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Short communication

Attraction of Female Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) to 1-butanol

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Abstract: The response of male and female Oriental fruit flies to 1-butanol was investigated in a screen cage under laboratory conditions. As a result, attractive responses were observed in females ten days after emergence and the rate of responded females increased with age. No positive response was observed in males. Although the rate of responded females was 30% at most in the tests, these results suggest that the response to 1-butanol may be closely related to the sexual maturity of females. To study how to utilize this phenomena, the effects of adding 1-butanol to a 2% beer yeast protein solution was investigated on the attractiveness to mature females (from 33 to 38 days old) in a screen cage. The beer yeast protein solution itself attracted 10% of the released sexually mature females, but 72% of released sexually mature females were attracted to the beer yeast protein solution with 1-butanol, and it was considered that 1-butanol could effectively increase the attractiveness of beer yeast protein solution for sexually mature females. This observation suggests that 1-butanol could be a stimulus for sexually matured females to induce searching behavior for host fruits to lay their eggs.

Key words: *Bactrocera dorsalis*, oriental fruit fly, female, attraction, butanol

Introduction

Bactrocera dorsalis (Hendel) (Diptera: Tephritidae) has been thought responsible for causing enormous losses to horticultural crops throughout Asia/Southeast Asia (Drew & Hancock, 1994). Although the import of host fruits is prohibited by the Japanese Plant Quarantine Law, this species was detected 296 times in confiscated fruits at airports in Japan in 2017 (PPS 2018). It is the most frequently detected species along with *Bactrocera latifrons* among fruit flies found at Japanese airports. Although *B. dorsalis* had been eradicated from Japan in 1986, adult flies are captured by monitoring traps baited with methyl eugenol every year in Okinawa Prefecture close to areas of occurrence such as Taiwan (Ohno *et al*., 2009; Otuka *et al*., 2018). Therefore, *B. dorsalis* is regarded as a pest of quarantine importance in Japan out of concern for its introduction into Japan. In the summer of 2015, this species invaded and became widespread across Amamioshima Island by the autumn of 2015. An emergency control program began on December 13 and eradication was achieved on July 14 of the following year after the seven-month campaign (Katoh, 2016).

It is well known that male adults of *B. dorsalis* are strongly attracted to methyl eugenol (Steiner, 1957). The male annihilation method using methyl eugenol was used for the eradication of the species (Yoshizawa, 1997). However, it may invade into uninfested areas and spread out in areas such as in the case of Amamioshima Island. With effective monitoring and control of females, it could be possible to reduce the risk of invasion and to eradicate this species more quickly in the event of its introduction.

Application of protein baits mixed with a killing agent is a common and effective attract-and-kill approach to fruit fly management targeting female populations (Roessler, 1989; Mangan, 2014). Currently, food-type attractants, such as fermenting sugars, hydrolyzed protein, and yeast, provide the best attractants for *B. dorsalis* females. However, these liquid lures lack potency, have limited field life, are difficult to handle, and attract non-target species (Siderhurst & Jang, 2006), so food-type attractants for *B. dorsalis* females need to be improved. Because there was a report that females were attracted to butanol on Chichijima in the Ogasawara Islands in 1937 (Ishii, 2015), we confirmed the attraction of female *B. dorsalis* to 1-butanol and examined how to utilize it for monitoring and controlling this species.

Materials and Methods

1. Test Insects

The insects used in the present study were originally from Malaysia and introduced to Japan with the permission from the Minister of Agriculture, Forestry and Fisheries of Japan in 1991 (import permit

No.3Y-968). After introduction into Japan, the flies were kept in a regular screen cage (30×30×45 cm, with a cloth-sleeved opening on one side) in a rearing facility (KOITOTORON-PCSH-2SP; Koito Electric Industries, Japan) at Research Division, Yokohama Plant Protection Station.

The flies were maintained under constant conditions [26±1°C, 60±10% relative humidity (RH), and a photoperiod of 14:10 light: dark (L: D), with two 1-h twilight phases at the beginning and end of the light phase]. The larval diet comprised 150 mL water, 0.25 mL HCL, 0.1 g Methyl p-hydroxybenzonate, 0.1 g sorbic acid, 5 g dried yeast (EBIOS; Asahi Food & Healthcare, Japan), 7.5 g sugar, 5 g toilet paper and 37.5 g wheat bran. The adult diet was a 4:1 mixture of sugar and hydrolyzed yeast (AY-65; Asahi Food & Healthcare, Japan). Fries were fed the diet and water provided ad libitum.

2. Traps

We used a simple trap consisting of a clear polyethylene terephthalate, cylindrical container measuring 40 mm in height with a 70-mm diameter top lid, and opened four square holes (10 × 10 mm) on the side of the container 30 mm in height (Fig.1).

3. Experiments and Observations

(1) Age-related response of male and female *B. dorsalis* to 1-butanol

The response of *B. dorsalis* two to 21 days after emergence to 1-butanol was studied under laboratory conditions. The age of flies for this study were 2, 4, 6, 8, 10, 12, 14 and 21 days after emergence. Test insects were fed an adequate diet and water, and were kept at the rearing facility until each day of the test.

20 pairs of males and females at the study age were released into separate regular screen cages with the diet and water at 9 AM. A simple trap with a filter paper dropped with 50 µL 1-butanol was placed in the center of the regular screen cage at 10 AM. Thereafter, the number of male and female flies attracted to 1-butanol within one hour was recorded. Trials were replicated three times for each age group of the fly.

Fig. 1. Simple trap for adult response of *Bactrocera dorsalis* to 1-butanol

(2) Effect of adding 1-butanol to beer yeast protein solution

As a result of the experiment (1), it was found that 1-butanol attracts sexually mature females. The effect of adding 1-butanol to beer yeast protein (Protein20E; Sankei Chemical Co., Ltd, Japan) was investigated to study how to utilize 1-butanol for the control of *B. dorsalis*. Test insects were fed an adequate diet and water, and were kept at the rearing facility until sexual maturity.

20 pairs of males and females were released into separate regular screen cages with the diet and water at 9 AM. Simple traps with 1) water (10 mL), 2) water (10 mL) and 1-butanol (50 µL), 3) beer yeast protein solution (2% protein) (10 mL) and 4) beer yeast protein solution (2% protein) (10 mL) and 1-butanol (50 µL) were placed in the corners of the regular screen cage at 10 AM. Thereafter, the number of male and female flies attracted in each trap three hours later was recorded. The installation position of each trap was rotated clockwise around the four corners of the screen cage and trials were replicated three times in each position.

4. Statistical analyses

Data on the number of males and females collected in each trap were analyzed with a general linear model. Data was transformed using the arcsine transformation prior to analysis to stabilize variances and normalize proportional data. Pairwise comparisons were made with Tukey's HSD test at the 5% probability level. Statistical analyses were conducted in SPSS® Statistics Version 22 (IBM®. 2013). All figures show untransformed data.

Results and Discussion

1. Age-related response of male and female *B. dorsalis* to 1-butanol

An attractive response was observed in females 10 days after emergence and the rate of responded females increased with age (Fig.2). The number of female flies attracted to 1-butanol reached 6 individuals per cage 21 days after emergence. 1-butanol was considered to be an effective attractant for sexually mature female *B. dorsalis*.

2. Effect of adding 1-butanol to beer yeast protein solution

It was apparently that many more females were attracted to Protein 20E and 1-butanol compared with Protein 20E only. Protein 20E at-

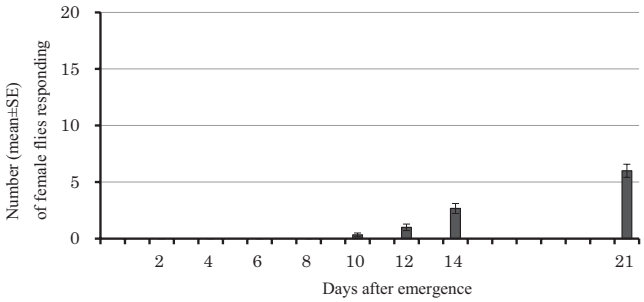

Fig. 2. Response of female *B. dorsalis* to 1-butanol

tracted 2 females per cage, but Protein 20E and *n*-BuOH (1-butanol) attracted 14.3 females per cage and 7.3 times more than Protein 20E only. The number of attracted females to *n*-BuOH was 2.4 individuals per cage. Females were attracted to water with added 1-butanol immediately after installation, but individuals attracted to water did not drown as with the protein, so thereafter most individuals escaped from the trap. Females in the trap of water were 0.08 individuals per cage after three hours (Table.1).

A three-way ANOVA was run on a sample of 480 flies to examine the effect of 1-butanol, sex of flies and Protein 20E on the proportion of flies caught in traps. The three-way interaction was significant: $F(1, 88) = 25.2$, $p < 0.0001$. All the main effects are statistically significant, i.e. 1-butanol attracted more flies, (*n*-BuOH: $F(1, 88) = 75.27$, $p < 0.0001$), more female flies are attracted than male flies (sex: $F(1, 88) = 96.33$, $p < 0.0001$) and Protein 20E attracted more flies (*n*-BuOH: $F(1, 88) = 56.83$, $p < 0.0001$); however, significant interactions qualify the main effects (Table 2).

The result of multiple comparison of simple-simple main effects indicated that the combination of *n*-BuOH and female is significant ($p < 0.001$, Table 3). The contribution ratios of "n-BuOH*Sex" is also high ($\rho = 12.9\%$, Table 2) and these results suggest the efficacy of the attractiveness of 1-butanol to female *B. dorsalis*. For female *B. dorsalis*, adding 1-butanol to protein can attract them more effectively than protein alone immediately after installation. (Test insects were 33 to 38 days old.)

Table 1. Mean number (± SEM) of male and female flies caught in traps with Protein 20E (Protein) and/or n-BuOH.

♂ *n*-BuOH / Protein	with	without
with	0.33±0.17	0.67±0.23
without	0.58±0.22	0.083±0.083

♀ *n*-BuOH / Protein	with	without
with	14.33±0.58	2.0±0.38
without	2.42±0.42	0.083±0.083

Table 2. Analysis of proportion of male and female flies (sex) caught in traps with Protein 20E (Protein) and/or n-BuOH using generalized linear model.

Source	Type Ⅲ Sum of Squres	d.f.	Mean Square	F	P	ρ (%)
n-BuOH	1.973	1	1.973	75.27	<0.0001	16.2
Sex	2.524	1	2.524	96.33	<0.0001	20.8
Protein	1.489	1	1.489	56.83	<0.0001	12.2
n-BuOH*Sex	1.576	1	1.576	60.15	<0.0001	12.9
n-BuOH*Protein	0.167	1	0.167	6.39	0.013	1.2
Sex*Protein	1.313	1	1.313	50.11	<0.0001	10.7
n-BuOH*Sex*Protein	0.66	1	0.66	25.2	<0.0001	5.3
Error	2.306	88	0.026			0.1
Total	17.77	96				
Corrected Total	12.01	95				

Table 3. Result of multiple comparison of simple-simple main effects.

(1) compared factor : *n*-BuOH

sex	female		male	
Protein 20E	with	without	with	without
P	< 0.001	< 0.001	-*	-

(2) compared factor : sex

n-BuOH	with		without	
Protein 20E	with	without	with	without
P	< 0.001	0.008	0.042	-

(3) compared factor : Protein 20E

n-BuOH	setted		unsetted	
sex	female	male	female	male
P	< 0.001	-	0.001	-

Bonferroni's multiple comparison test: P < 0.05

*The results of combinations didn't show significant difference were not indicated.

In addition, three isomers are known for 1-butanol, they are SBA (sec-butyl alcohol), i-BuOH (iso-butyl alcohol) and TBA (tert-butyl alcohol). As a result a comparative test for sexually mature female responding to beer yeast protein solution (2% protein) with added 1-butanol or one of isomers, none of the isomers more attractive than 1-butanol. (data not shown)

The main factors causing *B. dorsalis* to be captured in the southwestern islands of Japan are the possibility of flying from occurrence areas and the introduction of parasitic fruits. Otuka *et al*., (2016) analyzed the former natural factor by calculating backward trajectories for *B. dorsalis* complex males caught in monitoring traps from 1986 to 2012. In 65.8% of the total re-invasion events, trajectories could be traced to at least one of the insect's native regions. It suggests that a considerable number of males captured with the monitoring trap may have reached the location by flying across the sea. These individuals were all males captured in a trap with methyl eugenol; there is no information regarding flying females.

In an investigation of the flight ability of the wild strain of *B. dorsalis* introduced from Viet Nam and bred for 4 to 11 generations, the flight duration and flight frequency of mated females were significantly longer and higher than those of males, but there was no significant difference in flight duration per flight between males and mated females (Hirahara *et al*., 2016). This suggests that gravid females have a flying ability equal to or greater than males, so it might be possible for gravid females to fly to the southwestern islands of Japan like the males. If gravid females could be detected effectively after male detection in the trap, it would be possible to start emergency control against the invading population before becoming an outbreak as on Amamioshima in the autumn of 2015.

Female flies require protein for full ovarian development and egg production (Vergus and Prokopy, 2006). Protein/Fermentation-based attractants combined with toxicants have been used in traps for monitoring and sprayed on the orchard floor to suppress the fruit fly population (Prokopy *et al*., 2003). In the present experiments, the feeding

behavior of sexually mature females attracted to 1-butanol was not observed, but egg-laying site search behavior around 1-butanol was observed. It is likely that 1-butanol could be a stimulus for sexually matured females to induce searching behavior for their host fruits to lay eggs.

Although a laboratory strain was used in these experiments, it suggests that 1-butanol has the potential to improve the control of sexually mature female *B. dorsalis*. It is necessary to confirm the behavioral responses of wild *B. dorsalis* to 1-butanol in the field for the utilization of this phenomena.

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和　文　摘　要

ミカンコミバエ雌成虫のブタノールへの誘引

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ミカンコミバエのブタノール（1-ブタノール）への反応を実験室内のケージで調査した。誘引反応は羽化後 10 日令以降の雌で観察され、反応個体は日令とともに増加した。雄の誘引反応は観察されなかった。反応雌の割合は放飼数の最大 30% であったが、これらの結果はブタノールへの反応が雌の性成熟と密接に関わっていることを示唆している。ブタノールへの反応の活用方法を検討するため、ブタノールを添加した２% プロテイン水溶液の成熟雌（羽化後、33 日令から 38 日令）への誘引性をケージで調査した。プロテイン水溶液には放飼した成熟雌の 10% が誘引されたのに対し、ブタノールを添加したプロテイン水溶液には 72% が誘引され、ブタノールはプロテイン水溶液の成熟雌への誘引性を効果的に向上させることができると考えられた。また、試験結果は成熟雌にとってブタノールが産卵のための寄主果実を探索する刺激となっている可能性を示している。