

# AVAILABLE METHODS FOR ASSESSING VARIETAL RESISTANCE TO SUGARCANE STALK BORERS

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## Abstract

Sugarcane growing areas have a reasonably stable biological balance, and the use of chemicals to control stalkborers is undesirable. Varietal resistance could form an important component of an IPM strategy. In this paper, the main methods (field and laboratory) for assessing resistance to stalkborers are discussed, with some results obtained by the CIRAD team included.

## Introduction

Sugarcane is semiperennial and produces considerable biomass, forming an ideal habitat for insects. Most insects do not affect crops, but some compete with humans for sugar and by-products. Sugarcane stalkborers are key pests of this crop worldwide because they feed directly on the vegetative tissues that store sucrose, allowing the introduction of micro-organisms that affect yield and quality. Conversely, the crop habitat permits the development of many beneficial arthropods that limit borers and keep other pests in check. The regular use of insecticides is often undesirable if natural enemies are to have maximum impact and other means have to be used to improve the control of borers. Varietal resistance might well be one of the most valuable methods for implementing an integrated pest management (IPM) strategy. For example, in the USA, predation and varietal resistance have been used with success for the management of the sugarcane borer *Diatraea saccharalis* (Bessin & Reagan, 1993). Similarly, this method is often favoured in studies carried out by the main sugarcane producing countries (Macedo *et al.*, 1978; Leslie & Keeping, 1996; Allsopp *et al.*, 1996; Mukunthan and Mohanasundaram, 1998; Kakakhel *et al.*, 1999). This paper describes several methods for assessing varietal resistance, and results obtained by CIRAD entomologists with

two commercial varieties (R570 and R579) are presented. Methods for assessing the different mechanisms of varietal resistance, as described by Painter (1968) are presented according to their use in the field or laboratory.

Methods used in the field for overall evaluation of varietal resistance are discussed.

## Research station trials

### *Insecticide trials with two varieties*

The difference in production between a plot protected by an insecticide and a plot subject to significant pest attack, may serve as a measure of resistance. Using this method, two varieties were compared in a trial with complete blocks and four replications, where the protected blocks were sprayed with Decis® (25 g a.i./ha/treatment) every 10 days from the 3rd to the 10th month of the crop cycle. The treated plots showed yield increases of 22.7% and 1.8 % respectively for varieties R579 (susceptible) and R570 (resistant) when compared with the control plots (Table 1).

## Sampling variety trials

### *Row technique*

Under significant natural infestation, the % AIN in the central row of plots of variety trials can be estimated shortly before harvest. Each variety is awarded a rank according to the descending order of damage recorded. As a sufficient number of multisite trials are available, a simple overall analysis can be applied based on the average rank of each variety. An example is given in Table 2 for trials undertaken in Vietnam (Tay Ninh, 1995-98).

R570 was the variety least attacked in a geographical location with strong borer pressure (four major species). However, there were too few trials for statistical separation of the two varieties R570 and R579. Experience shows that variety trials such as this are not always useful for the evaluation of varietal resistance/tolerance because interference occurs when there are only a few rows per variety, hence the need to consider a large number of trials.

*CASIDI method* (Careful Attention and Scanning Illustration of Damaged Internodes).

This method is still at the experimental stage but has been used for the evaluation of a number of varieties or selected clones; it has the advantage of requiring little plant material to be attacked

**Table 1. Results of an insecticide trial comparing the two varieties R 570 (resistant) and R 579 (susceptible). UT = untreated blocks; T = treated blocks.**

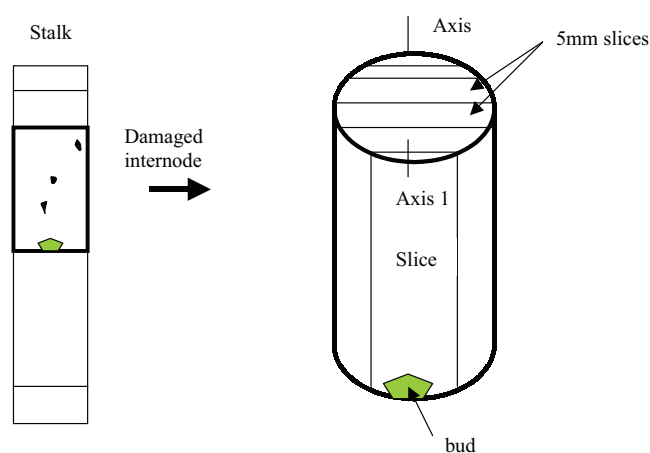
	Variety			
	R570		R579	
	UT	T	UT	T
% attacked stems (% AS)	61.3	9.8	92.0	25.3
% attacked internodes (% AIN)	5.7	0.5	14.1	1.6
Yield (tons cane/ha)	95.9	97.6	108.9	133.2

**Table 2. Ranking of varieties according to levels of borer damage in six trials in Vietnam.**

Trial No.	1995 – 1997 season			1997 – 1998 season			Sum of ranks (*)
	1	2	3	4	5	6	
Variety	rank						
R 570	1	1	1	2	1	1	7 (a)
R 573	6	2	3	5	2	5	23 (ab)
R 575	2	4	5	1	3	3	18 (ab)
R 579	5	3	2	3	4	4	21 (ab)
Roc 1	3	5	4	4	5	5	23 (ab)
Comus	4	6	6	6	6	6	34 (b)

Values followed by the same letter (a, b) are not significantly different (Friedmann's ranking test,  $T = 7.71$ ,  $LSD = 18.4$ )

by borers but has standard procedures for exhaustive studies. It requires separating the damaged internodes of stems and splitting each of them longitudinally into layers 5 mm thick (Figure 1).



**Figure 1. Procedure for splitting damaged internode.**

Several methods can be used to record the damage observed (tunnels and affected zones visualised by various stains: oxidation, red rot disease...) such as full-size tracing on sheets using pre-established graphic representation, or images taken using a digital camera or a scanner. A damage score can be awarded on a scale of 1 to 5. Several techniques can be used for processing the results (image analysis software, manual measurement of areas, etc.) with a large number of criteria, the final objective being that of obtaining reliable indexes such as measurement of the ratio of area of damage to internode volume (A/V) (Table 3).

#### Evaluation of damage level in commercial fields

This is a simple method but requires considerable logistical support and can only be applied to varieties that have already been released. It consists of applying a specific protocol to a panel of geographically varied fields. Two surveys were performed in Réunion with a 14-year interval (1981-83 and 1995-97) on the most common varieties grown on the island. In the first survey, the number of stems attacked and the number of healthy and attacked internodes per stem were counted in 15 sets 1 m long along a diagonal in selected fields. The average percentage of attacked stems (% AS) and attacked internodes (% AIN) was then calculated for each field. The counting system used in the second survey comprised four 10 m lengths of row (80 to

120 canes per length) chosen at random in a part of the field (1000 m<sup>2</sup>); this was mainly for practical reasons (availability of labour and time saving). In both cases, counting was performed shortly before harvest. Results in Table 4 show a difference in the distribution of the number of fields on either side of the 8% AIN threshold (the threshold beneath which the weight of R579 stems is not significantly affected) (Goebel, 1999).

Similarly, the CASIDI method can be used as mentioned above but also applied to a set of fields or to a plantation.

Different techniques used in the laboratory or under controlled conditions for identification of physical and/or chemical nature of resistance are outlined below.

#### Rearing on an artificial medium

The development of a semi-artificial medium for rearing stemborers enables useful comparisons when nutrient powder from different parts of healthy or damaged cane is incorporated in an artificial diet. The cane component of the medium used for *Chilo sacchariphagus* (Guennelon and Soria, 1973; Goebel, 1999) formed 25% by weight of the nutrient components of the medium. A preliminary experiment showed that powdered leaves of R570 or R579 inhibited full larval development and that the powder prepared from damaged cane enabled better development (at 21 days), whatever the variety. However, the survival of larvae until pupation was better on the medium incorporating with R579 powder (Table 5):

#### Artificial infestation

Developing an artificial rearing method allows the production of the life stages most suitable for artificially infesting stems in the field. All the methods used (cane infestation in the field or in the laboratory by eggs or larvae of various ages) have shown that for the criteria used (intensity of attacks and area of tunnels, survival of larvae, number of holes per stem, speed of entry in the stem, etc.) the greater susceptibility of R579 could only be demonstrated after introduction of the larvae into the stem (Table 6).

#### Oviposition tests

Whereas 'antibiosis' was central to evaluation using the preceding methods, females (reared as above) can also be used to study antixenosis, by recording the number of eggs laid on the leaves during tests in which a choice of variety is available. No significant difference was observed for this criterion in an ex-

**Table3. Summary of damage assessment using the CASIDI method (St-André, Réunion).**

Criterion	Variety	
	R570	R579
Number of stems	3	3
Total internodes	73	70
Number of attacked internodes (%AIN)	18 (24.7%)	32 (45.7%)
% attacks between top internodes		
- top 3	0/9	4/9
- top 5	2/15	8/15
Total slices	706	738
Total slices observed (%)	166 (23.5%)	330 (44.7%)
Slices bored (%)	85 (12.0%)	140 (19.0%)
Attack score (scale)		
1	57 (67 %)	72 (51.4 %)
2	21 (25 %)	42 (30.0 %)
3	6 (7 %)	19 (13.6 %)
4	1 (1 %)	5 (3.6 %)
5	0	2 (1.4 %)
Method 1	0,188	0,236
Method 2	0,395	0,649
Method 3	0,283	1,015

(\*) Method 1 = measurement of the surface area by an image processing software (Design Cad™) to give a reliable index based on ratio of damaged internode and the volume of internode (S/V).

Method 2 = measurement of the surface of main damage (width x length) with graduate rule

Method 3 = the same method based on a calculation of squared width (W<sup>2</sup>) of the main damage.

**Table 4. Count distribution in relation to the 8% AIN threshold.**

Threshold	Season			
	1981-1983 survey		1995-1997 survey	
	R570 (33 fields)	S17 (27 fields)	R570 (49 fields)	R579 (49 fields)
<8% AIN	81.8%	48.1%	96.9%	59.2%
>8% AIN	18.2%	51.9%	4.1%	40.8%

**Table 5. Survival of *C. sacchariphagus* to pupal stage with sugarcane powder in semi-artificial diet (25 larvae per assay) after 21 days.**

	Treatment							
	Leaf				Stem			
	R570		R579		R570		R579	
	undamaged	damaged	undamaged	damaged	undamaged	damaged	undamaged	damaged
Alive	0	0	0	0	1	1	7	13
Dead	25	25	25	25	24	24	18	12

**Table 6. Assessment of *C. sacchariphagus* damage (test 1, infestation by eggs)**

	Variety	
	R 570	R579
Test 1: infestation by eggs, observation after 15 days		
eggs per plant	33.1 ±1.2 (a)	37.6 ±1.3 (a)
hatching rate	88.4 ±1.4 (a)	86.2 ±1.8 (a)
leaf damage	1.6 ± 0.3 (a)	1.5 ± 0.4 (a)
Test 1: infestation by eggs, observation after 30 days		
larvae per stem	0.4 ± 0.1 (a)	1.3 ± 0.4 (b)
% attacked internodes	3.5 ±0.8 (a)	9.9 ±1.2 (b)
attack score (scale of 1 to 5)	0.5 ± 0.1 (a)	1.9 ±0.3 (b)

Mean followed by the same letter (a, b, c) are not significantly different at P=0.05 (t test) (Goebel, 1999)

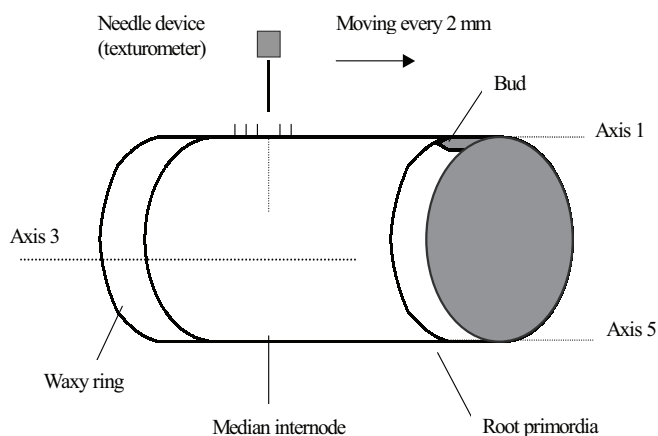
**Table 7. Oviposition responses of *C. sacchariphagus* females (choice available).**

variety	egg masses per plant	eggs per plant	eggs per egg mass
R570	1.9 ± 0.2 (a)	43.5 ± 5.3 (a)	22.5 ± 1.0 (a)
R579	2.2 ± 0.4 (a)	46.4 ± 9.3 (a)	20.9 ± 1.1 (a)

periment comparing R570 and R579. Trials were conducted with 10 replicates of 20 plants with 30 mated females (Table 7).

### Penetrometry

There has been a long period of co-evolution between *Chilo sacchariphagus* and the genus *Saccharum* and the two are closely associated. Young larvae (generally at the beginning of stage 3) have to overcome the plant's defences in penetrating the stem. Even when this is achieved, passage from one internode to the next is rare. A penetrometer (Texturometer®) was used to evaluate the hardness of an internode by calculating the energy required for the penetration of a standard needle to a depth of 15 mm every 2 mm along the longitudinal axes running through the bud (axis 1), the opposite side (axis 5) and one of the axes perpendicular to these (axis 3) (Figure 2). Results are summarised in Table 8.



**Figure 2. Penetrometer device for assessing the internode hardness.**

This makes it possible to understand the internode entry and exit behaviour of larvae that can easily bore into the harder zones—the root band, the growth ring and the waxy zone—whereas the force required in the bud zone falls to 200-300 kJ and that for the central part of the internode remains steady at between 300 and 400 kJ. With regard to varieties, the penetration of an R570 internode requires 15 to 50% more energy than R579, depending on the penetration site. An internal induration zone was characterised (2 to 10 mm beneath the epidermis) in the variety R 570; this may account for the difficulty larvae have in eating these tissues that protect the sap translocation zone (Morin, 2000). Further research may address the reasons why larvae do not enter via the bud (a weak part of a strong zone) to penetrate the internode.

### Use of Beam Identification by Non-destructive Grading (BING)

This non-destructive system for measuring the mechanical characteristics of wood was tested on two sugarcane varieties. The strength characteristics of wood (hardness, rigidity, etc.) are measured by a number of moduli weighted by the density of the timber appraised (apparent modulus of elasticity:  $E/\rho$ , etc.). Baillères *et al.* (1999) showed an excellent correlation between the measurement of this modulus and classic rheological methods (traction, compression or failure testing) and the BING method. Measurements performed on sugarcane cuttings in 1999 and 2000 showed a steady movement of the modulus  $E/\rho$  in the low, mid-height and upper parts of stems of R570 and R 579. While R570 showed a high value in the lower (+70%) and mid-height (+20%) parts in comparison with R579, the opposite was found in the upper part (-20%) whatever the type of force applied (flexion or compression) (Morin, 2000).

### Histochemistry

This method is used to determine the nature of the tissues playing a role in the behaviour of borers, especially in the induration zone. Work is being undertaken to check whether this phenomenon can be quantified, by evaluating the lignified tissues around the liber-ligneous rays from the epidermis to the centre of pith and by means of histological sections. Some stains used must be specific to tissue components (phloroglucinol for lignin for example).

### Chemical analysis

This is a complex field and measurements of mineral or organic compounds (silica, % lignin or soluble sugars, etc.) are often difficult to interpret because of insufficiently rigorous sampling. Numerous internal factors (redistribution of assimilates, daily variation of photosynthesis, etc.) and external factors (climate, diseases, etc.) that are linked to varying degrees affect sugarcane during development, making it difficult to collect homogeneous batches of stems. A stem that has grown lateral shoots after borer damage is no less affected in terms of sugar yield than an unbored stem (Van Dillewijn, 1960). Chemical analyses should therefore be targeted and linked to topographical analysis of tissues in order to be fully usable. Techniques such as NIRS should be developed (Rutherford, 1998; Baillères *et al.*, 2000).

### Conclusion

It is often necessary to combine several methods to be able to characterise the behaviour of a variety with regard to its response to attack by one or more borer species. Choice de-

**Table 8. Average energy measured for the penetration of a standard needle to a depth of 15 mm in an internode (kJ)**

Position of internode	Penetration site					
	root band		median internode zone		waxy zone	
	R 570	R 579	R 570	R 579	R 570	R 579
top of stem	600	500	300 to 400	200 to 300	600	500
middle of stem	600	500	300 to 400	300	500 to 600	500

depends on the objectives, funding and the technical resources available. Resistance can be exploited commercially but seems to have a 'cost': R579 is susceptible but has undoubted agronomic qualities (potential yield, ease of cutting, etc.) which make it preferred by certain growers. It is therefore important that varieties should be appraised by entomologists for resistance to borers before their release to farms. This is a rational response to economic and environmental issues.

### Acknowledgments

The authors wish to thank the CIRAD staff for technical assistance in this programme, particularly Jean-Marie Bègue, Richard Tibère, Jean-Claude Gauvin and Marie-Charlyne Gauvin.

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