AN INVESTIGATION INTO SUGARCANE BREEDING IN SOUTH AFRICA

By P. G. C. BRETT.

Under commercial cultivation the propagation of sugarcane asexually by cuttings has the advantage of keeping varieties true to type. Variation is needed, however, for selecting improved varieties, and this can be obtained by growing sugarcane from seed. Commercial types of cane do not breed true, and each seed formed after the fertilisation of an egg by a pollen grain gives rise to a new variety. Though fully formed eggs are nearly always produced, in some varieties the pollen grains are not capable of fertilisation. The latter are known as female varieties, and those with much fertile pollen as males. Varieties, however, may vary from male to female depending upon climatic conditions, for while the formation of viable pollen is rather easily inhibited, the ovules appear to be more hardy.

It was for a long time believed that sugarcane in Natal would not set seed as viable pollen was not produced. In 1944, however, germination of seed from commercial types of sugarcane was obtained for the first time, as has already been reported.[1] There would appear to have been no reports before this of sugarcane setting seed in Africa. Seedlings had been obtained four years previously from Amu Darya, which is, however, a type of wild cane belonging to the species *Saccharum spontaneum*.

Although it had been found that sugarcane seedlings could be raised in Natal, it was obvious that pollen production and seed setting were far less abundant than in cane breeding countries such as Mauritius and Java. Hence apart from the routine cross breeding work which was continued, an investigation was made into factors affecting fertility and seed setting in the hope that the scope of sugarcane breeding in Natal might be extended.

The preservation of Cut Canes in Various Solutions

In crossing sugarcane it is necessary for the male and female inflorescences, or tassels, to be brought together at the time of flowering; side by side planting of the varieties is of little use owing to the irregularity with which sugarcane flowers. If the tassels of the male are cut and brought to the female, they soon wilt and die even if their ends are placed in water. Hawaiian workers, however, found that a dilute solution of sulphurous acid prevented wilting. Later they used a solution containing 0.01 per cent. sulphurous acid and 0.01 per cent. phosphoric acid. In Hawaii both male and female tassels are cut and moved to a place isolated from other flowering canes. In some countries, however, it was found better not to cut the female tassel as the toxicity of the solution adversely affected the germination of the seeds.

When the effect of this solution upon sugarcane was first tried out here, the flowering season had not yet begun and ordinary cane stems had to be used. The toxic effects of the solution were soon shown. It was thought that under Natal conditions an improvement might be obtained by a change in concentration of the substances used, but the standard Hawaiian solution proved to be the best. A list of all substances investigated for their effect on cut canes is given in Table 1.

TABLE 1.

List of substances whose solutions were tested for preventing wilting of cut canes.

Substances without effect. (The concentration given is the highest tried out.)	Substances preventing wilting to a greater or less extent. (The concentration given is the lowest at which some effect was shown.)
1.0% ethyl alcohol.	0.1% benzoic acid
0.1% potassium permanganate.	0.1% citric acid.
0.1% suspension of bleaching powder	0.1% aconitic acid
0.01% suspension of bleaching powder and 0.01% phosphoric acid.	0.1% oxalic acid.
0.1% hydroxyquinoline potassium sulphate.	0.1% tartaric acid
0.01% p-nitrophenol.	0.1% nitric acid.
0.01% malachite green.	0.1% acetic acid.
0.1% hydroquinone.	0.1% formic acid.
0.01% ascorbic acid (Vitamin C).	0.1% lactic acid.
0.01% chloral hydrate.	0.1% hydrochloric acid.
0.1% boric acid.	0.01% formalin.
0.1% sodium acetate.	0.1% sodium benzoate.
0.1% potassium hydroxide.	0.05% Aretan.
0.1% sodium acetate.	
Saturated solution of carbon dioxide.	

Evans[2], in discussing the effect of sulphurous acid upon cut canes, says that its prevention of wilting is due, not to its inhibiting action on the growth of bacteria on the cut end (as has been stated by Hawaiian workers), but to its reducing action, which prevents blocking of the vessels otherwise produced by the enzymatic oxidation of organic substances. He found that a solution of oxalic acid

was as effective in preventing wilting as a solution containing sulphur dioxide, phosphoric acid and sulphuric acid, but that it also produced toxic symptoms. 0.5 per cent. or 1 per cent. solutions of invert sugar were found to delay wilting to some extent.

In this connection it is interesting to note that the suggestion has been made[3] that the ability of certain substances to antidote the wilt-producing toxins secreted by some plant pathogens is the result of their reducing properties. The effect of solutions of a number of these reducing substances upon cut canes was tried out, but at the concentrations used only 0.1 per cent. benzoic acid was found to prevent wilting, and even this was not as good as the standard solution. The reducing sugars maltose and lactose were also without effect at a concentration of 1 per cent. It appeared then that, though the prevention of wilting could not be dependent solely upon reducing action, it might be correlated with acidity: benzoic, oxalic, sulphurous and phosphoric acids had all at one time or another been found to prevent wilting to a greater or less extent. When a number of acids, both inorganic and organic, were tried out it was found that though none was as good as the standard solution, all except boric were effective to some extent at a concentration of 0.1 per cent. That acidity was not the sole factor involved was shown by the fact that 0.1 per cent. sodium benzoate delayed wilting to some extent; sodium acetate and potassium hydroxide at the same concentration had no effect. It would seem that a number of substances which delay or prevent the wilting of cut canes are also useful inhibitors of the decomposition of foodstuffs. This is not necessarily evidence in favour of the theory that the wilting of cut canes is the result of bacterial growth on their cut ends: bacteriocidal or bacteriostatic substances might inactivate the enzymes which according to Evans are responsible for the blocking of the plant's vessels. It would rather appear that to prevent wilting a substance must be capable of inhibiting both bacteria and plant enzymes. It was found that if the ends of cut canes were placed in boiling water for fifteen minutes (a process that would destroy the enzymes) and the canes then left standing in plain water, wilting did not occur till after a few days; bacterial decomposition then set in and wilting followed. Bacterial decomposition and the resulting wilting could be prevented by the addition to the water of chlorine produced by adding a little acid to a suspension of bleaching powder. A dilute solution of chlorine produced in this way had previously been found ineffective when the ends of the cane had not been placed in boiling water. The above method was, however, of little use for preserving canes because of the toxic effects of the chlorine.

Though none of the substances tried out were by themselves as effective as the standard solution, it appeared that a solution containing 0.008 per cent. of each of six acids (benzoic, acetic, nitric, sulphuric, phosphoric and oxalic) was nearly if not quite as good as the standard solution. At this time, however, another method of keeping cut canes alive was showing promise and work on the above lines was discontinued.

Rooting of Cut Canes.

The possibility was investigated of inducing cut canes to root in the preserving solution, after which they could be transferred to water and toxic effects thereby reduced. Rather surprisingly, it was found that canes which had been dug up to leave some of their roots attached were very much more inclined to wilt in the solution than canes cut in the ordinary way. Some success in rooting could be obtained if the preserving solution was not kept up to strength but allowed to become weaker; canes, however, were severely damaged by the time sufficient roots had been formed to enable them to be independent of absorption through the cut ends. The proprietary preparations Belvitan, Stimcut and Hortomone A, containing root-forming hormones, showed no effect when applied in solution with sulphur dioxide and phosphoric acid; and though rooting appeared to be stimulated to a slight extent when the canes were stood for sixteen hours in solutions of these substances alone, before being transferred to the standard solution, damage as a result of wilting was severe.

Aretan at a concentration of 0.05 per cent. delayed wilting to some extent but was toxic; when used with the standard solution at a concentration of 0.002 per cent. there was some indication that the development of the root primordia was stimulated, but only on that part of the cane immediately above the solution. It appeared that solutions capable of preventing wilting were too toxic to permit proper growth of roots. The possibility remained however of inducing the canes to root in pots placed above the solution. It had previously been noted that root primordia had tended to grow if covered by trash that dipped into the solution; and when five cut canes were surrounded by trash in Mitscherlich pots, four showed good rooting at the end of a week. The effect on root formation of a number of different rooting media was tried out; compost formed from well-rotted cane trash was found to be better than a rather heavy soil, which in turn was better than river sond, As it had been found that when canes, either cut or whole, were planted in the ground, the quickest rooting occurred from the region at the base of the lowest green leaf sheath, the Mitscherlich pots were raised to cover

this region. Rooting, however, was considerably reduced as a result. By using long pots, as suggested by Evans,[2] rooting was improved as a greater number of nodes were covered.

Canes are usually only slightly damaged when rooted by this method. Though they are not usually removed from the solution until after about a fortnight, the rooting which has occurred before this results in a considerably reduced intake of the toxic solution. Varieties, however, differ considerably both in the ease with which they root and their reaction to the solution. Though in India flowering canes in the field are rooted in pots placed around their bases before being cut and moved, the method is laborious and has in addition the disadvantage that canes cannot be moved till rooting has occurred. When the method described above is used, however, the cane can be moved at once, and if they are taken to a convenient place for rooting the amount of labour involved in the operation is considerably reduced. It would also appear that cut canes root more rapidly than canes left standing in the field.

Pollen Fertility.

As cut canes that were going to flower could now be kept alive for a considerable length of time, use was made of this method to investigate some of the factors affecting the production of viable pollen. Pollen of canes which had been kept under artificial conditions for some time before flowering was compared with that produced by canes left in the field. It was found that artificial conditions which included high temperatures and humidity and increased length of day gave rise to increased pollen fertility. It is not yet known which of these factors are of primary importance and these experiments will be continued when the flowering season again commences. A reliable method of increasing pollen fertility which was adaptable to a large number of varieties would be of great value in breeding sugarcane in South Africa, if the necessary facilities for using it on a large scale were available.

Technique of Crossing.

The method of crossing adopted in South Africa was that in which the female tassel is left standing in the field and the male tassels after cutting are brought to it and preserved in a solution of sulphurous acid and phosphoric acid. After it had been found possible to preserve cut canes for a long time, without much damaging them, a number of crosses were made in the glasshouse, the female tassel being cut as well. In Table 2 the number of seedlings from these crosses are compared with those from similar crosses made in the field.

TABLE 2.

A comparison between the numbers of seedlings obtained from crosses carried out in the glasshouse and those obtained from similar crosses made in the field.

Combination.	Method of preserving the cut female tassel in the glasshouse.	No. of seedlings. Crosses made in field.	No. of seedlings. Crosses made in glasshouse.
Selfed Co.205 seedling × Glagah	Previously rooted in pot	670	2,000
Co.205 × Glagah	Preserving solution	250	6,000
	In preserving solution while being rooted	—	1,070
N:Co.79 × Glagah	In preserving solution while being rooted	0	350

It would appear that glasshouse conditions had a very favourable influence on seed setting. It will be noticed that in crosses made between Co.205 and Glagah more seedlings were obtained from the female tassel that was simply preserved with the sulphurous acid solution than from the one which was being rooted at the same time. The variety Co.205 is, however, relatively little affected by the toxic action of the sulphurous acid, and is very slow in rooting. Small numbers of seedlings were obtained from other crosses made in the glasshouse, but owing to a shortage of tassels similar crosses could not be carried out in the field at the same time.

Flowering.

The factors influencing the amount of flowering from year to year in Natal are not well understood. One factor of importance in determining the amount of flowering during the following winter may be the total rainfall during the months of December, January and February. Since 1930 the highest rainfalls at the Experiment Station over these three months occurred during the years 1938 and 1932 with 21.57 and 19.47 inches respectively. These were also the outstanding years for flowering. Good flowering also occurred during the year 1943 with the next highest rainfall of 18.93 inches for the preceding months of December, January and February. 1944, 1945 and 1946 with corresponding rainfalls of 8.69, 10.03 and 10.01 inches respectively, were rather poor flowering years. On the other hand 1942, though a good flowering year, had a rainfall of only 11.71 inches for the preceding months of December, January and February. Unfortunately there are not sufficient records to determine the amount of flowering that occurred in other years during this period.

Attempts to produce flowering in sugarcane by injection of solutions have sometimes been made, but without success. The so-called "whip" produced in even very young canes attacked by the disease known as smut is supposed to be a malformed inflorescence; it was thought therefore that canes

might be induced to flower by the injection of sterile extracts from smut spores. No effect, however, was obtained on flowering although on one occasion it appeared as if a stimulation of growth occurred like that of smut-infected canes.

Seedlings.

In Table 3 is given a list of the various crosses made at the Experiment Station and the number of seedlings obtained. The list does not include attempts to obtain seedlings from intergeneric crosses, none of which were successful. One seedling, however, was obtained by using Co.356 as a male parent —this variety is itself the result of an intergeneric cross between sugarcane and sorghum.

TABLE 3.

List of Crosses made in Natal.

(1) Crosses with *Saccharum spontaneum*.

Combination.	No. of crosses made.	No. of seedlings obtained.
N:C0.310 × Amy Darya	2	17
N:Co.352 × Amu Darya	1	19
Co.205 × Glagah	4	7,890
Co.301 × Glagah	1	0
Co.312 × Glagah	1	25
N:Co.79 × Glagah	2	350
N:Co.310 × Glagah	1	22
N:Co.325 × Glagah	1	0
P.O.J.2725 × Glagah	2	0
P.O.J.2803 × Glagah	1	29
Selfed Co.205 seedling × Glagah	2	2,670

(2) Other crosses.

Combination.	No. of crosses made.	No. of seedlings obtained.
N:Co.310 × Co.205	1	0
N:Co.79 × Co.285	1	0
N:Co.310 × Co.285	1	6
N:Co.339 × Co.285	1	12
N:Co.349 × Co.285	1	0
P.O.J.2803 × Co.285	1	1
Co.281 × Co.301	1	14
N:Co.310 × Co.301	3	119
N:Co.349 × Co.301	2	1
N:Co.310 × Co.356	2	1
N:Co.324 × N.F.35	1	11
N:Co.339 × Uba Marot	1	0

(3) Selfings.

Variety.	No. of selfings.	No. of seedlings obtained.
Amu Darya	2	133
Co.205	3	9
Co.285	2	0
Co.301	35	546
Co.421	1	0
C.P.34/79	1	2
Glagah	1	1
N:F.35	1	0
Uba Marot	1	0
Total number of seedlings obtained		11,878

It is not yet known to what extent it will prove possible to breed sugarcane seedlings in South Africa, where the amount of flowering, pollen fertility and seed setting in general appears to be considerably less than in the well-established cane breeding countries. The one advantage South Africa would appear to enjoy is in the length of the flowering season. It has, for instance, been found possible in Natal to cross Amu Darya, which is a wild type of cane flowering in mid-summer, with commercial varieties, the usual flowering season of which is winter. In the United States when it was desired to make a similar cross, it was found necessary to send plants of Amu Darya from North to South America, where the seasons were reversed and commercial types of cane were in flower.

Though the number of seedlings raised at the Experiment Station has considerably increased (thirty-six were raised in 1944, over a thousand in 1945 and over ten thousand in 1946), many of these seedlings are of unpromising parentage; some, however, may prove of value in further breeding work.

Though it may never be possible to attempt as ambitious a breeding programme as those of well-established sugarcane breeding countries, and though the necessity for importing seed from other countries may never be removed, it is thought that there still remains a reasonable possibility of obtaining results of value from sugarcane breeding in South Africa.

Summary.

1. Though sugarcane does set seed in South Africa, fertility in general is low.

2. A solution containing 0.008 per cent. of each of six acids (benzoic, acetic, nitric, sulphuric, phosphoric and oxalic) was nearly if not quite as good as the Hawaiian solution of 0.01 per cent. phosphoric acid and 0.01 per cent. sulphurous acid for preserving cut canes. It would appear that to prevent wilting a substance must inhibit both plant enzymes and bacteria.

3. Some advantages of both Hawaiian and Indian methods of preserving canes can be combined by rooting the cut canes in pots placed above the preserving solution.

4. Using this method, cut canes that were going to flower were kept under artificial conditions and the production of viable pollen found to be thereby increased.

5. Crosses carried out in the glasshouse were found to give a larger number of seedlings than similar crosses made in the field.

6. The total rainfall during December, January and February may be of importance in determining the amount of flowering during the following winter.

7. A list is given of the crosses so far made at the Experiment Station, and the numbers of seedlings obtained.

8. It is not yet possible to say to how great an extent seed produced locally will be able to replace that imported from other countries.

References.

[1] Brett, P. G. C. (1946): Seed Setting of Sugarcane in South Africa. *Nature*, 157, 657.

[2] Evans, H. (1932): Research Botany. Part II of Third Annual Report of the Sugarcane Research Station, Dept. of Agric., Mauritius.

[3] Horsfall, J. G. (1945): Fungicides and their Action. Chronica Botanica Co., Waltham, Mass., U.S.A.

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The PRESIDENT said that he had found that years of abundant rainfall were always characterised by exceptionally wet months of November and December, which was, of course, an important growing period for cane; and it was interesting to see that rainfall during these months materially affected flowering of sugarcane.

Dr. McMARTIN said that the developments reported in this paper were important from the point of view of finding new varieties for Natal. The older method of introducing varieties from other countries through the quarantine glasshouse was slow, and had been augmented of recent years by importing fertilized tassles and raising the seedlings here. By this means a much larger number of types were available from which to choose, which led to a greater chance of securing a suitable variety. It had always been considered necessary to import such fertilized tassels, as no seed was obtained locally, due to non-viability of the pollen grains; from 1941 onwards, however, we have been finding some fertile pollen, which suggested the possibility of raising seed here. Intensive work on this has shown that this could be achieved, although at present only to a limited extent, as recorded in this paper.

Professor WARREN said that the fact that hydroquinone had proved ineffective in preventing wilt in cut canes disproved Dr. Evans' theory that wilt was prevented by a reducing substance. He suggested that hormones of the dichlorophenoxy acetic acid type be tried.

Dr. FISHER suggested that the age of the cane probably also played an important part in flowering. He was interested in the effects of December to February rainfall on flowering, but thought that a correlation might be found between cane flowering and the rainfall for some other period. It was found that unfavourable climatic conditions sometimes induced flowering.

It was rather disappointing that the parent canes used for breeding purposes were so unpromising. In animal husbandry, for example, no one would dream of using a wild boar as a male parent to produce good offspring.

Mr. BRETT agreed that the fact that hydroquinone was found ineffective indicated that the prevention of wilting was not dependent solely on reducing action; oxidising enzymes might, however, be concerned in the reaction, for it was possible that the wilting in this instance was produced by the oxidation of hydroquinone by relatively insoluble substances which blocked the vessels of the plant. As far as plant hormones of the dichlorophenoxy acetic acid type were concerned, it was intended to investigate their effect on sugarcane, though they had been found to have little effect upon members of the grass family as a whole.

He agreed that it was unlikely that many canes of value would be obtained from first crosses with wild canes, but most of the commercial canes now grown had as one of their ancestors a type of wild cane, and some of the seedlings raised at the Experiment Station might therefore be of use in a future breeding programme.

As far as the effect of rainfall on flowering was concerned, he had taken into account the rainfall during the whole season, but it was only for the months of December, January and February that there appeared to be any correlation between the amount of rainfall and the available data on flowering.

Dr. DODDS said that this paper dealt with a matter of fundamental potential importance, which would be followed up energetically. Table 3 showed, however, that, with a few exceptions, the male parents of our seedlings were not really suitable. The trouble was to procure pollen from good male parents, and this difficulty had been overcome in the U.S.A. by importing pollen by regular air-mail service from Central America. Pollen would only keep a few days in cold storage, however, but he thought a similar procedure might be followed here when a direct air-mail service was established between Natal and, say, Mauritius.