Bulletin of Insectology ***61*** *(2): 277-281, 2008*
ISSN 1721-8861

Detection of Asian rice gall midge (*Orseolia oryzae*) biotype 1 in the new locations of Karnataka, South India

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Abstract

Identification and virulence composition of Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) biotypes in Kodagu, Mysore and Hassan districts, South Karnataka, South India was studied during 2005 - 2006 under field conditions. Rice gall midge infestation in these three districts was detected for the first time in 2004 wet season. A set of 14 standard rice differentials representing four groups identified to characterize the prevailing rice gall midge biotypes in the country were evaluated against local gall midge populations in four locations. Based on the reaction pattern of standard differentials to gall midge populations, the biotype 1 gall midge was detected with R-R-R-S reaction pattern. The virulence composition was studied using three standard differentials viz., W1263 (*Gm1* gene for resistance), Phalguna (*Gm2* gene for resistance) and TN1 (susceptible without any gene). The local gall midge populations in all the test locations expressed their virulence only against susceptible group consisting TN1. The female to male sex ratio of their F_1 was close to 3:1 in TN1. These results confirmed the prevalence of genetically homogeneous biotype 1 population with avirulence to both *Gm1* (W1263) and *Gm2* (Phalguna) genes for resistance.

Key words: biotypes, virulence pattern, Cecidomyiidae, *Orseolia oryzae*, *Oryza sativa*.

Introduction

The Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera Cecidomyiidae) is a major insect pest of rice in several Asian countries (Bentur *et al.*, 2003). In India, rice gall midge has been reported from almost all the states except the Western Uttar Pradesh, Uttaranchal, Punjab, Haryana and Hill states of Himachal Pradesh and Jammu and Kashmir (Bentur *et al*., 1992). In India, crop losses ranging from 10-100% have been reported (Siddiq, 1991). Average annual yield loss due to the pest is estimated to be 80 million US$ (Ramaswamy and Jatileksono, 1996). The external symptom of damage caused by gall midge is the production of a silvery-white, tubular leaf sheath gall called a silver shoot. This renders the tiller sterile and causes the yield loss. Gall midge being endophytic, breeding resistant rice varieties has been a viable and ecologically acceptable approach for management this pest (Heinrichs and Pathak, 1981). Since 1970 more than 56 high yielding gall midge resistant rice varieties having different genes for resistance have been released for commercial cultivation (Bentur *et al*., 2003). After the widespread cultivation of high yielding gall midge-resistant rice varieties in farmer fields, different populations or biotypes were observed (Chatterji *et al*., 1975; Roy *et al*., 1971; Bentur *et al*., 1987; Nair and Devi, 1994; Srinivas *et al*., 1994; Singh, 1996). But the emergence of new virulent biotypes of gall midge in popular rice varieties is capable of overcoming resistance and this is a cause for concern. Six biotypes of rice gall midge have been so far identified and characterized in India (Bentur *et al*., 2003) based on the reaction of 14 standard differentials of four groups. Karnataka is one of the important rice producing state in India. The occurrence of gall midge on rice was first reported in 1927 by Hegdekatti, but more attentions were paid to this aspect during 1980s by All India Coordinated Rice Improvement Project (AICRIP). As a result the biotype 2 was identified in coastal parts of Karnataka (Kalode and Bentur, 1989). The rice gall midge infestation in southern districts of Karnataka viz., Kodagu, Mysore and Hassan was detected for the first time in wet and winter seasons of 2004. The level of infestation in these locations varied between 10-15 percent silver shoot. Since, southern parts of Karnataka form an important rice-growing tract, information on prevailing rice gall midge populations, their characterization and their virulence pattern are urgently needed. In many endemic locations of the country, the emergence of new virulent population capable of overcoming resistance has been recorded (Bentur *at al*., 1987; Prakasa Rao and Kandalkar, 1992; Singh, 1992; Nair and Devi, 1994; Srinivas *et al.,* 1994). Keeping this in view, the present investigation was undertaken in southern parts of Karnataka.

Materials and methods

Identification of biotypes

The investigations to identify prevailing rice gall midge biotypes in Kodagu, Mysore and Hassan districts, Karnataka, South India were made during wet and winter seasons of 2005 and 2006. Before conducting the investigation, a preliminary qualitative survey of gall midge infestation was made during early planting period (July-August) of 2004. A set of 14 standard differentials representing 4 groups identified to characterize the prevailing gall midge biotypes in the country by Directorate of Rice Research (DRR), Hyderabad under multi location trial of All India coordinated Rice Improvement Programme (AICRIP) were sown in test locations, coinciding with the peak population. In all the locations, each differential of 20-25 old seedlings were planted in 3 rows consisting 20 hills in each row with spacing of 20x15 cm between

rows and plants, respectively. To increase the level of infestation by creating higher relative humidity, constant water level of 5 inches in the field was maintained and 25 per cent excess nitrogenous fertilizer (urea) was applied. One row of each differential was also harvested at 30 days after planting to provide fresh sprouts for infestation (IRRI, 1981). The observations on damaged plants on hill basis and number of healthy and infested tillers (Silver shoots) in 20 hills were recorded at 30 and 60 days after transplanting (DRR, 2002). The percentage of damaged plants and silver shoots were recorded. Each entry under 4 groups was rated either resistant (R) with less than 10% plant damage or susceptible (S) with higher damage (Kalode and Bentur, 1989). Based on their pattern of resistance or susceptibility, the biotypes in test locations are differentiated as biotype 1 (R-R-R-S), biotype 2 (S-R-R-S), biotype 3 (R-S-R-S), biotype 4 (S-S-R-S), biotype 5 (R-R-S-S) and biotype 6 (R-S-S-S) (DRR, 1998). Further, the percentage silver shoot at 60 days of transplanting in each differential was converted to 0-9 scale using Standard Evaluation System (SES) for rice developed by International Rice Research Institute (IRRI), Los Banos, Philippines (IRRI, 2002).

Quantification of variations in virulence pattern

To quantify the composition of gall midge population in terms of virulence pattern in Kodagu, Mysore and Hassan district, Karnataka, South India, populations were monitored from August to December during 2005 to 2006 using three standard rice differentials viz., W1263 with *Gm1* gene for resistance (Reddy *et al.,* 1997), Phalguna with *Gm2* gene for resistance (Mohan *et al.,* 1994) and susceptible check TN1 (no gene for resistance) were received from DRR, Hyderabad and were sown separately in plastic tray (42 x 30 x 8 cm) two weeks prior to anticipated peak population of gall midge at the test locations. In each location 250 females (50 females/ location/ month) were tested from August to December.

Seedlings of one week old were transplanted to small plastic pots of about 10 cm height and 8 cm diameter holding 500 g of soil. In each pot, one hill of 3 differentials viz., W1263, Phalguna and TN1 were planted in triangular fashion. Each differential was represented by one hill containing 5 seedlings. Before infestation, precautions were taken to protect the plants from natural infestation by keeping the potted plants in a net cage (2.0 x 1.5 x 1.5 m). On the day of infestation, each pot containing one hill of 3 differentials were covered with the cylindrical plastic cage. Each cage was placed on the pot outside the differential and upper rim of the cage in each pot was covered by muslin cloth, tightened with rubber bands. The height of the cage was at least 15 to 20 cm to leave enough space above the plant. When the plants attained 3 leaf stage or two weeks old, each pot was infested with one female gall midge (presumed to be mated) collected during 7.30 to 11.30 PM near light source using aspirator in the rice farm and was released inside the pot through a small slit. Such gall midge infested pots were covered with cage for 2 days. On the third day, the cages were removed and plants were sprayed periodically with water using a clean hand atomizer at 2 hours intervals for 2 to 3 days to create extra relative humidity for egg hatching and maggot establishment. Alternatively, the pots were covered with plastic cage for 2 days after watering. All the plants were grown for 3 more weeks until galls in each differential developed. When differentials in all the pots show galls, observations on number of gall midge damaged plants for each of the differential and number of galls in W1263, Phalguna and TN 1 were recorded. Based on the reaction pattern of resistance (R) susceptibility (S) by single infested female, the biotypes in each infested pot are differentiated and confirmed as biotype 1 (R-R-S), biotype 2 (S-R-S), biotype 3 (R-S-S) and biotype 4 (S-S-S), and the number of females expressing reaction attribute of biotype 1, 2, 3, and 4 were counted in each tested pot and in each cropping month, percentage expression of each biotype pattern were calculated. Similarly, in each pot containing 3 differentials infested by single female, the sex of the emerging gall midge was recorded in each differential. This can be done by again covering the pots with plastic cages prior to adult emergence. The sex was identified by examining the pupae by dissecting under binocular microscope after 20 to 27 days of infestation. The male and female pupae were easily separated by their size and colour of the abdomen (Perera and Fernando, 1970; Panda and Mohanthy, 1970). Male pupae are small and brown in colour while the females are larger and pinkish in colour. Generally, if a single female infests each pot, all the emerging population (F_1) will be of one sex, if not the populations were genetically not homogenous (Sahu *et al*., 2004). Thus, reaction of offspring of a single female would help in identifying its biotype status. Reaction of all the females tested would help in quantifying the composition of gall midge population at the test location.

Results and discussion

Biotype identification

Surveys conducted during 2004 wet season in Mandya and adjacent districts viz., Kodagu, Mysore and Hassan revealed presence of rice gall midge infestation. Of the 14 standard differential rice genotypes evaluated during 2005 and 2006 wet and winter seasons, rice gall midge infestation was recorded only on TN1. The percent silver shoot on TN1 varied from 11.9 in Hassan to 13.7 in Madikeri, Kodagu. The rice gall midge was unable to feed on 13 other standard rice differentials representing first three groups. These results yielded reaction pattern of R-R-R-S. This reaction pattern to four groups of differentials was consistently observed at the four new locations consecutively for two years, both the seasons each year. These results confirmed the presence of biotype 1 of rice gall midge in the four new locations (table 1). Mandya, a major rice-growing district adjacent to Mysore, Hassan and Kodagu is an endemic area of rice gall midge (DRR, 2002). Biotype 1 of rice gall midge was detected in this district during 1998 (DRR, 1999). The presence of rice gall midge biotype 1 in adjacent district in wet and winter seasons of 2004 contributes the expansion of geographical distribution of rice gall midge in the area. Widespread continuous cultivation of gall midge

Table 1. Reaction of standard differentials to rice gall midge populations in southern Karnataka, 2005 and 2006 wet seasons; observations at 60 days after transplanting; R/S = Resistance/Susceptible reaction; SS = Silver Shoot; Score = 0- highly resistant (0% SS); 1- Resistant (<1% SS); 3- Moderately resistant (1-5% SS); 5- Moderately susceptible (6-10% SS); 7- Susceptible (11-25% SS); 9- Highly susceptible (>25% SS) (IRRI, 2002).

Serial Number	Group	Differential	Locations											
			Madikeri			Ponnampet			Mysore			Hassan		
			% SS	Score	R/S	% SS	Score	R/S	% SS	Score	R/S	% SS	Score	R/S
1	I	W 1263	0.00	0	R	0.00	0	R	0.00	0	R	0.00	0	R
2	"	ARC 6605	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
3	II	Phalguna	0.00	0	R	0.00	0	R	0.00	0	R	0.00	0	R
4	"	ARC 5984	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
5	"	Bhumansan	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
6	III	CR-MR 1523	0.00	0	R	0.00	0	R	0.00	0	R	0.00	0	R
7	"	Velluthacheera	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
8	"	Aganni	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
9	"	RP 2068-18-3-5	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
10	"	Abhaya	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
11	"	T 1477	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
12	"	INRC 202	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
13	"	INRC 1997	0.00	0	"	0.00	0	"	0.00	0	"	0.00	0	"
14	IV	TN 1	13.7	7	S	12.1	7	S	12.4	7	S	11.9	7	S

susceptible rice varieties such as Jaya, Mangala, IR20, IR64 and identical climatic conditions (all the five locations are under the Cauvery river basin) have contributed to the expansion of rice gall midge.

The adoption of rice varieties resistant to other insect pests has also resulted in a change in pest status of rice gall midge in certain regions. The large scale adoption of brown planthopper-resistant varieties, some of which were susceptible to gall midge, in the delta area of Krishna district of Andhra Pradesh witnessed an increase in gall midge incidence with damage reported to be as high as 32.5 per cent (Krishnaiah *et al*., 1986). A similar situation was observed in the Kuttanad area of Kerala, where predominantly brown planthopper tolerant varieties were popular, resulting in a yield loss of 90%, worth 1.8 million US$, from gall midge (Devi *et al*., 2004). Occurrence of gall midge biotypes in India was first suspected by Khan and Murthy (1955) even when no resistant varieties were developed. Subsequently, Roy *et al*. (1969) observed differential reaction pattern in some gall midge resistant donors/cultivars at two of the pest endemic locations viz., Sambalpur in Orissa and Warangal in Andhra Pradesh. Presence of two biotypes was further confirmed by results of evaluation of rice cultivars in national and international testing programs (Chatterji *et al*., 1975; Roy *et al*., 1971). Consequently a national program on monitoring reaction of these differentials at different pest endemic locations was launched in 1969. Since then, several regional, national and international collaborative studies have been undertaken to detect and characterize such variations in gall midge populations. The data generated through multiplication testing in India under All-India Coordinated Rice Improvement Programme (AICRIP) also indicated the existence of gall midge biotypes in different parts of the country. The geographical distribution of biotype 1 is continuous in south Karnataka. The presence of rice gall midge biotype 1 in south Karnataka specially Cauvery river basin and the susceptibility of currently cultivated rice varieties to rice gall midge underlines the urgent need for gall midge resistant varieties. In this context, the resistant genotypes viz., Abhaya, Aganni, ARC 6605, Phalguna and W1263 are recommended for use as parents in crop improvement programmes.

Virulence composition

The results of virulence spectrum of local gall midge populations in Kodagu, Mysore and Hassan districts during wet and winter seasons of 2005 and 2006 revealed the presence of homogeneous population of biotype 1. The number of virulent female tested for each location varied from 232 to 249. Except for TN1 (susceptible), the rice gall midge populations did not infest either W1263 (*Gm1* gene for resistance) or Phalguna (*Gm2* gene for resistance). Further investigations indicated that 100 percent of the test individuals reacted only as biotype 1 pattern (R-R-S) in all the test locations with greater virulence against TN1 containing absence of any gene for resistance. In all the test locations the gall midge population sex ratio in F_1 corresponded to 3:1 indicating homogeneous nature of biotype 1 (table 2). These results further confirmed the presence of homogeneous population of biotype 1 in all three new districts adjacent to Mandya. Meticulous monitoring of gall midge populations is urgently required to track the changing scenario of gall midge biotype populations in Southern Karnataka. This will help in developing rational management practices.

The data from Ragolu, Andhra Pradesh revealed that the gall midge population was mainly of biotype 3 (57%) and biotype 4 (39%) (DRR, 2003). The population at Mangalore, coastal Karnataka were found highly virulent against both W1263 (*Gm1* gene) and Phalguna (*Gm2* gene) indicating a mixture of population during wet 2003 and 2004 (DRR, 2004). Similarly the population was highly virulent against *Gm2* gene (Phalguna) followed by low virulence to *Gm1* gene (W1263). At Ragolu, Andhra

Table 2. Virulence spectrum of rice gall midge populations in four locations of South Karnataka, 2005 and 2006 wet seasons; GMB = Gall midge biotype; *Gm 1* and *Gm 2* are genes conferring resistance; F = Female; M = Male.

Location	No. of females tested	No. of females found infesting TN 1 (No gene)	W 1263 (*Gm 1*)	Phalguna (*Gm 2*)	*Gm1* + *Gm2*	% (No.) of females expressing virulence pattern of GMB 1	GMB 2	GMB 3	GMB 4	Sex ratio TN 1 F : M	W 1263 F : M	Phalguna F : M
2005												
Madikeri	249	249	0	0	0	100.0 (249)	0.00 (0)	0.00 (0)	0.00 (0)	3.22 : 1	0	0
Ponnampet	242	242	0	0	0	100.0 (242)	0.00 (0)	0.00 (0)	0.00 (0)	3.48 : 1	0	0
Mysore	232	232	0	0	0	100.0 (232)	0.00 (0)	0.00 (0)	0.00 (0)	3.07 : 1	0	0
Hassan	237	237	0	0	0	100.0 (237)	0.00 (0)	0.00 (0)	0.00 (0)	3.30 : 1	0	0
2006												
Madikeri	244	244	0	0	0	100.0 (244)	0.00 (0)	0.00 (0)	0.00 (0)	2.93 : 1	0	0
Ponnampet	244	244	0	0	0	100.0 (244)	0.00 (0)	0.00 (0)	0.00 (0)	3.20 : 1	0	0
Mysore	247	247	0	0	0	100.0 (247)	0.00 (0)	0.00 (0)	0.00 (0)	3.18 : 1	0	0
Hassan	245	245	0	0	0	100.0 (245)	0.00 (0)	0.00 (0)	0.00 (0)	3.45 : 1	0	0

Pradesh, the population was highly virulent against *Gm1* gene (W 1263) followed by *Gm2* gene (Phalguna) and 24.7% was virulent against both *Gm1* and *Gm2* gene and one fourth of the population were exhibited virulence against both genes *Gm1* and *Gm2* indicating pattern of biotype 4 (DRR, 2004). At Rapipur, Chattisgarh during wet 2004, 7.5% of the populations were found heterogeneous for biotype 1 (Modi *et al.,* 2004). This composition has been observed to vary from location to location and from year to year at the same test location (Bentur *et al.,* 2003). However, such phenomenon was not observed in the present study. Thus, the present study reveals the presence of gall midge biotype 1 in Madikeri and Ponnampet, Kodagu, Mysore and in Hassan districts with R-R-R-S reaction pattern. The similar populations were also reported at Mandya, southern Karnataka (Lingaraj *et al*., 2006), Hyderabad, Warangal and Maruteru in Andhra Pradesh, Sambalpur in Orissa, and Raipur in Chattisgarh (Kalode and Bentur, 1989; Pasalu *et al*., 2004).

Acknowledgements

The authors are grateful to Dr J. S. Bentur, Principle Scientist and Head, All India Coordinated Rice Improvement Programme (Rice Entomology) and Dr I. C. Pasalu, Principle Scientist, Directorate of Rice Research (DRR), Hyderabad for technical advice. We thank University Grants Commission (UGC), Government of India for financial support. Thanks are also due to Director of Research, UAS, Bangalore and farmers of Southern Karnataka for cooperation.

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Received December 20, 2007. Accepted August 26, 2008.