affects the rice blast disease caused by *Magnaporthe oryzae.* In this study herein, we tested four major GOCs, *trans*-2-hexenal (leaf aldehyde), *cis*-3-hexenol (leaf alcohol), *n*-hexanal, and *cis*-3-hexenal, to explore their biological efficacy against the rice blast fungus. The objectives of this study were to: 1) investigate the disease suppression efficacy of the GOCs against *M. oryzae*; and 2) identify the disease suppression mechanism(s). We hypothesized that some of the compounds would prevent *Magnaporth*e growth and would therefore aid in the development of chemical control systems of the organism.

Materials and methods

Green-odour compounds (GOCs)

Four major GOCs (more than 98% purity)—*trans*-2-hexenal (leaf aldehyde), *cis*-3-hexenol (leaf alcohol), *n*-hexanal, and *cis*-3-hexenal—were used in this study (Fig. 1). *trans*-2-hexenal and *n*-hexanal were provided from Chisso Petrochemical Co. (Chiba, Japan). *cis*-3-hexenol and *cis*-3-hexenal were provided from Nippon Zeon Co. (Kanagawa, Japan). Compounds were treated as vapours or in solution. When treated as vapours, 5, 10, 20, 40, and 80 μl of compound was applied to a

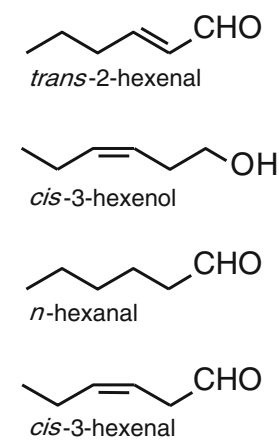

Fig. 1 Green-odour compounds (GOCs) used in this study

40-l bucket (see Fig. 2a). Maximum concentrations in the headspace were 0.11, 0.21, 0.43, 0.85, and 1.7 μg ml^{-1}, respectively. When treated in solution, 10 mg ml^{-1} stock solutions in DMSO were used.

Plant material and cultivation

We used a rice cultivar, Nipponbare (susceptible to *M. oryzae* isolate Kita 1), one of the popular rice crops in Japan. Rice seeds were germinated in the dark in a plastic Petri dish with 0.5-cm-deep water at 28°C for 3 d. Emerged seedlings were transplanted at 0.5 cm depth (approx.) in each black agri-pot (5× 15×10 cm) with "nursing soil" (pH 5.0; N: 0.029%; P: 0.071%; K: 0.036%) (Kureha Kagaku, Co., Ltd, Tokyo, Japan) by using sterilized forceps. The pots were then placed in a growth chamber (14 h light at 25°C:10 h dark at 20°C cycle) and grown for 4 wk until the 5th leaf expanded completely. The pots were watered with distilled water as and when necessary.

Fungal strain and cultivation

M. oryzae isolate Kita 1 (race 007), which is highly aggressive on the rice variety Nipponbare, was used in this study. *M. oryzae* was grown on OMA plates (7.25% oatmeal agar, Difco Co., Detroit, MI, USA), in PDB (2.4% potato dextrose broth, Difco Co.), or in YG (0.5% yeast extract, 2% glucose) at 25°C or 28°C. The conidial suspension was prepared as described previously (Motoyama et al. 1998).

Assays of the rice blast disease suppression efficacy of compounds

The experimental design is shown schematically in Fig. 2a. When the 5th leaf was fully expanded, 2 pots (14 rice seedlings) were put in a plastic pack with 1 l of water in the bucket (40-l), containing approx. 0.5 l of water to keep moist conditions for infection. Rice plants were then sprayed with 20 ml of spore suspension (1×10^5 ml^{-1} in 0.2% gelatin) with an artist's airbrush (Mr. HOBBY, Japan). After the spraying, compounds were added into the water of the bucket (see Fig. 2a) to treat the plants by their vapours. Rice plants that were sprayed with only spores were used as the control. The buckets were quickly covered with their lids and kept at 25°C for 24 h. After incubation, the rice plants were then transferred to plant growth chambers and continued to grow for 10 d for full symptoms to become

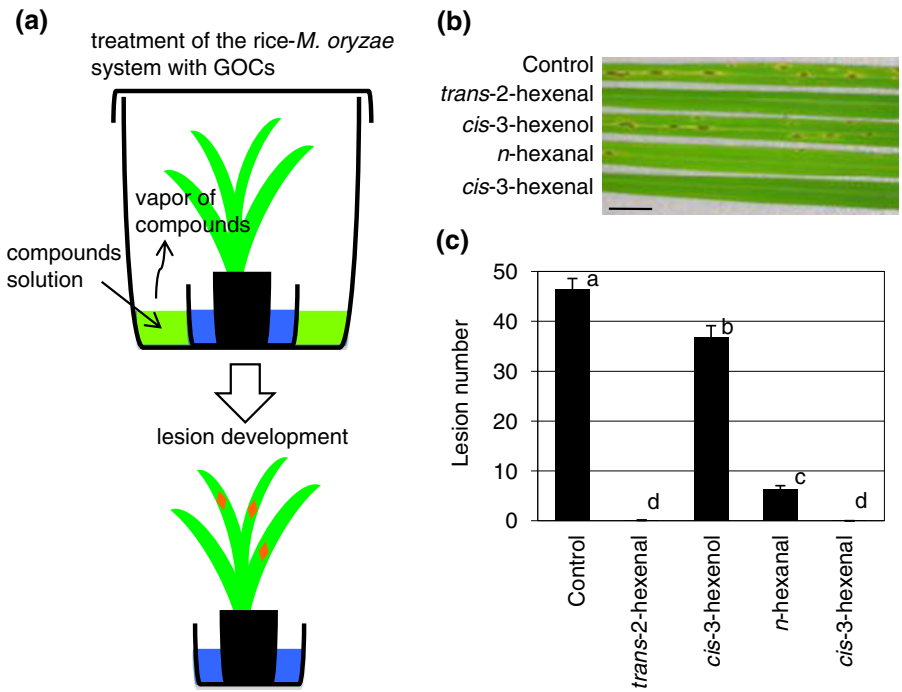

Fig. 2 Effect of GOCs on rice blast disease. Rice cv. Nipponbare was infected with *M. oryzae* isolate Kita 1 in the presence of 0.85 μg ml^{-1} compounds as vapours. **a** Experimental design. **b** Blast symptoms on 5th leaves of rice. Scale bar: 10 mm. **c** Lesion number (on the 3rd, 4th, and 5th leaves)/plant was shown as mean±SE of 3 experiments. Different letters above the bars indicate significant differences ($P<0.05$, Student's t test)

apparent. Disease incidence was recorded by counting the number of lesions (on the 3rd, 4th, and 5th leaves). Two pots (14 plants) were used for one experiment. We performed three experiments for one compound and statistically analyzed them. Lesion number (on the 3rd, 4th, and 5th leaves)/plant was shown as mean±SE.

Pre-application and post-application of compounds

Pre-application and post-application experiments were performed by modifying the method of the previous section. For pre-application experiments, rice plants were treated with GOCs for 2 d or 1 wk in the bucket shown in Fig. 2a before the spore suspension spray. After the spray, plants were incubated for 24 h in the bucket and cultivated in the growth chamber, as described in the previous section. For post-application experiments, rice plants were sprayed with spore suspension and incubated for 1 d or 2 d without compounds in the bucket. Then, plants were treated with compounds for 24 h in the bucket. After that, plants were cultivated in the growth chamber, as described in the previous section.

Observation of hypersensitive response (HR)-like reaction in rice plant

HR-like reaction-inducing activity of compounds was analyzed using the 4th leaf of the 5-leaf stage rice seedlings by a 96-well plate. Rice leaf (approx. 2 cm) was distributed into wells containing 50 μl of sterilized water plus the tested compound (10 μg ml^{-1}) or a spore suspension of *M. oryzae* (1×10^5 spores ml^{-1}). The plates were then put in a clear plastic box containing soaked paper towels, allowing them not to dry for at least 120 h, and placed in a growth chamber (phytotron) with a 12-h photoperiod and a day/night temperature regimen of 20°C. Three days after incubation, the brown part that was caused by an HR-like reaction was visually observed and recorded as no reaction (−), slight (+), moderate (++), or strong (+++). Cholic acid was used as a control. The "slight (+)" effect was equivalent to that of 200 μM cholic acid, "strong (+++)" was equivalent to that of 2000 μM cholic acid, and "moderate (++)" was between slight and strong. In the "slight" reaction, approximately 3% of the leaf area became brown. In the "strong" reaction, approximately 30% of the leaf area became brown.

Determination of ß-1, 3-glucanase activity in rice plant

For the measurement of β-1,3-glucanase activity, rice plants were treated with GOCs by using the same method as in the previous section, except that the incubation period was 2 d instead of 3 d. β-1,3-glucanase activity was assayed using azurine-crosslinked pachyman (AZCL-pachyman, Megazyme, Ireland) as the substrate by modifying the method described by Morohashi and Matsushima (2000). Briefly, treated rice leaves were broken with the SK Mill (Tokken, Kashiwa, Japan), and proteins were extracted with 0.4 ml 50 mM Na-acetate (pH 6.0). Protein concentration of this extract was determined with the Pierce BCA protein assay kit (Takara shuzo, Japan). Enzyme solution (200 μl) was mixed with 10 mg AZCL-pachyman and incubated at 25°C for 4 h. The reaction was terminated by adding 0.6 ml ethanol, and the amount of soluble dyed fragments that were released from AZCL-pachyman was determined colorimetrically at 590 nm. One unit of enzyme activity represents an increase in 1.0 absorbance units under the conditions used.

Observation of hyphal growth and appressorium formation in presence of the compounds

Minimal inhibitory concentration (MIC) values were determined as follows. *M. oryzae* strains were pre-cultured for 3 days in 5 ml of YG. Then, 100 μl of this pre-culture was mixed with 10 ml of PDB; 100 μl of this mixture was added to each well of a 96-well plate and mixed with 1 μl of compound solutions at 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0.078 mg ml^{-1} in DMSO. Final concentrations of the compounds were 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 μg ml^{-1}. The plate was sealed with a plastic tape to prevent volatilization of compounds and incubated at 28°C for 3 d. The lowest concentrations of compounds that caused complete growth inhibition were recorded as MICs.

In order to test the activity of the compounds on appressorium formation, 50 μl of compound solution (10 μg ml^{-1}) containing conidia (1×10^4 ml^{-1}) was put onto plastic coverslips lying in a Petri dish. To prevent volatilization of the compound, the Petri dishes were covered immediately with their lids and then sealed with vinyl tape. After a 24-h incubation at 25°C, the frequency of appressorium formation was analyzed. Appressorium formation efficiency was measured at least 3 times, using more than 100 spores per assay.

Results

Effect of green-odour compounds on rice blast disease

Effects of four green-odour compounds (GOCs) (Fig. 1) on rice blast disease suppression were analyzed (Fig. 2). Compounds were applied at a concentration of 0.85 μg ml^{-1} as vapour, as described in Fig. 2a. *trans*-2-hexenal and *cis*-3-hexenal substantially decreased the lesion number on rice leaves compared to control (Fig. 2b). Suppressive effects of *trans*-2-hexenal and *cis*-3-hexenal were 99.7% and 100%, respectively (Fig. 2c). On the other hand, *n*-hexanal was moderately effective (86.5% suppression), and *cis*-3-hexenol showed only a weaker effect (20.8% suppression). *trans*-2-hexenal and *cis*-3-hexenal were selected for further detailed investigation.

Effect of rate of application on the incidence of rice blast

A proper rate of application can provide control of the disease and increase the yield of plants. Here, we estimated appropriate application rates of *trans*-2-hexenal and *cis*-3-hexenal (Fig. 3). Concentrations of 0.11, 0.21, 0.43, 0.85, and 1.7 μg ml^{-1} as vapours were selected based on the outcomes of previous experiments. The number of lesions observed with the same compound varied markedly depending on the concentrations. Higher concentrations of *trans*-2-hexenal (0.85 to 1.7 μg ml^{-1}) showed potent disease suppression effects (Fig. 3a). Similar results were also observed in the rice plants treated with *cis*-3-hexenal (Fig. 3b,c).

To identify the potential effect of the compound mix on rice blast disease, equivalent amounts of the compounds were mixed with one another (0.43 μg ml^{-1}+0.43 μg ml^{-1}) to produce so-called 'green odour' and applied to rice plants (Fig. 4). The combination of *trans*-2-hexenal and *cis*-3-hexenal was most effective.

Effect of pre-application and post-application on the incidence of rice blast

To investigate whether the compounds can induce disease resistance in rice plants, a pre-application experiment was performed (Fig. 5a). Rice plants were treated with 0.85 μg ml^{-1} *trans*-2-hexenal or *cis*-3-hexenal 2 d and 1 wk before inoculation with the spore suspension. In the 0-d control, compounds and *M. oryzae* spores were applied simultaneously. Pre-

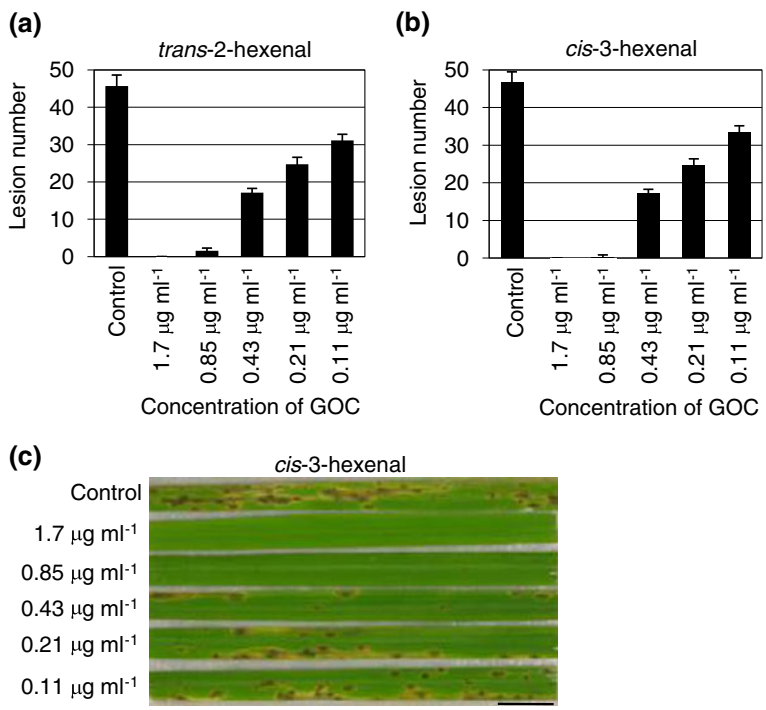

Fig. 3 Effect of GOC concentrations on rice blast. **a** Application of *trans*-2-hexenal. **b** Application of *cis*-3-hexenal. **c** Rice blast lesion in *cis*-3-hexenal-treated 5th leaves. Scale bar: 10 mm. In (**a**) and (**b**), lesion number (on the 3rd, 4th, and 5th leaves)/plant was shown as mean±SE of 3 experiments

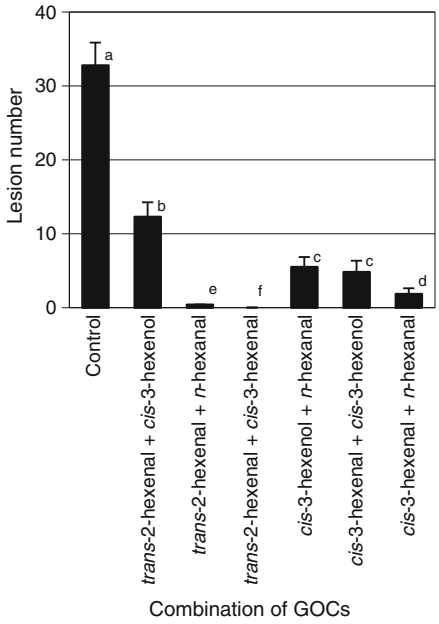

Fig. 4 Effect of compound mix (0.43 μg ml^{-1}+0.43 μg ml^{-1}) on rice blast incidence. Lesion number (on the 3rd, 4th, and 5th leaves)/plant was shown as mean±SE of 3 experiments. Different letters above the bars indicate significant differences ($P<0.05$, Student's t test)

application of *trans*-2-hexenal or *cis*-3-hexenal had little effect on disease suppression.

To investigate whether the compounds can affect *M. oryzae* growth in rice cells, a post-application experiment was done (Fig. 5b). Rice plants were treated with 0.85 μg ml^{-1} of *trans*-2-hexenal or *cis*-3-hexenal 1 and 2 d after inoculation with the spore suspension. In the 0-d control, compounds and *M. oryzae* spores were applied simultaneously. Post-application of *trans*-2-hexenal or *cis*-3-hexenal had little effect on disease suppression.

Effects of the volatile compounds on induced resistance in rice plant

To investigate the involvement of induced resistance in disease suppression, we observed the HR-like reaction-inducing activity of the compounds in rice plants (Table 1). Cholic acid, a known HR-inducing compound in rice plant (Koga et al. 2006), was used as a positive control. The 4 volatile compounds at 10 μg ml^{-1} in solution caused no or slight HR-like reactions in rice leaves in the absence of *M. oryzae* spores. In the presence of *M. oryzae* spores, HR-like reactions treated with GOCs except *cis*-3-hexenol were slightly higher than in the leaves in the absence of *M. oryzae.* In all the cases, HR-like reactions were much weaker than the positive control.

Next, we focused on β-1,3-glucanase, a marker enzyme of HR in rice plant. β-1,3-glucanase is also known as a pathogenesis-related protein (PR protein) involved in plant defence. We measured the β-1,3-glucanase activity in 4 GOC-treated (10 μg ml^{-1} in solution) plants as shown in Fig. 6. β-1,3-glucanase activity was higher in the compound-treated plants compared to the untreated control. However, we could not detect significant difference in the enzymatic activity between *cis*-3-hexenal-treated plants and *n*-hexenal-treated plants although *cis*-3-hexenal shows

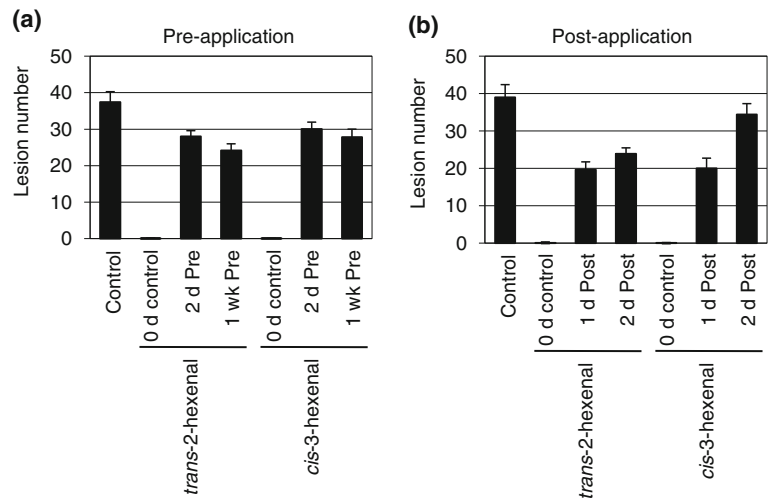

Fig. 5 Effect of (**a**) pre-application and (**b**) post-application time of *trans*-2-hexenal and *cis*-3-hexenal on rice blast. Compounds (0.85 μg ml^{-1}) were applied before *M. oryzae* inoculation (Pre) or after *M. oryzae* inoculation (Post). "0 d control" means that compounds and *M. oryzae* spores were applied simultaneously. Lesion number (on the 3rd, 4th, and 5th leaves)/plant was shown as mean±SE of 3 experiments

Table 1 Hypersensitive response (HR)-like reactions in rice plant treated with different GOCs at 10 μg ml^{-1}. The results of 3 experiments are presented here

Treatment	Replication	HR-like reaction (3 d after incubation)	
		without *M. oryzae*	with *M. oryzae*
Control(water)	1	−	−
	2	−	−
	3	−	−
Control (2 mM cholic acid)	1	+++	+++
	2	+++	+++
	3	+++	+++
trans-2-hexenal	1	−	+
	2	−	+
	3	−	+
cis-3-hexenol	1	−	−
	2	−	−
	3	−	−
n-hexanal	1	−	+
	2	−	+
	3	−	+
cis-3-hexenal	1	+	+~++
	2	+	+~++
	3	+	+~++

− no reaction, + slight, ++ moderate, and +++ strong reaction

significantly higher disease-suppressive activity than *n*-hexenal. In all the cases, β-1,3-glucanase activities were much lower than in the positive control.

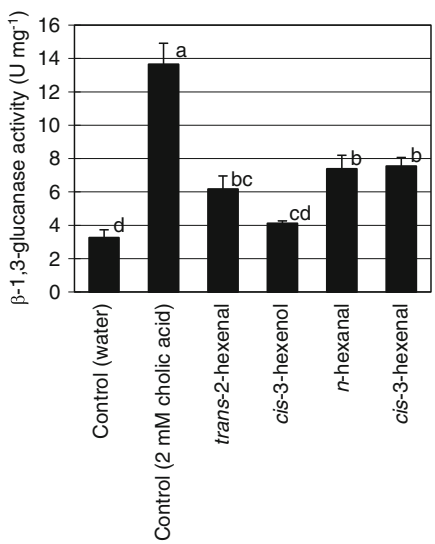

Fig. 6 Activity of β-1,3-glucanase in the presence of *M. oryzae* in rice plants treated with different GOCs at 10 μg ml^{-1}. The results are expressed as the mean±SE of 3 experiments. Different letters above the bars indicate significant differences ($P<0.05$, Student's *t* test)

Effects of the volatile compounds on hyphal growth and appressorium formation

To test for an effect on hyphal growth, we determined the MIC values for the compounds in solution (Table 2). *cis*-3-hexenal showed the most potent activity (MIC: 25 μg ml^{-1}). *trans*-2-hexenal also showed growth inhibitory activity (MIC: 50 μg ml^{-1}). *cis*-3-hexenol and *n*-hexenal could not cause complete growth inhibition up to 100 μg ml^{-1}.

cis-3-hexenal at 10 μg ml^{-1} in solution completely suppressed appressorium formation of *M. oryzae* (Table 3). In contrast, *trans*-2-hexenal had a moderate effect (appressorium formation efficacy 37.6%), while *n*-hexanal and *cis*-3-hexenal had very little effect on appressorium formation (efficacy ranging from 71.9 to 80.2%) compared with the untreated control treatment (appressorium formation efficacy 85.3%).

Discussion

We found that two GOCs, *trans*-2-hexenal and *cis*-3-hexenal, have remarkable suppressive effects against rice blast disease. Similar findings were made by Neri

Table 2 Effect of GOCs on hyphal growth of *M. oryzae*

Compound	MIC ($\mu g\ ml^{-1}$)
trans-2-hexenal	50
cis-3-hexenol	100<
n-hexenal	100<
cis-3-hexenal	25

et al. (2006) in pear plants. They reported that *trans*-2-hexenal was the only compound of the four that were tested to show control of pear disease that was caused by *Penicillium expansum*. *trans*-2-hexenal and *cis*-3-hexenal occur naturally in many edible plants, such as tomato, endive (Whitfield and Last 1991), strawberries (Latrasse 1991), apples, pears, stone fruits (Berger 1991), kiwifruits (Witerhalter 1991), and olive oil (Kubo et al. 1995). These compounds may also be involved in defence against pathogens in natural environments.

GOCs are volatile C6-aroma compounds that are emitted from plants. One interesting nature of GOCs is the difference in biological activity as a vapour and in solution. In this paper, GOCs appeared to show higher activity as vapours than in solution. For example, 0.85 $\mu g\ ml^{-1}$ *trans*-2-hexenal and *cis*-3-hexenal as vapours could suppress lesion formation nearly completely (Fig. 2). In contrast, in solution, higher concentrations were required for hyphal growth inhibition (25–50 $\mu g\ ml^{-1}$, Table 2) and appressorium formation inhibition (around 10 $\mu g\ ml^{-1}$, Table 3). A similar phenomenon was observed in the inhibition of bacterial growth by GOCs (Hatanaka, unpublished data; Nakamura and Hatanaka 2002). Elucidation of the reasons for this difference is important for the development of efficient application methods for the compounds.

Table 3 Effects of GOCs (10 $\mu g\ ml^{-1}$) on appressorium formation of *M. oryzae*. The results are expressed as the mean±SE of 3 experiments

Compound	Appressorium formation efficiency (%)
control	85±15
trans-2-hexenal	38±9
cis-3-hexenol	80±17
n-hexenal	72±12
cis-3-hexenal	0±0

Plants protect themselves from pathogens by inducing a specific reaction, called a hypersensitive reaction (HR), in response to pathogens. Therefore, HR-inducing compounds can control rice blast disease (Koga et al. 2006). However, the reduced blast incidence in *trans*-2-hexenal- and *cis*-3-hexenal-treated plants did not coincide with the induction of the HR-like reaction and HR marker enzyme (β-1,3-glucanase) activity. A pre-application experiment (Fig. 5a) also supports that the induced resistance in plant is not the major factor in the disease suppression mechanism of the compounds.

Disease suppressive compounds, *cis*-3-hexenal and *trans*-2-hexenal, had comparatively stronger inhibitory effects on hyphal growth and appressorium formation of the pathogen. Post-application of compounds had little effect on disease suppression, suggesting that the inhibition of appressorium formation appears to be the basis for the protection against *M. oryzae*. This hypothesis is also supported by the fact that the compounds have higher inhibitory activity against appressorium formation (Table 3) than hyphal growth (Table 2). Although some studies have reported the antifungal activity of GOCs (Boue et al. 2005; Kishimoto et al. 2008), the exact mechanism of action of such compounds is poorly understood. Studies of effects of GOCs on the morphology and gene expression of the fungus may reveal the mechanisms.

The timing and rate of application are important for practical rice blast disease control. Good control of blast was obtained when *trans*-2-hexenal and *cis*-3-hexenal were treated just after spore inoculation. Pre-application and post-application of the compounds had little effect on disease suppression. The effects of *trans*-2-hexenal and *cis*-3-hexenal on disease suppression were different, depending on the application rate. Higher concentrations (0.85 and 1.7 $\mu g\ ml^{-1}$) of *trans*-2-hexenal and *cis*-3-hexenal had excellent disease suppressive effect. In addition, the 1.7 $\mu g\ ml^{-1}$ application rate of *trans*-2-hexenal and *cis*-3-hexenal had no inhibitory effects on the growth of rice plants (data not shown). Therefore, the proper timing of application and the higher application rate of compounds (0.85–1.7 $\mu g\ ml^{-1}$) are critical factors in efficient disease control.

Four major GOCs were used in this study. We analyzed the effects of all six possible combinations of compound mixes on rice blast disease suppression (Fig. 5). As expected, the combination of *trans*-2-hexenal and *cis*-3-hehenal was most effective. We

could not observe apparent synergistic effects on disease suppression.

We conclude that *trans*-2-hexenal and *cis*-3-hexenal could provide an alternative strategy to fungicides in the control of *M. oryzae* infections in rice, as a 'green-chemical' fumigant. The European Commission in the FAIR program has actively stimulated the development and commercial implementation of such natural anti-fungal agents. Therefore, the potential use of GOCs for disease management should be the focus of future research. Furthermore, investigation is required, however, to test the safety and mammalian toxicity of *trans*-2-hexenal and *cis*-3-hexenal and to enable practical application of these compounds.

Acknowledgements We thank Laboratory Head Dr. Y. Arimoto (Arimoto Laboratory, RIKEN, Japan) for providing the growth room space. This work was supported by the Japan Society for the Promotion of Science (JSPS), the Ministry of Education, Science, Sports and Culture (FY2007, JSPS/FF1/30, ID No.: P 07626), Japan, and a Universiti Putra Malaysia (UPM) Research University Grant (01-04-10-1040RU).

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