Combined on-farm effect of plot size and sorghum genotype on sorghum panicle-feeding bug infestation in Mali

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Abstract The reaction of four sorghum cultivars to panicle-feeding bugs was assessed in small (15 m^2) and large (0.5-1.0 ha) plots for 2 years in three villages of the Kolokani region (Mali). The aim was to explain the somewhat contradictory earlier observations of pest infestation and damage in small experimental plots (on-station and on-farm) as well as in farmers' field surveys. Irrespective of the plot size, the local guinea sorghum cultivar Bibalawili was consistently the least infested and damaged, followed by bug-resistant compact-headed cv Malisor 84-7, whereas the improved caudatum cultivar Gadiabani, which had been disseminated for nearly a decade in

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P. Letourmy CIRAD, UPR Biostatistique, Montpellier, F-34398, France the region, and the improved hybrid ICSH 89002, were the most heavily damaged. When located along the border of large plots of a susceptible cultivar, small plots of the four cultivars overall were less infested and damaged than when located along the border of plots of resistant cultivars. However, they were more infested and damaged when located in the centre of large plots of susceptible cultivars than when they were in the centre of resistant cultivar plots. In large plots, bug populations and damage decreased from the border to the centre. These results suggest that, in addition to the mere plot size, plant breeders should take the genotypic environment of their experimental plots into account, namely the vicinity of large plots of pest-susceptible or -resistant cultivars, and the position of the test plots (border or centre) relative to these large plots.

Keywords *Eurystylus oldi* \cdot Host–plant resistance \cdot Dilution of infestation \cdot Concentration of infestation

Introduction

Sorghum [Sorghum bicolor (L.) Moench] is the most important food crop in savanna areas of the West and Central African region, including Mali, where sorghum production was 0.66 Mt in 2005 (FAO 2006). Mirid panicle-feeding bugs, particularly *Eurystylus oldi* Poppius, have recently become key pests of this cereal in most countries of this region (Ratnadass and Ajayi 1995). Bug feeding and oviposition on maturing sorghum grains result in severe quantitative and qualitative losses, including higher grain mold incidence. This is noted particularly on improved compact-headed types which, although better yielding, are more susceptible to panicle-feeding bug damage than local loose-panicled guinea landraces (Ratnadass et al. 1995a, 2003). Yield losses of more than 80% have been attributed to *E. oldi* damage in on-station trials (Ratnadass et al. 1995b) and results of onstation and on-farm surveys indicated that *E. oldi* occurs on all varieties of sorghum in much of West and Central Africa, being equally important on farmers' fields (Ajayi et al. 2001).

Improved caudatum sorghum cultivars have not been widely adopted in Mali (Yapi and Debrah 1998). However, it was found that some of these cultivars, including ICSV 1063 and ICSV 1079, were introduced in the Kolokani area (about 130 km north of Bamako) by Catholic missionaries in the late 1980s. They have since spread and are being cultivated under the name "Gadiabani" by many farmers in over 100 villages (S.K. Debrah and D. Sanogo, unpublished data). These compact-panicled varieties are prone to bug damage. Earlier onstation screening at the ICRISAT-CIRAD Samanko Research Station (12°32'N; 8°25'W) showed that both ICSV 1063 (ICRISAT seeds) and Gadiabani (Kolokani seeds) were susceptible to panicle-feeding bugs (Ratnadass et al. 1995a).

However, during a preliminary survey conducted in the Kolokani area in 1995, it was found that panicle-feeding bug infestation levels in farmers' fields cropped with Gadiabani were higher than in those cropped with local guinea cultivars, but these levels were still quite moderate (ca. five bugs per panicle vs three on local checks) (unpublished data). On the other hand, dramatically high bug infestation levels were observed on improved caudatum cultivars in small plots in an on-farm test conducted the same year in the village of Tioribougou (ca. 20 km south of Kolokani). Up to 500 bugs per panicle were recorded on a sorghum hybrid (compared to ca. 30 bugs per panicle on the local guinea check) (Somboro 1995). This type of decrease in pest infestation and damage with a parallel increase in field size was also previously noted by Vaissayre and Hau (1985) with respect to beetle pests on glandless cotton cultivars in Côte d'Ivoire.

We therefore conducted on-farm studies in the area in 1997 and 1998 to determine whether the results of on-farm varietal tests with small plots of paniclefeeding bug susceptible cultivars could be considered to accurately predict the reaction of these cultivars when cultivated in larger plots, along with the genotypic environment factors to take into account.

Materials and methods

Trial design

A trial was conducted in 1997 and 1998 in three Malian villages (Tioribougou, Wenia and Ntiobougou), located within a radius of 30 km from the town of Kolokani ($13^{\circ}55'$ N; $8^{\circ}2'$ W), to evaluate the effect of sorghum genotype on panicle-feeding bug infestation and damage by comparing two bug resistant (local & Malisor 84-7) and two susceptible (Gadiabani & ICSH 80002) cultivars.

The local resistant cultivar was Bibalawili, a guinea landrace which is photoperiod sensitive, with a lax drooping panicle and long glumes that cover the grain up to maturity. Malisor 84-7 is a genotype that was bred by the Malian Institut d'Economie Rurale through random mating of a Malian population (Shetty et al. 1991)-this cultivar's high-level and stability of panicle-feeding bug resistance has been confirmed over the years at many locations (Ratnadass et al. 1995a, 2003; Showemimo, 2003). Gadiabani served as the "local" improved caudatum cultivar whose panicle-feeding bug susceptibility had been established on-station (Ratnadass et al. 1995a). ICSH 89002 is a high-yielding hybrid whose super-susceptibility to bugs was established on-farm (Somboro 1995).

At each location, the four cultivars were compared both in small plots $[15 \text{ m}^2]$ and large plots [0.5 ha (atTioribougou and Ntiobougou) or 1.0 ha (at Wenia)], in a nested design, with scattered blocks and a replicated control (i.e. the local cultivar Bibalawili) (Fig. 1).

The small plots were adjacent, forming blocks that were nested within the large plots, and the relative positions of the large and small plots were randomized. Small plots consisted of four rows of 5 m each, with 0.75 m inter-row spacing and 0.30 m intra-row spacing. Plants were thinned to two plants per hill.



Fig. 1 Field layout designs in 1997 and 1998

In 1997, small plots were placed along the border of the large plots, while in 1998, they were placed in the centre of the large plots. The field design was thus altered based on 1997 significant results, to check whether it made a difference to have small plots "exposed" at the border of large plots, vs to have them "hidden" at the centre of the same.

For cultivar comparison in large plots, observations were obtained in 15 m² (3 m \times 5 m) subplots set up within the large plots. Care was taken that they had exactly the same stand density and management as the small plots. There were two reps (subplots) for the two levels of distance from plot border (DPB: namely "border" and "centre") in 1997, and four reps in 1998. All subplots (even of the same DPB level) were located at least 10 m from each other, and remote from the small plots. Border subplots were located alongside the large plot, which was not adjacent to any other cultivated plot (Fig. 1).

Both years, the plots were fertilized with 50 kg ha⁻¹ of urea (46%N) applied at the plant growing stage. In 1997, the cultivar in a given large plot and the four cultivars in the small bordering plots were sown on the same date, while in 1998, a given cultivar was planted on the same date, irrespective of the plot size. Based on the cycle lengths observed in 1997, the plots were sown in 1998 so as to obtain quasi-synchronized flowering and maximal infestation, while reducing the risk of substantial panicle-feeding bug population movements between plots (Ratnadass and Butler 2003). All experiments were conducted under natural mirid bug infestation.

Data collection

Both years, five panicles at the grain maturing stage (3 weeks after half-anthesis) were randomly chosen from the first central row of each plot, and successively shaken in a polyethylene plastic bag so as to dislodge all insects present (Sharma et al. 1994). The total number of *Eurystylus* bugs (adults and nymphs) was then determined. However, data from Tioribougou in 1998 were not considered since the sampling was not conducted in time. In addition, five panicles randomly chosen from the second central row of each plot were visually scored for bug damage, using a 1–9

rating scale, where 1 = all grains fully developed with less than 10% showing a few bug feeding punctures, and 9 = more than 75% grains remaining undeveloped and barely visible outside the glumes (Sharma et al. 1994; Ratnadass et al. 2002).

Statistical analysis

In small plots, the model (1) below was used for the analysis of variance:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_{ikl} + \varepsilon_{ijkl}$$
(1)

where: μ is the grand mean; α_i is the effect of cultivar i in a large plot nesting a set of small plots; β_j is cultivar j in a small plot; γ_k is the effect of locality k; δ_{ikl} is the effect of repetition l of cultivar i nesting a set of small plots in locality k, and corresponding interactions; Y_{ijkl} is the analysed variable and ε_{ijkl} is the residual error.

In large plots, the model (2) below was used for the analysis of variance:

$$Y_{ijklm} = \mu + \alpha_{i} + \beta_{j} + \gamma_{k} + \delta_{l} + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\alpha\delta)_{il} + (\beta\gamma)_{jk} + (\beta\delta)_{jl} + (\gamma\delta)_{kl} + (\alpha\beta\gamma)_{ijk} + (\alpha\beta\delta)_{ijl} + (\alpha\gamma\delta)_{ikl} + (\beta\gamma\delta)_{ikl} + (\alpha\beta\gamma\delta)_{ijkl} + \varepsilon_{ijklm}$$
(2)

where: μ is the grand mean; α_i is the effect of cultivar i in a large plot nesting a set of small plots; β_j is the effect of distance j from the plot border; γ_k is the effect of locality k, δ_l is the effect of year l, and corresponding interactions; Y_{ijklm} is the analysed variable and ε_{iiklm} is the residual error.

Individual and combined analyses of variance were performed using SAS software (SAS Institute Inc. 1999–2001). Results regarding small plots were analyzed separately for each year due to the change in design, while a combined analysis of 1997 and 1998 data was possible for large plots. Panicle-feeding bug numbers were analysed after square root transformation. Differences between cultivars and treatments were determined with the *F* test, and mean values were compared using LSD at the P < 0.05 threshold.

Results

In small plots

In our analysis of the 1997 small plot data, the cultivar effect in small plots was highly significant for panicle-feeding bug infestation and damage scores (Table 1). The cultivar effect in large plots was significant (although to a lesser extent) for these parameters. The effect of locality was not significant for bug infestation, while it was significant for damage scores. With bug infestation, there was a significant interaction between cultivar in large plots and locality, and with bug score, there were significant interactions between locality and both cultivars in small and large plots (Table 1). The significant interaction between locality and cultivar in small plots for the panicle-feeding bug damage score in 1997 highlighted that Gadiabani plants were slightly more damaged than the hybrid at Wenia.

In our analysis of the 1998 small plot data, the effect of cultivar in small plots was highly significant for panicle-feeding bug infestation and damage (Table 1). The cultivar effect in large plots was highly significant for bug infestation but not for the damage score. The locality effect was significant for bug infestation and damage. There were significant interactions between cultivars in small plots and cultivars in large plots for bug infestation (Table 1).

For both years at all three localities, the overall ranking of the three cultivars was the same, with the hybrid and the guinea landrace being, respectively, the most and least infested and damaged cultivars. However, while differences between susceptible cultivars (ICSH 89002 and Gadiabani) and resistant cultivars (Bibalawili and Malisor 84-7) were always significant, this was not always the case within the groups (Tables 2, 3, 4, 5).

The infestation level and damage scores in small plots were greatest when they were next to the large plots of the resistant cultivar Bibalawili, and least when adjacent to the susceptible cultivar Gadiabani in the 1997 trial (with ICSH 89002 and Malisor 84-7 being intermediate) (Tables 2, 4).

Conversely, in 1998, the small plots overall were significantly more infested and damaged when located in the centre of large plots of the susceptible cultivars Gadiabani and ICSH 89002 than when located in the centre of large plots of resistant Bibalawili and Malisor 84-7 (Tables 3, 5).

In large plots

A combined analysis of the 1997 and 1998 large plot data was conducted on means for the two DPB levels (Table 6). Since panicle-feeding bug numbers were not determined at Tioribougou in 1998 (see above), this locality was not taken into account in the combined analysis.

Table 1 Mean squares (MS) and their significance from a combined analysis of variance of panicle-feeding bug populations and bugdamage scores in small plots in 1997 and 1998

Source of variation	df ^a	Panicle-feeding	Panicle-feeding bug population ^b MS		Panicle-feeding bug damage score MS	
		1997	1998	1997	1998	
Residual	9 (6)	1.216	1.966	0.1339	0.3586	
F1 (cultivar in small plot)	3	200.66***	227.41***	8.601***	37.784 ***	
F2 (cultivar in large plot)	3	12.28**	82.21***	0.896*	1.074 (ns)	
F3 (locality)	2 (1)	0.83 (ns)	184.93***	0.627*	3.360**	
$F1 \times F2$	9	3.77 (ns)	18.68**	0.278 (ns)	0.786 (ns)	
$F1 \times F3$	6 (3)	2.45 (ns)	8.27 (ns)	0.783**	1.143 (ns)	
$F2 \times F3$	6 (3)	10.56**	3.82 (ns)	0.646*	0.273 (ns)	
$F1 \times F2 \times F3$	18 (9)	1.85 (ns)	4.34 (ns)	0.215 (ns)	0.278 (ns)	

Significance in the F-test: *significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level; ns not significant at the 5% level

^a df of n° of head bugs are given in parentheses

^b Square root of the number of panicle-feeding bugs per five panicles

Cultivar in large plots	Cultivar in small plots						
	Bibalawili	Gadiabani	ICSH 89002	Malisor 84-7	Mean		
Bibalawili	2.2	134.0	145.8	45.5	81.9 a		
Gadiabani	6.0	64.0	65.3	28.3	40.9 c		
ICSH 89002	11.0	99.7	104.7	36.7	63.0 ab		
Malisor 84-7	3.0	95.7	106.7	41.0	61.6 b		
Mean	4.9 C	105.5 A	113.7 A	39.4 B	65.9		

Table 2 Panicle-feeding bug populations (number per five panicles) observed in small plots: combined analysis of 1997 data fromTioribougou, Wenia and Ntiobougou

Data were analyzed after square-root transformation

Mean values within columns followed by the same lower case letters are not significantly different, Bonferroni test, P = 0.05Mean values within rows followed by the same letter are not significantly different, Bonferroni test, P = 0.05

Table 3 Panicle-feeding bug populations (number per five panicles) observed in small plots: combined analysis of 1998 data fromWenia and Ntiobougou

Cultivar in large plots	Cultivar in small plots					
	Bibalawili	Gadiabani	ICSH 89002	Malisor 84-7	Mean	
Bibalawili	2.0	25.0	63.0	9.0	24.8 c	
Gadiabani	29.5	190.5	336.0	16.0	143.0 a	
ICSH 89002	12.0	205.5	110.0	30.0	89.4 ab	
Malisor 84-7	4.5	145.5	174.5	2.5	81.8 b	
Mean	12.0 B	141.6 A	170.9 A	14.4 B	84.7	

Data were analyzed after square-root transformation

Mean values within columns followed by the same lower case letters are not significantly different, Bonferroni test, P = 0.05Mean values within rows followed by the same letter are not significantly different, Bonferroni test, P = 0.05

Table 4 Panicle-feeding bug damage scores observed in small plots: combined analysis of 1997 data from Tioribougou, Wenia andNtiobougou

Cultivar in large plots	Cultivar in small plots						
	Bibalawili	Gadiabani	ICSH 89002	Malisor 84-7	Mean		
Bibalawili	1.63	3.47	4.10	2.43	2.91 a		
Gadiabani	1.73	2.73	2.93	2.07	2.37 b		
ICSH 89002	1.80	3.07	3.13	2.13	2.53 ab		
Malisor 84-7	1.67	3.40	3.40	2.27	2.68 ab		
Mean	1.71C	3.17 A	3.39 A	2.23 B	2.68		

Damage assessed on a 1–9 scale where 1 = grains fully developed with < 10% showing bug feeding punctures, and 9 = >75% grains undeveloped and barely visible outside the glumes

Mean values within columns followed by the same lower case letters are not significantly different, Bonferroni test, P = 0.05Mean values within rows followed by the same letter are not significantly different, Bonferroni test, P = 0.05

Cultivar in large plots	Cultivar in small plots						
	Bibalawili	Gadiabani	ICSH 89002	Malisor 84-7	Mean		
Bibalawili	2.10	4.50	5.52	3.38	3.88 ab		
Gadiabani	1.70	4.67	5.93	3.73	4.01 a		
ICSH 89002	1.97	4.37	6.87	2.67	3.97 a		
Malisor 84-7	1.80	3.93	4.80	2.93	3.37 b		
Mean	1.89 D	4.37 B	5.78 A	3.18 C	3.82		

 Table 5
 Panicle-feeding bug damage scores observed in small plots: combined analysis of 1998 data from Tioribougou, Wenia and Ntiobougou

Damage assessed on a 1–9 scale where 1 = grains fully developed with < 10% showing bug feeding punctures, and 9 = >75% grains undeveloped and barely visible outside the glumes

Mean values within columns followed by the same lower case letters are not significantly different, Bonferroni test, P = 0.05Mean values within rows followed by the same letter are not significantly different, Bonferroni test, P = 0.05

 Table 6
 Mean squares (MS) and their significance, from a combined analysis of variance of panicle-feeding bug population and bug visual scores in large plots in 1997 and 1998

Source of variation	df	Panicle-feeding bug damage score ^a MS	Panicle-feeding bug population ^b MS
Residual	8	0.062	0.2186
F1 (cultivar)	1	15.59***	226.80***
F2 (distance from plot border)	1	3.3258***	45.25***
F3 (locality)	1	0.3544*	100.863***
F4 (year)	3	12.03***	21.6380***
$F1 \times F2$	3	0.2640*	5.3130***
$F1 \times F3$	1	0.5790**	24.719***
$F1 \times F4$	1	1.7303***	23.7562***
$F2 \times F3$	1	0.1222 (ns)	1.5355*
$F2 \times F4$	3	0.0322 (ns)	0.0041 (ns)
$F3 \times F4$	3	0.3108 (ns)	139.359***
$F1 \times F2 \times F3$	3	0.1060 (ns)	2.2796**
$F1 \times F2 \times F4$	1	0.1620 (ns)	0.4752 (ns)
$F1 \times F3 \times F4$	3	0.1376 (ns)	43.881***
$F2 \times F3 \times F4$	1	0.0072 (ns)	9.6953***
$F1 \times F2 \times F3 \times F4$	3	0.0160 (ns)	5.0863***

Significance in the F-test: *significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level; ns not significant at the 5% level

^a Damage assessed on a 1–9 scale where 1 = grains fully developed with < 10% showing bug feeding punctures, and 9 = >75% grains undeveloped and barely visible outside the glumes

^b Square root of the number of panicle-feeding bugs per five panicles

For both years at both remaining localities, the ranking of the four cultivars was the same in terms of bug infestation and damage, with significant differences between cultivars—ICSH 89002 and Bibalawili were the most and the least damaged, respectively (Tables 7, 8).

DPB level	Large plots						
	Bibalawili	Gadiabani	ICSH 89002	Malisor 84-7	Mean		
Border	1.86	3.93	5.03	3.17	3.50 a		
Centre	1.65	3.30	4.08	2.55	2.89 b		
Mean	1.76 D	3.61B	4.55 A	2.86 C	2.91		

Table 7 Effect of distance from plot border (DPB) and cultivar in large plots on panicle-feeding bug damage (combined analysis of1997 and 1998 data from Wenia and Ntiobougou)

Damage assessed on a 1–9 scale where 1 = grains fully developed with < 10% showing bug feeding punctures, and 9 = >75% grains undeveloped and barely visible outside the glumes

Mean values within columns followed by the same lower case letters are not significantly different, Bonferroni test, P = 0.05Mean values within rows followed by the same letter are not significantly different, Bonferroni test, P = 0.05

Table 8 Effect of distance from plot border (DPB) and cultivar in large plots on panicle-feeding bug infestation (number per fivepanicles): combined analysis of 1997 and 1998 data from Wenia and Ntiobougou

DPB level	Large plots						
	Bibalawili	Gadiabani	ICSH 89002	Malisor 84-7	Mean		
Border	7.5	78.4	278.8	59.3	106.0 a		
Centre	3.5	52.0	144.8	31.6	58.0 b		
Mean	5.5 D	65.2 B	211.8 A	45.4 C	82.0		

Data were analyzed after square-root transformation

Mean values within columns followed by the same lower case letters are not significantly different, Bonferroni test, P = 0.05Mean values within rows followed by the same letter are not significantly different, Bonferroni test, P = 0.05

Panicle-feeding bug infestation and damage were also significantly higher along the border of large plots than at their centre (Tables 7, 8).

Discussion

The results showed a significant genotypic effect on panicle-feeding bug infestation and damage in both large and small sorghum plots, a significant border effect in large plots, and a significant effect of the cultivar cropped in large plots on those cropped in small plots, which differed depending on the position of the latter (i.e. at the border or centre of the large plots). For both years and all three locations, the overall ranking of the four cultivars was consistently the same, with the hybrid and the local guinea cultivar being, respectively, the most and least infested and damaged.

For the four cultivars and both years, paniclefeeding bug infestation and damage were significantly higher along the border than at the centre of large plots, indicating that infestation originated from outside the sorghum field, and that the overall damage on a given variety should decrease as the field size increases.

Small plots were less damaged when located along the border of a large plot of a susceptible cultivar as compared to a resistant cultivar. Conversely, they were more damaged when in the centre of a large plot of a susceptible cultivar as compared to a resistant cultivar. Attractiveness and barrier effects (physical obstruction and visual camouflage, according to Finch and Collier, 2000) seemed to be the major phenomena involved, while there was little evidence of infestation from plots of early maturing to later maturing cultivars.

In 1997, flowering in the large plots might have influenced infestation of the bordering small plots. However, only Bibalawili, whose cycle was substantially longer than the other cultivars, could have been burdened by higher infestation. Malisor 84-7 had the shortest cycle and could have escaped peak infestation and contributed to higher infestation in neighbouring plots of Gadiabani and the hybrid. In 1998, however, adjustment of planting dates had the reverse effect, with Bibalawili flowering earlier than other cultivars, and Malisor 84-7 later.

The "small-plot effect" that we highlighted through these studies could partly explain the high infestation observed in the on-farm tests at Tioribougou in 1995 (Somboro 1995). Plant breeders should therefore not be deterred by high infestation levels observed in on-farm tests with improved cultivars in small plots, but rather conduct confirmation tests in larger plots.

They should, however, be aware that counteracting effects could prevail over time, with antixenosis resistance mechanisms (non-preference) becoming of little use when a single cultivar is cropped in large stands and the pest insect is thus placed in no-choice conditions.

The conclusions of this study may also apply to other crops and environments. Small plots are characterized by a high perimeter-to-area ratio, which could enhance immigration by invading species. In this respect, further to observations on glandless cotton cultivars in Côte d'Ivoire (Vaissayre and Hau 1985), our results are in line with those of Schmidt et al. (2004), who reported a reduction in herbivory (% destroyed buds) by the rape pollen beetle (*Meligethes aeneus*) in landscapes where the oilseed rape (*Brassica napus*) crop had expanded, indicating a dilution effect resulting from a change in food resource availability at the landscape level.

Wilsey and Polley (2002) reported such dilution and concentration effects of the spittle bug (*Clastoptera xanthocephala*) on *Solidago* plants in an experiment on the effect of grassland species evenness on dicot seedling invasion and spittle bug infestation. Namely, spittle masses were diluted in high evenness plots that had more *Solidago* stems, while in low evenness plots, they were concentrated on the fewer *Solidago* stems.

However, in our study on caudatum sorghum, the exceptionally high infestation level observed in 1995 could probably also be ascribed to another factor (found a posteriori), namely the presence of castor bean (*Ricinus communis*), an alternate host of *E. oldi* (Ratnadass et al. 1997), which was cropped in fields close to the test plots. Experiments were therefore designed to verify this assumption (Ratnadass et al. 2001).

Notwithstanding this particular context, our results indicate a variety of possible effects at play, either synergistic or counteracting, regarding crop infestation by pests in experimental test plots: dilution; concentration; contamination or protection. Therefore, in addition to mere plot size, plant breeders should take the genotypic environment of their onfarm tests into account, namely the vicinity of large plots of either pest-susceptible or -resistant cultivars, and the position of test plots (border or centre) relative to these large plots.

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