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Developmental biology of *Chilo partellus* (Swinhoe) on diverse sorghum genotypes

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Abstract

We studied the effects of different sorghum genotypes on developmental biology, and larval and adult behaviors of spotted stem borer, *Chilo partellus*. The test maize genotypes had significantly lower pupal weight, larval survival, adult emergence and fecundity as compared to susceptible check, Swarna. There was significant increase in pre-mating period, decrease in pre-oviposition and oviposition periods, decrease in numbers of egg clusters and eggs per cluster, and increase in egg incubation period in *C. partellus* on these test genotypes as compared to those from susceptible check, Swarna. The studies on establishment and feeding behaviors of *C. partellus* larvae on different sorghum genotypes revealed that the neonate larvae took longer time to establish on the resistant sorghum genotypes, and the establishment on resistant sorghum genotypes was also significantly lower than on the susceptible genotype. Thus, the sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046 and IS 18551 impart detrimental effects on the growth, development and progeny production of *C. partellus* and can be utilized in breeding to derive *C. partellus* resistant varieties of sorghum.

Keywords: Sorghum, *Chilo partellus*, biology, behavior, resistance

Introduction

Sorghum (*Sorghum bicolor* Moench) is an essential crop of semi-arid tropics (SAT). It provides livelihood to millions of people in Asia, Africa, USA, Latin America and Australia. It is a fifth major cereal crop after rice, wheat, maize and barley^[1]. Sorghum is grown on 45 million ha area with production of 68.9 million tons of grains around the world. In India, it is cultivated on 5.65 million ha with annual production of 4.41 million tones^[2]. It is mainly used for livestock and poultry feed, malt industry and for human consumption^[3]. About 150 insect species have been reported to sorghum from sowing to harvest, and even in storage. Of these, spotted stem borer, *Chilo partellus* (Swinhoe) is most devastating pest of sorghum in Asia and Africa^[1, 4]. The *C. partellus* alone causes about US\$ 334 million annual crop loss in the SAT^[5]. Spotted stem borer attacks sorghum from 2 week aged seedlings till crop harvest. It damages all plant parts, except the roots. The damage by *C. partellus* can be diagnosed through damage symptoms viz., leaf feeding, exit holes, stem tunneling, chaffy grain and deadhearts^[6, 7, 8]. Young larvae feeds on the leaf whorls and causes pinholes and elongated lesions. At early stage, *C. partellus* causes “deadheart”. The older larvae goes down inside the leaf whorls and bore through the stem of the plant. The concealed feeding habit provides protection to the developing *C. partellus* larvae from the insecticidal sprays on the crop. The hiding nature of larvae is the main cause of less effectiveness of pesticides against this pest^[6]. The host plant resistance (HPR) can effectively minimize losses due to insect pests in sorghum^[9]. It is also compatible with other pest management tools including chemical and biological control. Three mechanisms viz., antixenosis, antibiosis and tolerance are operational for *C. partellus* resistance in sorghum^[4, 7, 9]. Antibiosis affects biology of the pest through reduction of pest abundance and fecundity which cause mortality. The neonate larvae choose appropriate substrate whether to accept or reject the plants^[10, 11], and then orient towards suitable host and get settled. Antixenosis mechanism of resistance influence larval orientation, settling and feeding response due to presence of chemical and/or morphological factors^[12]. This behavioral response could be used as a tool for the management of stem borer in sorghum. Considering importance of insect behavioral and biology, we studied the adult oviposition, larval feeding and establishment behaviour, in response to damage by *C. partellus* in sorghum.

Materials and Methods

Laboratory rearing of *Chilo partellus*

The nucleus culture of *C. partellus* was collected from the field of research fields of ICAR-Indian Agricultural Research Institute, New Delhi and reared on fresh sorghum stalks till pupation. The males and females thus obtained were collected in pairs and transferred to wire mesh oviposition cages (16 cm ht. and 8 cm dia.) covered with circular butter paper for egg laying. The butter papers were changed every day till adult mortality. Butter papers bearing eggs of *C. partellus* were stored at 30 °C in plastic bucket (20 lit. capacity) having water at the bottom to avoid desiccation till the eggs turned black. The black head stage eggs were kept at room temperature for hatching. The newly hatched larvae were transferred to plastic jars (1000 ml capacity) containing artificial diet^[13] at 27 ± 2 °C temperature and 75-85% relative humidity at Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi. After completion of one generation on artificial diet, the *C. partellus* neonates thus obtained were used for further experiments.

Plant materials

Six sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046, IS 18551, IS 2205 (resistant check), and Swarna (susceptible check) were used in experimentation. Each genotype was sown in 2 row plots of 4 m row length. The rows were 75 cm apart from each other. The seeds were sown at 5 cm depth with dibbling. There were three replications in randomised block design. Recommended agronomic practices, except insecticide application were followed to raise test sorghum genotypes under field conditions.

Developmental biology of *C. partellus* on test sorghum genotypes

The studies on biology of spotted stem borer, *C. partellus* were done on the above mentioned test sorghum genotypes. The experiment was carried out at 27 ± 2 °C temperature, 75-85% relative humidity and 12L: 12D photoperiod under controlled conditions in the laboratory. The seedlings of the test sorghum genotypes (starting at 14 days after their germination) raised under field conditions were utilized for *C. partellus* biological studies. The *C. partellus* neonates obtained from stock culture as mentioned above were inoculated on leaf whorls of test sorghum genotypes with camel hair brush in the plastic jars (10×8 cm). Since the *C. partellus* neonates have phototactic behavior, these jars were wrapped with black paper and kept under laboratory conditions at 27 °C ± 2 °C and 75-85% relative humidity. Twenty five neonates of *C. partellus* larvae were released in a jar per replication. There were five replications of each test sorghum genotype in a completely randomized design (CRD). The food was changed on alternate days, and every day whenever required. After attaining second instar, the larvae were offered stems of test sorghum genotypes as food till pupation. Larval mortality was recorded during food change and dead larvae were discarded. Observations were recorded on larval period, larval survival, pupal weight, pupal period, adult emergence and fecundity per female. The larval period was recorded separately for each insect, and the mean larval period per replication was calculated for the surviving larvae. The data on the number of larvae survived was expressed as percentage larval survival. The pupal period was recorded separately for each insect, and the mean pupal period per replication was calculated for the surviving pupae. The pupal

weight was measured for individual pupa on an electronic balance (Contech, CB-120) within 24 h after pupation. The data on number of adults emerged from each replication was expressed in percentage adult emergence based on the number of larvae released per replication. The data on fecundity from each replication was expressed as total number of eggs laid per female.

Female oviposition behaviour

The male and female *C. partellus* adults obtained, where the larvae were fed on different test sorghum genotypes were used in this study. Fifteen freshly emerged pairs of *C. partellus* adults were released individually in the oviposition cages for each test sorghum genotype under laboratory conditions at 12D:12L photoperiod, 27 ± 2 °C and 75-85% relative humidity, thus making 15 replications per test genotype. The observations were recorded on pre-mating, mating, post-mating, pre-oviposition, oviposition and post-oviposition periods. The number of egg masses laid per female, numbers of eggs in each egg mass and incubation period were also recorded for each test genotype.

Larval feeding behaviour

To know orientation and settling behaviour that involves the process of selection of a suitable site in which the larva have an option to accept or reject the plant was measured by no and multi-choice tests^[12]. The leaf discs measuring 3 cm and 5 cm diameter were prepared from the whorl leaves of two week old seedlings of test sorghum genotypes for no-choice and multi-choice tests, respectively. For no-choice test, 10 neonate *C. partellus* larvae were released in the center of a petri-dish having leaf disc of each genotype separately. However, one leaf disc of each test sorghum genotype were placed on the sides in a petri-dish at equidistant and 60 neonate *C. partellus* larvae were placed in the center for multi-choice test. There were five replications for no-choice test and 10 replications for multi-choice test in a completely randomized design. After five days of release, the numbers of larvae recovered from each leaf disc per replication were recorded on all the test sorghum genotypes under no-choice test. For multi-choice test, the numbers of *C. partellus* larvae settled on each leaf disc were recorded after 24h, and expressed as numbers of larvae recovered from the leaf disc of each test sorghum genotype. The *C. partellus* larval settlement behaviour was carried out on two week old seedlings of test maize genotypes. The sorghum seedlings of test genotypes were grown in the plastic pots using recommended crop growing practices. The neonate of *C. partellus* was released on tip and under side of the 1st and 3rd leaf from the top of each test sorghum genotype, respectively. There were six replications for each test sorghum genotype in a completely randomized design. The observations on time taken by the *C. partellus* larvae to establish in the leaf whorl from the 1st and 3rd leaf of each test sorghum genotype were recorded separately.

Statistical analysis

The data on various biological parameter, and adult and larval behaviour of *C. partellus* were subjected to analysis of variance (ANOVA) using completely randomized design. The significance of differences among genotypes were tested by *F*-test and the treatment means were compared.

Results

Developmental biology of *C. partellus* on test sorghum genotypes

Biological performance of *C. partellus* on different sorghum genotypes found that the larval period, larval survival, pupal period, pupal weight and adult emergence varied between 20.1 to 23.7 days, 56.0% to 76.0%, 4.5 to 5.5 days, 50.7 to 114.1 mg/pupa, 37.8 to 64.8%, respectively (Table 1). These studies further revealed significant differences in larval survival ($F = 53.24$; $df = 5, 20$; $P < 0.001$), pupal weight

($F=45.64$; $df=5, 20$; $P < 0.001$) and adult emergence ($F=81.57$; $df = 5, 20$; $P < 0.001$) when the larvae of *C. partellus* were fed on different test sorghum genotypes. However, the differences for larval ($F=1.22$; $df=5, 20$; $P=0.338$) and pupal ($F=1.96$; $df=5, 20$; $P=0.129$) periods were non-significant (Table 1). The pupal weight was significantly higher on susceptible check, Swarna than on test sorghum genotypes. However, the pupal weight of *C. partellus* on test sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046 and IS 18551 was on par with resistant check, IS 2205 (Table1).

Table 1: Biological performance of *Chilo partellus* on different sorghum genotypes

Genotypes	Larval period (days)	Pupal period (days)	Pupal weight (mg/pupa)	Larval survival (%)	Adult emergence (%)
ICSV 1	20.7	5.3	71.4	64.8	48.0
ICSV 93046	23.7	5.4	67.0	65.6	46.4
ICSV 700	20.1	5.3	67.4	68.0	50.4
IS 18551	23.4	4.5	68.0	63.2	39.2
IS 2205	22.2	5.3	50.7	45.6	28.8
Swarna	20.5	5.5	114.1	85.6	73.6
LSD ($P = 0.05$)	NS	NS	9.32	5.15	4.87
P-value	0.338	0.129	<0.001	<0.001	<0.001

NS = Values non-significant $P = 0.05$.

C. partellus adult oviposition behaviour

The oviposition behaviour of *C. partellus* adults was studied in terms of effects of larval food on the pre-mating, mating and post-mating periods, pre-oviposition, oviposition and post-oviposition periods, number of egg clusters/female, number of eggs/cluster, fecundity and incubation period of the eggs obtained from these females when their larvae were fed on different sorghum genotypes. The pre-mating, mating and post-mating periods of *C. partellus* adults varied from 6.20 to 8.40 h, 4.33 to 5.93 h and 5.07 to 7.47 h, respectively when their larvae were reared on different sorghum genotypes (Table 2). There were significant differences in pre-mating ($F=4.58$; $df=5, 70$; $P < 0.001$), mating ($F=4.47$; $df=5, 70$; $P < 0.001$) and post mating ($F=7.07$; $df=5, 70$; $P < 0.001$) periods of *C. partellus* adults when their larvae were reared on different sorghum genotypes (Table 2). The pre-mating period of *C. partellus* adults was significantly longer when their larvae were reared on resistant check, IS 2205 as compared to other sorghum genotypes. Further, the pre-mating period was significantly longer and mating period was shorter when their larvae were reared on the test sorghum genotypes as compared to susceptible check, Swarna. However the post-mating periods of *C. partellus* adults from ICSV 93046 were on par with those from susceptible check, Swarna (Table 2). The pre-oviposition, oviposition and post-oviposition periods of *C. partellus* females varied from 1.40 to 2.10 days, 2.87 to 4.13 days and 1.30 to 1.70 days, respectively when their larvae were reared on different sorghum genotypes (Table 2). There were significant differences in oviposition ($F=4.85$; $df=5, 70$; $P < 0.001$) periods of *C. partellus* females, while the differences for pre-oviposition ($F=1.66$; $df=5, 70$; $P=0.157$) period and post-oviposition ($F=0.76$; $df=5, 70$; $P=0.583$) were non-significant when their larvae were reared on different sorghum genotypes (Table 2). The oviposition period of *C. partellus* females was significantly shorter when their larvae were reared on resistant check, IS 2205 as compared to other sorghum genotypes. Further, the oviposition period was significantly shorter when their larvae were reared on the test

sorghum genotypes as compared to susceptible check, Swarna. However, no significant differences were found among the sorghum genotypes for pre-oviposition and post-oviposition periods of *C. partellus* females (Table 2). The oviposition behavior further revealed that the fecundity, number of egg clusters/female and numbers of eggs/cluster laid by *C. partellus* females varied from 297.27 to 367.33 eggs/female, 12.27 to 14.73 egg clusters/female and 23.53 to 27.07 eggs/cluster, respectively when their larvae were reared on different sorghum genotypes (Table 2). There were no significant differences in fecundity ($F=1.15$; $df=5, 70$; $P=0.34$), numbers of egg clusters/female ($F=0.81$; $df=5, 70$; $P=0.548$) and numbers of eggs/cluster ($F=1.76$; $df=5, 70$; $P=0.133$) laid by *C. partellus* females, when their larvae were reared on different sorghum genotypes (Table 2). The incubation period of eggs laid by *C. partellus* females varied from 3.30 to 4.80 days when their larvae were reared on different sorghum genotypes (Table 2). There were significant differences in incubation period of *C. partellus* eggs obtained from the females when their larvae were reared on different sorghum genotypes ($F=5.23$; $df=5, 70$; $P < 0.001$). The eggs obtained from susceptible check, Swarna reared *C. partellus* took significantly shorter time to hatch as compared to those from other sorghum genotypes. However, the incubation period of the eggs obtained from the test sorghum genotypes and the resistant check, IS 2205 were on par with each other (Table 2). These findings thus reveal significant increase in pre-mating and post mating periods, decrease in mating and oviposition periods, and decrease in number of egg/cluster in *C. partellus* when their larvae were reared on test sorghum genotypes than those from susceptible check (Swarna). These findings indicate deleterious effect of larval food on the progeny production of spotted stem borer. These findings thus reiterate that the spotted stem borer larvae fed on test sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046, IS 18551 and IS 2205 impart detrimental effects on the progeny production, thus can be utilized in breeding for resistance to *C. partellus* in sorghum.

Table 2: Effects of larval food on the oviposition behavior of *Chilo partellus* adults when reared on different sorghum genotypes

Genotypes	Premating period (h)	Mating period (h)	Post-mating period (h)	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	No. of egg clusters/female	No. of eggs/cluster	Fecundity (eggs/female)	Incubation period (days)
ICSV 1	7.53	4.33	6.53	2.07	4.00	1.40	13.33	23.53	314.93	4.80
ICSV 93046	7.27	4.40	5.67	1.67	3.60	1.70	13.40	26.13	342.00	4.10
ICSV 700	7.60	4.80	6.53	1.40	4.07	1.40	13.07	24.33	316.20	4.60
IS 18551	8.20	4.73	6.33	2.13	3.40	1.40	14.73	23.73	351.67	4.80
IS 2205	8.40	4.73	7.47	1.67	2.87	1.60	12.27	24.93	297.27	4.20
Swarna	6.20	5.93	5.07	1.67	4.13	1.30	13.53	27.07	367.33	3.30
LSD (P = 0.05)	1.03	0.77	0.87	NS	0.62	NS	NS	0.72	NS	NS
P-value	<0.001	<0.001	<0.001	0.157	<0.001	0.583	0.34	<0.001	0.548	0.133

NS = Values non-significant P = 0.05.

Establishment and feeding behavior of *Chilo partellus* larvae

Time taken by the neonate *C. partellus* larvae to establish in the central leaf whorl of different sorghum genotypes when released on the tip of top 1st leaf varied from 43.2 to 142.8 min, while from top 3rd leaf it varied from 67.7 to 176.3 min (Table 3). There were significant differences in time taken by the neonate *C. partellus* larvae to establish in the central leaf whorl of different sorghum genotypes when released on the tip of top 1st leaf (F=10.03; df=5, 24; P<0.001) and when released on the top 3rd leaf (F=11.64; df=5, 23; P<0.001). The neonate *C. partellus* larvae released on the tip of top 1st leaf and on the top 3rd leaf of resistant check, IS 2205 took more time to establish in the central leaf whorl than on other test sorghum genotypes (Table 3). However, the neonate *C. partellus* larvae released on test sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046 and IS 18551 took more time to establish in the central leaf whorl than on susceptible genotype, Swarna (Table 3). The number of *C. partellus* larvae recovered from the leaf discs of different sorghum

genotypes varied between 2.0 to 7.2 larvae/disc under no-choice condition, while under multi-choice condition it varied between 3.8 to 11.8 larvae/disc (Table 3). There were significant differences in numbers of *C. partellus* larvae recovered from the leaf discs of different sorghum genotypes both under no-choice (F=9.16; df=5, 20; P<0.001) and multi-choice (F=14.55; df=5, 45; P<0.001) conditions under laboratory conditions. The numbers of *C. partellus* larvae recovered from susceptible check, Swarna were significantly higher than those from test sorghum genotypes under both no-choice and multi-choice conditions. However, *C. partellus* larval recovery from the leaf discs of test sorghum genotypes and resistant check, IS 2205 were on par under no-choice and multi-choice conditions (Table 3). These studies indicate that neonate *C. partellus* larvae take longer time to establish on the resistant sorghum genotypes. The larval establishment on resistant sorghum genotypes was also significantly lower than on the susceptible genotype. These findings thus have implications for devising suitable pest management techniques for both the cultivation scenarios.

Table 3: Establishment and feeding behaviour of neonate *Chilo partellus* larvae on different sorghum genotypes

Genotypes	Time taken by neonate <i>C. partellus</i> larvae to establish in the central leaf whorl (min)		Number of <i>C. partellus</i> larvae recovered	
	From top 1 st leaf	From top 3 rd leaf	No-choice condition (n =10)	Multi-choice condition (n = 60)
ICSV 1	85.2	88.3	3.4	3.9
ICSV 93046	106.5	97.0	2.6	4.7
ICSV 700	113.0	87.5	4.2	5.8
IS 18551	112.5	110.8	2.4	5.4
IS 2205	142.8	176.3	2.0	3.8
Swarna	43.2	67.7	7.2	11.8
LSD (P = 0.05)	30.91	32.32	1.87	2.24
P-value	<0.001	<0.001	<0.001	<0.001

NS = Values non-significant P = 0.05

Discussion

These studies revealed significant variation in larval survival, pupal weight and adult emergence of *C. partellus* on test sorghum genotypes. The sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046, IS 18551 and IS 2205 had significantly lower pupal weight, larval survival and adult emergence as compared to susceptible genotype, Swarna. Various componential characters impart resistance to insects in plants [14, 15]. Host plant quality parameters like plant nutritional and anti-nutritional biochemical factors are the main factors, which determine performance of herbivores [16, 17]. The nutrients and biochemical substances in host plants plays major role in feeding, survival, development, growth and oviposition preference in herbivores and determine the resistance or susceptibility of host plants against pests [18]. These nutritional and biochemical factors have also been

reported to determine resistance or susceptible reaction against insect pests in sorghum [19, 20]. Nutritional quality of host plants and variation in genetic makeup of the insect species varies the differential effects of various genotypes on insect biological attributes [21, 22]. Long-term genetic differentiation, direct physiological response to host genotypes and the environmental factors determines the behavioral, physiological and genetic differences in different pest populations [23]. The differences in biological performance of *C. partellus* on test sorghum genotypes in the present studies could be due to variation in their biochemical composition. The results of no-choice and multi-choice tests showed that higher numbers of larvae were recovered on Swarna as compared to other sorghum genotypes. Similar trend in choice and no-choice tests of insect behavioral response [24]. The results of no-choice tests indicate that in the

presence of preferred host plants, the first instar larvae were likely to settle on susceptible genotype. Further, *C. partellus* larvae could stay longer than 24 h on resistant check, IS 2205, could possibly be used as an alternative tool for management of *C. partellus*. The results of multi-choice tests indicate that in the presence of other host plants, the first instar larvae preferred to settle on susceptible genotype than other sorghum genotypes. Similar results reported by various workers on host selection by various insect pests on different host plants like *Hyparrhenia tamba* (Steud.), *Zea mays* L., *Sorghum bicolor* L., *Pennisetum glaucum* (L.), and *Pennisetum purpureum* Schumach^[24, 25, 26]. The colonization process in larvae of *C. partellus* appears to be critical for feeding^[27]. At this stage, the larvae mainly depend on limited resources^[28]. Present studies revealed that the neonate *C. partellus* larvae took more time to establish in the whorls of sorghum genotypes ICSV 1, ICSV 700, ICSV 93046, IS 18551 and IS 2205 as compared to susceptible genotype, Swarna. The findings on larval establishment and feeding behavior thus conclude that the *C. partellus* larvae took longer time to establish and their recovery was also lower on resistant sorghum genotypes than on susceptible check, Swarna. These results suggest antixenosis mechanism of resistance to *C. partellus* in the test sorghum genotypes. Earlier studies revealed that plant morphological characteristics cease the larval movement and force larvae to leave the host plant^[29] for long exposure to predators, dehydration and harsh environmental conditions^[30].

Conclusion

Present study revealed significant differences in larval survival, pupal weight and adult emergence of *C. partellus* when reared on different sorghum genotypes. The test sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046, IS 18551 and IS 2205 had significantly lower pupal weight, larval survival and adult emergence as compared to susceptible check, Swarna. These findings thus reveal significant increase in pre-mating and post-mating period, decrease in mating period, decrease in oviposition period and decrease in number of egg/cluster in *C. partellus* when their larvae were reared on test sorghum genotypes as compared to those from susceptible check (Swarna). These suggesting the deleterious effect of larval food on the progeny production of spotted stem borer. The neonate *C. partellus* larvae released on sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046, IS 18551 and IS 2205 took more time to establish in the central leaf whorl than on susceptible genotype, Swarna. The numbers of *C. partellus* larvae recovered from these test sorghum genotypes were also significantly lower than those from susceptible genotype, Swarna under both no-choice and multi-choice conditions. The findings on larval establishment and feeding behavior thus conclude that the *C. partellus* larvae took longer time to establish and their recovery was also lower on resistant sorghum genotypes than on susceptible check, Swarna, thus having implications for devising suitable pest management techniques for both the cultivation scenarios. These findings thus recapitulate that the sorghum genotypes viz., ICSV 1, ICSV 700, ICSV 93046, IS 18551 and IS 2205 impart detrimental effects on the growth, development, oviposition, and larval feeding and establishment behaviors of *C. partellus*.

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