

Orientation of Sorghum Midge, *Stenodiplosis sorghicola*, Females (Diptera: Cecidomyiidae) to Color and Host-Odor Stimuli¹

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ABSTRACT Sorghum midge, *Stenodiplosis sorghicola* (Coquillett), is one of the most important pests of grain sorghum worldwide. Sorghum midge adults emerge in the morning, mate at or near the site of emergence, and then the females proceed in search of sorghum crop at flowering for oviposition, and some visual and odor stimuli play an important role in host finding and oviposition process. We used a glass apparatus with two (Y-tube) arms to study the orientation of sorghum midge females to visual and odor stimuli under laboratory conditions. Most sorghum midge females were attracted to yellow (30%), followed by green (26%), red (23%), and blue (10%). Sorghum midge females responded more quickly to yellow, followed by red, green, and blue. However, under dual-choice conditions, differences in numbers of sorghum midge females attracted to yellow versus green, red versus blue, and blue versus green were not significant. More sorghum midge females were attracted to sorghum panicle odors plus red (47%) or yellow (40%) colors than to host odors alone (31%). Information on the color preference of sorghum midge females could be exploited for developing suitable traps to monitor its abundance in combination with kairomones or pheromones.

KEY WORDS sorghum midge, *Stenodiplosis sorghicola*, color stimuli, host odor, plant resistance, attraction, *Sorghum bicolor*

Sorghum, *Sorghum bicolor* (L.) Moench, is one of the most important cereal crops in Africa, Asia, Australia, and the Americas. Nearly 150 species of insects have been recorded as pests of sorghum (Jotwani et al. 1980), of which sorghum midge, *Stenodiplosis sorghicola* (Coquillett), is the most important pest worldwide (Harris 1976). Sorghum midge adults emerge in the morning from infested sorghum panicles at the milk to dough stages of development, mate at or near the site of emergence, and then the females search for flowering sorghums for oviposition. The males hover around the site of emergence, mate with the newly emerged females, and die soon after (Harris 1976). Sorghum midge females lay eggs in flowering sorghum spikelets during the morning hours, and usually die

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within 4 to 6 h. Thus, sorghum midge females have a limited time to locate a flowering sorghum crop for oviposition. Odors emanating from flowering sorghum panicles attract sorghum midge females (Sharma et al. 1990). Yellow- and white-colored traps are more attractive than blue or black traps to sorghum midge females in the field (Wiseman et al. 1972, Sharma et al. 1990). However, there is no information on the relative attraction of different colors alone and in combination with sorghum odors to sorghum midge females. Therefore, we used dual-, multi-, and no-choice tests to study the attraction of sorghum midge females to different colors alone and in combination with sorghum odors to gain an understanding of the host-finding behavior of this insect.

Materials and Methods

Sorghum midge females were obtained from sorghum panicles collected from farmers' fields in Darling Downs, Queensland, Australia, and were kept in 30 × 45-cm brown paper bags at 27°C ± 2°C, 60% ± 5% RH, and a 12:12-h (L:D) photoperiod in the laboratory. An inverted transparent plastic jar (21.5-cm long × 10.5-cm in diameter) with three wire mesh-screened windows (4-cm diameter), two on the sides and one at the top, was placed over the paper bag and tied to it by a 1-cm-wide rubber band. The rubber band was twisted at the rim of the jar and pulled onto the upper end of the handle of the jar to keep the jar upright without support. Upon emergence, sorghum midges moved upward into the plastic jar because of their positive phototactic behavior. Sorghum midges were retained in the plastic jar until 1,000 h, where they presumably mated during this period. Each jar containing sorghum midges was covered on the sides with a sheet of black polyethylene. Sorghum midges were collected in a 20-mL glass vial attached to an opening (2.5 cm in diameter) in the lid of the jar. A small piece of clay was used to hold the vial to the lid. The sorghum midges moved into the glass vial as a result of attraction to light. The vial containing 40 to 50 sorghum midges was removed from the jar and was replaced with a new one. Twenty sorghum midge females were collected in each 20-mL glass vial. Attraction of these sorghum midge females to different color and odor stimuli was studied under laboratory conditions.

Attraction of sorghum midge females to color and odor stimuli under dual-choice conditions was studied in a Y-shaped glass olfactometer with an insect-holding chamber (6-cm diameter × 23-cm length; Fig. 1). One end of the glass chamber was blocked with sintered glass followed by an 18-cm-long glass joint tapering into a 2-cm-diameter tube. A plastic tube (1.8-cm diameter × 15-cm length) was connected to the glass tube. The other end of the plastic tube was connected by a T-joint to an airtight vacuum chamber (17-cm diameter × 15-cm height). A plastic hose (1.5-cm diameter × 3-m length) was connected to the vacuum chamber at one end and to a vacuum pipe inlet (connected to the central vacuum system) at the other. The knob of the vacuum inlet was adjusted carefully to create a steady airflow (nearly 1 cm sec⁻¹) through the glass apparatus. A bifurcated T-joint to which two glass arms (3.5-cm diameter, 30-cm length) were attached was connected to the insect-holding chamber at the other end. The glass arms were blocked with sintered glass at 18 cm, leaving an 11-cm portion to hold flowering rachis branches of sorghum panicle (the stage at which sorghum midge females lay eggs in sorghum panicles). Five rachis branches from a flowering

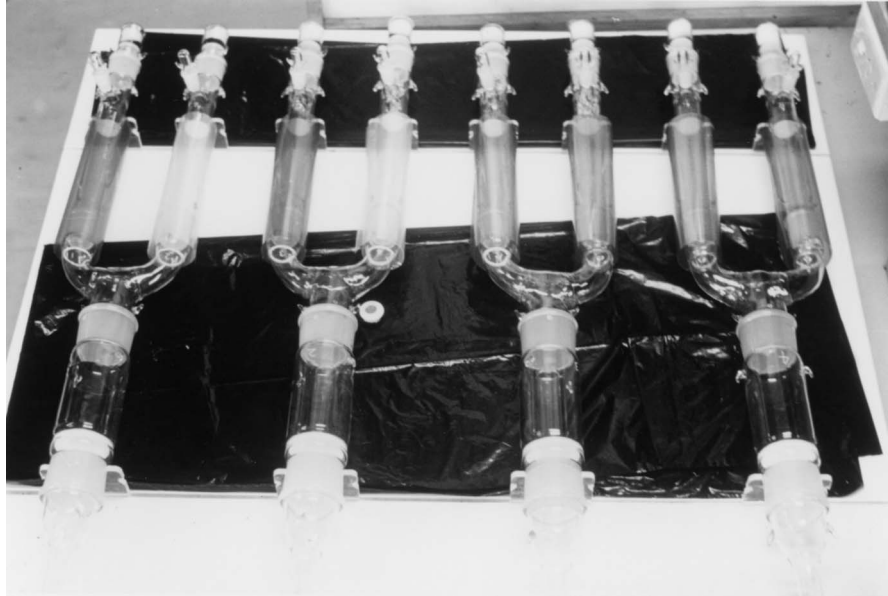


Fig. 1. Glass apparatus used to assess response of sorghum midge females to different colors and odor stimuli.

sorghum panicle were placed in this section and were used to measure the attraction of sorghum midge females to odors alone or in combination with visual stimuli. A 9-cm glass tube containing charcoal and cotton wool to filter the incoming air was attached at the end of this section. Four glass units (Y-tubes) were placed horizontally on a wooden board in the laboratory. The four glass units were used for comparing combinations of color and odor stimuli. The light intensity was uniform on all sides of the glass apparatus. A black polyethylene sheet was placed under the glass apparatus to avoid reflection of light from the white wooden board. Twenty sorghum midge females were released into the holding chamber, and the glass apparatus were joined together immediately. The insect-holding chamber and 5-cm portions of the glass arms were covered with a black polyethylene sheet to provide directional visual stimuli to the sorghum midge females. The glass apparatus was cleaned with soap, rinsed with alcohol, and dried at 105°C after each experiment.

Movement of sorghum midge females towards light and sorghum odors. Movement of sorghum midge females towards the two arms of the Y-tube from the holding chamber of the glass olfactometer was studied by covering the entire glass apparatus with a black polyethylene sheet, by covering the holding chamber and 5-cm portion of the Y-tube arms with a black sheet to provide a directional light stimuli, and by leaving the entire glass apparatus uncovered. Five flowering rachis branches of sorghum cultivar 'ICSV 197' were placed in one arm, and a cotton swab soaked in 2 mL of water was kept in the other arm as a blank. Twenty sorghum midge females were released into the holding chamber. The numbers of sorghum midge females moving to two arms of the Y-tube were

recorded after 30 min. The experiment was repeated four times, and the three treatments (flowering rachis branches and water in combination with covered, partially covered, and uncovered glass apparatus) were compared in a completely randomized design. The data were subjected to analysis of variance (ANOVA), and significance of differences between the treatments was judged by paired *t* test at $P = 0.05$.

Attraction of sorghum midge females to different colors in relation to normal light. In this experiment, one arm of the dual-choice Y-tube was wrapped with a green, red, blue, or yellow transparent plastic sheet (30 × 30 cm), whereas the other arm was left uncovered (normal light). This provided a choice to the sorghum midge females between normal light and light passing through colored transparent plastic sheets (green, red, blue, or yellow). Twenty freshly emerged sorghum midge females were released in the holding chamber, and the holding chamber and 5-cm portion of the Y-tube were covered with a black sheet. Numbers of sorghum midges moving to the two arms of the Y-tube were recorded after 30 min. The insects were discarded after each test. The treatments were tested in pairs, and there were eight replications over time for each comparison. The significance of differences between the treatments was compared by paired *t* test at $P = 0.05$ for each comparison.

Relative attraction of sorghum midge females to different colors under dual-choice conditions. Relative attraction of different colors to sorghum midge females was studied in all possible combinations (yellow versus blue, yellow versus green, yellow versus red, red versus green, red versus blue, and blue versus green). The two arms of the Y-tube were covered with transparent sheets of the two colors being compared. The holding chamber and 5-cm portion of the glass arms were covered with a black polyethylene sheet. This provided the sorghum midge females a dual-choice between the colors being compared. Twenty sorghum midge females were released into the holding chamber, and the numbers of sorghum midge females that had moved to the glass arms were recorded 30 min later. Different colors were tested in pairs as described above, and there were 10 replications over time for each comparison. The treatment means were compared with a paired *t* test at $P = 0.05$ for each comparison.

Relative attraction of sorghum midge females to different colors under no-choice conditions. In this experiment, both arms of the Y-tube of the glass olfactometer were wrapped with transparent plastic sheets of the same color. The holding chamber and 5-cm portion of the glass arms were covered with a black polyethylene sheet as described before. Twenty sorghum midge females were provided only one choice, i.e. to respond to the same color in both the arms of the olfactometer. Numbers of sorghum midge females moving to the glass arms were recorded at 5, 10, 15, and 30 min after initiating the experiment to get an idea of the speed at which sorghum midge females respond to different colors. The numbers of sorghum midge females moving to both the arms of the glass apparatus at different time intervals was used as a measure of the attractiveness of a particular color to sorghum midge females. The experiment was repeated three times, and the four-color treatments were arranged in a randomized complete block design. The data were subjected to ANOVA using GENSTAT 5.0. The significance of differences between the treatments was judged by *F* test, and the treatment means were compared by least significant difference (LSD) at $P = 0.05$.

Relative attraction of sorghum midge females to different colors under multi-choice conditions. Relative attraction of sorghum midge females to red, yellow, green, and blue transparent plastic sheets wrapped on the four sides of the cage (30 × 30 × 30 cm) was also studied under multi-choice conditions, i.e. the sorghum midge females were offered a choice between the four colors being compared at the same time. The cage frame was made of aluminum, and the four sides and top were covered with a thin, transparent polyethylene sheet. Red, yellow, green, and blue transparent plastic sheets (30 × 30 cm) were placed on the four sides of the cage. Twenty sorghum midge females were released in the center of the cage, and the numbers of sorghum midge females that settled on the four sides of the cage with different colors were recorded at 15, 30, 45, and 60 min after initiating the experiment. The position of colors was changed after each test to avoid the directional effect, if any, of the normal light. The treatments were arranged in a completely randomized design and there were three replications. The data were subjected to ANOVA using GENSTAT 5.0. The significance of differences between the treatments was judged by *F* test, and the treatment means were compared by LSD at *P* = 0.05.

Relative attraction of sorghum midge females to different colors plus odor stimuli. Dual-choice conditions were used to study the attraction of sorghum midge females to odors alone and in combination with yellow, green, or red colors. Five rachis branches from flowering panicles of the sorghum genotypes 'QL 12' or 'QL 39' were placed at the ends of the arms of the glass Y-tube. Both arms of the Y-tube were covered with yellow, red, or green transparent plastic sheets. Twenty sorghum midge females were released into the holding chamber. The holding chamber and 5-cm portions of the glass arms were covered with a black polyethylene sheet. The Y-tube then was connected to the vacuum chamber. This resulted in a steady stream of air passing over the sorghum rachis branches placed at the ends of the glass arms. The air moved from the ends of the glass arms toward the holding chamber, where the sorghum midge females were released. Numbers of sorghum midge females moving to the arms of the Y-tube were recorded after 15, 30, and 60 min after initiating the experiment. The experiment was repeated three times, and the treatments were arranged in a completely randomized design. The data were subjected to ANOVA using GENSTAT 5.0. The significance of differences between the treatments was judged by *F* test, and the treatment means were compared by LSD at *P* = 0.05.

Results and Discussion

Attraction to normal light and odor stimuli. More sorghum midge females (60%) moved into the arms of the Y-tube having a light and odor stimuli (HC + O) than to the arms providing only light stimulus (HC; 30%) when the holding chamber and 5-cm portion of the glass arms was covered with a black polyethylene sheet (which provided a directional light stimulus to the sorghum midge females; Fig. 2). The differences between these treatments were not statistically significant (*P* > 0.05) when the glass apparatus was fully covered with a black polythene sheet, indicating that visual stimuli are important in the orientation behavior of this insect. When the entire glass apparatus was left uncovered, there were no significant differences in the numbers of sorghum midge females that moved into the arms of the Y-tube in response to light and odor

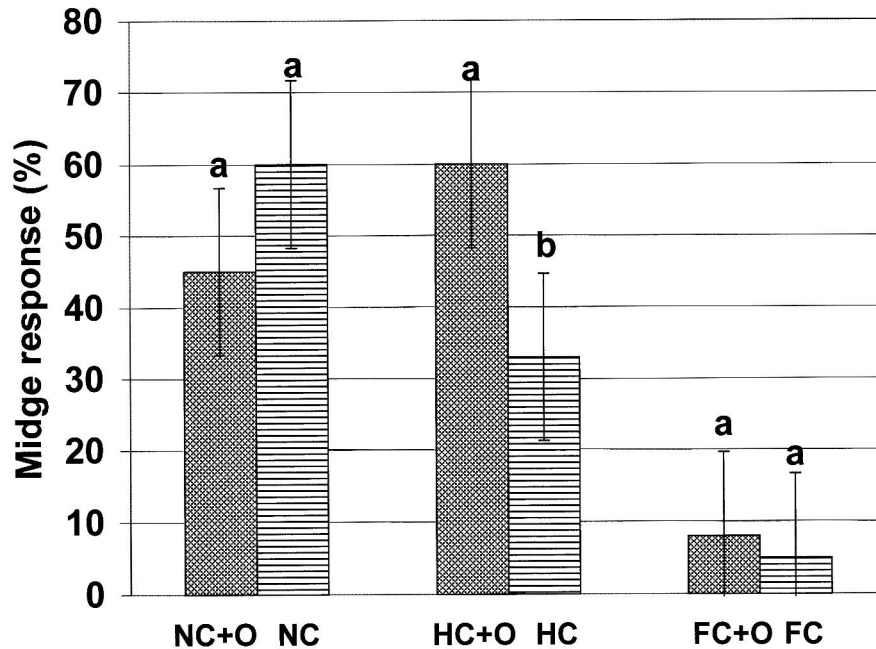


Fig. 2. Attraction of sorghum midge females to normal light and odor from the panicles of sorghum cultivar 'ICSV 197.' NC, No black polythene cover on the glass apparatus; O, odors from the sorghum panicle; HC, one-half of the glass apparatus covered with a black polythene sheet; FC, glass apparatus fully covered with a black polythene sheet. A pair of bars with the similar letters are not significantly different at $P = 0.05$ ($df = 15$) based on paired t test.

stimuli. Covering the insect-holding chamber and 5-cm portions of the glass arms with a black polyethylene sheet resulted in maximal response of sorghum midge females in the Y-tube, and this was used as a standard procedure to study the response of sorghum midge females to different colors.

Attraction of sorghum midge females to different colors in relation to normal light. When the sorghum midge females were offered a choice between normal light and the light passing through different colored polythene sheets, significantly ($df = 7, P \leq 0.05$) more sorghum midge females responded to yellow and blue colors than to the normal light. Maximum numbers of midges responded to yellow (62%), followed by blue (43%), green (30%), and red (26%; Fig. 3). Differences in numbers of sorghum midge females attracted to red and green versus normal light (blank) were not significant at $P = 0.05$ based on paired t test.

Relative attraction of sorghum midge females to different colors. When sorghum midge females were offered a choice between the light passing through different colored transparent polyethylene sheets in a dual-choice assay, significantly ($df = 9; P \leq 0.05$) more sorghum midges responded to yellow than to blue (38% versus 22%) or red (48% versus 24%) colors (Fig. 4). Also, more sorghum

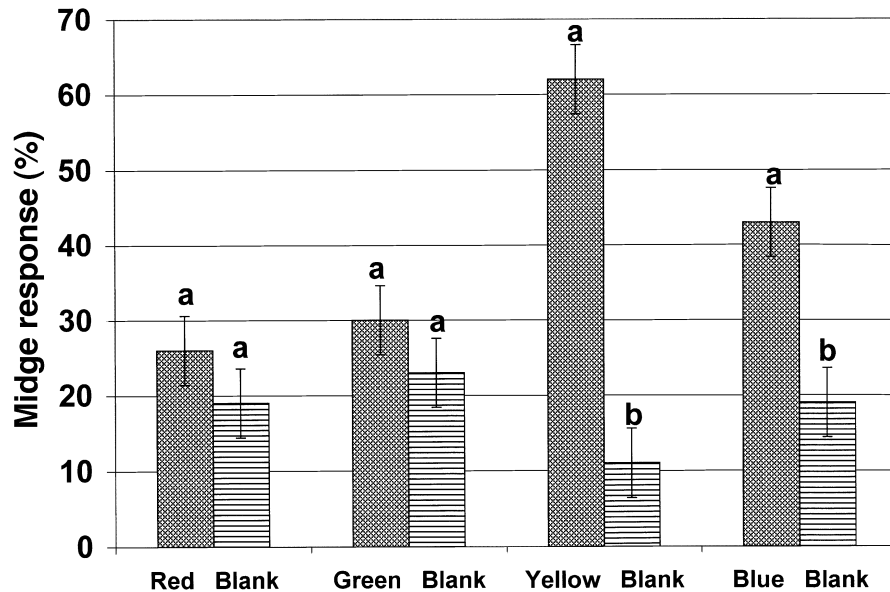


Fig. 3. Attraction of sorghum midge females to different colors versus normal light (Blank) under dual-choice conditions. A pair of bars with similar letters are not significantly different at $P = 0.05$ based on paired t test.

midges were attracted to red (39%) than to blue (25%). Differences in attraction of sorghum midge females to yellow versus green, red versus green, and to blue versus green were not significant at $P = 0.05$ based on paired t test. In general, the sorghum midge females preferred yellow over the other colors tested, and red to green and blue colors.

Relative attraction of sorghum midge females to different colors under no-choice conditions. Attraction of sorghum midge females to different colors under no-choice conditions was used as a measure of the promptness with which sorghum midge females respond to different colors. Sorghum midge females moved fastest toward yellow, followed by movement toward red, green, or blue (Fig. 5). Five minutes after initiating the experiment, significantly ($df = 7$, $P = 0.06$) more midges responded to yellow and red colors (80% and 85%, respectively) than to green and blue colors (65% and 67%, respectively) based on LSD comparison. Ten minutes after initiating the experiment, 88% of the midge females responded to yellow, 83% to green, 80% to red, and 77% to blue, although the difference in midge response to different colors were not significant ($P > 0.05$). The movement of sorghum midge females was relatively slower towards blue and green colors initially, but was similar to red and yellow colors at 10 to 30 min after initiating the experiment, and there were no significant differences in midge response to different colors at 30 min.

Relative attraction of sorghum midge females to different colors under multi-choice conditions. Under multi-choice cage conditions, more sorghum midge females responded to yellow (40% to 53%) than to the other colors

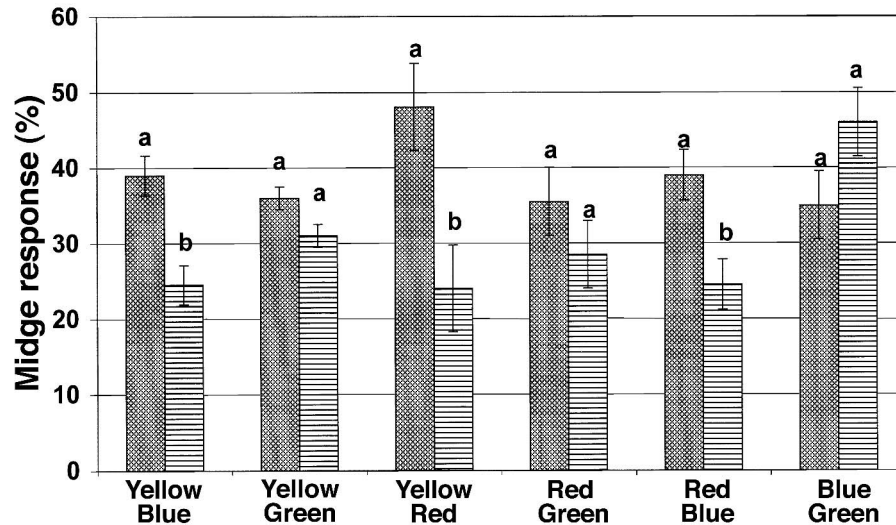


Fig. 4. Relative attraction of sorghum midge females to different colors in dual-choice tests under laboratory conditions. A pair of bars with similar letters are not significantly different at $P = 0.05$ based on paired t test.

tested (10% to 28%; Fig. 6). However, based on F test and LSD comparison, the differences in midge response to different colors were significant ($df = 6$) only at $P = 0.09$. At 15 min after initiating the experiment, more sorghum midge females were attracted to yellow (40%), followed by red (25%), green (13%), and blue (12%) colors. Throughout the experiment, most sorghum midge females were attracted to yellow, followed by red, green, and blue. Maximum response of the sorghum midge females to different colors was observed 45 min after initiating the experiment.

Attraction of sorghum midge females to color plus odor stimuli. More sorghum midge females were attracted to yellow color plus odor (34%) 15 min after initiating the experiment than to red (25%) or green (33%) plus odor or to odor alone (26%; $df = 10$; $P = 0.07$; Fig. 7). However, differences in midge response to different colors plus odor, and odor alone were not significant at $P = 0.05$. Similar trends in midge response were also observed at 30 and 60 min after initiating the experiment. Thus, color plus odor stimuli seemed to be slightly more attractive to sorghum midge females than host odor alone.

Color, contact, and odor stimuli influence host selection behavior of insects. However, we do not understand fully how insects detect their host plants. Some insects have strong color preferences (Prokopy & Owens 1983, Harris et al. 1993, Barker et al. 1997). In general, phytophagous insects prefer yellow, and darker colors such as blue and black, are least preferred. Both hue and intensity influence insect response to different colors. Traps with fluorescent yellow paint are more attractive than traps with non-fluorescent yellow paint that reflects the same wavelength (550 nm; Meyerdirk et al. 1979).

Detailed information on the role of visual stimuli in host selection behavior is available only for a few insect species, whereas the influence of chemical stimuli

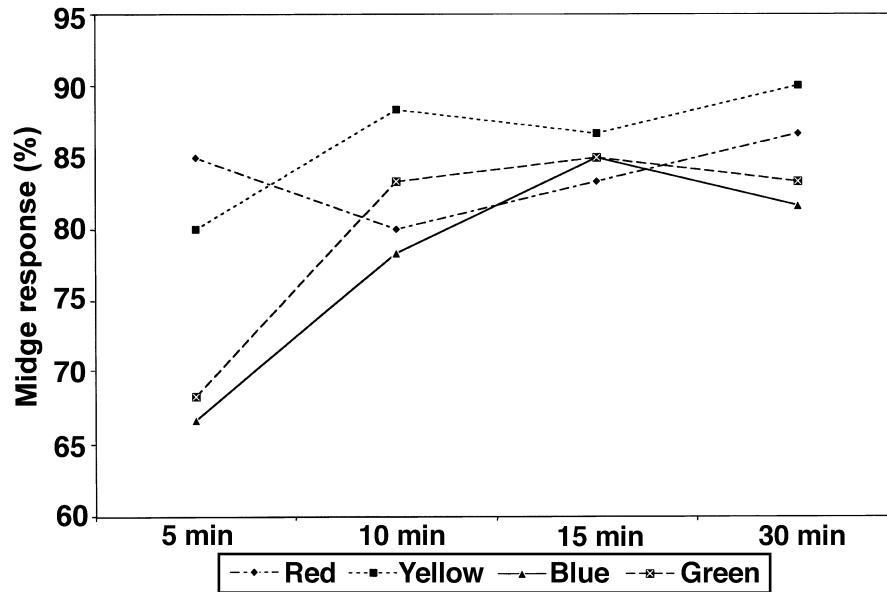


Fig. 5. Attraction of sorghum midge females over time to different colors in a glass olfactometer under no-choice conditions. The SEM for comparing mean midge response to different colors is 4.3, 5.1, 4.2, and 3.1 at 5, 10, 15, and 30 min after initiating the experiment, respectively.

in host plant selection has been studied in considerable detail (Harris et al. 1993). Visual cues and odor stimuli together elicit greater response from Hessian fly, *Mayetiola destructor* (Say), than do visual stimuli alone (Harris et al. 1993), and egg-laying response is stimulated by the hue rather than intensity of colors (Harris & Rose 1990).

Spectral quality and patterns of individual plants or their components serve as visual cues to insects (Prokopy & Owens 1983). Spectral transmission curves of foliage under different light conditions are consistent for several plant species because of absorption properties of chlorophyll (hue of 500 to 580 nm). Light reflectance is affected by plant surface characteristics such as glossiness or glume hairiness (Sharma & Nwanze 1997), high cellular water content, or lack of chlorophyll. Most of these characteristics result in greater total reflectance between 350 to 650 nm (Prokopy & Owens 1983). Spectral characteristics of foliage can enable insects to distinguish between living plants and other objects. In many plants, spectral quality seems to be the principal stimulus eliciting alightment (Kennedy et al. 1961, Coombe 1981), and intensity of reflected or transmitted light by foliage is a more variable parameter than spectral composition (Prokopy & Owens 1983). The diversity of insect species that are known to respond positively to yellow color (Kennedy et al. 1961, Walker 1974, Cross et al. 1976, Coombe 1981) has led to the speculation that for many insects, yellow constitutes a supernormal foliage stimulus emitting energy in the same band-width as that of the insect vision spectrum.

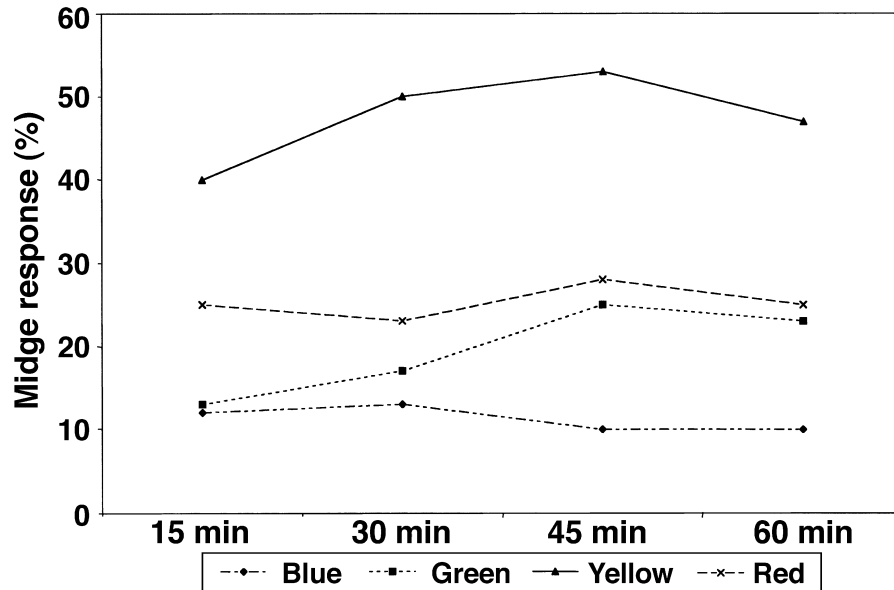


Fig. 6. Attraction of sorghum midge females to different colors over time in a cage (multi-choice conditions). The SEM for comparing the mean midge response to different colors is 12.9, 14.9, 11.9, and 11.6 at 15, 30, 45, and 60 min after initiating the experiment, respectively.

Genotypic variation may be as great as phenotypic variation in composition of the spectral light reflected from the plants at the intraspecific level. Individuals of a plant species may vary greatly morphologically between genotypes: e.g. sorghum genotypes have different intensities of trichomes, waxiness, glossiness, chlorophyll content, and hairiness of the glumes (Sharma & Nwanze 1997). These characteristics may influence the quality and intensity of light reflected from the sorghum plant, and ultimately influences the host-plant selection by the sorghum midge females. Sorghum midge-resistant and sorghum midge-susceptible genotypes also differ in the intensity of light reflected at the flowering stage of plant growth (H. C. S., unpublished data), and this may influence the host plant selection by sorghum midge females.

Sorghum midge females are attracted to yellow sticky traps (Wiseman et al. 1972, Sharma et al. 1990) and lay fewer eggs in spikelets of sorghum panicles covered with yellow or white cloth bags than in panicles covered with blue or black cloth bags (Sharma et al. 1988, 1990, Sharma & Vidyasagar 1994). In the present studies, sorghum midge females showed greater preference to light passing through yellow than to light passing through red, green, or blue colors. More sorghum midge females were attracted to odors from a sorghum panicle in combination with red or yellow than to host odors and the normal light. Thus, both color and odor stimuli are important in host finding by the sorghum midge females. Many insects use combined visual and chemical information when selecting their host plants (Prokopy & Owens 1983). Visual cues have a greater effect

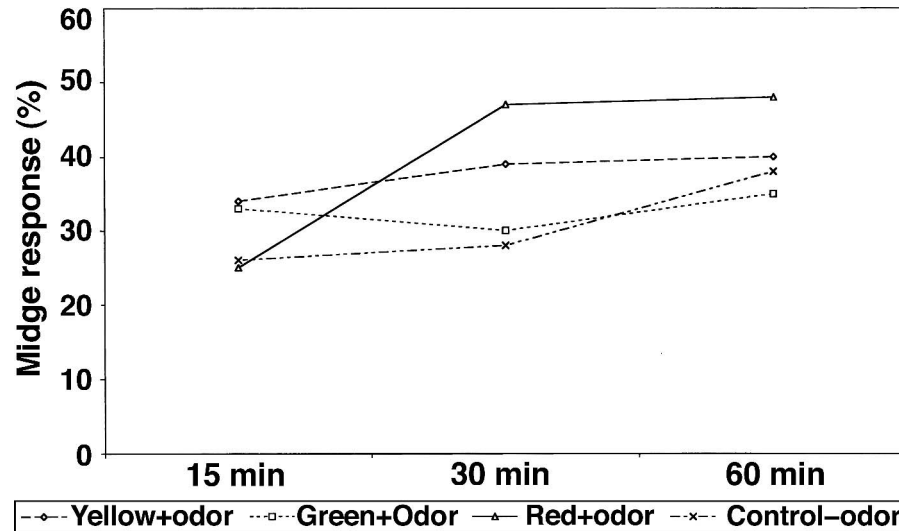


Fig. 7. Attraction of sorghum midge females to sorghum panicle odor alone and in combination with color stimuli ($df = 10$; $P = 0.07$). The SEM for comparing the midge response to different treatments is 5.1, 8.1, and 11.1 at 15, 30, and 60 min after initiating the experiment, respectively.

on flight orientation responses than plant odors for the Hessian fly (Harris et al. 1993). Similar responses were observed with sorghum midge, where different colors in combination with plant odors elicited a greater response than the plant odors alone.

Information on the attraction of sorghum midge females to visual and chemical stimuli may be useful for designing appropriate techniques to study population dynamics under field conditions. Monitoring sorghum midge density by visual counts is time consuming, must be carried out during the morning hours, and often is difficult with the unaided eye. However, the color preference of sorghum midge females could be exploited for developing suitable traps for monitoring its abundance in combination with sex pheromones or other odor stimuli. Development of suitable color traps to monitor sorghum midge abundance in the field could play an important role in understanding the behavior and population dynamics of this insect.

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