



Role of rice plant and its extracts in attracting predatory mirid bugs, *Cyrtorhinus lividipennis* Reuter and *Tytthus parviceps* (Reuter) (Hemiptera: Miridae)

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ABSTRACT: Healthy and hopper (BPH, WBPH and GLH) infested rice plants, different plant parts (leaf, stem and panicle) and rice plants of different ages (15, 40, 75 day old and harvesting stage plants) and their extracts were evaluated as the sources of synomones for attracting predatory mirid bugs viz., *Cyrtorhinus lividipennis* Reuter and *Tytthus parviceps* (Reuter) to the plant ecosystem. Rice plant and its extracts served as source of synomone for the mirid bugs. Mirid bugs exhibited preference towards hopper damaged rice plants and their extracts. Brown planthopper (BPH) damaged rice plants and extracts were more attractive to the mirid bugs than white backed planthopper (WBPH) and green leafhopper (GLH) damaged plants and their extracts. Among plants of different ages, 75 day old plants and their extracts were preferred by mirid bugs to 40 day old, 15 day old and harvesting stage plants and their extracts. Rice leaf extracts was more attractive to the mirid bugs compared to stem and panicle extracts. Rice plant plays an important role in attracting mirid bugs to the plant system and mirid bugs were able to distinguish the insect damaged and undamaged plants and recognize plants of suitable age.

KEY WORDS: Brown planthopper, *Cyrtorhinus lividipennis*, green leafhopper, rice, synomones, *Tytthus parviceps*, whitebacked planthopper

INTRODUCTION

Among various insect pests damaging rice crop, planthoppers including brown planthopper (BPH), *Nilaparvata lugens* (Stål), whitebacked planthopper (WBPH), *Sogatella furcifera* (Horváth) and green leafhopper (GLH), *Nephotettix virescens* (Distant) are very important causing both direct damage by sucking plant sap and indirect damage by acting as vectors of virus diseases. Mirid bugs, *Cyrtorhinus lividipennis* Reuter and

Tytthus parviceps (Reuter) are effective biocontrol agents against rice hoppers (Liquido and Nishida, 1983). The information available on how mirid bugs locate their hosts is very scanty. Synomones from the host plant play an important role in mediating host habitat and host location of the natural enemies. An attempt has been made to identify the sources of volatile chemicals originating from the host plant (rice) to attract the predatory mirid bugs to the rice ecosystem.

MATERIALS AND METHODS

TN1 rice plants were grown in the greenhouse at $30 \pm 5^\circ\text{C}$ and 60 ± 10 per cent relative humidity. BPH, WBPH and GLH were reared on 60-day-old rice plants in wooden cages in the greenhouse. Mirid bugs were reared on BPH oviposited rice plants. Adult insects were confined to these plants for 2-3 days for oviposition. The nymphs hatched were maintained in separate cages to obtain nymphs or adults of specified age.

Bioassay methods used to identify the sources of volatiles

The following plant materials were bioassayed for their role as synomones to the mirid bugs in Petri-dish, olfactometer and greenhouse bioassays. Undamaged rice plants, BPH, WBPH and GLH damaged rice plants, and their acetone extracts were evaluated by olfactometer and greenhouse bioassays. In the olfactometers, undamaged plant extracts, BPH, WBPH and GLH damaged rice plant extracts were tested in no choice tests. In the greenhouse cages, choice was given among (a) undamaged plants, and BPH, WBPH and GLH damaged rice plants, (b) rice plants sprayed with plant extracts of BPH, WBPH and GLH damaged plants and unsprayed plants. Rice plants of different ages *viz.*, 15, 40, 75-days-old seedlings and harvesting stage plants and their extracts were evaluated in olfactometer and greenhouse bioassays. In the olfactometers, plant extracts of different ages were evaluated in no-choice tests. In the greenhouse cages, choice was given among 4 different ages of intact rice plants. Extracts of different parts of rice plant *viz.*, leaf, stem and panicle were evaluated in olfactometer no choice tests. Nymphs, females and males of mirid bugs *viz.*, *C. lividipennis* and *T. parviceps* were tested separately for their response to plant material.

Preparation of rice plant extracts

Rice plants of required age were cut into pieces and 100 g of the plant material was soaked in 300 ml of acetone overnight. Next day the material was filtered through Whatman No. 1 paper using

charcoal powder to remove chlorophyll content and the filtrate was concentrated in rotavapor to a volume of 20 ml. In the case of infested plants, the insects were allowed to feed on plants for one week, and after removing insects, the damaged plants were used for extraction of plant volatiles. Different plant parts like leaf, stem and panicle were separately cut and they were extracted in acetone.

In the laboratory, no choice experiments were conducted using 'Y' tube olfactometer with 35cm arm length and 4cm diameter. Air was passed through cylinders containing distilled water, charcoal powder and honeydew source at one end and control at other end to get humid and odourless air passed through both arms of the olfactometer. Sterilized, absorbent cotton treated with one ml of extract served as the source and cotton treated with solvent served as the control. Nymphs, females and males of mirid bugs were released separately at the base of the olfactometer in-groups of 10 and replicated 6 times. In another experiment, they were released singly. Observations like number of mirid bugs present at the extract source up to a distance of 2 cm, at the centre and at control end were recorded 10 minutes after their release. Test material and control solvent were alternated between the arms to avoid bias. Between experiments all glass apparatus was cleaned with acetone and distilled water. Number of mirid bugs attracted were converted into percentages and analyzed in Completely Randomized Block Design after arcsine transformation and means were separated by DMRT.

Greenhouse bioassays

The plants required for testing were kept along with control plants at four corners of the wooden cage in the green house. There were 6 replications. Fifty mirid bug nymphs or females or males were released separately in the centre of the cage and number of insects settled on different plants was recorded after 5 hours. Numbers of mirid bugs attracted were converted into percentages and were analyzed in Completely Randomized Block Design after arcsine transformation and means were separated by DMRT.

RESULTS AND DISCUSSION

Attraction of mirid bugs to damaged and undamaged plant extracts

In the olfactometer no choice tests, mirid bugs were attracted in highest numbers to BPH damaged rice plant extract followed by undamaged plant extract and WBPH damaged rice plant extract whereas GLH damaged rice plant extract attracted least number of mirid bugs. Nymphs preferred and oriented to undamaged plant extract whereas females and males were attracted to BPH damaged plant extract (Table 1). *T. parviceps* preferred WBPH damaged and undamaged plant extracts followed by BPH damaged and GLH damaged plant extracts. Mirid bugs behaved in a similar manner when they were released singly in the olfactometers (Table 1).

In the greenhouse choice tests, BPH damaged rice plants and plants sprayed with the BPH damaged plant extract attracted highest number of both *C. lividipennis* and *T. parviceps* followed by WBPH damaged and GLH damaged rice plants and plants sprayed with their extracts whereas undamaged rice plant attracted least number of mirid bugs. Individual stages like nymphs, females and males were also attracted in highest numbers to BPH damaged plant and plants sprayed with its extract. (Tables 2 and 3).

The results are in conformity with those of Rapusas *et al.* (1996) and Lou and Cheng (2003) who observed that *C. lividipennis* was attracted to the odours of healthy rice plants compared to clean air in the olfactometers. Similar observations were recorded by Vinson (1981) and Sato and Osaki (1987) in other parasitoids and predators. In the present

Table 1. Per cent mirid bugs attracted to damaged and undamaged TN1 rice plant extracts in no choice tests using olfactometer

Plant extract	Mirid bugs attracted (%) when							
	Released in groups of 10				Released singly			
	Nymphs	Females	Males	Mean	Nymphs	Females	Males	Mean
<i>C. lividipennis</i>								
Undamaged	88.14 (70.49) ^a	72.26 (58.34) ^{ab}	80.88 (64.3) ^a	80.42 (64.38) ^a	68.50 (57.87) ^a	80.00 (68.8) ^a	82.45 (68.82) ^a	76.98 (65.17) ^a
BPH Damaged	79.71 (63.63) ^{ab}	85.54 (68.01) ^a	81.83 (64.93) ^a	82.36 (65.52) ^a	54.26 (46.93) ^{ab}	60.52 (50.83) ^{ab}	67.4 (61.03) ^{ab}	60.73 (52.93) ^{ab}
WBPH Damaged	68.16 (55.75) ^b	74.58 (60.23) ^{ab}	69.55 (56.65) ^b	70.76 (57.54) ^b	55.30 (50.08) ^{ab}	46.65 (43.04) ^{bc}	53.50 (50.09) ^b	51.82 (47.74) ^{ab}
GLH Damaged	29.29 (32.64) ^c	22.88 (28.01) ^c	22.13 (27.54) ^c	24.76 (29.39) ^c	13.65 (14.1) ^c	26.75 (25.04) ^c	24.70 (21.89) ^c	21.70 (20.34) ^d
<i>T. parviceps</i>								
Undamaged	45.01 (42.09) ^b	78.72 (62.78) ^a	71.72 (58.19) ^a	65.15 (54.35) ^a	67.32 (57.88) ^b	52.70 (50.09) ^a	68.20 (57.87) ^a	62.74 (55.28) ^a
BPH Damaged	49.54 (44.71) ^{ab}	66.40 (54.74) ^{ab}	69.21 (56.59) ^a	61.72 (52.01) ^a	58.40 (52.09) ^b	52.50 (45.99) ^{ab}	62.14 (55.99) ^a	57.68 (51.36) ^a
WBPH Damaged	57.90 (49.55) ^a	71.15 (57.8) ^a	67.29 (55.3) ^a	65.44 (54.22) ^a	74.50 (68.08) ^a	53.40 (50.09) ^a	45.40 (42.89) ^b	57.77 (53.69) ^a
GLH Damaged	50.87 (45.49) ^{ab}	56.48 (48.76) ^b	67.29 (56.12) ^a	58.21 (50.12) ^a	66.70 (57.88) ^b	6.58 (7.05) ^b	46.74 (43.04) ^b	40.00 (35.44) ^b

Figures followed by same letter in a column are not significantly different at 5 % level (DMRT).

Figures in parentheses are arcsine-transformed values.

Table 2. Attraction of mirid bugs to insect damaged plants in multiple-choice tests under greenhouse conditions

Damaged plant	Mirid bugs attracted (%)			
	Nymphs	Females	Males	Mean
<i>C. lividipennis</i>				
BPH damaged plant	49.34 (44.63)a	44.05(41.53)a	47.79(43.72)a	47.06(43.29)a
WBPH damaged plant	26.81(30.87)b	24.07(29.22)b	29.28(32.69)b	26.72(30.93)b
GLH damaged plant	15.89(23.41)c	17.99(24.9)c	15.54(22.69)c	16.47(23.67)c
Undamaged plant	5.19(11.61)d	12.38(20.37)d	8.15(16.38)d	8.57(16.12)d
<i>T. parviceps</i>				
BPH damaged plant	59.29(50.48)a	46.08(42.72)a	44.49(41.82)a	49.95(45.01)a
WBPH damaged plant	26.72(30.69)b	30.79(33.67)b	32.23(34.44)b	29.92(32.93)b
GLH damaged plant	7.68(14.05)b	16.75(23.58)c	16.08(23.15)c	13.5(20.26)c
Undamaged plant	5.62(11.17)b	3.88(8.03)d	7.19(13.22)d	5.56(10.81)d

Figures followed by same letter in a column are not significantly different at 5% level (DMRT).

Figures in parentheses are arcsine-transformed values.

Table 3. Attraction of mirid bugs to plants sprayed with damaged plant extracts in Greenhouse cages under multiple choice tests

Plant extract	Mirid bugs attracted (%)			
	Nymphs	Females	Males	Mean
<i>C. lividipennis</i>				
BPH damaged	36.48(37.04)a	47.62(43.73)a	42.86(40.85)a	42.32(40.54)a
WBPH damaged	25.02(29.88)ab	20.92(27.19)b	20.97(27.17)b	22.30(28.09)b
GLH damaged	21.81(27.74)b	17.36(24.81)b	21.87(27.83)b	20.35(26.79)b
Unsprayed	16.68(23.94)c	16.99(23.94)b	14.30(22.05)c	15.99(23.31)c
<i>T. parviceps</i>				
BPH damaged	37.61(37.71)a	41.51(40.05)a	39.89(39.02)a	39.67(38.93)a
WBPH damaged	28.06(37.92)ab	22.76(28.21)b	27.16(31.17) b	25.99(32.44)b
GLH damaged	21.90(27.85)b	23.61(28.91)b	24.04(29.25)b	23.18(28.67)b
Unsprayed	12.43(20.46)c	12.12(17.16)c	8.91(17.16) c	11.15(18.95)c

Figures followed by same letter in a column are not significantly different at 5% level (DMRT).

Figures in parentheses are arcsine-transformed values.

investigation, insect damaged rice plant extracts were more attractive to mirid bugs compared to undamaged plants. Rapusas *et al.* (1996) also recorded similar results and stated that plants naturally infested with insect pests apparently release chemicals that elicit a response by *C. lividipennis*. In the greenhouse choice tests, mirid bugs preferred BPH damaged plants and their extracts compared to WBPH and GLH damaged plants and their extracts, and undamaged plants and their extracts. These results are in conformity with the observations of Obata (1986) and Schaller and Nentwing (2000) that natural enemies have the ability to differentiate between insect damaged and undamaged plant volatiles and this helps them to save time and energy by avoiding searching on the plants where host is not present.

Synomonal activity of rice plant extracts of different ages

In olfactometers, in no choice tests, extract

from 75-day-old rice plant was preferred by *C. lividipennis* to plant extracts of other ages. *T. parviceps* did not exhibit any significant difference in its preference to plant extracts of different ages. Individual stages like nymphs, females and males also behaved similarly as that of group releases (Table 4). In greenhouse choice tests (Table 5), 75 day old plant was more attractive to *C. lividipennis* and *T. parviceps* compared to plants of other ages. In the present studies, mirid bugs could distinguish different ages of rice plant. The preference of mirid bugs to the rice plants of 75 days age is due to the suitability of the plant for population development and multiplication. The mirid prey, BPH also prefer rice plants above 60 days (Bac and Pathak, 1966). The seedlings and harvesting stage plants do not harbour any hoppers and they are not suitable for egg laying and development of the predator.

Synomonal activity of extracts of plant parts

Leaf extract was more attractive to both *C.*

Table 4. Attraction of mirid bugs to rice plant extracts of different ages in no choice tests using olfactometers

Plant extract	Mirid bugs attracted (%) when							
	Released in groups of 10				Released singly			
	Nymphs	Females	Males	Mean	Nymphs	Females	Males	Mean
<i>C. lividipennis</i>								
15 days old	43.09 (40.94)c	47.47 (43.48)c	74.59 (60.13)b	55.05 (48.18)c	19.99 (21.15)b	34.50 (32.09)b	73.50 (64.92)a	42.70 (39.39)b
40 days old	72.33 (58.75)ab	60.19 (51.14)b	71.67 (58.33)b	68.06 (56.07)b	80.00 (71.97)a	67.50 (57.87)ab	72.02 (61.77)a	73.20 (63.87)a
75 days old	87.59 (69.79)a	75.42 (60.59)a	82.09 (65.24)a	81.70 (65.20)a	75.40 (64.95)a	81.20 (68.82)a	80.50 (68.82)a	79.00 (67.53)a
Harvesting stage	44.31 (41.59)c	45.86 (42.51)c	69.06 (69.06)b	53.08 (51.05)c	66.60 (57.87)ab	31.60 (28.94)b	40.00 (35.99)b	46.00 (40.93)b
<i>T. parviceps</i>								
15 days old	29.35 (32.35)b	46.24 (43.07)a	24.10 (29.27)c	33.23 (34.89)b	46.53 (43.04)b	45.10 (42.5)ab	66.67 (61.02)ab	52.76 (48.85)b
40 days old	55.51 (48.18)a	47.47 (43.61)a	57.75 (49.96)a	53.58 (47.25)a	66.73 (61.03)a	60.00 (50.83)a	73.50 (64.92)ab	66.74 (58.93)a
75 days old	61.36 (51.83)a	43.74 (41.35)a	46.44 (42.92)b	50.51 (45.37)a	64.20 (57.87)ab	53.33 (50.1)a	80.00 (68.81)a	65.84 (58.93)a
Harvesting stage	13.23 (21.26)c	6.61 (14.72)b	10.00 (18.34)d	9.93 (18.11)c	53.34 (46.93)b	46.67 (43.04)ab	40.00 (35.99)b	46.67 (41.99)bc

Figures followed by same letter in a column are not significantly different at 5 % level (DMRT).

Figures in parentheses are arcsine-transformed values.

lividipennis and *T. parviceps* compared to stem and panicle extracts. All stages of *T. parviceps* oriented in large numbers to leaf extract followed by stem and panicle extracts. *C. lividipennis* nymphs preferred to move towards leaf extract and

females equally preferred leaf and stem extracts whereas males preferred leaf and panicle extract. When mirid bugs were released singly in the olfactometers, they behaved in a similar manner as in the case of group releases (Table 6).

Table 5. Attraction of mirid bugs to plants of different ages in greenhouse cages under choice tests

Plant age	Mirid bugs attracted (%)			
	Nymphs	Females	Males	Mean
<i>C. lividipennis</i>				
15 days old	14.63(22.31)c	8.60(16.94)c	8.38(16.06)c	10.54(18.44)c
40 days old	33.07(35.03)b	26.11(30.46)b	34.07(35.47)b	31.08(33.65)b
75 days old	50.03(44.97)a	56.76(48.92)a	53.28(46.88)a	53.36(46.92)a
Harvesting stage	2.275(8.67)d	6.77(15.07)c	4.29(11.94)c	4.45(11.89)c
<i>T. parviceps</i>				
15 days old	13.23(21.21)b	13.22(21.08)b	10.39(18.26)b	14.93(20.18)ab
40 days old	37.28(37.55)a	39.01(38.56)a	42.97(40.91)a	39.76(39.0) a
75 days old	33.44(35.25)a	40.22(39.51)a	41.48(39.88)a	38.38(38.21) a
Harvesting stage	16.05(23.49)b	7.22(12.77)c	5.13(10.72)b	9.46(15.66) b

Figures in a column followed by same letter are not significantly different at 5% level (DMRT).

Figures in parentheses are arcsine-transformed values.

Table 6. Attraction of mirid bugs to rice plant extracts of different plant parts in no choice tests

Plant extract	Mirid bugs attracted (%) when							
	Released in groups of 10				Released singly			
	Nymphs	Females	Males	Mean	Nymphs	Females	Males	Mean
<i>C. lividipennis</i>								
Leaf	83.21 (66.53)a	83.29 (66.00)a	83.78 (66.30)a	83.43 (66.29)a	68.12 (59.23)a	76.19 (67.04)a	57.14 (51.41)b	67.15 (59.23)ab
Stem	62.68 (52.45)b	80.87 (64.58)a	49.36 (44.61)b	64.31 (53.88)b	70.20 (64.79)a	63.92 (56.44)b	76.19 (67.04)a	70.10 (62.76)a
Panicle	36.07 (36.78)c	73.61 (59.50)ab	78.79 (62.79)a	62.83 (53.02)b	14.28 (15.11)b	19.04 (20.67)c	15.32 (15.11)c	16.21 (16.96)c
<i>T. parviceps</i>								
Leaf	59.83 (50.76)a	57.06 (49.17)a	58.88 (50.06)a	58.51 (49.99)a	62.50 (51.94)a	40.19 (33.52)b	38.09 (38.03)b	46.92 (41.16)ab
Stem	43.87 (41.43)b	48.32 (44.01)b	41.93 (40.32)b	44.71 (41.92)b	58.22 (49.16)a	57.35 (46.90)a	61.95 (51.94)a	59.17 (49.33)a
Panicle	9.75 (26.32)c	22.61 (27.73)c	34.90 (35.87)bc	19.17 (29.97)c	0.00 (0.00)b	9.52 (10.07)c	15.2 (15.11)c	8.24 (8.39)c

Figures followed by same letter in a column are not significantly different at 5 % level (DMRT).
Figures in parentheses are arcsine-transformed values.

From the study, it can be concluded that rice plant serves as a source of synomone for attracting mirid bugs to the plant ecosystem. The bugs could differentiate the pest infested and uninfested plants and select the plants with suitable age.

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