Attractancy of Alcohols Isolated from Culture Filtrates of *Fusarium* Fungi for the Robine Bulb Mite, *Rhizoglyphus robini* CLAPARÈDE (Acari: Acaridae), in Sand

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Five alcohols (ethanol, *n*-propanol, iso-butanol, iso-pentanol, and 2-methyl-1-butanol), extracted and identified from culture filtrates of *Fusarium oxysporum*, were examined for their attractiveness for *Rhizoglyphus robini* in sand. Mites preferred the mixed-alcohols (90:1:4:4:1 in the order named) to sterile water, and they were attracted to the alcohols from a distance of 3 cm within 24 hr. Although each alcohol showed a varying degree of attractancy for mites, the effect was most extensive in ethanol, iso-butanol and iso-pentanol. Furthermore, a piece of healthy rakkyo bulb attracted mites through sand, but its intensity was strongly enhanced by pouring the mixed-alcohols on the piece, which was similar to mite preference for *Fusarium*-infected rakkyo bulbs over healthy bulbs.

INTRODUCTION

Members of the acarid genus *Rhizoglyphus* attack decaying as well as healthy plant tissues throughout the world, and are often associated with ornamental and vegetable bulbs such as tulips and crops of the genus *Allium* in Japan. Among these, *R. robini* CLAPARÈDE has been considered as one of the most popular species and several biological studies have been conducted (BAKER and KRANTZ, 1985; GERSON et al., 1985).

Attractiveness to mites of various substances extracted from healthy and decaying foods have been documented for several taxa, mold mite (YOSHIZAWA et al., 1970, 1971; VANHAELEN et al., 1980 a, b) being one example. We showed in our previous papers (SHINKAJI et al., 1988 a, b) that the Robine bulb mite was strongly attracted to culture filtrates of *Fusarium oxysporum* and to five identified alcohols with the low boiling point as well as *Fusarium*-infected rakkyo plants. These alcohols were ethanol, *n*-propanol, iso-butanol, iso-pentanol and 2-methyl-1-butanol. Most of this result was obtained by observations of mite behaviour on petri dishes to allow free movement of mites and ease of observation, but may not have precisely reflected behaviour in the natural environment.

The purpose in this paper is to determine if this attractiveness of alcohols to bulb mites persists in the sand and if the extent is influenced by the presence of host plants.

MATERIALS AND METHODS

Mites were originally obtained from the Stored-Product Entomology Laboratory,

Fig. 1. Diagrammatic representation of experimental system used for the preference (A) and distance (B) experiments.

National Food Research Institute and, since then, have been maintained in the laboratory (25°C) using artificial foods for rats (CE-2, Clea Japan Inc.) (SHINKAJI et al., 1986). Adult females were selected from the culture and used in the experiments. Five extra-pure alcohols, namely ethanol, *n*-propanol, iso-butanol, iso-pentanol and 2-methyl-1-butanol, were purchased from the chemical company, and whenever it was necessary they were mixed with a ratio of 90:1:4:4:1, respectively, to simulate the natural ingredients in the culture filtrate of fungi (SHINKAJI et al., 1988 b).

Rakkyo plants (bulbs), var. Rakuda, were obtained from the Tottori Fruit, Vegetable & Flower Experimental Station prior to the experiments, and the culture of *Fusarium oxysporum* f. sp. *allii* (SUF1251) used was donated by the Laboratory of Plant Pathology, Faculty of Horticulture, Chiba University. Fungi were cultured on PDA plate medium, and their conidia suspension was obtained after 5 to 7 days at 25°C. This suspension was mixed with sandy soil and given to the rakkyo plants to be infected.

In the experiments, behaviour of mites was measured by their movement through the sands contained in transparent acrylic rings (3 cm in inside diameter and 1 cm in height) as shown in Fig. 1. To investigate the preference of mites for different substances, four rings were prepared (Fig. 1A). The bottom side of the lowest ring was covered with Parafilm®, and a piece of rakkyo bulb or alcohols were placed inside this ring with sand sterilized in a hot air oven, and to which 6% distilled water by weight was subsequently added. When applying alcohols, cotton was soaked in 0.2 ml of alcohol solution, then placed inside the Tygon® tube (0.5 cm in inside diameter and 1.5 cm in length). This tube was placed in the ring with sand. The second and third lower ring contained only sand. However, 50 mature female mites were released between these rings. In the last and highest ring, the Tygon® tube with control materials was buried as the lowest ring. The top of the last ring and the ring junctions were covered with Parafilm® and scotch tape, respectively.

In the experiment to determine travelling behaviour of the mites, distance was controlled by the number of rings inserted between the mites and alcohols (Fig. 1B). Rings were placed horizontally in an incubator maintained at 25°C and approximately 100% RH. After 24, 48 and 72 hr, respectively, the ring at the distant end was detached from the rest with a knife and the number of mites inside was counted. All experiments were repeated three times.

Table 1. Persistency of mites in the tube buried in the ring

Sources in the tube	Sterile water			10% mixed-alcohol			20% mixed-alcohol		
Replicate	1	2	Total	1	2	Total	1	2	Total
Initial number of mites in the tube	13	12	25	17	21	38	18	10	28
Number of mites remaining in the tube after 1 week	13	10	23	11	21	32	17	10	27
% of mites remaining	92			84			96

Fig. 2. Preference of mites for sterile water and mixed-alcohols of various concentrations: * for attractancy, refer to the text. ●: 10%, ○: 20%, ▲: 50%.

Fig. 3. Preference of mites for sterile water and individual alcohols each with the concentration contained in the 20% mixed-alcohols: * for attractancy, refer to the text. ●: ethanol 18%, ■: iso-butanol 0.8%, ○: iso-pentanol 0.8%, ▲: 2-methyl-1-butanol 0.2%, □: n-propanol 0.2%.

RESULTS

Prior to the series of main experiments, preliminary observations were conducted to ascertain behavioural stability of the mites after reaching the ring with source materials. Three rings containing sand were connected and placed horizontally. A piece of Tygon® tube with mites and substance-soaked cotton was buried in the middle ring, and after 1 week, the number of mites remaining in the tube was counted. Table 1 shows the results of two replicates. Regardless of the substances (sterile water, 10% and 20% mixed-alcohols) used, the majority of mites remained in the tube, suggesting that the mites in the main experiments would remain inside the tube for the full observation period of 72 hr, once they had entered.

Attractancy to the mixed-alcohols

Three concentrations of 10%, 20% and 50% mixed-alcohols were prepared by diluting with sterile water. Results of attractancy in each solution are shown in Fig. 2.

Table 2. Attractancy of mites at various distances and observation times

Distance (cm)		1			2			3		
Time (hr)		24	48	72	24	48	72	24	48	72
Sources										
None		3.3±1.2	0.7±0.7	2.3±1.5	2.3±1.5	1.7±0.3	3.3±0.7	0.3±0.3	2.3±1.2	2.7±0.3
Water		16.0±2.1	10.0±4.0	10.3±3.7	2.3±0.7	10.3±3.5	12.7±5.2	10.3±5.8	3.7±3.2	20.7±5.2
Mixed-alcohol										
10%		24.7±5.5	21.7±4.5 ^{aa}	21.7±1.7 ^{aa,b}	5.7±1.5	7.3±1.2 ^{aa}	7.7±3.7	16.0±0.6 ^{aa}	7.7±2.0 ^a	11.7±4.8
20%		11.0±2.9	8.0±2.5 ^a	17.3±2.7 ^{aa}	19.3±3.8 ^{a,b}	10.7±2.6 ^a	7.0±2.6	11.3±3.8 ^a	14.7±3.2 ^a	12.3±4.5
50%		14.7±2.8 ^a	5.7±1.9	6.0±0.6	5.7±1.2	6.3±1.5 ^a	7.0±0.6 ^a	2.3±0.3 ^{aa}	2.3±0.3	3.3±0.3 ^b

Values are the average ± SE of numbers of mites reaching the ring with the source tube, after releasing 50 mites initially: a (b) and aa (bb) indicate statistically significant differences from None (or Water) at the 5% and 1% levels, respectively, by the *t*-test.

Fig. 4. Preference of mites for sterile water and the 20% solution of individual alcohols: * for attractancy, refer to the text. ○: iso-pentanol, ●: ethanol, ■: iso-butanol, □: n-propanol, ▲: 2-methyl-1-butanol.

Table 3. Preference of mites for bulbs with and without alcohols

Time (hr)	24				48				72			
Replicate	1	2	3	Total	1	2	3	Total	1	2	3	Total
Healthy bulb	9	3	10	22	3	11	5	19	5	4	1	10
Healthy bulb with 20% mixed-alcohols	12	12	22	46	23	31	20	74	17	33	44	94
χ^2 -value ^a	8.47**				32.5**				67.8**			

^a The χ^2 -test was conducted to examine fit to a 1:1 ratio between totals, and ** indicates a statistical difference at the 1% level.

Table 4. Preference of mites for healthy and *Fusarium*-infected bulbs

Time (hr)	24				48				72			
Replicate	1	2	3	Total	1	2	3	Total	1	2	3	Total
Healthy bulb	6	4	0	10	30	15	1	46	11	0	6	17
<i>Fusarium</i> -infected bulb	17	27	30	74	20	35	49	104	39	50	40	129
χ^2 -value ^a	48.8**				22.4**				85.9**			

^a The χ^2 -test was conducted to examine fit to a 1:1 ratio between totals, and ** indicates a statistical difference at the 1% level.

Attractancy for mites was shown by a percentage of the difference in numbers of mites between two substance-containing rings: $\text{attractancy} = 100 \times (A - B) / (A + B)$, where A and B represent the numbers of mites in the ring with alcohols and with water, respectively. The 10% mixed-alcohols failed to show a significant attractancy to mites throughout the observation period. In comparison, the results of χ^2 -test indicated that significantly more mites preferred the 20% (at 24 and 48 hr) and 50% (at 24 hr) mixed-alcohols to sterile water, but this preference was reversed over time.

Movement of mites was investigated for distances of 1, 2 and 3 cm, and the results are shown in Table 2. Mites generally travelled well to water and low concentrations of alcohols. Even for a distance of 3 cm, a considerable number of mites reached the source within 24 hr.

Attractancy to the individual alcohols

Experiments of preference for sterile water and individual alcohols were conducted using following 5 alcohols with their concentrations expressed in parentheses: ethanol (18%), *n*-propanol (0.2%), iso-butanol (0.8%), iso-pentanol (0.8%), and 2-methyl-1-butanol (0.2%). These concentrations are the components of each alcohol contained in the 20% mixed-alcohols which seemed to show a consistently high attractiveness to mites in the above-described experiment. Figure 3 shows the results. Regardless of the kind of alcohols, more mites were attracted to alcohols than to sterile water at 24 hr. However, the effect decreased in 2-methyl-1-butanol and *n*-propanol over time. When the concentration of each alcohol increased to 20% (Fig. 4), it seemed that two groups of alcohols were recognized based on their attractiveness to mites. Iso-pentanol, iso-butanol and ethanol attracted more mites than water, but *n*-propanol and 2-methyl-1-butanol showed only a similar effect to water.

Synergistic effect of alcohols and host plant

An experiment was conducted to study mite preference for pieces of healthy rakkyo bulbs and those on which 1 ml of the 20% mixed-alcohols had been poured. The results shown in Table 3 clearly indicate that more mites were attracted to the bulbs with alcohols and that the difference increased over time. For comparative purposes, mite preference was also tested using pieces of healthy and *Fusarium*-infected bulbs in the same manner. Tendency and intensity in preference by the mites (Table 4) were similar to those for alcohol-poured bulbs.

DISCUSSION

SHIBATA (1958) demonstrated that bulb mites travelled 13.8 cm on average to reach their host plants in the glass tube within 16 hr. Our observation also showed that many mites passed through 3 cm of sand within 24 hr under laboratory conditions. In the experiment of preference for sterile water and the mixed-alcohols (Fig. 2), the 20% solution attracted mites with the best efficiency within 48 hr. The 10% solution lacked extra attractiveness compared to water. When the experiment lasted longer, more mites were found at the water side than at the 20% solution, probably due to diffusion of alcohol odour in entire rings and to some alcohol-associated negative effect such as environmental pollution.

Bulb mites are known to select a habitat with a high humidity (SEKIYA, 1948). Our experiment also showed that mites were attracted well to water alone, but their attractancy was strongly enhanced by an addition of alcohols identified in *Fusarium* fungi.

In regard to attractiveness of each alcohol, our previous paper (SHINKAJI et al., 1988 b) demonstrated that mites were attracted to 30 μ l (but not 1 and 5 μ l) ethanol in the petri dish. The 0.2 ml of 20% ethanol solution used in this experiment corresponds to 40 μ l of pure ethanol and this attracted mites well in the sand, similar to the case of 30 μ l in the dish. Iso-butanol also showed a slight improvement in attractancy when its contents increased from 0.8 to 20%. On the contrary, *n*-propanol and 2-methyl-1-butanol exhibited somewhat lowered attractiveness when they increased in concentration from 0.2 to 20%. Iso-pentanol did not show any attractancy at 1, 5 and 30 μ l in the petri dish (SHINKAJI et al., 1988 b), but attractancy greatly increased in the sand. It was difficult to specify the reason for loss of attractiveness at 48 hr for ethanol. In this study,

the observation period was extended, compared to the previous dish experiments, in consideration of possible difficulty encountered by mites in moving freely through the sand. Therefore, the differences in volatility of each alcohol may have influenced the behaviour of mites more significantly during this longer observation period in the present experiment. Some of the discrepancies found in the results of the two experiments could be explained by this reason.

Nevertheless, mites showed chemotactic movements in response to alcohols in the dish experiments (SHINKAJI et al., 1988 a), and the results in this experiment further displayed that many mites were attracted to alcohols through the sands at distances used within 24 hr. It seemed probable that mites, after being attracted to alcohol sources, started to disperse in accordance with the dispersion of alcohols all over the rings.

No alcohol exhibited a negative effect against mites, which was also shown by the dish experiments. Individually or mixed, these 5 alcohols have a certain degree of attractiveness to bulb mites. It was shown that the alcohols 3-methylbutanol and *cis-trans*-octa-1, 5-dien-3-ol individually attracted mold mites (YOSHIZAWA et al., 1970; VANHAELEN et al., 1980 a). Volatile alcohols probably play an important role in introducing mites to food sources due to their higher rates of dispersion than any other substances contained in the food.

Pouring mixed alcohols on a piece of healthy rakkyo bulb attracted more mites (Table 3), and this result resembled that of mite preferences for healthy and *Fusarium*-infected bulbs (Table 4). Synergistic effects in attraction were also shown for mold mites (YOSHIZAWA et al., 1971) where more mites were attracted to the mixture of 3-methylbutanol and methyl ketones. Alcohols are effective individually, but their attractiveness may increase in cooperation with other odorous materials contained in, or associated with, foods. IKESHOJI et al. (1980) reported that onion maggots were attracted by dipropyl disulfide (the major odorous substance of onions), but the attractancy increased by three times after additions of tetramethylpyrazine and *n*-heptanal (identified and extracted from decaying onions). This observation seems to be one example of strong attractancy of insects, from a rotting habitat, to healthy crops treated with volatile substances which are produced by pathogenic microorganisms. Similarly, the bulb mites in the present experiment were attracted to alcohol-poured bulbs similar to their response to *Fusarium*-infected bulbs.

The Robine bulb mites were attracted more intensively to decaying than healthy rakkyo bulbs, and their preferences for alcohols which were produced by *Fusarium* fungi were clearly shown even in the sand. Although it has not yet been confirmed that these alcohols are actually produced on *Fusarium*-infected bulbs and that they are the only attractants in decaying bulbs for bulb mites, the synergism between alcohols and host plants was proved in their attractiveness to mites. Bulb mites strongly associate with fungi in their habitat, and thus understanding of relationships between mites, fungi and host plants is important for the future development of mite control.

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