UPDATE ON METHODOLOGY USED IN SCREENING FOR RESISTANCE TO *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE) IN POTTED SUGARCANE

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Introduction

Routine screening of fourth (primary variety trial) and fifth (secondary variety trial) stage sugarcane clones for susceptibility to the stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae), has been carried out as a part of the SASEX sugarcane selection programme since 1985 (Anon, 1988; Leslie and Nuss, 1992; Nuss, 1991). This report is an update on modifications and new additions to the screening methodology originally developed by Nuss and Atkinson (1983) and Nuss (1991), and includes results of screening trials conducted between 1999 and 2001. Changes in methodology and statistical analysis have aimed at both enhancing the discrimination of resistance among test clones and discerning progress in selection for resistance. Recently, the number of clones screened per year increased from about 208 to 360.

Keywords: varietal resistance; sugarcane; stalk borer; screening trials, selection programme

Methodology and Analysis

Unreleased clones are screened for susceptibility to *E. saccharina* in five potted-cane trials annually, with seed material collected from four selection sites in spring and from three sites in autumn (total of six selection sites) (Table 1). All trials from 2000 onwards have included six control varieties, ranging from the most susceptible to the most resistant. The mean susceptibility rating across the controls is therefore a good approximation of the mid-point of susceptibility of commercial varieties to *E. saccharina* and can be used as a relatively static reference point for calculating the susceptibility ratings of test clones (selections). Trials are conducted in large 'shade houses' (walls of 40% green shade cloth and transparent fibre-glass roofing) in order to: (1) enable controlled water stressing of plants, which is not possible if plants are grown in an outdoor situation; (2) protect the plants from feral infestations of *E. saccharina*, which would otherwise result in non-uniform damage by an unknown number of borer generations.

Table 1. Screening trials for susceptibility/resistance of sugarcane clones (selections) to *Eldana saccharina* conducted annually in pot trials at SASEX. Clones are selected at six sites, one of which (Gingindlovu) is split into two programmes.

Trial code	Season	Selection stage	Selection site*	No. clones screened
ER 1	Autumn	Secondary	T, U	30
ER 2	Spring	Secondary	B, G, K, S	60
ER 3	Autumn	Secondary	F	30
ER 4	Autumn	Primary	T, U	90
ER 5	Spring	Primary	B, G, K, S	150
Total	-	-	-	360

* B: Bruyns Hill; F: Pongola; G: Gingindlovu (long cycle); K: Kearsney; S: Glenside; T: Empangeni; U: Gingindlovu (short cycle)

Six seedlings of each variety are planted into 25 litre plastic pots (one pot = one replicate) filled with sieved river sand, and laid out in a randomised lattice design with rows of 24 pots each. Primary trials are replicated four times and secondary trials six or eight times. Single 'guard pots' of variety NCo376 are placed at the ends of rows to reduce end effects. Pots are fertilised monthly with 14 g 4:1:1 (45) N:P:K and every other month with Hygrotech® Seedling Mix (200 g/25 l water at 500 ml/pot) to provide micronutrients. Plants are drip irrigated at a rate of between 0.33 and 1.0 litre/pot/day, depending on stage of plant growth. TVD leaf samples are taken at four months to determine if plant nutrient levels are within acceptable limits. From three months, plants are sprayed monthly with insecticide (chlorpyriphos at 20 ml/10 l water) to exclude pests and potential *E. saccharina* egg predators. Spraying is halted one month before inoculation with *E. saccharina* eggs to ensure no residue remains on plants at the time of inoculation.

Results of pre-1995 trials have shown that the number of borer-damaged internodes per pot is moderately positively correlated (r=0.56 – 0.73 over five trials) with the number of stalks per pot. Consequently, strongly tillering varieties are likely to accrue more damage by the end of a trial than weakly tillering varieties, thereby confounding the assessment of resistance with an unrelated agronomic trait. To standardise data across trials, the number of tillers per pot is equalised to five as far as possible, by removing one primary tiller plus all secondary tillers immediately before moisture stressing.

After seven to eight months of growth the water supply is reduced in a staged fashion to induce water stress, such that at the end of one month plants have approximately (and no less than) five green leaves each. Stress increases the susceptibility of sugarcane to *E. saccharina* as well as the quantity of nitrogen in the stalk, both of which improve borer survival and mass (Atkinson and Nuss, 1989). A typical stressing programme is as follows: week one: 1 litre/day; week two: 0.67 litre/day; week three and four: 0.33 litre/day. The final stress level is maintained until trial harvest, when irrigation is returned to the normal (pre-stress) level to slow the progress of the infestation.

Trials are artificially inoculated with *E. saccharina* eggs provided by the SASEX Insect Rearing Unit. Fertilised eggs laid by the moths on sheets of paper towel are cut out in small sections bearing batches of *ca.* 100 eggs each. The paper sections are placed behind a lower leaf sheath on the cane plant, which is a position similar to the preferred egg-laying site of *E. saccharina* females (Atkinson, 1979). The level of infestation achieved is critically dependent on the numbers of eggs used for inoculation, as well as the degree of water stress. Inoculations have varied between 100 and 300 eggs per pot, but the optimum appears to be 200 per pot.

Infestations are allowed to develop over a period sufficient to accumulate 500 degree-days, by which time the majority of individuals have developed to the late larval (fifth to sixth) instar or pupal stage (Way, 1995). Degree-days are measured with pre-programmed Tempest® units supplied by Insect Investigations, Ltd. (Cardiff, UK), set at a developmental threshold temperature of 10°C.

Trials are harvested by removing and dissecting all stalks from all pots. Data recorded per pot include: length of stalk; total length of borer tunnels; number of internodes; number of internodes bored; number of larvae, pupae and pupal cases; total fresh (live) mass of larvae, pupae, and pupal cases. Four variables are used in estimating overall plant susceptibility to *E. saccharina*: (a) number of internodes bored; (b) length of stalk bored; (c) number of larvae + pupae (+ empty pupal cases if present); (d) mass of larvae + pupae (+ empty pupal cases). Values of (a) to (d) for each variety were originally converted to percentages of the *overall trial mean* (=100%). However, the *controls mean* (also=100%) is now used for this purpose. A weighted mean of the four percentages so produced is computed (see Keeping and Meyer, in press) and converted into units on a one (resistant) to nine (susceptible) rating scale. This is achieved by making a 68% confidence interval

equivalent to a unit rating (linearly) and the 100% rating (i.e. the controls mean) equivalent to 5. The data are subjected to analysis of variance, as well as to analysis of covariance and REML analysis to reduce the effects of residual variation in stalk number and spatial effects across the trial, respectively.

Results and Discussion

Table 2 presents summarised results of 10 trials conducted on 1999 and 2000 selections. Good discrimination of genetic differences in resistance between selections is reflected in DGD (degree of genetic determination) values (Bond, 1988) as close to 1.0 as possible. Infestations that are too heavy, due either to excessive stress and/or a high inoculation rate, produce low DGDs, as in trials 1/00 and 3/00 (Table 2). Inclusion of a larger number of control varieties, together with good discrimination between controls, increases the 'spread' of selection ratings across the full one to nine range and improves confidence in the ratings (e.g. 5/99, 2/00 and 5/00, Table 2; where larger numbers of resistant and susceptible clones were identified). Ratings calculated on the basis of the overall trial mean (e.g. 1/99, 3/99, 4/99; Table 2) are likely to vary according to the selections included in the trial. By contrast, use of the controls mean, as a relatively static reference point, should allow for assessment of progress in selection from stage to stage and year to year.

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Table 2. Summarised results and statistics from *Eldana saccharina* resistance screening trials of 1999 (1-5/99) and 2000 (1-5/00) selections. Means and SEDs are per pot values. DGD = Degree of Genetic Determination (selections only).

Trial	No. control varieties	No. (%) suscep.	No. (%) inter.	No. (%) resist.	Stalk length damaged (% len. dam.)				Internodes damaged (% int. dam.)				Total no. of larvae + pupae				Total mass of larvae + pupae (g)			
					Mean	SED	DGD	CV	Mean	SED	DGD	CV	Mean	SED	DGD	CV	Mean	SED	DGD	CV
1/99[ad]	4	1 (4)	26 (92)	1 (4)	338 (50)	65.1	0.48	38.6	38 (53)	6.5	0.38	34.3	30	6.9	0.45	45.7	2.06	0.51	0.56	49.5
2/99[bd]	5	4 (6)	62 (94)	0 (0)	115 (20)	54.4	0.24	74.6	14 (25)	5.27	0.39	56.6	18	9.5	0.02	83.6	1.16	0.71	0.02	96.7
3/99[ad]	4	4 (14)	23 (82)	1 (4)	314 (52)	59.4	0.68	37.7	37 (54)	5.99	0.72	32.1	38	7.95	0.53	41.1	3.08	0.68	0.62	44.0
4/99[ac]	5	3 (5)	54 (92)	2 (3)	128 (15)	54.5	0.20	67.4	79 (18)	5.27	0.29	57.7	10	3.99	0.32	65.3	0.73	0.32	0.51	68.6
5/99[bd]	5	3 (3)	73 (82)	13 (15)	143 (18)	59.2	0.49	58.4	17 (25)	4.52	0.62	37.1	21	8.9	0.63	61.0	1.16	0.55	0.63	61.3
1/00[be]	6	0 (0)	29 (91)	3 (9)	448 (86)	75.3	0.0	33.6	53 (86)	7.3	0.0	27.6	68	16.1	0.34	47.5	4.28	1.02	0.38	47.7
2/00[bd]	6	2 (5)	16 (41)	21 (54)	99 (21)	32.7	0.47	66.4	17 (26)	4.3	0.73	49.5	17	5.3	0.43	60.9	0.97	0.33	0.52	67.4
3/00[be]	5	0 (0)	25 (96)	1 (4)	423 (78)	85.5	0.0	26.6	47 (78)	1.2	0.08	22.8	47	3.3	0.08	66.0	2.70	0.21	0.05	74.6
4/00[be]	5	4 (4)	78 (86)	9 (10)	373 (50)	110.9	0.39	42.0	37 (56)	8.7	0.47	33.6	33	11.2	0.43	47.9	2.32	11.2	0.56	47.9
5/00[be]	6	8 (5)	86 (57)	56 (37)	88 (13)	39.5	0.59	63.8	12 (19)	3.8	0.71	43.6	15	3.8	0.61	61.5	0.81	0.45	0.52	78.9

[a] Ratings based on percent of trial mean; [b] Ratings based on percent of controls mean
[c]100-egg inoculation; [d]200-egg inoculation; [e]300-egg inoculation