An Ovicidal Substance Produced by Rice Plants in Response to Oviposition by the Whitebacked Planthopper, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae)

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Benzyl benzoate was identified in extracts of the watery oviposition lesion formed by rice plants in response to oviposition by *Sogatella furcifera*. The water solution of benzyl benzoate exhibited ovicidal activity against *S. furcifera* eggs at concentrations of ≥ 6.4 ppm at 25°C. This ovicidal substance was not detected in either intact rice plant tissues or in non-watery oviposition sites, where the mortality rate of *S. furcifera* eggs is much lower than it is in watery oviposition lesions.

Key words: *Sogatella furcifera*, ovicide, rice, benzyl benzoate, plant response

INTRODUCTION

The whitebacked planthopper, *Sogatella furcifera* (Horváth) is a major insect pest of rice. It lays eggs in the intercellular air spaces of the parenchymal tissue of leaf sheaths and midribs of leaf blades. Sogawa (1991) and Suzuki et al. (1993, 1994) revealed that *S. furcifera* eggs suffer a high physiological mortality on *japonica* rice varieties as a result of plant reaction to oviposition. The physiological egg mortality depends on the growing stages of rice and is highest at the maximum tillering stage on the Hinohikari variety (Suzuki et al., 1993). We recently found that the physiological death of *S. furcifera* eggs is associated with the watery lesion formed in most of the oviposition sites within 12 h after egg-laying (Suzuki et al., 1996). Since *S. furcifera* eggs can complete embryonic development in distilled water, the involvement of an ovicidal substance was believed to be the reason for the death of *S. furcifera* eggs in the watery oviposition lesion.

The present paper reports the detection of benzyl benzoate in the watery lesion tissues as an ovicidal substance produced in response to *S. furcifera* oviposition.

MATERIALS AND METHODS

Plants. Seedlings of a *japonica* rice variety, Reiho, were individually transplanted in 220 ml plastic cups after 3-d germination at 25°C, and cultivated in an outdoor growth cabinet controlled at 14L24°C : 10D20°C. Fertilizer was applied once 4 weeks after transplanting. Plants at the maximum tillering stage were used throughout the experiments after the small tillers were removed because the ovicidal reaction against *S. furcifera* is weak on small tillers (Suzuki et al., 1996).

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Sampling plant tissues. Gravid *S. furcifera* females were obtained from a stock colony maintained at the Kyushu National Agricultural Experiment Station on small Reiho seedlings since 1989. It was confirmed that Reiho seedlings do not form the watery lesion in response to *S. furcifera* oviposition. The insects were released at a rate of 16 females per plant to rice plants which were individually covered with a clear plastic cylinder with a tetron gauze cap, and allowed to oviposit for 1 d in a rearing chamber at 25°C, 16L : 8D. After removing the insects, the plants were kept in the same chamber for 1 d prior to sampling plant tissues. Leaf sheaths bearing oviposition-induced watery lesions were sampled by removing the sheaths with scissors and cutting them into pieces of ca. 1.8 cm each. For comparison, the corresponding position of the leaf sheaths was also sampled from intact *S. furcifera*-free plants (intact plant tissues) as described above.

Extraction and separation of ovicidal substance. Sample tissues were immersed in a tenfold volume of 70% MeOH in water (70% MeOH) for 1 d at 25°C, and the extract was collected by filtration. The residue of samples was again extracted with 70% MeOH for 1 d. Both filtrates were combined and MeOH was removed under reduced pressure. The water layer was then extracted 3 times with the same volume of ether. The ether layer was dehydrated over anhydrous Na_2SO_4. After the solvent was removed under reduced pressure, the ether extract was separated on a Florisil column (Wako Florisil PR, 10 g in 1 cm i.d. × 20 cm glass column) by eluting with 10 ml of each of the following solvents; 40% ether in hexane, 5 times (fraction No. 1–5), ether, 5 times (fraction No. 6–10) and, finally, acetone, 3 times (fraction No. 11–13).

Bioassay. *S. furcifera* eggs were obtained within 6 h after oviposition by dissecting small seedlings of Reiho on which gravid females were allowed to oviposit for 3 h. Test samples were transferred into the glass vials (1.5 cm i.d. × 4 cm) and, after removing the solvent, 250 μl of distilled water was added. Thirty eggs were put on a piece of tetron gauze (6 × 6 mm) and immersed into a water solution or distilled water (for control) in the vial. The vials were capped and kept at 25°C for 7 d. Egg mortality was evaluated by checking for eye-spot formation.

Synthetic benzyl benzoate was purchased from Wako Pure Chem. Industries Co., Ltd. Distilled water was saturated with benzyl benzoate (BB) and the saturated solution (25.4 ppm) was diluted to prepare a series of test solutions. BB concentration was determined by GC analysis. The bioassay was carried out in 250 μl of water solution in the vials.

Fisher's exact test with Bonferroni-adjusted probabilities was employed in pairwise comparisons for the egg mortality rate between controls and each treatment.

Instrument analyses. A Hewlett-Packard (HP) 5890 series II gas chromatograph equipped with an HP5972 mass detector and an HP-5 fused silica capillary column (Hewlett-Packard, 0.25 mm i.d. × 30 m, 0.25 μm film thickness) was used under the following conditions: Column oven temperature program (TP), 50°C for 1 min, 50 to 300°C at 10°C/min, and 300°C for 4 min; carrier gas, helium at a column head pressure of 20.7 kPa; mass detector, EI mode at 70 eV.

For certain GC analyses, a Shimadzu GC-14B gas chromatograph equipped with an FID and a TC-1 column (GL Science, 0.32 mm i.d. × 30 m, 0.25 μm film thickness) was operated under the same TP as the GC-MS analyses.

A liquid chromatograph consisting of a Jasco PU-987 pump unit and a Jasco MD-910 multiwavelength detector was equipped with an ODS-2 column (GL Science, 4.6 mm i.d. × 250 mm). As the eluting solvent, 90% MeOH in water was flowed at a rate of 0.5 ml/min. For injection, substances were dissolved in the same solvent as the eluting solvent.
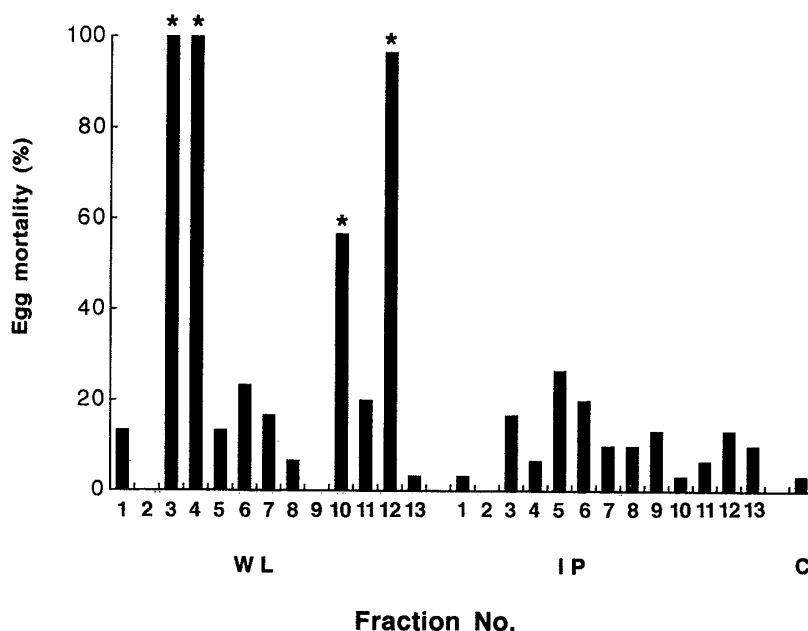
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Fig. 1. Mortality of *S. furcifera* eggs immersed in water solution of fractionated ether extracts obtained from 70% MeOH layer of watery oviposition lesion tissues (WL) and intact plant tissues (IP). For control, eggs were immersed in distilled water (C). Asterisk (*) indicates significant difference from control at $p = 0.01$.

Quantification of BB in plant tissues. For determining BB contents, ether extracts were prepared using 70% MeOH extracts from 22 pieces of sample tissues (38.7 cm long) of watery and non-watery oviposition lesion tissues, as described for the treatment for bioassay and GC-MS analysis. The amounts of BB in these ether extracts were measured using the GC-MS without subjecting it to Florisil column chromatography.

RESULTS

Identification of ovicidally active substance

For the bioassay, an ether extract corresponding to 85 pieces of sample tissues (150 cm long; 2.24 g of the watery oviposition lesion tissues and 1.50 g of intact plant tissues) was used. Each fraction from the Florisil column chromatography was submitted to a bioassay for ovicidal activity.

The egg mortality rate was significantly higher at $p = 0.01$ in 4 of the 13 fractions of watery oviposition lesion tissue extract than it was in the control (Fig. 1). In fractions 3 and 4 that were eluted with 40% ether in hexane, all eggs died without showing embryonic development, which was similar to the typical death occurring in the watery oviposition lesions. In fractions 10 and 12, the mortality rate to eye-spot formation stage decreased to 56.7–96.7% (Fig. 1). In contrast, the egg mortality rate in every fraction of intact plant tissue extract was less than 30% and not significantly different from that of the control.

GC-MS analyses of active fractions 3 and 4 showed the presence of a conspicuous peak at $t_R = 18.72$ min that was not observed in the corresponding fractions from intact plants. The mass spectrum of the substance showed a molecular ion peak at m/z 212 and characteristic fragment ion peaks at m/z 105, 91 and 77 (Fig. 2), which were considered to
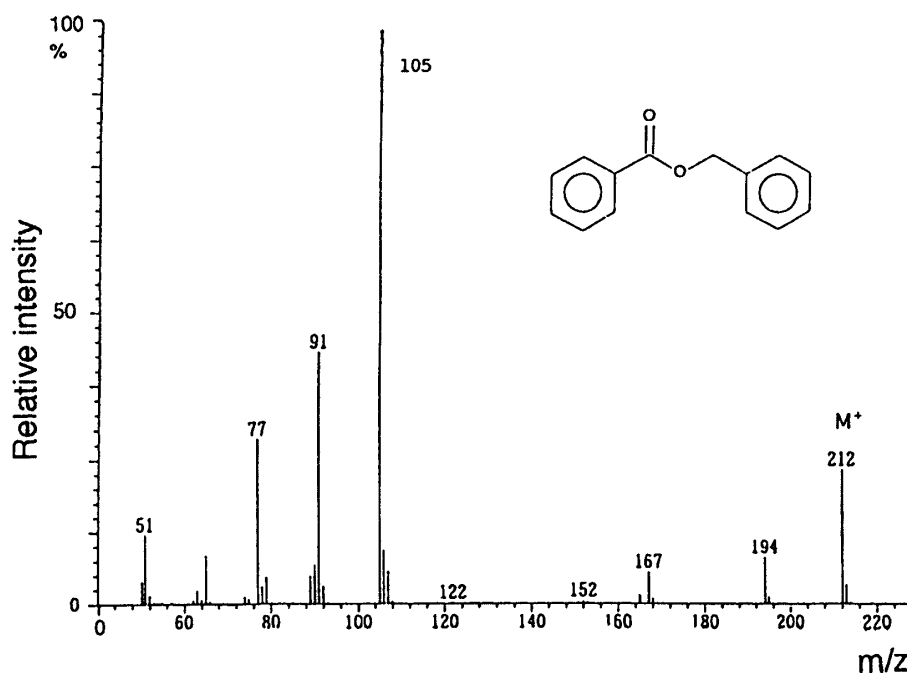
Fig. 2.   Mass spectra of the conspicuous compound, benzyl benzoate, in watery oviposition lesion tissue extracts (EI 70 eV, $t_R = 18.72$ min).

correspond to $C_6H_5\text{-}CO^+$, $C_7H_7^+$ and $C_6H_5^+$, respectively. By taking molecular size into consideration, the conspicuous substance was suggested to be BB. This interpretation was confirmed by injecting authentic BB into the GC-MS under the same conditions. The mass spectrum and retention value ($t_R = 18.72$ min) of BB were identical to those of the conspicuous substance in the active fractions.

For further confirmation, active fraction 3 and authentic BB were analyzed with GC and LC. By the GC analyses using a different apolar column (TC-1), the conspicuous substance and authentic BB showed the same retention value ($t_R = 14.73$ min). By the LC analyses, these compounds also showed the same retention value ($t_R = 9.44$ min) and UV spectra ($\lambda_{max} = 231$ nm, $\varepsilon_{max} = 3.0 \times 10^4$). These results confirmed that the conspicuous substance in the ovicidally active fractions was BB.

Ovicidal activity of BB

The embryonic development of *S. furcifera* eggs was completely inhibited in a water solution of synthetic BB at 25.4 ppm (Fig. 3). The egg mortality was significantly higher at 25.4, 12.7 and 6.4 ppm than it was in the control (0 ppm).

This result was consistent with the 100% egg mortality in fractions 3 and 4 which contained 22.9 µg and 10.6 µg of BB, respectively, in 250 µl distilled water prepared for the bioassay.

For comparison, the ether extracts of watery oviposition lesions were also assayed by the same method. A water solution of the ether extract was prepared so that its BB concentration corresponded with that of the synthetic BB. The egg mortality in the water solution of the ether extract of the watery oviposition lesion tissues was significantly higher only at
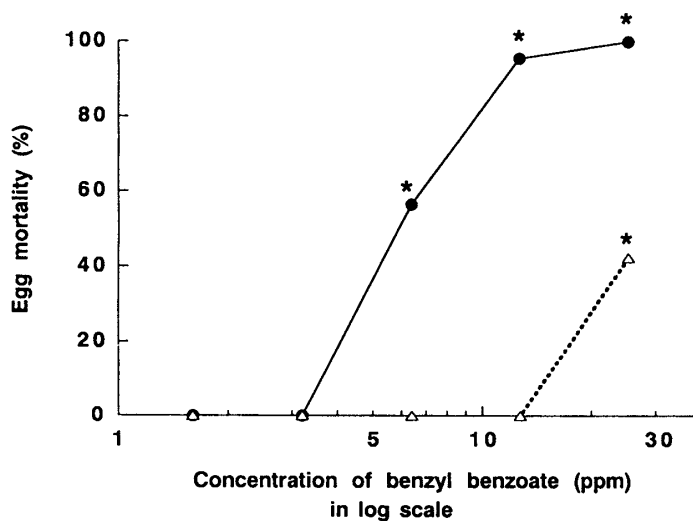
Fig. 3. Mortality of *S. furcifera* eggs in synthetic BB solution (solid line) and ether extracts of watery oviposition lesion tissues (hatched line). ABBOTT correction was applied to the mortality rate. Asterisk (*) indicates significant difference from control (0 ppm) at $p = 0.01$.

25.4 ppm of BB concentration than it was in the control and much lower than that in the water solution of the synthetic BB at ≥ 6.4 ppm (Fig. 3). This suggests the existence of substances that suppress the effect of BB on *S. furcifera* eggs in the ether extract.

Amount of BB in watery and non-watery oviposition lesion tissues

The amount of BB in the ether extracts was measured at 18.1 μg per 22 pieces of watery oviposition lesion tissues (0.64 μg/piece, or 4.67 μg/10 cm long) by GC analyses. In contrast, BB was not detected from the ether extracts of 22 pieces of non-watery oviposition lesion tissues (the lower limit detection: 0.02 μg). It follows that the BB content in non-watery oviposition lesion tissues was negligible compared with that in watery oviposition lesion tissues.

DISCUSSION

Benzyl benzoate (BB) was identified as an ovicidal substance against *S. furcifera* eggs from watery oviposition lesion tissues of the rice plant. BB showed ovicidal activity against *S. furcifera* eggs at ≥ 6.4 ppm in water (Fig. 3). It was also demonstrated that the ovicidal activity of BB decreases in water solutions of ether extracts from watery oviposition lesion tissues. The results of GC-MS analyses showed that hydrocarbon and ester compounds were also found in the ether extracts. These substances may prevent BB from acting on *S. furcifera* eggs. However, the mechanism remains unknown.

The production of BB is associated with the formation of a watery lesion at the oviposition site, which corresponds to a much higher physiological mortality of *S. furcifera* eggs in watery oviposition lesions than in non-watery oviposition lesions (SUZUKI et al., 1996). This suggests that BB is produced exclusively in watery oviposition lesion tissues as an ovicidal substance after oviposition by *S. furcifera*. We have found that BB is also detected in watery oviposition lesion tissues at oviposition sites of the brown planthopper, *Nilaparvata lugens* (STÅL), whose eggs suffer a high mortality there (SEINO et al., unpublished). This discovery is

important not only because it opens the way for incorporating the ovicidal reaction as a new source of planthopper-resistance into the breeding of resistant rice varieties but because it provides the first evidence for the involvement of ovicidal substances in the interaction between plants and insects.

BB is known as a major oil constituent in some plants such as *Uvaria* sp. and *Cinnamomum* sp. (OGUNTIMEIN et al., 1989; NKUNYA et al., 1990; JANTAN, 1990; HISHAM et al., 1991). BB contained in flowers of *Taraxacum officinale* WEBER is suggested to serve as an ingredient of a kairomone for the scarab beetle, *Anomala octiescostata* BURMEISTER (LEAL et al., 1994). However, it has never been detected in rice plants. Ovicidal activity of BB against insect eggs is also new to us, though BB is known as a scabicidal (SATO et al., 1989) and acaricidal (HAYDEN et al., 1992) compound.

There are two similarities between the ovicidal reaction of rice plants against *S. furcifera* oviposition and plant reaction against the infection of microorganisms. One is that plant tissues become watery at the reaction site and the other is that harmful organisms are killed by a biochemical reaction by plants. BB may be categorized as a post-infectional compound; post infectional compounds are classified into post-inhibitins and phytoalexins in INGHAM's classification of disease resistance factors (INGHAM, 1973). Several compounds have been identified as phytoalexins produced by rice plants (CARTWRIGHT et al., 1977; AKATSUKA et al., 1983; SEKIDO et al., 1986), yet as far as we are aware, BB has never been detected in rice plants or any other plants as a phytoalexin. This suggests that the ovicidal reaction of rice plants is distinct from antifungal reaction. It should be noted, however, that yeast-like intracellular symbionts play an essential role in the development of *S. furcifera* and some other rice planthoppers and leafhoppers (SANDER, 1976; LEE and HOU, 1987). SCHWEMMLER (1974) has shown that symbiont-free eggs can not complete embryonic development. It is therefore unclear whether BB affects the embryo directly or indirectly through affecting the symbionts in *S. furcifera* eggs.

Further study is also needed to examine the involvement of substances other than BB in the ovicidal response of rice plants.

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