Ratoon Stunting Disease of Sugarcane: Possible Correlation of Resistance with Vascular Anatomy

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ABSTRACT

When water was sucked through single-node cuttings of sugarcane, the average flow rates were 1.4 ml/minute for a clone "immune" to ratoon stunting disease (RSD), 2.6-8.4 ml/minute for three "tolerant" clones and 13.0 - 19.6 ml/minute for six "susceptible" clones. A "field resistant" clone had a flow rate of 19.1 ml/min, similar to that of the "susceptible" clones.

When india ink was sucked for a period of 30 seconds into double-node cuttings of sugarcane, the average number of ink-discolored vascular bundles in the third (distal) internode was one for the "immune" clone, 2-29 for the three "tolerant" clones, and 36-41 for the two "susceptible" clones and the one "field resistant" clone.

It is concluded that vascular anatomy, possibly in the form of a low number of large, continuous vessels passing through the nodes, may be a factor in the RSD resistance of the "immune" and "tolerant" clones, but possibly not the "field resistant" clone.

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Ratoon stunting disease (RSD) is a major disease of sugarcane, which is probably caused by a small bacterium (5,6,9,10). The disease can be controlled by heat treatment of planting material, by the avoidance of spread on contaminated knives, and to a lesser extent by the use of resistant clones (8). The nature of host resistance to RSD has not been studied in detail, but preliminary reports indicate that three types of resistance occur. Firstly, some clones, such as CP29-116, CP52-68, and Q50 are "tolerant". Although they can be infected, they usually do not suffer heavy losses (8). Secondly, some clones, such as Badila and Q44, appear to be "field resistant". They often escape infection in the field, although when artificially inoculated they may be severely stunted (8). Thirdly, one clone, H60-6909, is reported to be "immune", because attempts to infect it have been unsuccessful (11).

Following the observation that healthy single-node cuttings of the "immune" clone, H60-6909, were much more resistant to the passage of water than were those of the susceptible clone, Q28, we became interested in the possibility that vascular anatomy was involved in resistance to RSD. We hypothesized that resistance to water movement would be paralleled by resistance to movement of the RSD agent, and that this would restrict its colonization of the plant. It seemed feasible that all three types of resistance, "immunity", "tolerance", and "field resistance", might be based on different levels of resistance to the movement of the RSD agent.

To investigate the above hypothesis we selected eleven different clones of known RSD reaction, and tested stalk cuttings of each for their resistance to water movement. In addition, we did preliminary tests on the ability of india ink (a colloidal suspension of carbon black particles) to

pass through sugarcane stalk cuttings. Some particles in india ink are similar in size to those of the bacterium which is associated with RSD (5,6,9,10).

METHODS.—*Movement of water through sugarcane stalk cuttings.*—Three healthy one-year-old stalks, each of about 2 m of mature cane, were collected from each of ten different clones of sugarcane growing at the Pathology Farm of the Bureau of Sugar Experiment Stations, Eight Mile Plains, Brisbane, Australia. Three healthy nine-month-old stalks of clone Q44, each about 1.5 m long, were also collected. The base of each stalk was sliced and examined to check that symptoms of RSD and other diseases were absent. Four 60-mm long "single-node cuttings" (comprising a node and a part of each of the two adjacent internodes) were taken from the middle portion of each stalk, and the diameter of each cutting was measured. In addition, occasional 60-mm long internode cuttings were also taken.

Ease of movement of water into and through each cutting was determined in the following manner. One end of a freshly prepared cutting was fitted with one or more rubber sleeves, then wedged tightly into the neck of a 2-liter conical vacuum flask. A vacuum of about 27 mm Hg was applied to the flask by means of an electric pump, while at the same time sterile distilled water was pipetted onto the upper end of the cutting. The rate of pipetting was adjusted so that the upper cut end was continuously covered with water, but so that little or no water spilled over the edge. The volume of water used was usually 10 ml, but only 3 - 8 ml was used with cuttings which greatly resisted the movement of water. Compared with 10 ml, these smaller volumes give a higher water flow rate since the rate gradually decreases with time.

Movement of india ink through sugarcane stalk cuttings.—This was tested in a manner generally similar to that described above, but only seven representative clones were included, and double-node cuttings (comprising two nodes, the intervening internode, and a part of each of the two adjacent internodes) were used instead of single-node cuttings. India ink instead of water was pipetted onto the upper end of the cutting wedged in the vacuum flask, and evacuation of the flask was continued for a set time of 30 seconds. An absorbent paper pad was pinned to the end of the cutting in the vacuum flask, so that any ink passing completely through the cutting was not sucked back into the cutting when the vacuum was released.

The ability of the india ink to move through the nodes was assessed on the basis of the number of blackened vascular bundles in the two internodes furthest from the upper end of the cutting where the ink was applied. During examination, the internodes were sliced tangentially with a sharp knife to show the blackened bundles more clearly.

RESULTS.—*Movement of water through sugarcane stalk cuttings.*—The rate of water movement through single-node cuttings of the 11 clones is shown in Table 1. The "immune" clone, H60-6909, was highly resistant to the movement of water, while the three "tolerant" clones, CP29-116, Q50, and 60C644, were moderately resistant to movement of water. The "field resistant" clone, Q44, and six "susceptible" clones, Q82, Q91, Q71, Q87, H66-6921, and Q28, were all relatively low in resistance to movement of water. Average diameter of the cutting was not correlated with rate of water movement (Table 1).

Rate of water movement through internode cuttings was always very high, 42-80ml/min, whatever the RSD resistance of the clone.

Movement of india ink through sugarcane stalk cuttings.—The number of blackened bundles in the

TABLE 1. Movement of water and india ink through healthy stalk cuttings of some sugarcane clones

Clone	Ratoon stunting disease class	Stalk diameter (mm)[a]	Water flow rate (ml/min)[a]	No. of ink-discolored vascular bundles[b]	
				Second internode	Third internode
H60-6909[c]	"Immune"	25	1.4 A	8	1[d]
CP29-116	"Tolerant"	23	2.6 B	14	2
Q50	"Tolerant"	25	4.3 BC	73	29
60C644	"Tolerant"	25	8.4 C	53	14
Q44	"Field-resistant"	37	19.1 C	57	36
Q82	"Susceptible"	26	13.0 C		
Q91	"Susceptible"	25	13.2 C		
Q71	"Susceptible"	23	14.0 C		
Q87	"Susceptible"	25	14.8 C		
H66-6921	"Susceptible"	27	15.4 C	63	40
Q28	"Susceptible"	27	19.6 C	67	41

[a]Average of 12 single-node cuttings. Data followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's new multiple range test.

[b]Average of 10-20 double-node cuttings.

[c]H60-6909 and H66-6921 were bred in Hawaii, CP29-116 was bred in Canal Point, Florida, and the other clones were bred in Queensland, Australia.

[d]Statistical analysis using stalk means showed that the differences between varieties were not significant. However, using the *t*-test it was shown that there was a significant difference between some of the classes, namely immune and field-resistant, tolerant and field-resistant, and tolerant and susceptible.

second (middle) and third (distal) internodes of the double-node cuttings of the seven clones is shown in Table 1. In the "immune" clone, H60-6909, india ink moved through one node to the second internode in an average of only eight vascular bundles per cutting. The ink usually failed to move through the second node into the third internode.

In the "tolerant" clone, CP29-116, india ink moved through the first node to the second internode in a moderate number of vascular bundles, and only a few or no vascular bundles were blackened in the third internode.

In the "tolerant" clones, Q50, and 60C644, the "field resistant" clone, Q44, and the "susceptible" clones, H66-6921, and Q28, india ink moved through the cuttings to the second internode in many vascular bundles and reached the third internode in a moderately large number of vascular bundles.

DISCUSSION.—The correlation between resistance to RSD and resistance to water movement through single-node cuttings was good for all ten of the "immune", "tolerant", and "susceptible" clones. Rate of movement of water was least through the "immune" clone, was intermediate through the "tolerant" clones, and was greatest through the "susceptible" clones. Since water movement is largely in the vascular bundles, RSD reaction also may depend on a property of the vascular bundles.

An indication of the nature of this property is given by the work with india ink. In some clones tested the number of vascular bundles carrying india ink through the nodes of the cuttings was negatively correlated with resistance to RSD. Thus, with these clones the number of large bundles traversing the nodes without barriers or undue constriction may be a factor determining the ability of the RSD agent to move between internodes.

The "field resistant" clone, Q44, resembled the "susceptible" clones in having little resistance to the passage of water and india ink through the nodes. Possibly its ability to escape infection in the field has a basis other than vascular anatomy. However, the results with Q44 must be accepted with caution because of the younger age and greater diameter of this clone.

We have found few reports of diseases where an internal anatomical feature apparently influences resistance to a pathogen. With most diseases, either physiological resistance is considered to be of major importance, or alternatively, the relative contributions of physiological and morphological resistance are difficult to evaluate. Exceptions to this generalization include one bacterial disease and several fungal diseases. With the bacterial wilt disease of alfalfa, caused by *Corynebacterium insidiosum,* roots and stems of resistant varieties had fewer vascular bundles, shorter vessel elements and a thicker cortex than did those of susceptible varieties (3). Reduced infection and spread of the bacterium in resistant varieties was considered to be due partly to anatomical features restricting its movement and partly to a cell sap inhibitory to its multiplication.

The fungal diseases in which vascular anatomy apparently influences resistance include the Dutch elm disease, in which movement of conidia of the pathogen, *Ceratocystis ulmi,* is restricted by the relatively narrow, short vessels of resistant elms (4,7). Also, in the sugarcane red rot disease, conidia of *Physalospora tucumanensis* cannot move from the initially infected internode in resistant clones, such as CP29-116, which have mainly discontinuous vessels through the nodes. On the other hand, those sugarcane clones with a large number of continuous vessels allow the ready migration of conidia up or down the stalk (1,2). We consider it significant that both Atkinson (2) and ourselves, working with different diseases, have concluded that a relative lack of large, continuous vascular bundles in the sugarcane clone CP29-116 may be a basis of morphological resistance to disease.

Resistance based on morphological features, such as vascular anatomy, may be particularly valuable because of its stability. Cho et al. (3) reported that several varieties of alfalfa which have morphological resistance to bacterial wilt have been grown extensively over vast areas of the U.S.A. and Canada for 20 years, apparently without losing any of their original resistance. RSD resembles bacterial wilt of alfalfa in that the pathogen is largely restricted to the vascular bundles, and possibly in that a coryneform bacterium is involved (10). Morphological resistance to RSD introduced into sugarcane by means of a breeding program might prove to be long lasting. In any such breeding program, a preliminary screening of sugarcane clones might be made on the basis of a rapid and objective test, such as, the rate of water flow through single-node stalk cuttings.

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