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Preference by Adult Female *Riptortus Clavatus* Thunberg for Plants from the Fabaceae and Other Plant Families

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Abstract

To obtain basic knowledge regarding the preference of adult female *Riptortus clavatus* for particular host plants, we investigated the preference of *R. clavatus* for green soybean, common bean, and cow pea as host plants, and between cow pea and either bell pepper, lettuce, cabbage, or cucumber. *R. clavatus* indicated a preference for cow pea over green soybean, followed by common bean. In addition, it was indicated that *R. clavatus* can distinguish among host plants. *R. clavatus* preferred cow pea over various non-host plants to a highly significant degree. These results clarified that adult female *R. clavatus* have strong ability to distinguish among host and non-host plants in the Fabaceae.

Keywords: *Riptortus clavatus* Thunberg, Fabaceae plants, cow pea, preference

INTRODUCTION

Recently, there have been misgivings about the use of chemical pesticides and fertilizers due to concerns about environmental pollution and adverse effects on human health. Both consumers and producers are interested in chemical-free vegetables and organic farming. Without the use of chemicals, however, crops suffer from severe pest damage. Plant species in the Fabaceae, including *Glycine max*, which is one of the world's most important crops, are often damaged by stink bugs, in particular *Riptortus clavatus* Thunberg. *R. clavatus* selectively feeds and oviposits on plants in this family, in soybean fields (Naito et al., 1986; Ikeda and Fukazawa, 1983; Ouchi and Setokuchi, 1982; Kobayashi, 1979). More than 50% of damage to soybeans harvested in autumn has been caused by stink bugs, including *R. clavatus* and *Piezodorus hybneri* Gmelin, among others (Kadosawa and Santa, 1981).

At present, the most common control methods for *R. clavatus* are periodic chemical pesticide treatments, pheromone traps, and bait crops (Naito et al., 1986; Ikeda and Fukazawa, 1983; Osakabe and Honda, 2002). Chemical pesticide treatment has been effective 30 d after flowering, during the early stages of soybean seed development. Pheromone traps and bait crops have been less effective than chemical pesticide treatments, but the use of soybeans and cowpeas (*Vigna unguiculata*) as bait crops can reduce the number of *R. clavatus* landing on soybean, and the insects land on cow pea more often than on soybean (Naito et al., 1986; Tabuchi et al, 2005).

R. clavatus propagate on Fabaceae plants in weedy areas adjacent to crop fields during seasons in which crop plants from the Fabaceae are unavailable (Ito, 1982) and subsequently enter the crop fields to feed on developing Fabaceae crop host plants (Natsuhara, 1985; Ito, 1984). In addition, it is known that adult female *R. clavatus* search for and oviposit on host plants on which their larvae can mature (Natsuhara, 1985). Thus, it is thought that adult female *R. clavatus* can distinguish among potential host plants from various species in the Fabaceae at the appropriate time for oviposition. However, there are no reports regarding the specific preference of *R. clavatus* for particular host plant species in this plant family.

R. clavatus has been confirmed in soybean fields that bear mature soybean pods, where the females had oviposited on plant leaves and stems when the diameter of soybean pods were greater than 6 mm (Yushima et al., 1991; Natsuhara, 1985). In addition, populations of *Trigonotylus caelestialium* Kirkaldy and *Stenotus rubrovittatus* Matsumura, which are major pests of rice, drastically increased during rice grain filling (Niiyama et al., 2007; Jin and Dae, 2003). Volatile scent compounds emitted and emanate throughout the field during different growth stages of rice are important attractants for *R. clavatus*, serving as host plant identification factors during early rice panicle development (Niiyama et al., 2007; Jin and Dae, 2003). Prior to soybean podset, *R. clavatus* lives in weedy fields that contain plants from the Fabaceae, after which the number of landings by *R. clavatus* increases during soybean pod development (Natsuhara, 1985). Therefore, it is thought that volatile compounds emitted by the soybean pods may be a factor promoting incursion of *R. clavatus* from the weedy fields to the soybean fields. *R. clavatus* may be able to detect aroma compounds emitted at different growth stages by host plants and subsequently land on the most attractive plants. However, there have been no reports to date regarding the preference of *R. clavatus* for various growth stages and organs of host plants.

In this study, we investigated the preference of *R. clavatus* for green soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and cow pea as host plants, and between cow pea and either bell pepper (*Capsicum annuum* L. var. 'Grossum'), lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea* var. *capitata*), or cucumber (*Cucumis sativus* L.), which each belong to different plant families.

MATERIALS AND METHODS

Insect collection and rearing

Adult females of *R. clavatus* were collected from the field at Meiji University. They were fed as described by Yushima et al. (1991). Adults and hatched larvae were reared on dry soybeans as feed and cotton wool perfused with water in a plastic case (13 cm i.d. × 7 cm) at 25 ± 1°C under a L16:D8 photoperiod, and 16.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) in an incubator (LTI-700, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) until used in the experiments.

Test crop and culture methods

Seedlings of the test host plants from the Fabaceae , green soybean 'Fusamidori' (Sakata Seed Corporation, Kanagawa, Japan), cow pea 'Kegon no Taki' (Sakata Seed Corporation) and common bean 'Maisugata' (Sakata Seed Corporation), and non-host plants, bell pepper 'Suigyoku No. 2' (Sakata Seed Corporation), lettuce 'Falcon' (Sakata Seed Corporation), cabbage 'Ajihosi' (Sakata Seed Corporation), and cucumber 'Wasehusinari' (Tohoku Seed Co., Ltd., Utsunomiya, Japan) were grown in a greenhouse at Meiji University from April 29 to September 26, 2008.

Twenty seeds each of green soybean, cow pea, common bean, bell pepper, cucumber, lettuce, and cabbage were sown into plastic pots (9 cm i.d.) containing a soil mix composed of equal proportions of propagation medium (N:P:K =150:3000:150 mg L^{-1}, Cope Chemical Co., Ltd., Tokyo, Japan) and vermiculite (Showa Vermiculite Co., Ltd., Kanagawa, Japan) in a greenhouse at Meiji University on April 29, 2008. Subsequently, seedlings were transferred to plastic pots (24 cm i.d., 8 L) containing a growth medium mixture of 3 L of vermiculite, 4.5 L of soil, 50 g of compost (Fujimi Group, Shizuoka, Japan), and 2 g of chemical fertilizer (N:P:K=8:8:5, Mitsubishi Chemical Agri. Inc., Tokyo, Japan) on May 14, 2008.

Sowings were conducted at 15-d intervals until September 26, 2008. Additional fertilization with 2 g of chemical fertilizer was conducted depending on growth status. The distance between plants of each species was 40 cm. Experimental plants were used at harvest stage.

Adult female landing preference experiments

In the experiments, white shading net cages (1.6 m × 1.6 m × 1.8 m) were installed in a glass greenhouse, with each cage containing two test plants that were each placed 60 cm from the center of the net cage. Thirty post-mating females were released into the cage. Combinations of test plants consisted of green soybean and common bean, cow pea and green soybean, cow pea and common bean; and cow pea with either bell pepper, lettuce, cabbage, or cucumber.

In each of the experiments, the number of landings by females on each plant was counted every 30 min per 3 h period. Preference (%) was calculated as: (number of landings on a plant from one species – number of landings on a plant of the other species) / (total landings on both species of plant) × 100. There were 10 replications for each experimental treatment. Experimental light and temperature conditions were >84 μmol $m^{-2} s^{-1}$ of PPFD and >25 °C, respectively.

Statistical analysis

The significance of the experimental data was tested using the χ^2 test. For this statistical analysis the expected values were equal selection by adult females of *R.clavatus* for each treatment. The χ^2 test was conducted between the actual count of number of individual selections and the expected value, and a statistically significant difference was assumed when $P < 0.01$.

RESULTS AND DISCUSSION

After a period of 3 h, preference for the combination of green soybean and common bean was 35.5% and 64.5%, for the combination of cow pea and green soybean was 54.5% and 45.6%, and for the combination of cow pea and common bean was 75% and 25%, respectively (Figure 1). *R. clavatus* preferred cow pea over green soybean, followed by common bean. There was no significant difference in preference between green soybean and common bean over the course of the experimental period, but *R. clavatus* tended to prefer common bean to green soybean. The number of *R. clavatus* on cow pea increased with time, the number on common pea increased slightly with time, and the species significantly preferred cow pea to common bean (Table 1). These results indicated that adult female *R. clavatus* considered these three cultivars to be suitable host plants, because the number of landings increased with time and they tended to stay on the feeding site once they had discovered an attractive host (Table 1). Natsuhara (1985) investigated the number of landings by *R. clavatus* on green soybean, mottled kidney beans, and common bean in the field. Adult *R. clavatus* that had just emerged settled longer (6 d) on soybean than on mottled kidney beans (3 d), or on common bean (2 d). Immature pods of common bean were found to be unsuitable for feeding. However, there are reports about the relationship between cow pea and *R. clavatus*. Naito et al. (1986) compared soybean and cow pea as bait crops and found that cow pea was not a suitable bait crop. Oviposition by the species on cow pea was found to be weak, landing was higher on 'Tachisuzunari' soybean than on 'Kintoki' cow pea, and soybean was still frequently damaged by the insects when plants had reached the flowering stage, despite cow pea being regarded as an appropriate bait crop for *R. clavatus* (Naito et al., 1986). The results are supported by those of the present study. Taken together, it appears that adult female *R. clavatus* can select suitable host plants for oviposition from among plants belonging to various species in the family Fabaceae.

Next, the preference among combinations of cow pea and either bell pepper, lettuce, cabbage, or cucumber was 92.8%, 98.1%, 98.1%, and 77.1%, respectively (Figure 2). *R. clavatus* significantly preferred cow pea to other plants. The number of landings by *R. clavatus* on cow pea was >90% of the expected value for combinations with cucumber (77.1%) and infers that adult females have extremely high ability to distinguish between host and non-host plants. There were more landings by *R. clavatus* on cucumber than on other non-host plants (Table 2). *R. clavatus* was thought to have landed on cucumber only by chance, simply because cucumber is longer than cow pea and the volume of space occupied by cucumber plants is greater due to their larger leaf area. In addition, *R. clavatus* might use cucumber, as well as bell pepper, leaves as a background site for estivation. On the other hand, *R. clavatus* was observed to be feeding on cow pea only, not on other non-host plants, revealing that *R. clavatus* could distinguish between host and non-host plants (Table 2). These results indicate that adult female *R. clavatus* are able to distinguish the most appropriate host plant for reproduction.

In summary, our aim was to obtain basic knowledge regarding the preference by adult female *R. clavatus* for particular host plants, and to investigate the preference specificity of the species among green soybean, common bean, and cow pea as host plants, and between cow pea and either bell pepper, lettuce, cabbage, or cucumber, which each belong to different plant families. *R. clavatus* preferred cow pea to green soybean, followed by common bean. In addition, the number of landings on any combination of Fabaceae plants increased with time, so Fabaceae plants are suitable feeding for *R. clavatus*. However, their preference differed between Fabaceae species, clarifying that the species can distinguish among host plants. *R. clavatus* preferred cow pea significantly over various non-host plants, confirming that it can clearly distinguish host from non-host plants.

In the near future, it will be important to investigate the preference of *R. clavatus* for differenr growth stages and organs of cow pea, which will hopefully lead to the development of new bait crop applications.

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Table 1 A number of landing of *R. clavatus* among three pairs of plants in the Fabaceae.

Time	A number of landing ±SE					
	common bean	green soybean	green soybean	cow pea	common bean	cow pea
0.5	4.0 ± 0.6 ab[z]	0.3 ± 0.3 a	1.3 ± 0.7 bc	6.3 ± 0.5 a	1.7 ± 0.7 a	4.0 ± 0.5 ab
1.0	5.5 ± 0.3 abc	1.0 ± 0.5 a	4.7 ± 1.4 bc	8.0 ± 0.0 b	3.0 ± 0.8 ab	7.0 ± 0.0 bc
1.5	8.5 ± 1.4 abc	2.7 ± 0.7 ab	8.7 ± 1.5 bc	9.7 ± 0.5 bc	3.3 ± 0.7 ab	10.0 ± 0.5 cde
2.0	9.5 ± 0.9 bc	3.7 ± 1.2 abc	8.3 ± 1.4 c	12.3 ± 0.5 bc	3.7 ± 1.2 ab	13.0 ± 1.2 de
2.5	10.0 ± 0.0 bc	4.3 ± 1.4 abc	10.3 ± 1.4 c	11.7 ± 1.0 bc	3.7 ± 0.7 ab	14.0 ± 0.8 e
3.0	11.0 ± 0.6 c	5.7 ± 1.1 abc	11.0 ± 1.9 c	12.7 ± 0.5 bc	5.0 ± 1.2 ab	14.0 ± 0.9 e

[z] Different letters indicate a significant difference at the 1% level for the χ^2 test (n=10).

Table 2 A number of landing of *R. clavatus* among cow pea and various other plants.

Time	A number of landing ±SE							
	cucumber	cow pea	cabbage	cow pea	lettuce	cow pea	bell pepper	cow pea
0.5	1.3 ± 0.7 a[z]	4.3 ± 0.5 b	0.0 ± 0.0 a	3.0 ± 0.5 b	0.0 ± 0.0 a	4.3 ± 1.4 b	1.3 ± 0.7 a	4.0 ± 1.2 bc
1.0	1.3 ± 0.3 a	5.3 ± 1.0 bc	0.0 ± 0.0 a	6.7 ± 0.7 bc	0.0 ± 0.0 a	6.0 ± 0.9 bc	2.0 ± 1.2 ab	4.7 ± 1.5 bc
1.5	0.7 ± 0.3 a	8.0 ± 0.5 bc	0.0 ± 0.0 a	10.7 ± 1.2 bc	0.0 ± 0.0 a	5.7 ± 1.0 bc	3.3 ± 2.3 ab	6.7 ± 1.9 bcd
2.0	1.7 ± 0.5 a	9.7 ± 0.5 cd	0.3 ± 0.3 a	13.7 ± 1.1 cd	0.0 ± 0.0 a	8.3 ± 1.0 cd	0.7 ± 0.5 a	9.7 ± 1.5 bcd
2.5	2.0 ± 0.5 a	10.0 ± 0.0 cd	0.3 ± 0.3 a	15.7 ± 0.7 de	0.0 ± 0.0 a	10.0 ± 0.5 de	1.7 ± 0.7 a	11.3 ± 1.4 cd
3.0	3.7 ± 0.3 a	12.3 ± 0.3 e	0.3 ± 0.3 a	17.0 ± 0.5 e	0.3 ± 0.3 a	13.7 ± 0.7 e	1.0 ± 0.0 a	13.3 ± 1.4 d

[z] Different letters indicate a significant difference at the 1% level for the χ^2 test (n=10).

Figure Legends

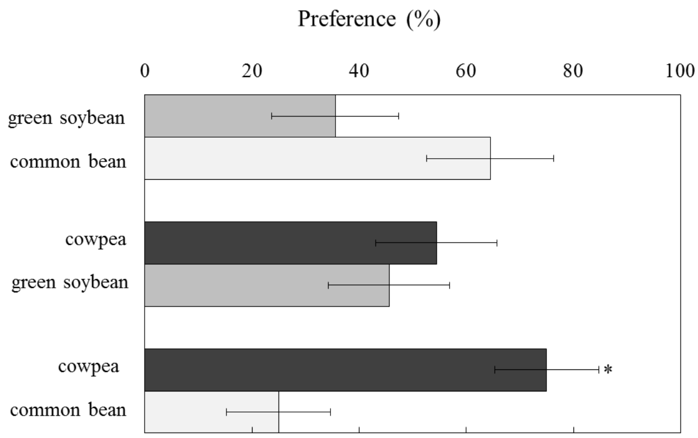

Figure 1 Preference of *R. clavatus* among three pairs of plants in the Fabaceae

Parallel bars represent the standard error of the mean percentage.

*Significant at p<0.05, χ^2 test (n=10)

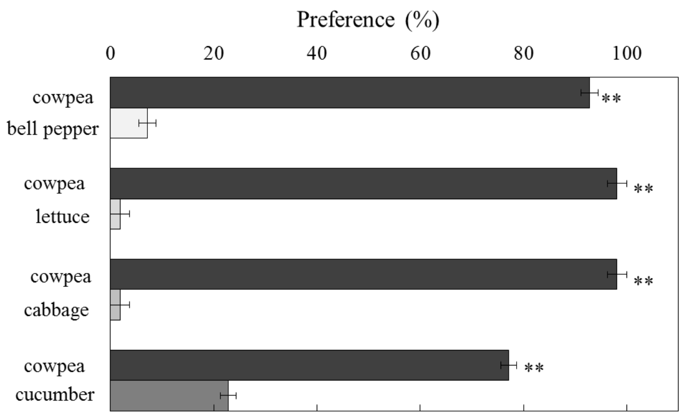

Figure 2 Preference of *R. clavatus* among cow pea and various other plants

Parallel bars represent the standard error of the mean percentage.

**Significant at p<0.01, χ^2 test (n=10)