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PHAGOSTIMULANT ACTIVITY OF SUCROSE, STEROLS AND SOYBEAN LEAF EXTRACTABLES TO THE CABBAGE LOOPER *TRICHOPLUSIA NI* (LEPIDOPTERA: NOCTUIDAE)

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Abstract—Phagostimulant activities of sucrose, sitosterol, stigmasterol, campesterol, and soybean leaf extractables were evaluated towards the third-instar larvae of the cabbage looper, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) on elderberry-pith discs under laboratory conditions. There was a linear relationship between sucrose dosage and larval feeding on the pith discs. Water fraction from PI 227687 soybean leaf extractables at dosages > 400 μg/disc inhibited feeding by *T. ni* larvae. Ethyl acetate fraction showed phagostimulant properties comparable to sucrose. Sterols (5 μg/disc) and soybean leaf extractables (40 μg/disc) in combination with sucrose (400 μg/disc) showed synergistic effect as phagostimulants. Implications of genotypic differences in sterol content in relation to host plant resistance are discussed.

Key Words: Sucrose, sitosterol, stigmasterol, campesterol, phagostimulants, host plant resistance, soybean extractables

Résumé—Les activités appétitrices du sucrose, du sitostérol, du stigmastérol, du campestérol et d'extraits de feuille du soja ont été évaluées au laboratoire contre le 3éme stade larvaire de l'arpenteuse du chou, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) sur des disques en moëlle de baie de sureau. Une relation linéaire s'est dégagée entre concentration du sucrose et consommation par la larve des disques de moëlle. La fraction aqueuse de l'extrait de feuille du soja "PI 227687" aux concentrations supérieures à 400 μg/disque a inhibé la consommation chez *Trichoplusia ni*. La fraction avec l'éthyl acétate a montré des propriétés appétitrices comparables au sucrose. Les stérols (5 μg/disque) et les extraits de feuille de soja (400 μg/disque), associés au sucrose (40 μg/disque) ont produit un effet synergétique en tant qu'appétents. Les implications des différences génotypiques dans le contenu en stérol en rapport avec la résistance à la plante-hôte sont discutées.

Mots Clés: Sucrose, sitostérol, stigmastérol, campestérol, extraits de feuille du soja, agents appétents, résistance à la plant-hôte

INTRODUCTION

Nutritionally important constituents of a host plant play a significant role in the feeding behaviour of phytophagous insects (Thorsteinson, 1960). At physiological concentrations, sugars, amino acids, lipids, salts and some secondary plant substances act as phagostimulants. A combination of some of these components quite often produces synergistic phagostimulant effects (Beck and Hanec, 1958; Thorsteinson and Nayar, 1963; Gothilf and Beck, 1967; Doss et al., 1982; Doss, 1983; Shanks and Doss, 1987).

In addition to being phagostimulants, sterols are important for insect growth and development. Insects are incapable of *de novo* synthesis of the steroid skeleton, which they require to synthesise the moulting hormone, ecdysone. To meet the sterol requirements, the phytophagous insects depend on

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their host plant or symbionts. Insect-resistant soybeans (PI 227687 and PI 229358) have been reported to have a lower sterol content at the seedling stage, but these lines accumulated greater amounts of sterols at pod-filling as compared with the insect-susceptible genotypes Ransom and Coker Hampton 266A (Tester, 1977). Grunwald and Kogan (1981) did not observe such differences in sterol content between insect-resistant and susceptible soybean cultivars. Over the growing season, the total and bound sterols increased, while the free sterols decreased. Sitosterol, stigmasterol, and campesterol are the major sterols in soybean. Over the season, sitosterol increased, while stigmasterol decreased. The role of sterols in host plant preference and feeding by the cabbage looper, *Trichoplusia ni* Hubner remains unclear. In our continuing efforts to elucidate various aspects of insect host plant relationships involving soybean, this paper reports findings on the role of sucrose, sterols and soybean leaf extractables, alone and in combinations, in feeding preferences of the third-instar larvae of the cabbage looper, *T. ni*.

MATERIALS AND METHODS

Test chemicals

Sucrose, sitosterol, stigmasterol, and campesterol were obtained commercially (Sigma Chemical Co, St. Louis, MO, USA). Sitosterol, stigmasterol, and campesterol are the major sterols in soybean leaves (Tester, 1977), and hence were selected for studying their phagostimulant properties towards third-instar larvae of the cabbage looper, *T. ni*. Soybean leaf extractables were obtained by the process described below.

Soybean leaf extractables

Insect-resistant plants of the PI 227687 and susceptible, Davis genotypes were grown in the greenhouse at the United States Department of Agriculture (USDA) Dairy Forage Research Center, University of Wisconsin, Madison. Plant growing conditions were the same as described by Sharma and Norris (1991). Seedlings germinated in moistened vermiculite were transplanted in 20-cm-diameter earthen pots containing a sterilised mixture of soil, sand, and vermiculite (2:1:1). Plants were fertilised with 50 ml solution of 1% Miracle-Gro (Stern's Nursery Inc., Geneva, N.Y., USA) every fortnight. Pots were kept under Metalarc high intensity light (1000 watt) for 16 h photophase. Leaves from the 8-week-old plants (V8 stage of development) (Fehr et al., 1971) were removed and stored at -5°C or lyophilised and stored in a desiccator.

Chemical constituents of soybean leaves were extracted by a sequential extraction with ethyl acetate and methanol. Green leaves (@100 g) were homogenised with 850 ml of ethyl acetate in an ice-cooled Waring blender for 5 min. The homogenate was filtered through Whatman filter paper no. 1. The residue was re-extracted with 500 ml of ethyl acetate on an automatic shaker for 8 h, and then filtered. The re-extraction was repeated five times per leaf sample. Each time, the filter paper was put back in the residue. After such ethyl acetate extraction, the residue was extracted three times with 500 ml of 60% methanol as described above. Ethyl acetate and 60% methanol leaf extractables were combined and rotoevaporated at 50 ± 1°C.

Polar and nonpolar components of the leaf extractables were separated by the following process to identify fractions possessing phagostimulant and/or antifeedant properties towards the third-instar larvae of *T. ni*. The dried leaf extractables were dissolved in 200 ml of ethyl acetate and 60% methanol (1:1), and transferred into a separatory funnel. This mixture was then extracted with 200 ml of distilled water. To the above mixture, 50 ml ethanol was added to achieve clear and quick separation between the ethyl acetate and aqueous phases. The ethyl acetate fraction was repeatedly extracted with 200 ml water + 50 ml ethanol. This process was repeated six times. Water and ethyl acetate fractions were rotoevaporated to dryness at 50 ± 1°C. Ethyl acetate and water fractions were re-dissolved in benzene: ethyl acetate : methanol (4:3:3) and 40% ethanol, respectively, as 10% stock solutions and stored in a refrigerator at 4°C.

Elderberry pith discs

Elderberry, *Sambucus canadensis* pith discs (400 µ thick and cut to size with a no.7 cork borer) were prepared as described by Sharma and Norris (1991), and stored in ethanol. Before use in bioassays, pith discs were allowed to dry on a filter paper. Each pith disc was mounted singly 1-cm high on an insect pin above a Whatman no.1 filter-paper covering a 0.5-cm-thick bed of paraffin wax in a 9-cm-diameter petri dish. Dry pith discs were treated with sucrose and/or sterols and soybean leaf extractables as described under each experiment. Treated discs were dried for 1 h under a slow stream of air from a table fan. Discs treated with different chemicals/leaf extractables were exposed to the third-instar larvae of the cabbage looper in a double- or multi-choice assay as described later.

Insects

Larvae of the cabbage looper, *T. ni*, were reared under laboratory conditions (27 ± 1°C and 65 ± 5% R.H.) on a pinto bean-based artificial diet (Shorey and Hale, 1965). Newly moulted third-instar larvae were used in the bioassays. Larvae were starved for 4 h, but kept water-satiated in a 9-cm-diameter petri dish before being confined with the assay discs in a double- or multi-choice assay as described below.

Double-choice assay

Two discs were pinned centrally in an appossed arrangement on a filter paper covering a 0.5-cm-thick bed of paraffin wax in a 9-cm-diameter petri dish. A 9-cm-diameter filter paper was moistened with 2 ml water and attached to the inner surface of the petri dish. A single 4-h-starved, but water-satiated larva was released in each petri dish arena for 12 h or as indicated in a given experiment. There were 10 replications for each comparison. After 12 h, the larvae were removed, and the remaining area of insect-exposed discs was measured using an automatic leaf-area meter (LI 3100, LI-COR, Inc, Lincoln, NE). Treatment means were compared using a paired *t*-test.

Multi-choice assay

The discs treated with different chemicals/leaf extractables in an experiment were arranged in a circle in a 9-cm-diameter petri dish. A control disc, treated with sucrose and appropriate solvent, was placed within the circle of treated discs in the petri dish. The order of the treated discs was randomised. There were 10 replications for each test. Four-hour-starved, but water-satiated larvae were released in each petri dish. The number of larvae released in each dish equaled the number of treatments under comparison. Larvae were confined on the discs for 12 h. Larvae-exposed discs were measured for the remaining area using an automatic leaf-area meter, as described above. Significance of difference between treatment means was determined using analysis of variance, and the treatment means were compared using least significant difference (LSD).

Phagostimulant activity of sucrose: Alone and in combination with leaf extractables and sterols

Sucrose dosages at 0, 40, 400, 2000, and 4000 µg/disc were tested alone and in combination with water fraction (400 µg/disc) from PI 227687 leaf extractables in a multi-choice assay. In another experiment, two dosages of sucrose (400 and 4000 µg/disc) were tested in combination with four dosages (40, 400, 2000, and 4000 µg/disc) of the water fraction from PI 227687 in a multi-choice assay, to determine whether sucrose affects the antifeedant activity of the water fraction of leaf extractables from the insect-resistant soybean line PI 227687.

Ethyl acetate and water fractions from PI 227687 and Davis soybean cultivars were evaluated at 400 µg/disc, alone and in combination with sucrose (400 µg/disc), in a multi-choice assay. Discs treated with sucrose plus the appropriate solvent were used as a control. Sucrose (400 µg/disc) alone and in combination with sitosterol, stigmasterol, campesterol (5 µg/disc) or leaf extractables were also tested for phagostimulant activity in double- and multi-choice assays. There were 10 replications. Significance of difference between treatment means was determined by paired *t*-test in the double-choice assays and by least significant difference in the multi-choice assays.

RESULTS

There was a linear component in dosage/response relationship between sucrose dosage and the pith disc area consumed by the third-instar larvae of the cabbage looper, *T. ni* ($Y = 1.38 - 0.00015X$, Coefficient of determination (R^2) = 94% for unconsumed disc area, and $Y = 6.31 + 0.10X$, $R^2 = 94\%$ for % disc area consumed) (Fig. 1). Larvae fed most on the pith discs treated with 4000 µg sucrose, followed (in order) by those treated with 2000, 400, and 40 µg sucrose. The least feeding was observed in untreated control pith discs. When different dosages of sucrose were offered in combination with 400 µg of water fraction from PI 227687, there was an increase in disc feeding by the larvae. Increase in disc feeding was greater at higher dosages of sucrose ($Y = 1.28 - 0.001X$, $R^2 = 84\%$ for unconsumed disc area, and $Y = 5.43 + 0.008X$, $R^2 = 84\%$ for % disc area consumed). Larval feeding on pith discs was inversely proportional to the dosage of the water fraction of soybean leaf extractables from PI 227687 (Fig. 2). The antifeedant effects of water fraction were more apparent in combination with 4000 µg of sucrose ($Y = 1.06 + 0.0001X$, $R^2 = 74\%$ for unconsumed disc area, and $Y = 2.62 - 0.007X$, $R^2 = 68\%$ for % disc area consumed), than with 400 µg of sucrose ($Y = 1.25 + 0.00003X$, $R^2 = 12\%$ for unconsumed disc area, and $Y = 10.18 - 0.003X$, $R^2 = 12\%$ for % disc area consumed). Sucrose did not inhibit the antifeedant effects of water fraction of leaf extractables from PI 227687 at dosages >400 µg/disc. Under multi-choice conditions, larval feeding was significantly ($P <$

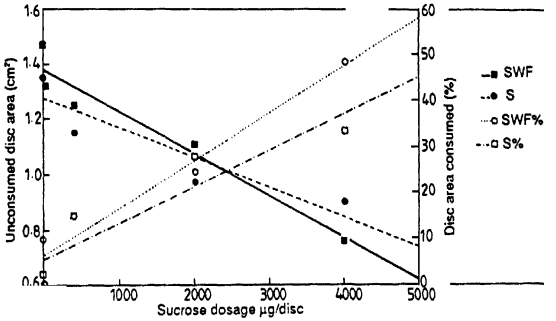

Fig. 1. Phagostimulant activity of sucrose to third-instar larvae of cabbage looper, *T. ni*. SWF = different dosages of sucrose + 400 µg of water fraction (WF) of PI 227687, and S = different dosages of sucrose alone. SWF% and S% refer to the % disc area consumed in relation to control disc for SWF and S, respectively

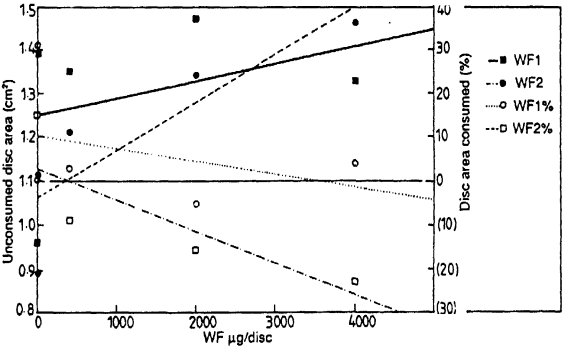

Fig. 2. Effect of two dosages of sucrose on antifeedant activity of water fraction of PI 227687. WF1 = different dosages of water fraction of leaf extractables + 400 µg sucrose/disc, and WF2 = different dosages of water fraction of leaf extractables + 4000 µg sucrose/disc. WF1% and WF2% refer to the % disc area consumed in relation to the control disc for WF1 and WF2, respectively

Table 1. Phagostimulant activity of soybean leaf extractables alone and in combination with sucrose to third-instar larvae of the cabbage looper, *T. ni* in a multi-choice assay

	Unconsumed disc area (cm^2)			
Cultivars	Water fraction+ sucrose	Ethyl acetate fraction + sucrose	Water fraction	Ethyl acetate fraction
PI 227687	0.94 a	1.11 b	1.05 b	1.16 b
Davis	1.15 a	0.95 a	1.17 b	1.24 c
Sucrose + solvent (control)	1.42 b	1.18 b	0.87 a	0.61 a
S. E.	± 0.14	± 0.06	± 0.09	± 0.06

Means followed by the same letter within a column are not statistically different at $P \leq 0.05$.

Table 2. Phagostimulant activity of sterols alone and in combination with sucrose to third-instar larvae of the cabbage looper, *T. ni* in a double-choice assay

	Unconsumed disc area (cm^2)					
Sterol	Sterol + sucrose	Sucrose	Sterol + sucrose	Sterol	Sterol	Sucrose
Campesterol	1.19 ± 0.08 a	1.19 ± 0.05 a	0.76 ± 0.17 a	1.41 ± 0.02 b	1.27 ± 0.03 a	0.87 ± 0.18 b
Stigmasterol	1.14 ± 0.06 a	1.29 ± 0.05 b	0.77 ± 0.13 a	1.39 ± 0.02 b	1.29 ± 0.03 a	0.67 ± 0.16 b
Sitosterol	1.13 ± 0.08 a	1.27 ± 0.05 b	0.88 ± 0.07 a	1.36 ± 0.03 b	1.26 ± 0.06 a	0.75 ± 0.12 b
Ethyl acetate fraction	0.89 ± 0.15 a	1.30 ± 0.02 b	0.70 ± 0.16 a	1.27 ± 0.06 b	1.05 ± 0,06 a	1.30 ± 0.06 a
Water fraction	1.08 ± 0.09 a	1.27 ± 0.04 b	0,78 ± 0.13 a	1.36 ± 0.63 b	1.22 ± 0.04 a	0.83 ± 0.13 b

Means followed by the same letter in a row (within a comparison) are not statistically different at $P \leq 0.05$.

Table 3. Phagostimulant activity of sterols alone and in combination with sucrose to third-instar larvae of the cabbage looper, *T. ni* in a multi-choice assay

Treatment	Unconsumed disc area (cm^2)
Campesterol + sucrose	0.94 ± 0.08 a
Campesterol	1.34 ± 0.02 b
Sitosterol + sucrose	0.99 ± 0.06 a
Sitosterol	1.28 + 0.04 b
Stigmasterol + sucrose	0.99 ± 0.08 a
Stigmasterol	1.28 ± 0.04 b
Sucrose (control)	1.04 ± 0.04 a
S. E. (pooled)	± 0.063

Means followed by the same letter are not statistically different at $P \leq 0.05$.

0.05) greater on pith discs treated with soybean leaf extractables + sucrose than those treated with sucrose alone (Table 1). When the pith discs were treated with leaf extractables alone, greater feeding was recorded on discs treated with sucrose. Thus, leaf extractables (at 400 µg/disc) in combination with sucrose showed a greater phagostimulant activity towards the larvae of *T. ni*.

Larvae fed more on pith discs treated with sterols + sucrose than on those treated with sucrose alone (Table 2). Also, the sterol-treated discs were *significantly* ($P < 0.05$) *less preferred than those* treated with sterols + sucrose. When the pith discs were treated with sterols or sucrose only, greater feeding was recorded on those treated with sucrose alone. Thus, sucrose is a better phagostimulant to *T. ni* larvae than the sterols. Leaf extractables at 400 µg/disc in combination with sucrose showed greater phagostimulant activity than sucrose or leaf extractables alone. There were no differences between ethyl acetate fraction and sucrose in phagostimulant activities. However, pith discs treated with water fraction alone were less preferred than on those treated with sucrose. Under multi-choice conditions, significantly greater feeding was recorded on the pith discs treated with sterols + sucrose, than on those treated with a sterol alone (Table 3). Sucrose in combination with sterols showed greater phagostimulant activity towards the third-instar larvae of the cabbage looper, *T. ni*.

DISCUSSION

Sucrose has largely been used experimentally as a phagostimulant in antifeedant assays involving non-host substrates (Norris and Baker, 1967; Shanks and Doss, 1987; Sharma and Norris 1991). However, the relative proportion of the dosages of antifeedants and sucrose seems to be quite important in bioassays. Lower dosages of soybean leaf extractables in combination with sucrose were preferred to sucrose alone. Sucrose (4000 µg/disc) did not mask the antifeedant effects of the water fraction from PI 227687 at dosages >400 µg/disc. Therefore, bioassay of candidate antifeedants on non-host substrates should include a range of concentrations of the test materials.

Larvae of the cabbage looper, *T. ni* preferred the pith discs treated with sterols + sucrose to those treated with sucrose or sterols alone. Sucrose and sterols have been reported to be phagostimulants to a number of insects (Beck, 1965; Shanks and Doss, 1987). Sucrose and individual sitosterol are phagostimulatory to obscure root weevil, *Sciopithes obscurus* Horn, and in combination are synergistic (Doss et al., 1982; Doss, 1983). These chemicals at physiological concentrations are also phagostimulants to the black vine weevil, *Otiorhynchus sulcatus* (F.). Combinations of compounds are frequently more effective phagostimulants than single compounds alone (Schoonhoven, 1972). Synergistic phagostimulant activity of sugars + amino acids has been shown for *Ostrinia nubilalis* Hubner (Beck and Hanec, 1958) and *Cannula pellicida* Scudder (Thorsteinson, 1960), potassium salts + lipids for *T. ni* (Gothilf and Beck, 1967), sucrose + sterols for *O. sulcatus* and *S. obscurus* (Doss, 1983; Shanks and Doss, 1987), and sinigrin + sucrose for *Plutella maculipennis* Curtis (Thorsteinson, 1953).

Sitosterol, stigmasterol and campesterol are the major sterols in soybean (Grunwald and Kogan, 1981). Quantitative differences have been reported in sterol content over growth stages, and between early- and late-maturing genotypes. Sitosterol decreases and stigmasterol increases with soybean plant growth. Also, both free and bound sterols increase with soybean plant growth (Grunwald and Kogan, 1981). These differences in sterol content may affect the acceptance of and feeding on soybean leaves by the insects. Insect moulting hormones (derived from plant sterols) often occur in greater quantities in plants than they do in insects. It has been suggested that mode of multiple resistance in some soybean introductions may result from the presence of hormone analogues (Tester, 1977). Specific extracts from insect-resistant and susceptible cultivars should be subjected to bioassay to determine the role of sterols in soybean in insect plant interrelationships.

CONCLUSION

Sucrose can be used as a standard phagostimulant in bioassays of antifeedants/phagostimulants on inert substrates. However, these interactions should be studied over a range of concentrations of the compounds/extracts being bioassayed. Sterols and soybean leaf extractables (<400 μg/disc) in combination with sucrose showed greater phagostimulant activity than sterols or leaf extractables alone. These interactions may play an important role in host plant selection by the cabbage looper, *T. ni*. The present studies indicate that sterols in combination with other chemical constituents may act as phagostimulants towards insects.

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REGISTRATION OF PARENTAL LINES

Registration of ICS 88019 and ICS 88020 Midge-Resistant Grain Sorghum A and B Parental Lines

ICSA 88019 and ICSB 88019 (Reg. no. PL-254, PI 592505) and ICSA 88020 and ICSB 88020 (Reg. no. PL-255, PI 592506) are sorghum midge [*Contarinia sorghicola* (Coquillet)] resistant sorghum [*Sorghum bicolor* (L.) Moench] seed parents based on the A_1 cytoplasmic–genetic male-sterility system, and were developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) at ICRISAT Asia Center, Patancheru, AP, India. They were selected for resistance to sorghum midge during 1988. ICSB 88019 (PM 7061B) was derived by pedigree selection; its pedigree is IS 152 × DJ 6514-8-1-1-1-1. ICSB 8020 (PM 7068B) was similarly derived, and its pedigree is FLR 101 × DJ 6514-13-1-1-2-1. DJ 6514 is a stable source of resistance to sorghum midge from India (2). IS 152 is a locally adapted germplasm accession from India, while FLR 101 is an elite line derived from the FLR population. The B-lines were crossed to 296A (A_1 cytoplasm) for conversion into male-sterile lines. Six backcrosses with continued selection for midge resistance and agronomic desirability were made during 1984–1988.

ICSB 88019 and ICSB 88020 have tan plant color, with non-juicy stems of medium thickness. Leaves are erect, narrow, long, with light-green midrib, and leaf sheaths cover the next internode. Flag leaves are short and slightly drooping. These lines flower in 59 to 60 days during the rainy season, and 72 to 74 days during the postrainy season at ICRISAT Asia Center. Panicles are compact and elliptical. Glumes are short, straw colored, and cover about 25% of the caryopsis. Seeds are white, lustrous, without subcoat, and have a beak and a thin pericarp. At ICRISAT Asia Center, plant height is 151 to 155 cm during the rainy season and 116 to 120 cm during the postrainy season. During 1993, ICSB 88019 yielded 1.214 t ha^{-1} and ICSB 88020 yielded 1.365 t ha^{-1}, compared with 0.664 t ha^{-1} in ICSB 42 and 1.135 t ha^{-1} in 296B under midge infestation (Table 1). Several hybrids using these male-sterile lines have yielded more than the commercial hybrid CSH 11 (296A × CS 3541) over two sowing dates (1). In combination with midge-resistant restorer lines, these lines have good potential for producing high yielding midge-resistant hybrids.

ICSB 88019 and ICSB 88020 have shown high levels of resistance to sorghum midge over locations and seasons. ICSB 88019 and ICSB 88020 suffered 8 to 18% midge damage, compared with 60 to 82% in ICSB 42 and 296B under no-choice panicle cage screening. Under natural infestation, ICSB 88019 and ICSB 88020 suffered midge damage rating (DR) of 1.7 to 3.3 (1 = <10% damage and 9 = >80% damage), compared with a DR of 7.0 to 9.0 in ICSB 42 and 296B (1). They are as susceptible to spotted borer (*Chilo partellus* Swinhoe) as ICSB 42 and 296B, and moderately susceptible (DR 4.0 to 5.3, compared with 4.7 to 6.7 in ICSB 42 and 296B) to head bug (*Calocoris angustatus* Lethiery).

These lines are less susceptible to rust (caused by *Puccinia purpurea* Cooke), leaf blight [caused by *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs], zonate leaf spot [caused by *Gloeocercospora sorghi* Bain & Edgerton ex Deighton], and anthracnose [caused by *Colletotrichum graminicola* (Cesati) G.W. Wilson] (DR 4.3 to 5.3, compared with a DR of 6.7 to 8.0 in ICSB 42 and 296B). These lines showed moderate susceptibility to grain molds (DR 3.3 to 5.7, compared with 6.0 to 8.3 in ICSB 42 and 296B).

Seed of these lines will be maintained and distributed by the Genetic Resources Division of ICRISAT Asia Center, Patancheru PO, Andhra Pradesh, 502 324, India, and seed of the two B-lines has been stored under quarantine conditions at the U.S. National Seed Storage Laboratory, 1111 S. Mason St., Fort Collins, CO 80521-4500.

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Table 1. Performance of midge-resistant lines ICSB 88019 and ICSB 88020 (ICRISAT Asia Center, 1990 to 94).

	Plant height		Time to 50% anthesis		Grain yield		Midge damage: Headcage		Midge damage: Natural infestation‡	
Cultivar	R† 1991	PR 1991–1992	R 1991	PR 1991–1992	R 1993	PR 1993–1994	R 1990	PR 1990–1991	R 1990	PR 1990–1991
	cm		d		t ha^{-1}		%			
ICSB 88019	151	115	63	82	1.21	1.99	18	8	3.3	1.7
ICSB 88020	155	120	63	79	1.37	2.17	14	12	3.3	2.0
Controls										
ICSB 42	135	104	70	74	0.66	0.95	71	82	9.0	7.3
296B	124	98	70	74	1.14	0.98	62	60	7.5	7.0
SE	11.1	3.5	—	—	0.207	0.343	8.8	5.8	0.9	0.7

† R, rainy season; PR, postrainy season.
‡ Midge damage rating under natural infestation: 1 = <10% damage, 9 = >80% damage.