Joint Progress Report
SORGHUM PHYSIOLOGY-3/SORGHUM ENTOMOLOGY-3

NATURE AND OCCURRENCE OF TRICHOMES IN SORGHUM LINES WITH RESISTANCE TO THE SORGHUM SHOOTFLY

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International Crops Research Institute for the Semi-Arid Tropics
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The authors are Physiologist, Sorghum Program; Physiologist, Pearl Millet Program; Entomologist and former Research Scholar Sorghum Program; and Associate Director, ICRISAT.

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INTRODUCTION

The sorghum shootfly (Atherigona soccata Rond.) is a major pest of sorghum (Sorghum bicolor Moench) in much of the Indian subcontinent and in many parts of Africa. Eggs are laid by the shootfly on the abaxial surface of seedling sorghum leaves. The larvae hatch, migrate down the leaf into the whorl, and feed on the young leaf tissue and cut the growing point of the shoot. This produces wilting of the growing shoot and the typical 'dead heart' symptoms. Under high levels of infestation, the sorghum shootfly can cause severe loss of plant population and hence yield losses.

The identification of sources of genetic resistance to this pest and their incorporation into elite materials is an important objective of the ICRISAT Sorghum Improvement Program. Currently this effort relies on field screening techniques, in which test materials are exposed to high populations of the shootfly by a combination of late planting of screening nurseries, shootfly attractants, and the use of early sown spreader rows of a susceptible cultivar.

Work at a number of centers including ICRISAT has established the existence of three general mechanisms of resistance to the sorghum shootfly (Starks, 1972; Blum, 1972).

1) Oviposition non-preference: This appears to be the major form of resistance in Indian landrace materials (Jotwani, 1976; Soto, 1972).

2) Antibiosis or seedling resistance: Certain cultivars show a relatively low incidence of dead hearts despite a fairly high level of egg laying. Little is known of the mechanisms of this type of resistance (Blum 1972) and its potential has not been studied.

3) Recovery resistance: This type occurs mainly in East African lines, in which heavy tillering after shootfly attack replaces the lost shoots (Doggett et al 1970).

An initial survey of the leaf epidermal morphology of a small set of cultivars possessing some field resistance to the sorghum shootfly indicated that many of the resistant lines were trichomed (R.K. Maiti, 1977). Experience in other crops suggested that the presence or the nature of the trichomes could be a mechanism of either oviposition non-preference or seedling resistance. Based on this possibility, a series of cooperative experiments were initiated in 1977 by the Sorghum Physiology, Entomology and Breeding subprograms.

This report is the first of a series from those studies. It deals with the nature, occurrence and variability of leaf trichomes in sorghum. Subsequent reports will cover studies on the role of trichomes and of a seedling morphological trait in shootfly resistance.

NATURE OF LEAF TRICHOMES IN SORGHUM

In order to learn something of the nature and the variation in trichomes in sorghum, microscope studies were made of the trichomes on a number of lines from the Sorghum Entomology field screening program. Observations included the morphology, length and angle of the trichomes (from the horizontal plane of leaf surface) and their density and distribution on the leaf surface. These findings have been summarized in this section

Materials and methods

Standard procedures with some modifications for the clearing of leaves for microscopic observation were adopted for the observation of leaf trichomes. Leaf segments about 1-2 cm^2 were heated in 20 cc of water in small glass vials (2.0 cm diameter by 7.5 cm high) for 15 minutes in an incubator set at 85^{o} C. The water was poured off and 20 cc of 95% ethyl alcohol was added and the leaves boiled for approximately 20 minutes in the 85^{o} C incubator. This alcohol was poured off, fresh alcohol added, and the boiling procedure repeated to completely remove the chlorophyll from the leaf. The alcohol was again poured off and 20 cc of concentrated (90%) lactic acid was added, the vials stoppered, and heated again at 85^{o} C until the leaf segments cleared (approximately 45 minutes). The vials were cooled and stored for observation. Leaf segments could be stored indefinitely in this manner.

For examination, the segments were mounted on slide in a drop of lactic acid and observed with a compound microscope (160 x magnification). Counts of trichome numbers were made on randomly selected fields of 0.8 mm^2 and results converted to a 1.0 mm^2 basis for reporting. Trichome lengths were estimated with the help of an occular micrometer, usually on five randomly selected trichomes per microscope field. Trichome angle (from the horizontal) was estimated visually to the nearest 5 degrees, by comparison to a set of standard angles.

Results and discussion

Trichome occurrence

Trichomes are of infrequent occurrence in sorghum; of approximately 550 entries selected from the germplasm to represent all taxonomic groups in the collection only 16 were found to have trichomes. Trichomes are found on both surfaces of the leaf, but tend to be more numerous on the adaxial surface (Table 1). They are also more numerous near the tip of the leaf than at the middle or the base (Table 2). The trichomes are generally concentrated along the main vascular bundles but are also found in the interveinal areas of the leaf. They vary in density from as many as 45 to as few as five per square mm (Table 3). When leaves which are less than fully expanded are sampled, trichomes number per unit area of microscope field will appear more numerous and under these conditions they should be related to epidermal cell numbers rather than to unit leaf area for valid comparison among cultivars.

Trichome morphology

Trichomes in sorghum are single celled projections or hairs, easily visible at 160x magnification, on the epidermis of the leaf (Fig. 1-3). They are frequently, but not necessarily pointed at the tip. They form an acute angle with the surface of the leaf, an angle which in our observations varies between 17 and 32^{0} among different cultivars. Trichomes are generally directed towards the base rather than the tip of the leaf. On the cultivars we observed they ranged from 20 to 55 microns in length. (For a general description of trichomes, see Esau, 1965).

There are a number of variations in the morphology of trichomes in sorghum. Some of these are illustrated in Figure 4. Note differences in size, shape, length and orientation (relative to the epidermal cells) in the drawings.

Leaf anatomy of trichomed and trichomeless genotypes

We have made a number of observations of the anatomy of the leaves of trichomed and trichomeless genotypes to determine if there were differences in mechanical barriers associated with the presence of trichomes which could be important in shootfly resistance. No such differences in cuticle thickness or in degree of lignification have been observed in either the leaf sheath, leaf lamina or the vascular bundles in any of the lines examined.

Table 1 : Trichome density per mm^2 on the center portion of the adaxial and abaxial surfaces of the fifth leaf. Means are of ten leaves per cultivar and two microscope fields per leaf.

Genotype	Adaxial surface	Abaxial surface
IS 1054	17 ± 1.3	6 ± 1.0
IS 2146	45 ± 3.8	27 ± 4.4
IS 2314	22 ± 1.6	4 ± 0.6
IS 5604	28 ± 1.6	9 ± 2.2
IS 5484	37 ± 5.1	22 ± 4.1

Table 2 : Trichome density per mm^2 at base, middle and tip of the abaxial surface of the fifth leaf. Means are of 10 leaves and 2 microscope fields per leaf.

Genotype	Base	Middle	Tip
1. IS 5604	2 ± 0.2	5 ± 0.6	15 ± 1.9
2. IS 1054	2 ± 0.2	3 ± 0.3	6 ± 1.0

Figure 1A [illegible] surface of the [illegible] stomata (x 2).

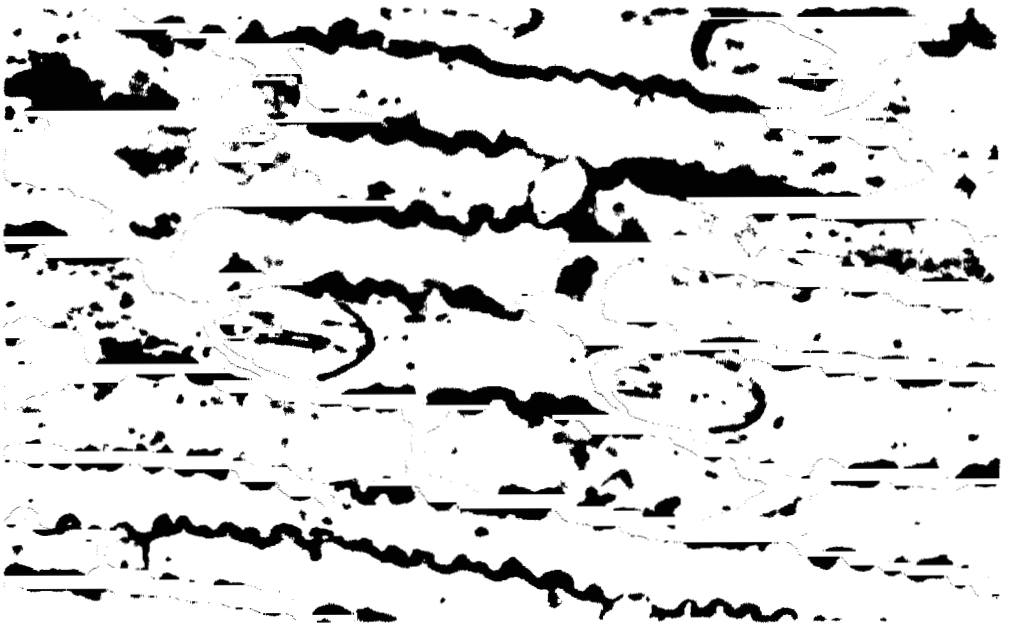
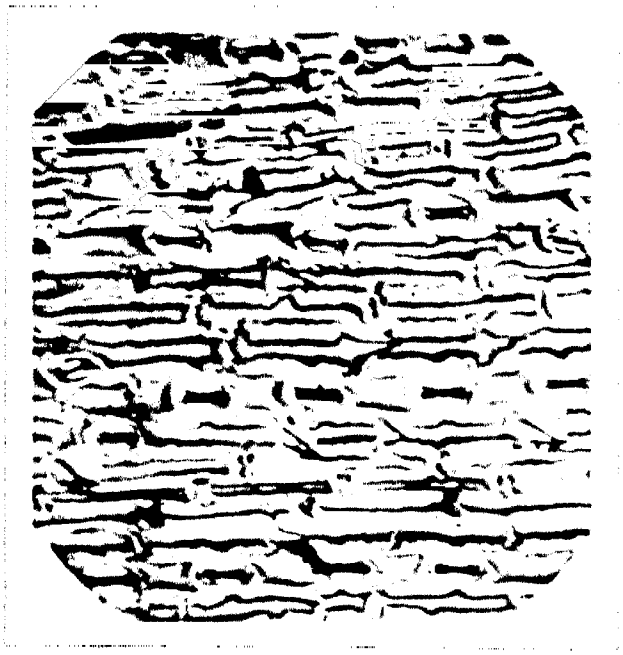


Figure 1B : Enlargement of a section of figure 1A showing clearly the location of the trichomes relative to the rows of stomata (x 20).

2B

Figure 2A : Scanning electron micrograph of the surface of cultivar Mr-1. Double rows of trichomes are clearly visible, alternating with rows of stomata (120 x).



2A

Figure 2B : Detail of figure 2A (720x).

NB: Figures 2A and 2B are used by courtesy of Dr. A. Shiv Raj, Plant Physiologist, Andhra Pradesh Agricultural University.

3A

3B

Figures 3A & : Leaf clearing of the abaxial surface of the fifth leaf of IR 1082 (A) and IS 18654 (B) two lines with field resistance to the shoot-fly (~50x).

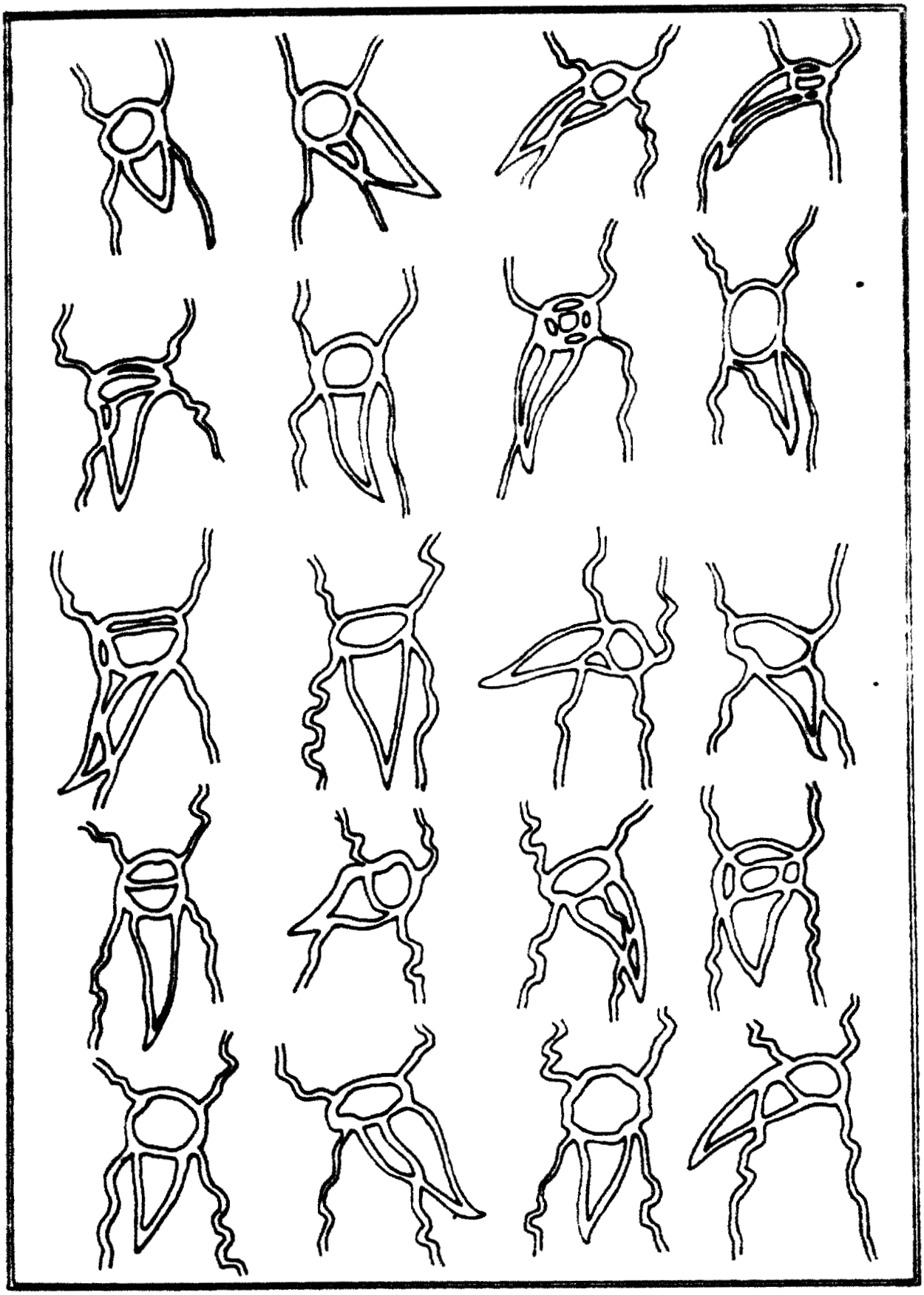


Figure 4 : Differences in trichome morphology in sorghum. Drawings taken from microscope slides of the abaxial leaf surface of the lines used in the study of the frequency of occurrence of trichomes in section V of this report.

Table 3 : Range of trichome density on the abaxial leaf surface of the fifth leaf in selected sorghum cultivars. Means are of 10 leaves per cultivar and 2 microscope fields per leaf.

Cultivar	Trichomes/mm^2
IS 1119	46 ± 4.7
IS 5613	45 ± 4.7
EN 3342-4	45 ± 4.1
IS 2146	45 ± 4.4
IS 2205	30 ± 4.1
IS 18588	28 ± 2.5
IS 5622	28 ± 4.4
IS 1054	8 ± 0.9
NCL-3	4 ± 1.3
IS 5067	4 ± 0.9

VARIATION IN TRICHOME NUMBER (EXPERIMENT 1)

Because the possibility existed that trichome density may be related to shootfly resistance, studies were undertaken to determine the magnitude of variation in trichome density within cultivars and the factors affecting density. (Subsequent work has indicated that the presence or absence of trichomes on the leaf is more important for shootfly resistance than the density of trichomes. These studies on trichome density are reported as a matter of general information on the nature and occurrence of trichomes).

Materials and methods

From a preliminary experiment conducted during 1977, it appeared that trichome number per mm^2 varied with the leaf chosen for observation, the stage of growth of the leaf and the part of the leaf sampled. The decision was taken to restrict the portion of the leaf sampled to the center portion of the abaxial leaf surface, as this is the region in which shootfly eggs are usually laid (Davies and Seshu Reddy, unpublished data). It was decided however to investigate in more detail the variation of trichome number among individual leaves and among different stages of growth of the plant (upto 4 weeks from emergence).

Ten cultivars of which seven were known to have trichomes were planted in single row plots on July 11, 1977. Three trichomeless lines were included in order to determine if the absence of trichomes was consistent

in all the samples. All expanded, but not visually senescing, leaves were sampled on 10 randomly selected plants per row at 7, 14, 21 and 28 days after seedling emergence. Trichome number, angle and length were determined in two microscope fields of the abaxial surface of a section of the leaf taken near the center.

Results and discussion

Trichome number per unit leaf area appears to be a highly variable characteristic; the analyses of variance for this experiment indicated significant effects of the leaf sampled, stage of growth at sampling and an interaction between the leaf sampled and cultivar. The data for all cultivars in Experiment 1 (mean trichome number for each leaf at each date of sampling) is presented in Appendix Table 1. It has been summarized in tables 4 to 7 illustrate the general effects of the two main variables, the leaf sampled and the time of sampling.

There was a general tendency for trichome number to increase in the later leaves, upto a maximum on leaves 5 and 6 (Table 4). There was variation among cultivars in this however; for example, maximum trichome number in the two lines derived from crosses involving 23/2 as a parent was attained in leaf 3 rather than the later leaves. Differences in trichomes/mm^2 among different leaves sampled at 21 days after emergence (at which time leaves 3 to 6 were sampled) were tested for three cultivars using leaf x plant mean square as an error term (Table 5). Leaf to leaf variation was significant for two of the three cultivars. Compared to variation within a leaf (microscope field mean square), leaf x plant effects were also significant.

In addition to the differences between individual leaves sampled on a single date, there was also variation in trichome number for a single leaf sampled on different dates (Table 6). This effect was tested for two selected leaves, the third and the fifth (which were sampled on days 7, 14, and 21 and days 14, 21 and 28 respectively). Date of sampling was highly significant in both leaves (Table 7), and the interaction between date and cultivar significant for third leaf. The apparently higher values for leaf five on the first date of sampling (14 days after emergence) may be due to the fact that this leaf was not fully expanded at this time, and counts of trichomes per unit microscope field gave abnormally high values. There is no obvious reason for the differences in trichome number among dates for leaf three. This effect (and the interaction) was due mainly to two cultivars IS 5604 and IS 18584 (IS 5604 x 23/2) (Appendix Table 1).

The three trichomeless cultivars in the study, CSH-1, Swarna and IS 130 were found to be trichomeless on all leaves and on all dates sampled indicating that trichomes are constently either present or absent in sorghum.

Because of the variability in trichome number within cultivars for both time of sampling and leaf sampled, characterizations of individual cultivar trichome density will be subject to some imprecision and results may not be independent of time and method of sampling. As our purpose was to attempt to relate the presence and the density of trichomes to resistance to shootfly, a sampling procedure was adopted based on the pattern

Table 4 : Mean trichome number per mm^2 (for all dates of sampling) for leaves 1 to 7. Experiment 1.

Cultivar	Leaf numbers 1	2	3	4	5	6	7
IS 1082	6.6	6.9	6.3	10.0	16.1	22.4	16.4
IS 18584 (5604 x 23/2)	8.3	10.0	21.5	18.1	16.8	12.5	8.1
IS 18652 (1052 x 23/2)	7.6	14.1	20.5	14.6	14.4	12.8	9.4
IS 18588 (5642 x R960)	16.6	11.6	13.2	18.2	23.7	22.6	11.2
IS 18654 (1082xWABC 4062)	7.4	8.0	9.7	16.7	28.7	26.0	19.8
5604	5.8	12.0	21.8	19.2	18.7	20.4	14.8
IS 1084	7.5	6.0	4.7	5.6	11.8	10.0	11.6
X	8.5	9.8	14.0	14.6	18.6	18.1	13.0

Table 5 : Analyses of variance for effects of plant and leaf sampled on trichome density of the abaxial leaf surface at 21 days. Experiment 1.

	df	Mean squares IS 1054	IS 1082	IS 5604
Plant	9	52	298	312
Leaf	3	293*	2989**	721
Plant x leaf	27	67	273	226
Microscope field (pxl)	40	11	39	50

* $p < .05$

** $p < .01$

Table 6 : Mean trichome number per mm^2 (for all cultivars) for each time of sampling, Experiment 1.

Day	Leaf number						
	1	2	3	4	5	6	7
7	7.0	8.6	11.8				
14	10.0	10.9	13.4	15.2	22.7		
21			16.7	13.9	19.4	24.1	
28				14.7	13.7	12.2	13.0

Table 7 : Analysis of variance for trichome number as a function of cultivar and date of sampling for the third and fifth leaves*, Experiment 1.

		Mean squares	
	df	Leaf 3	Leaf 5
Genotype	6	1617**	1036**
Date	2	438**	1405**
Genotype x date	12	109**	101
Plant(genotype x date)	189	37	71

Dates sampled are 7, 14 and 21 days from emergence for leaf 3 and 14, 21 and 28 days from emergence for leaf 5.

** $p < .01$

of egg laying of the shootfly. Counts of egg numbers on large number of plants have identified the fourth and fifth leaves as the major sites for oviposition (Davies and Seshu Reddy, unpublished data). Therefore, these two leaves were selected for sampling and approximately 21 days after emergence was recommended as the time for sampling, as the fifth leaf is generally expanded by this time. This procedure was field tested in Experiment 2, using proper field replication and larger set of cultivars.

FIELD SAMPLING METHODOLOGY FOR TRICHOME NUMBER DETERMINATION(EXPERIMENT 2)

Based on the results of Experiment 1, a sampling scheme (described above) was fixed with respect to the leaves to be chosen for sampling and the time of sampling. A replicated field experiment was carried out using this sampling scheme, and the contributions to variance for trichome number from the following sources were estimated: microscope fields per sample, leaf sampled on a plant (4th and 5th leaf from the base only), plants sampled per plot, and replications in a test.

Materials and methods

Thirty eight lines (of which 27 were trichomed) were planted in 1 row x 5 m long plots in 4 replications on August 1, 1978. At 21 days after seedling emergence, three fourth and three fifth leaves (from different plants) were sampled from each plot for the 27 trichomed lines. Three microscope fields per sample were counted. The data were analysed as a factorial experiment with genotype and leaf number as main factors and with plants nested within the genotype by leaf interaction and microscope fields nested within plants. Variance components were estimated using expected mean squares for each effect.

Results and discussion

Genotype means for trichome density in this test ranged from 9-45 trichomes/mm^2 with an overall experimental mean of 24. Genotypes, leaf

position, genotype x leaf position and plant within leaf position were all highly significant effects (Table 8). Leaf position effects were expected from the results of Experiment 1 and significant plant effects (as tested by within plant variation) should not have been unexpected (Table 5). Similarly, the significant interaction between leaf position and genotype was suggested in Experiment 1 (Table 4). These results confirm the earlier conclusion that trichome number varies among plants within a cultivar and among leaves within an individual plant and that the sampling of a single, standard leaf is not sufficient to compare genotypes.

The variance component analysis (Table 9) indicated major contributions to the variance were from plants and microscope fields within plants. Leaf position was omitted from the analysis as it was considered a fixed effect. The contribution from plot differences was negligible and that from genotype x leaf position interactions intermediate. A number of sampling strategies (different combinations of plants, leaves, etc.) were then evaluated as means of reducing the variance of mean trichome number (Table 10).

As the two largest variance components were plants and microscope fields per leaf sample, the first attempts were made to reduce these two components. Increasing the number of microscope fields with a constant number of plants does not reduce the variance of a genotype mean significantly (compare line Nos. 1-3, Table 10). Increasing the number of plants

however, reduces both the plant and microscope field components of the variance, as well as substantially reducing the variance of a genotype mean (see lines 4-7, Table 10).

The number of plants sampled is clearly the most important single factor in determining the variance of a genotype mean. Approximately 20 total plants are required to reduce the standard deviation of a genotype mean to an acceptable level - approximately ten percent of the mean (lines, 7, 10 and 11, Table 10). Plant numbers can be increased either by increasing the number of plants within a replication or by increasing the number of replications. For reasons of general experimental technique, at least two replications should be used. Using more than two replications, however, results in only a small decrease of variance if the total number of plants is kept constant (compare lines 10 and 11, Table 10).

Sampling two different leaf positions reduces the variance only slightly, (compare lines 5-7 to lines 8-10, Table 10), but is desirable because there is a substantial genotype x leaf position interaction (Tables 8 and 9). It is advantageous to sample different leaf positions from different plants, rather than the same plant. This procedure doubles the number of plants sampled without increasing the total number of samples, and thereby halves the contribution of the plant component to the total variance (compare lines 11 and 15, Table 10). Observing two microscope fields per leaf sample when plant numbers are sufficient, decreases variance only slightly (lines 14 vs. 16, 15 vs. 17, Table 10), but might be worthwhile since it does not involve much additional effort.

Table 8 : Analysis of variance for trichome number. Experiment 2. Three-fourth and three-fifth leaves were sampled (from different plants) from 27 trichomed cultivars at 21 days after emergence. Three microscope fields were counted for each sample.

Source	df	ss	MS	EMS (Leaf fixed, others random)
Rep	3	363	121^{NS}	
Genotype	26	150402	5785**	$\sigma^2F+3\sigma^2p+9\sigma^2E+72\sigma^2G$
Leaf position	1	67157	67154**	$\sigma^2F+3\sigma^2p+9\sigma^2E+36\sigma^2GL+972KL$
Gen. x leaf position	26	19058	7335**	$\sigma^2F+3\sigma^2p+9\sigma^2E+36\sigma^2GL$
Error	159	29688	187	$\sigma^2F+3\sigma^2p+9\sigma^2E$
Plants within leaf	432	69346	161**	$\sigma^2F+3\sigma^2p$
Fld/pl/Lf[a/]	1296	39345	30	σ^2F
	1943	375354		

** $p < .01$

a/ Microscope fields within plants within leaves.

Table 9 : Variance components for trichome number, Experiment 2.

$\sigma^2 F = \underline{30.36}$	Variation among different microscope fields of the same leaf.
$\sigma^2 P = \underline{43.39} = (160.52 - 30.36)/3$	Variation among plants in the same plot.
$\sigma^2 E = \underline{2.91} = (186.71 - 160.52)/9$	Plot to plot variability.
$\sigma^2 GL = \underline{15.17} = (733.00 - 186.71)/36$	Genotype x Leaf position interaction.
$\sigma^2 G = \underline{77.75} = (5784.69 - 186.71)/72$	Variation among pure line genotypes in this study.

Table 10: Sampling strategies for determining trichome density(using variances from Experiment 2)

# Line	# Reps	# plants/ rep	# LF	# Fields	Total obs.	Contribution of components			S^2G^a	$S\bar{x}^b$	Detectable difference^c (trichomes/mm^2)
						$\sigma^2 E/r$	$\sigma^2 p/rp$	$\sigma^2 p/rflp$			
1	1	1	1	1	1	2.91	43.39	30.36	76.66	8.76	34.70
2	1	1	1	2	2	2.91	43.39	15.18	61.48	7.84	31.07
3	1	1	1	3	3	2.91	43.39	10.12	56.42	7.51	29.76
4	1	2	1	1	2	2.91	21.70	15.18	39.79	6.31	25.00
5	1	5	1	1	5	2.91	8.68	6.07	17.66	4.20	16.65
6	1	10	1	1	10	2.91	4.34	3.04	10.29	3.20	12.71
7	1	20	1	1	20	2.91	2.17	1.52	6.60	2.57	10.18
8	1	5	2	1	10	2.91	8.68	3.04	14.62	3.82	15.15
9	1	10	2	1	20	2.91	4.34	1.52	8.77	2.96	11.73
10	1	20	2	1	40	2.91	2.17	0.76	5.84	2.42	9.58
11	2	10	2	1	40	1.46	2.17	0.76	4.39	2.10	8.30
12	3	10	2	1	60	0.97	1.45	0.51	2.93	1.71	6.78
13	4	10	2	1	80	0.73	1.08	0.38	2.19	1.48	5.86
14	2	10*	2	1	20*	1.46	2.17	1.52	5.15	2.27	8.99
15	2	20*	2	1	40*	1.46	1.08	0.76	3.30	1.82	7.20
16	2	10*	2	2	40*	1.46	2.17	0.76	4.39	2.10	8.30
17	2	20*	2	2	80*	1.46	1.08	0.38	2.92	1.71	6.77

a Variance of a genotype mean (= $\sigma^2 E/r + \sigma^2 p/rp + \sigma^2 rflp$)

b Standard deviation of genotype mean

c The procedure for calculating detectable difference is explained in the text

* Different plants used for sampling at different leaf positions

It is possible to calculate a 'detectable difference' in trichome density from the variance of a genotype mean. These calculations were done assuming a significance level of 5% ($\alpha_0 = 0.05$) and the desirability of obtaining a statistically significant difference 80% of the time ($\beta = 0.8$). The applicable formula is:

$$d \geq SG \sqrt{2}(t_0 + t_1)^2$$

where d = the minimum difference which one can be confident of detecting β x 100% of the time

SG = Standard deviation of a genotype mean

t_0 = t value for $\alpha_0 = 0.05$

t_1 = t value for $\alpha_1 = 2(1-\beta)$

For simplicity, a large genotype set was assumed, so the degrees of freedom associated with t_0 and t_1 are effectively equal to infinity For small numbers of genotypes, different t values would have to be used.

As an example, if an experimenter wishes to detect a difference of 10 trichomes/mm^2, he can do so with 2 reps, 10 plants/rep, 2 leaf positions, and 1 microscope field per leaf sample (line 11 or 14, Table 10) The necessary sampling scheme can be determined from Table 10 for any desired level of detectable difference.

Recommendations for sampling

From the results of the above experiments, we recommend the following

procedure for sampling to obtain genotype trichome density.

1. Two field replicates are sufficient
2. Single row plots are large enough to provide sufficient plants for sampling
3. Time of sampling should be approximately 21 days from emergence (by which time the fifth leaf is fully expanded)
4. Both fourth and fifth leaves should be sampled because of the interaction of cultivar and leaf number
5. Ten plants (if possible) should be sampled per plot, taking one leaf per plant rather than two
6. One microscope field per leaf is sufficient. Two could be done with little more work, but the advantage is small.

FREQUENCY OF OCCURRENCE OF TRICHOMES IN SHOOTFLY RESISTANT LINES (EXPERIMENT 3)

In order to evaluate the role of trichomes in resistance to the sorghum shootfly, 74 cultivars and lines supplied by the Entomology program were sampled for the presence and density of trichomes in the post monsoon season, 1977. The material included lines with a range of susceptibility to the shootfly, as well as lines whose reaction to the shootfly was not known.

Materials and methods

The lines were planted in single row, unreplicated, 5 meter long plots on 17th October, 1977 and irrigated up. No spreader rows were used, but the shootfly population was moderately high because of the late planting date.

Sixteen days after emergence the fifth leaf of seven randomly selected plants was sampled in each plot.* A section from the center of the leaf was cleared and mounted as previously described. Abaxial surface trichomes were counted on two microscope fields per sample and trichome length and angle (from the horizontal) estimated on ten randomly chosen trichomes in each field. Sampling was done 'blind' - without knowing the identities of the individual lines - in order to remove any bias in observation.

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* The experiment was carried out before the methodology experiment reported in the previous section, therefore the recommended sampling procedure was not yet established.

Field counts were taken of the percentage of plants with eggs at 21 days after emergence and of the percentage of plants showing dead hearts at 21 and 28 days after emergence. Because of the lack of field replication, no estimates of error variance for egg and dead heart counts were possible. For the trichome data, individual plant data were used to estimate sampling error and this was used to test for differences in trichome density, angle and length among the lines. This method is justifiable, since Experiment 2 data indicate that plot to plot variability in trichome number is small. Data on all cultivars is contained in Appendix Table 3.

Results and discussion

Forty-three of the 76 lines sampled were found to have trichomes, with mean densities ranging from 4 to 46 per mm^2 of abaxial leaf surface (Appendix Table 2). Cultivar differences in trichome density for the subset of 43 trichomed lines were highly significant (Table 11). Trichome lengths ranged from a minimum of 26 μ to a maximum of 48 μ and trichome angle from a minimum of 19^0 from the horizontal to a maximum of 29^0. There were significant cultivar differences for both parameters (Table 11).

There was thus considerable genetic variability for the density of trichomes on the leaf, and therefore an opportunity to evaluate the role of this factor in shootfly resistance. Variability for trichome length and particularly for trichome angle was less (as a proportion of the mean) than for density, although the ratio of the among-lines mean square to the

within-lines mean square is similar in all three cases (Table 11). The role of trichomes in resistance was estimated in two ways: using a subset of the 74 lines with known reaction to shootfly and using the field data for the entire set of 74.

For the first estimate, 38 lines which had been entered in multiple shootfly resistance screening tests were selected from the set of 74 and rated as either resistant, intermediate or susceptible. These are listed in Appendix Table 3, along with their trichome number as determined in this study. In the resistant and the intermediate lines 14 of 15 and 9 of 10 lines, respectively, were trichomed, compared to only 1 of 13 lines in the susceptible class (Table 12). There were no differences among the trichomed lines in the resistant and intermediate classes, however, in terms of either mean trichome density or in the range of trichome densities (Table 12).

Two types of comparisons were made utilizing the field data from the entire set of 74 lines. The first comparison was of trichomed vs. trichomeless lines (Table 13) and the second was among sub-classes of the trichomed lines (Table 14). Sub-classes were established by ranking the trichomed lines in order of increasing frequency of trichomes/mm^2, increasing trichome length, etc. and then dividing the ranked lines into five equal classes. Mean percent plants with eggs (21 days) and mean percent dead hearts (28 days) were calculated for each of the sub-classes and these were tested for differences by 't' test.

Table 11 : Means, ranges and variances for trichome number, angle and length, Experiment 3. Data are from the fifth leaf from seven plants, sampled 16 days after emergence.

	Trichome number/mm^2		Trichome* length(μ)	Trichome angle*
	All lines	Trichomed lines		
Mean	16	27	37	24
Range	0-46	4-46	26-48	19-29
Among lines				
mean square		1025	165	40
degrees of freedom		42	42	42
Within lines				
mean square		96	19	4
degree of freedom		258	258	258

* Trichomed lines only.

Table 12 : Presence of trichomes in lines with known shootfly response, Experiment 3.

	Number of lines		Trichome density/mm^2	
	Total	With trichomes	Mean*	Range
Resistant	15	14	31.6	0-45.5
Intermediate	10	9	30.5	0-46.0
Susceptible	13	1	-	0-21.7

* Lines with trichomes only

Table 13 : Comparison of trichomed and trichomeless lines for percentage dead hearts (%DH) and percentage plants with eggs (%PWE), Experiment 3.

	N	% DH 28 days		% PWE 21 days	
		Mean	Range	Mean	Range
Trichomed lines	43	30	6-60	18	0-60
Trichomeless lines	31	50	18-80	45	18-73
't' statistic		5.64	$p < .001$	7.30	$p < .001$

Table 14 : Comparison of sub-classes of trichomed lines for percentage dead hearts (% DH) and percentage plants with eggs (% PWE), Experiment 3.

Class*	Trichome trait		% DH 28 days		% PWE 21 days	
	Mean	Range	Mean	Range	Mean	Range
	Trichome number/m^2					
1	43.5	40-46	26	6-48	10	2-32
2	35.6	32-39	31	14-41	13	0-33
3	27.4	23-32	29	7-60	17	0-40
4	20.1	15-22	31	16-49	21	4-60
5	8.8	4-15	33	17-60	28	11-38
	Trichome length (μ)					
1	44.6	41-48	30	7-60	18	2-40
2	39.5	38-41	35	14-60	25	0-60
3	36.6	35-38	28	16-50	14	0-36
4	34.5	33-35	32	16-41	17	4-33
5	30.9	26-33	23	6-40	15	2-38
	Trichome angle (o)					
1	27.1	26-29	23	6-40	9	0-24
2	25.8	25-26	38	24-60	17	2-40
3	23.5	23-24	27	16-41	13	5-29
4	21.9	21-22	32	10-60	23	4-38
5	20.5	19-21	30	17-50	25	11-60

* Classes 1 and 5 n = 8
  Classes 2,3 & 4 n = 9

There were significant differences between the trichomed and trichomeless lines for both percent of plants with eggs (18 vs. 45%) and for percent dead hearts (30 vs. 50%), indicating that the presence of trichomes is associated with reduced shootfly damage, possibly via a reduction in egg laying (Table 13). The range of values in each group of lines was very broad, however (Table 13). Part at least of this variability is undoubtedly a reflection of the lack of field replication (a particular problem in insect work) but it also suggests that other factors, independent of trichomes, are influencing the degree of resistance/susceptibility of a genotype to shootfly.

There were no differences in percent dead hearts among the sub-classes of the trichomed lines, whether the sub-classes were established on the basis of density, length or angle of the trichomes (Table 14). There was a suggestion of differences among the sub-classes of trichome density and trichome angle for percentage egg laying at 21 days. Mean percentage egg laying, for example, ranged from 10 eggs/plant for the class with a mean density of 43.5 trichomes/mm^2 to 28 for the class with 8.8 trichomes/mm^2 (Table 14). Ranges within each class were equally broad however, and the reduced egg laying did not result in reduced percent dead hearts. It is, therefore difficult to attach any particular significance to the within class differences at this point. Thus differences in either trichome number, angle or length within the trichomed lines do not seem to have any relationship to the degree of shootfly resistance/susceptibility of a genotype.

However, both comparisons involving trichomed vs. trichomeless lines (their respective frequencies in known resistant and susceptible lines and the mean percent dead hearts in this test) do indicate that the presence of trichomes on the leaf is associated with a definite reduction in damage to a cultivar from the sorghum shootfly. More definitive evidence for the advantage, the means by which it appears to act, and its value under varying levels of shootfly pressure will be presented in a subsequent report.

SUMMARY AND CONCLUSIONS

Detailed studies were undertaken on the nature and occurrence of leaf trichomes in sorghum, following initial observations that many lines having some field resistance to the sorghum shootfly had trichomes on the leaves. These studies included the variability in trichome morphology and the frequency of trichomes on the leaf surface, methodology of field sampling for trichome density, and the occurrence of trichomes in lines with differential response to the shootfly. The following points summarize the findings of the study:

1. Trichomes appear to be of rather infrequent occurrence in sorghum and vary in number per unit area of the leaf surface, and in length, angle and morphology, in those genotypes in which they occur,
2. The presence or absence of trichomes on the leaf is a stable varietal characteristic. Trichome frequency on the leaf surface is variable however, influenced by the plant and leaf sampled, the time of sampling, etc.
3. A field sampling methodology for trichome frequency designed to minimize the variance of cultivar means is presented. This method covers time of sampling from emergence, number of plants to sample, leaves to sample and number of microscope field to observe per leaf sample.
4. The presence of trichomes on the leaf surface is related to a lesser frequency of both egg laying by the adult shootfly and plants destroyed by the shootfly larvae. Neither the density of trichomes, trichome angle, nor trichome length were related to differences in shootfly damage, however.

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Appendix Table 1 Mean trichome number per mm^2 by date of sampling and by leaf number for all lines in Experiment 1

Day	Leaf number						
	1	2	3	4	5	6	7
	IS 1082						
7	5.3	6.5	4.7				
14	7.9	7.3	5.5	5.5	18.6		
21			8.6	10.7	18.0	35.5	
28				13.8	11.7	9.2	16.4
	IS 18584 (IS 5604 x 23/2)						
7	3.2	8.3	16.9				
14	13.4	11.6	21.6	17.8	20.2		
21			25.9	21.2	20.7	15.7	
28				15.4	9.6	10.2	8.1
	IS 18652 (IS 1054 x 23/2)						
7	8.8	12.2	21.6				
14	6.4	15.9	16.2	17.7	17.5		
21			23.7	11.6	14.6	13.7	
28				14.6	11.1	11.8	9.4
	IS 18588 (IS 5642 x R-960)						
7	14.1	11.3	12.0				
14	19.0	11.9	15.2	19.8	30.1		
21			12.4	17 1	23.8	30.8	
28				17.7	17.2	14.4	11.2
	IS 18654 (IS 1082 x WABC 4062)						
7	6.8	6.5	7.3				
14	7.9	9.5	11.1	17.4	34.6		
21			10.7	15 4	31.5	36.3	
28				17.4	19.9	15.6	19.8
	IS 5604						
7	4.5	9.0	14.8				
14	7.0	15.0	20.6	22.6	18.9		
21			30.1	17.0	18.3	24.0	
28				17.9	18.9	16.9	14.8
	IS 1054						
7	6.2	6.4	5.3				
14	8.8	5.5	3.4	6.1	19.4		
21			5.3	4.5	8.5	12.9	
24				6.2	7.6	7.0	11.6

CSH-1, Swarna and IS 130 were also sampled in an identical fashion as the above lines. They were found to be without trichomes on all dates of sampling and on all leaves.

Appendix Table 2 : Trichome number, angle and length and shootfly incidence, Experiment 3.

Genotype	Trichome No/sq.mm (±Se)	Trichome angle (o)	Trichome length in (u)	% plants with eggs (21 days)	% plants with dead hearts (28 days)
IS 1119	46.0 ± 5.6	25.4	43.1	9	38
IS 5613	45.5 ± 5.7	24.4	35.4	5	24
EN 3342-4	45.0 ± 4.8	24.7	36.6	2	38
IS 2146	44.9 ± 5.5	26.7	30.0	10	6
IS 5642	42.8 ± 1.7	25.1	41.1	32	48
IS 4664	42.7 ± 4.7	23.6	33.1	7	20
IS 3962	40.4 ± 4.5	27.1	36.9	8	18
IS 5604	40.3 ± 3.9	23.0	36.0	6	16
IS 5480	38.6 ± 2.8	26.9	40.9	0	14
EN 3337-4	38.5 ± 4.0	24.7	38.9	4	30
IS 2162	37.7 ± 2.4	25.4	46.3	2	24
EN 3337-1	35.4 ± 3.1	26.6	32.6	7	40
IS 18629 (IS1082 x WABC 3101)	35.3 ± 2.6	22.1	33.7	33	41
IS 4646	34.8 ± 5.4	23.6	38.3	29	41
IS 5648	34.1 ± 3.4	22.1	37.4	11	28
IS 5615	32.5 ± 5.5	20.9	33.4	20	35
IS 18654 (IS1082 x WABC 4062)	32.1 ± 3.1	22.6	45.7	14	22
IS 2312	31.8 ± 2.0	28.1	46.5	17	7
IS 2176	31.0 ± 6.1	29.4	48.3	24	37
IS 2205	29.5 ± 4.8	23.0	35.4	16	24
IS 5642 x R960	28.4 ± 2.9	25.1	41.3	40	60
IS 5622	27.7 ± 5.2	20.4	34.0	16	27
IS 5566	26.9 ± 3.3	25.4	39.4	24	26
IS 18584 (IS 5604 x 23/2)	25.0 ± 5.6	26.1	36.3	0	35
IS 18628 (IS1054 x WABC 4142)	23.4 ± 3.6	24.1	42.3	16	39
IS 2122	23.3 ± 2.5	22.1	32.3	2	10
EN 3332-2	21.7 ± 3.5	21.7	35.4	25	29
IS 18585 (IS 5604 x 453)	21.4 ± 2.2	25.9	33.4	5	25
IS 1082	21.1 ± 3.9	21.6	34.6	4	38
IS 2195	21.1 ± 1.7	23.0	35.1	5	16
IS 5469	20.8 ± 2.6	25.6	38.9	13	49
EN 3332-3	20.3 ± 3.2	21.1	40.0	60	34
IS 4553	20.1 ± 3.8	25.3	43.1	23	28
IS 5470	19.7 ± 3.3	20.9	38.0	16	23

Appendix Table 2 continued ...

1	2	3	4	5	6
IS 18652 (IS 1054 x 23/2)	15.0 ± 2.8	21.6	32.6	36	40
EN 3257-3	14.7 ± 2.9	20.0	35.7	36	50
IS 1034	11.1 ± 1.0	22.3	37.3	28	22
Local (White)	11.0 ± 2.7	22.1	26.0	38	22
EN 3332-1	8.5 ± 2.7	21.4	38.2	33	60
EN 3255	8.5 ± 2.2	21.1	34.3	28	35
IS 1054	8.5 ± 1.2	20.3	27.0	11	17
NCL 3	4.5 ± 1.7	19.4	39.7	30	20
5067	4.1 ± 1.2	23.8	34.4	21	41
IS 5383	0	0	0	16	52
IS 4799	0	0	0	48	77
IS 4829	0	0	0	48	73
IS 5113	0	0	0	18	76
IS 8311	0	0	0	50	70
IS 5642 x 23/2	0	0	0	28	60
IS 18641 (IS 5622 x WABC 1121)	0	0	0	31	52
IS 18653 (IS 1082 x WABC 1092)	0	0	0	37	43
IS 18630 (IS 1082 x R 960)	0	0	0	28	56
IS 5359	0	0	0	19	17
IS 9317-1	0	0	0	17	54
IS 18651 (IS 8315 x WABC 4022)	0	0	0	36	47
3-P-5-1-1	0	0	0	64	12
EN 3362-1	0	0	0	63	69
Serena	0	0	0	59	47
211-P-1-2-1	0	0	0	73	44
CSH1	0	0	0	62	51
EN 3363	0	0	0	28	46
Swarna	0	0	0	69	80
37-P-3-2-1	0	0	0	58	40
V-99-1-1-1	0	0	0	51	36
IS 8776	0	0	0	36	34
IS5604 x WABC 3111	0	0	0	20	46
IS 18659 (IS 5383 x R 960)	0	0	0	67	69
V-70-1-1-1	0	0	0	54	44
EN 3518	0	0	0	66	28
IS 8315	0	0	0	20	50
V-63-1-1-2	0	0	0	52	52
V-2-1-1-1	0	0	0	64	36
V-20-1-1-2	0	0	0	52	32
EN 3308	0	0	0	49	57