Expl Agric. (1985), volume 21, pp. 233-240
Printed in Great Britain

A DESIGN AND METHODS OF ANALYSIS TO MONITOR CROP GROWTH CONDITIONS ILLUSTRATED WITH SORGHUM SCREENING TRIALS FOR RESISTANCE TO *STRIGA*†

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(Accepted 6 December 1984)

SUMMARY

Checker-board field layouts are used to screen sorghum genotypes for resistance to *Striga*. A systematic check entry is used to monitor crop growth conditions and methods to analyse the results are assessed.

Major difficulties confronting biological scientists in the tropics are the sources of variation encountered in field experiments. Experimental procedures often used to account for variability require careful site selection, so that plant growth conditions in all plots within each block are as similar as possible with respect to nutrients, water, light, disease pressures, etc. Much work has been done over the years in temperate areas to devise experimental designs to take account of as much variation as possible. Row and column designs to eliminate trends in two directions, designs with a small number of treatments in a block and procedures using additional information from plots within each block have all been successfully used for this purpose.

In all these designs, it is assumed that conditions in a block are homogeneous for all plots and when possible concomitant information from plots is used to assist assessments of treatment effects. However, many problems have been encountered in trials in the tropics, and selection of homogeneous blocks is sometimes impossible (Kang and Moormann, 1977).

In this paper a technique is considered in which a known susceptible genotype is included in numerous systematic check plots to monitor the *Striga* intensity within a field in which the resistance of new varieties to *Striga* is being assessed. The technique, incorporating check plots, has been suggested in the past as a way to monitor plant growth conditions (Yates, 1936) but may also be useful when extreme variability in pest, disease or parasitic intensity is expected.

Striga, a root parasite of cereals, is a serious problem for farmers. Sorghum and millet grain losses due to *Striga hermonthica* have assumed economic proportions in many African countries. *S. asiatica*, which is more widespread than

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† ICRISAT Journal Article 346.

S. hermonthica, has also been identified as an important problem in southern Africa, parts of the United States of America and the less fertile soils of the semi-arid regions of India. The most economical way of preventing grain yield losses due to *Striga* is to breed resistant genotypes using reliable and reproducible field screening methods (Vasudeva Rao *et al.*, 1983). A resistant variety is one which has relatively few *Striga* plants in its plots even though the potential infestation at that site is high.

This paper presents trial layouts used for assessing sorghum resistance to *Striga* and methods of analysing data from experiments with a checker-board layout.

METHODS AND RESULTS

Assessment of field designs

In the past block designs were often used to screen sorghum genotypes, in a field naturally or artificially infested with *Striga*. But unreliable and non-uniform *Striga* infestation restricted classification of genotypes because it was impossible to measure the intensity of the weed pressure or the area of infestation within any block. Row and column designs were also used with very limited success. Because of the likelihood of an uneven distribution of the parasitic weed, it was impossible to distinguish between low infestation in a plot and genotype resistance. It is important, therefore, to measure the weed infestation level to which genotypes are subjected. For this reason check plots of a known *Striga*-susceptible entry were grown adjacent to all test entry plots in a checker-board layout (Fig. 1).

It is appreciated that a one to one, test to check plot ratio appears extravagant in land use, but it is essential to measure the parasitic intensity to which each test entry is subjected.

The association between the number of *Striga* plants on a test entry with that recorded on the check entry provides an answer to the question 'Did the resistance hold in spite of an increased number of *Striga* plants on the adjacent susceptible check?'

Relationships of *Striga* plant numbers on check plots with those on test

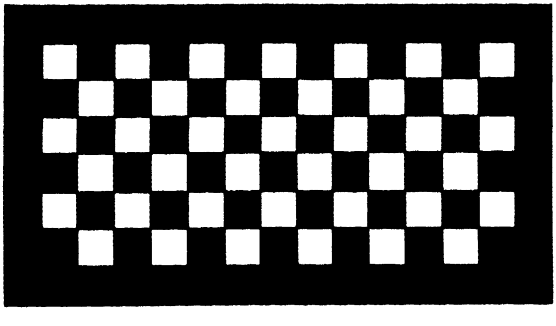

Fig. 1. Checker-board design illustrating field layout with alternate check (■) and test (□) entries.

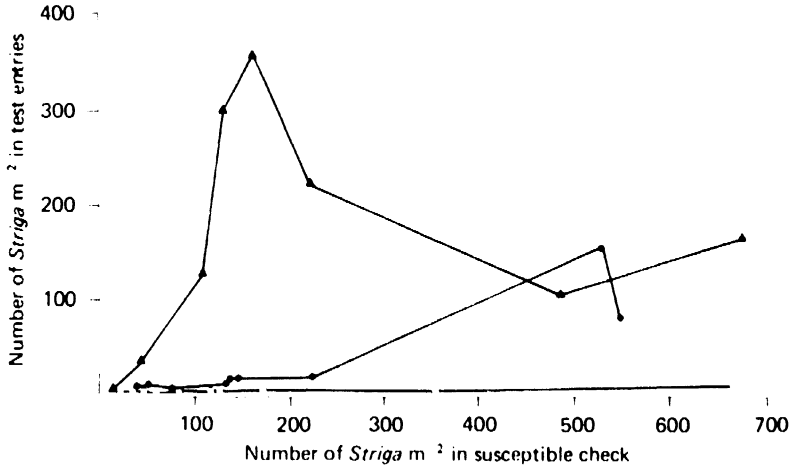

Fig. 2. Association between *Striga* numbers in test entries and *Striga* intensity in the soil measured by the number of *Striga* in a susceptible check, for o——o N-13 (a stable resistant line), ●——● SRN4841 (a moderately resistant line) and ▲——▲ T233B (a susceptible line).

entries with varying resistance to *Striga* from a series of trials are shown in Fig. 2. The stable resistant line N-13 held its resistance even at the greatest recorded *Striga* pressure. The moderately resistant line SRN4841 held its resistance under smaller pressures from *Striga*, but failed to hold its resistance under larger pressures. The susceptible line T233B showed increasing susceptibility as the *Striga* intensities increased. The drop in the number of *Striga* plants in this line at larger *Striga* intensities probably occurred because the plants could not sustain the number of *Striga* plants to which they were exposed. This indicates the need to have a further concomitant variable to measure the effects of *Striga* on the sorghum plant, either in terms of reduced growth or of damage symptoms such as wilting and leaf rolling (Ramaiah *et al.*, 1983). Such information was not available for the trials considered in this paper, but the entries had previously been screened through two preliminary stages of testing (Vasudeva Rao *et al.*, 1983) and were unlikely to have been included if they were very susceptible to *Striga*. Hybrid CSH-1 was used as the susceptible check.

The importance of using a susceptible check able to respond to a high intensity of *Striga* plants is indicated in Fig. 2. A situation might otherwise arise where the numbers of *Striga* plants measured on the susceptible checks were low because of damage to their roots before they could sustain the very high *Striga* load. Hence the relationship between *Striga* load and *Striga* plant numbers on test entries depends on their genetic resistance. In this study the susceptible check was used only to measure the intensity of *Striga* plants and selections of test entries were only made when a sufficient pressure was present.

Sorghum plant growth conditions and *Striga* intensity are confounded. Hence test entry results adjusted for either *Striga* plant numbers or neighbouring check results need careful interpretation because a bias may have been introduced. However, the main objective of the field layout was to be able to select, with some measure of confidence, test entries very resistant when subjected to a high level of *Striga* attack.

The results quoted here are *Striga* counts recorded from all test and check entry plots at three Indian locations in 1981. The trials at Akola (Maharashtra) and Bhavanisagar (Tamil Nadu) were conducted during the rainy season and that at Bijapur (Karnataka) on stored soil moisture during the post-rainy season. A common set of twenty test entries was assessed. Numbers 1 to 14 were breeding lines derived from crosses of *Striga*-resistant source lines with adapted lines. Number 15 was a known susceptible entry and 16 to 20 were source lines reported to be resistant to *Striga*. Each trial consisted of two replicates. The results were assessed by three methods: plot assessment, covariance analysis and nearest neighbour analysis.

(i) Plot assessment. The resistance of each test entry was assessed from the number of *Striga* plants emerging on the test entry plot relative to numbers on the adjacent checks. Given stable high resistance as the main criterion for selection, we require low *Striga* counts in test entry plots adjacent to check entries

Table 1. *Actual and log.* Striga *counts of test and check entries at Akola, Maharashtra, with regression coefficients from the nearest neighbour analysis*

	Counts			Log. of counts				
		Checks adjacent to					Ends and sides	
Entry no.	Test entry	Ends	Sides	No local control	Ends	Sides	Separate	Together
1*	35	946	581	3.56	3.60	3.75	3.76	3.72
2*	58	998	771	4.07	3.99	4.07	4.04	3.97
3	190	651	1196	5.25	5.29	5.12	5.15	5.20
4	111	996	910	4.68	4.63	4.56	4.56	4.54
5*	27	818	1057	3.29	3.29	3.13	3.15	3.18
6	100	883	950	4.53	4.51	4.42	4.43	4.43
7	392	777	731	5.96	5.96	6.00	6.00	5.97
8	810	1259	991	6.65	6.50	6.54	6.49	6.39
9	225	702	643	5.04	5.11	5.12	5.15	5.16
10	227	1002	1405	5.33	5.26	5.00	5.00	5.03
11	153	632	653	5.03	5.12	5.16	5.20	5.21
12*	64	974	864	3.75	3.70	3.69	3.69	3.65
13*	30	582	1018	3.19	3.31	3.14	3.20	3.28
14*	55	764	834	3.42	3.48	3.38	3.41	3.46
15	214	780	1029	5.05	5.08	4.98	5.00	5.03
16*	32	910	1024	3.49	3.46	3.38	3.38	3.37
17	96	923	1590	4.48	4.43	4.08	4.09	4.16
18*	33	802	881	3.21	3.21	3.12	3.13	3.14
19	204	1365	976	5.16	5.04	5.02	4.98	4.92
20*	43	687	739	3.52	3.60	3.54	3.58	3.62
Check mean				6.53	6.53	6.56	6.56	6.56
SE ±				0.66	0.64	0.58	0.58	0.58
CV(%)				11.3	11.0	10.0	10.0	10.0
Regression coefficients				–	0.31	0.55	0.13 0.51	0.70

* Highly resistant entries.

with very large numbers of *Striga*. The results from the three trials are given in Tables 1, 2 and 3.

When the *Striga* pressure was considered too low, test entries were not assessed for stable resistance. At Akola, the *Striga* intensity was judged to be sufficient and eight entries with *Striga* counts less than 10% of the neighbouring check values, most with much less, and one other entry close to this classification were selected for further assessment. Whenever a large count of *Striga* was observed in a plot, irrespective of what happened in other replications, the test entry was rejected. Also in Tables 1, 2 and 3 are test entries with *Striga* counts much larger and nearer the order of magnitude of the neighbouring checks. These entries would certainly be rejected by any but the most lax of selection criteria.

We know that this method is subjective and have considered other assessment methods such as covariance analyses, assuming some form of relationship between adjacent plots. Two such relationships are considered below.

(ii) Covariance analysis. Methods of analysis for check-test data assuming different error structures have been used (Van der Reyden, 1954). Covariance analysis has also been advocated (Sprent, 1955) and is considered here.

Table 2. *Actual and log.* Striga *counts of test and check entries at Bijapur, Karnataka with regression coefficients from the nearest neighbour analysis*

	Counts			Log. of counts				
		Checks adjacent to					Ends and sides	
Entry no.	Test entry	Ends	Sides	No local control	Ends	Sides	Separate	Together
1	2	98	81	0.69	0.81	0.51	0.69	0.65
2	0	79	62	0.00	0.24	0.25	0.26	0.26
3	2	74	88	0.69	0.72	0.88	0.79	0.80
4	1	67	49	0.35	0.76	0.50	0.70	0.65
5	1	112	113	0.35	0.05	0.09	0.03	0.03
6	0	108	150	0.00	−0.52	−0.26	−0.48	−0.44
7	8	125	107	2.12	2.00	1.73	1.87	1.83
8	65	128	133	4.19	3.82	3.82	3.77	3.77
9	8	131	86	1.45	1.43	1.34	1.39	1.38
10	2	75	72	0.90	1.01	1.06	1.04	1.04
11	14	152	114	2.62	2.34	2.01	2.17	2.13
12	8	118	162	2.09	1.52	1.71	1.53	1.56
13	3	195	142	0.97	0.50	0.16	0.30	0.26
14	0	131	100	0.00	−0.10	−0.27	−0.19	−0.21
15	0	36	46	0.00	0.61	1.31	0.97	1.03
16	0	94	98	0.00	−0.11	−0.10	−0.13	−0.13
17	8	115	104	1.98	1.87	1.68	1.78	1.75
18	4	56	56	1.50	1.82	1.91	1.89	1.89
19	1	102	106	0.35	0.33	0.22	0.28	0.26
20	3	91	112	1.24	1.11	1.24	1.14	1.16
Check mean				4.30	4.33	4.34	4.34	4.34
SE ±				0.87	0.58	0.62	0.56	0.55
CV (%)				26.8	17.9	19.2	17.2	17.0
Regression coefficients				–	0.86	0.92	0.38 0.61	0.98

Table 3. *Actual and log.* Striga *counts of test and check entries at Bhavanisagar, Tamil Nadu with regression coefficients from the nearest neighbour analysis*

	Counts			Log. of counts				
		Checks adjacent to					Ends and sides	
Entry no.	Test entry	Ends	Sides	No local control	Ends	Sides	Separate	Together
1*	7	135	146	1.35	1.21	1.09	0.83	0.72
2	18	54	159	2.89	2.80	3.05	2.97	2.87
3	20	91	108	2.85	2.72	2.69	2.49	2.40
4	42	91	131	3.63	3.72	3.51	3.68	3.75
5	20	88	209	2.69	2.53	2.56	2.29	2.17
6*	4	180	67	1.45	1.41	1.13	1.05	1.03
7	27	198	118	3.32	3.20	2.88	2.62	2.55
8	51	116	58	3.95	3.96	3.87	3.91	3.92
9	32	185	84	2.95	2.90	2.56	2.44	2.41
10	12	97	37	2.09	2.19	1.89	2.06	2.13
11	74	110	52	3.57	3.62	3.48	3.58	3.61
12	16	104	92	2.74	2.86	2.54	2.75	2.85
13	24	80	75	3.11	3.12	3.30	3.40	3.38
14	30	62	99	2.40	2.36	2.55	2.55	2.50
15	69	80	178	3.15	2.98	2.98	2.70	2.56
16	6	56	130	1.87	1.72	1.87	1.65	1.53
17	18	160	123	1.79	1.73	1.41	1.27	1.24
18	34	121	70	3.54	3.50	3.70	3.70	3.64
19*	4	162	206	1.10	0.85	0.94	0.52	0.33
20	50	132	55	3.18	3.19	3.01	3.04	3.05
Check mean				3.83	3.85	3.89	3.93	3.95
SE ±				1.17	1.17	1.14	1.11	1.07
CV (%)				34.0	34.0	33.1	32.2	31.2
Regression coefficients				–	0.18	0.39	0.48 0.31	0.91

* Retained for future assessment.

Variates obtained from plots can be represented by mathematical models. For 'v' varieties tested in a randomized block with 'b' blocks, a model can be assumed to be:

$$Y_{ij} = B_i + V_j + e_{ij} \tag{1}$$

with e_{ij} assumed $N(0, \sigma^2)$, $i = 1, \ldots, b; j = 1, \ldots, v$ and y_{ij} the variate value for variety j in block i. When the neighbouring check values are used in a covariance analysis, Equation 1 can be represented by:

$$Y_{ij} = B_i + V_j + \beta(X_{SL,\,j} + X_{SR,\,j})/2 + e_{ij} \tag{2}$$

where $X_{SL,\,j}$ and $X_{SR,\,j}$ are the check values at the left and right sides of variety j. For double covariance, taking into account end as well as side neighbours, Equation 2 becomes

$$Y_{ij} = B_i + V_j + \beta_1(X_{SL,\,j} + X_{SR,\,j})/2 + \beta_2(X_{ET,\,j} + X_{EB,\,j})/2 + e_{ij} \tag{3}$$

where $X_{ET,\,j}$ and $X_{EB,\,j}$ are the check values at the ends of variety j.

Using logarithmic conversions of the *Striga* count data the association between the test entry values and those of their neigbouring checks were calculated for the following four arrangements: side plots alone, end plots alone, side and end plots together, and side followed by end plots. The analysis of covariance gave variance ratios of approximately one for ends, sides, and ends plus sides used as the concomitant variable, at all three sites. Hence association between test and neighbouring check values was poor at all sites.

On reflection the poor associations between check and test values are not surprising. The lack of association between the logarithms of *Striga* counts on test entries with their neighbouring checks implies that some test entries are more susceptible to *Striga* than others. This we have already observed by the plot assessment method. Since highly resistant and more susceptible entries were deliberately included in the trials, the results were predictable.

(iii) Nearest neighbour. A nearest neighbour technique was first proposed by Papadakis (1937) and investigated by Bartlett (1978) and has since been used by a number of applied statisticians in an attempt to account for site variation (Pearce and Moore, 1976; Kempton and Howes, 1981). In this analysis it is assumed that the environmental effect on a plot is closely related to effects on its neighbours, unlike the covariance method described above, where the assumed association is between actual neighbouring values. The same four conditions as tested in the covariance analysis, namely ends alone, sides alone, ends + sides together and ends + sides separately, were tested by the nearest neighbour technique. When no adjustments were made, i.e. no local control, the analysis was based on a complete randomized design.

The mathematical model for the adjustment by side neighbours is:

$$Y_{ij} = \mu + V_j + \beta_1(e_{SL,\,j} + e_{SR,\,j})/2 \tag{4}$$

where Y_{ij} is the log count of the jth variety, $e_{SL,\,j}$ and $e_{SR,\,j}$ the environmental effects associated with the check values at the sides of variety j, and β_1 is a regression coefficient. For sides and ends taken together as neighbours the relationship is

$$Y_{ij} = \mu + V_j + \beta_1(e_{SL,\,j} + e_{SR,\,j})/2 + \beta_2(e_{ET,\,j} + e_{EB,\,j})/2 \tag{5}$$

where EB and ET are the 'end' values associated with variety j. The logarithm of counts was used and estimates of the parameter(s) obtained from the above models are shown in Tables 1, 2 and 3.

At Bijapur the *Striga* pressure was small and this restricted the assessment of resistance but allowed rejection of very susceptible entries. Thus entry 8 in Table 2 was rejected and other entries regarded with suspicion. The *Striga* pressure at Bhavanisagar in Table 3 was higher than at Bijapur but not so great as at Akola. Several entries were rejected at this site and three retained for future assessment. The *Striga* pressure at Akola was much greater than at the other two sites so that more confidence should be given to the results obtained there (Table 1). With both the plot assessment and nearest neighbour techniques the classification of entries was similar.

No significant differences were found between the analyses with and without the values from the surrounding border check plots. Similar classifications of the entries were also obtained from the logarithm and square-root transformed analyses.

Nine entries were classified as very resistant at Akola and three at Bhavanisagar, with only one entry common to both sites. It should be emphasized that the *Striga* intensity at Bhavanisagar was not great and that the numbers of *Striga* associated with 'highly resistant' entries, as classified at Akola, were much smaller than the check values. It may be that the very small numbers of *Striga* at Bhavanisagar correspond to test entries which are less susceptible but which cannot be judged because of the low intensity.

CONCLUSION

The use of frequent check plots throughout the trial area enables the intensity of *Striga* pressure at different locations in the field to be monitored. This then allows neighbouring test entries to be assessed with more confidence. The checker-board layout, and the two methods of analysis, plot assessment and nearest neighbour comparison, need further extensive testing. However, our initial reaction is that the checker-board layout is promising for the *Striga* screening work and may have a wider application in screening for other biotic and abiotic stress factors.

Acknowledgements. The authors thank the editor and a referee for their constructive comments on an earlier version of this paper.

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