STUDIES OF THE EFFECT ON SUGARCANE OF DAMAGE CAUSED BY FROST AND ASSOCIATED MICRO-ORGANISMS

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Introduction

In the winter of 1964, sugarcane suffered severely from frost damage in the higher altitude areas of Natal. Damage also occurred in scattered areas at lower elevations, where frost had seldom been known previously. In these instances, damage was relatively serious, particularly where frost pockets formed.

The injury caused by frost is characterised by browning of the canopy and the leaf sheaths, but the growing point may also be severely damaged by destruction of some or all of the meristematic tissue. Experience gained in previous years has served to indicate that frosted cane does not in all cases deteriorate with the same rapidity. Thus, when rain falls on cane damaged by frost, or conditions become humid, then deterioration is rapid. However, deterioration of the cane is also influenced by the degree of frost injury suffered, and this must be taken into account when judging whether a frost-damaged crop should be cut immediately, or left.

To clarify the nature and scope of the damage caused by frost, the injuries it causes have been studied in some detail. The investigation embraces:

(*a*) A study of the role of micro-organisms involved in the deterioration of cane, when this has been damaged by frost.

(*b*) Cataloguing of symptoms associated with frost damage at different levels of severity, so that the grower can determine the significance of the damage caused, and decide whether and when to harvest.

(*c*) Determining the effect of frost on the quality of cane juice.

Data used were obtained from cane damaged by frost both in the field and in cooling chambers, where frost conditions were simulated. The nature of this damage, the regeneration of the cane, the relative susceptibility of different varieties, the effect of frost and the microflora associated with the damaged tissue on purity, brix and sucrose percentage, were all examined in the course of this study.

Field Observations

During the winter of 1964, cane in the field, which was exposed to frost, displayed varying degrees of injury. These ranged from slight symptoms of damage on the canopy, to total destruction of both the terminal growing point and all the visible lateral buds. Symptoms in the field compared closely with the standards established in 1960 by J. Wilson, Director of the Experiment Station of the South African Sugar Association. Four categories of frost damage were listed at that time and details are quoted in the South African Sugar Journal, 44, pp. 837-839. These categories are:

"(*a*) Where the fully developed or exposed parts of leaves are killed, but the innermost and covered parts of the spindle leaves are uninjured and remain green.

(*b*) Where the innermost leaves of the spindle are killed, but the apical growing point and the basal parts of other leaves in the spindle are unaffected.

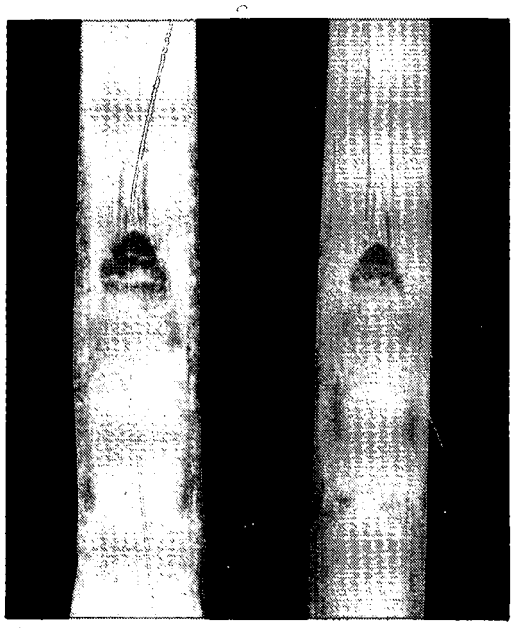
(*c*) Where the apical growing point is killed in addition to (*a*) and (*b*).

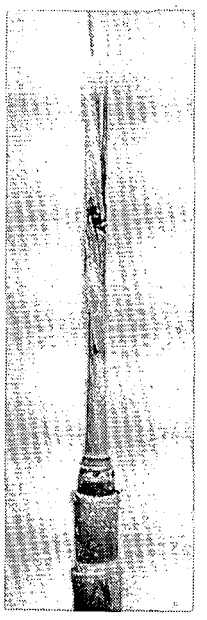
(*d*) Where the lateral buds on the main stem are also killed, in addition to (*a*), (*b*) and (*c*)."

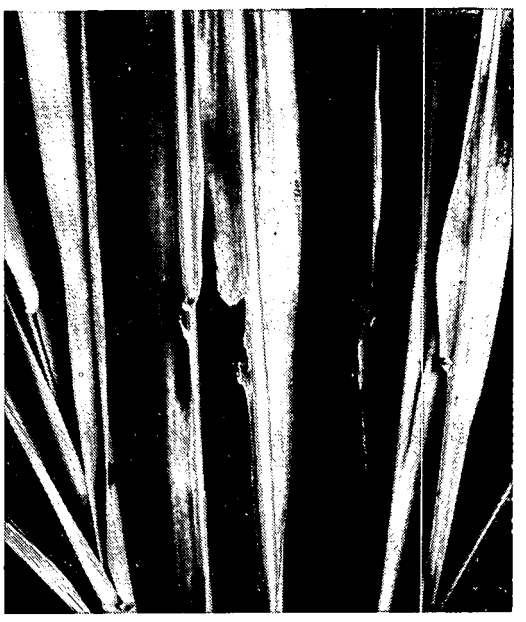
In 1964 it was found that slight damage caused by frost may result in wilting of the leaf tips or parts of the youngest leaves soon after exposure to freezing temperatures, and that these symptoms may in turn extend slowly over the whole canopy. These symptoms are usually found following exposure to temperatures down to − 2° C. for a short period, but in no case for longer than about one hour. In all save a very few cases, the susceptible growing point is not affected (Fig. 1), and no damage was caused to the protected rolled leaves of the spindle.

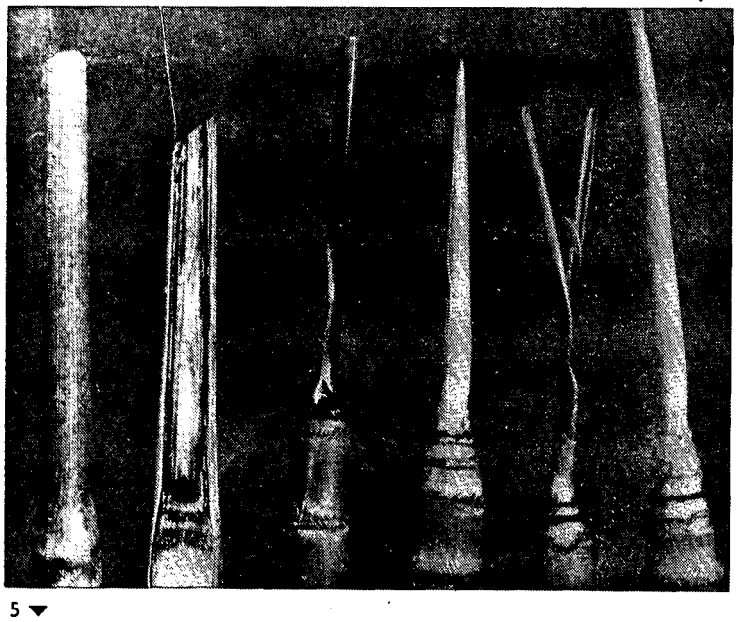
Yet another phenomenon which appears in cane exposed to mild frosts for a short period, is the evidence of damage found on leaves within the rolled spindle. These symptoms are illustrated in Fig. 2 and in this case they occurred at temperatures of not lower than - 1° C. They are invariably associated with the presence of small quantities of free water within the rolled spindle, and the damage is presumably caused by the formation of ice crystals. Extension of the damaged areas depends on the population of microbes present and on environmental conditions following frost. High humidity favours the development of micro-organisms within the damaged plant tissue, and a typical spindle rot may develop.

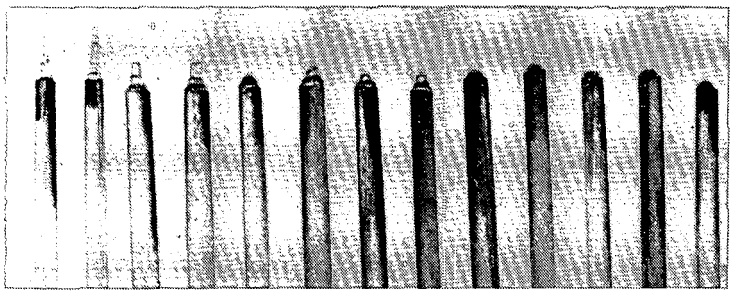
Damage to the spindle is influenced by groups of micro-organisms. In cases where saprophytic microbes alone are present, damage may be localised, but in other cases spindle rot can develop rapidly, reaching parts of the growing point. When this happens, the leaves of the spindle rot and the youngest leaves wilt. At the time, however, the canopy as a whole does not show any obvious symptoms of frost injury and, provided the growing point has not been completely killed by micro-organisms, the plants may still recover without serious ill effects.











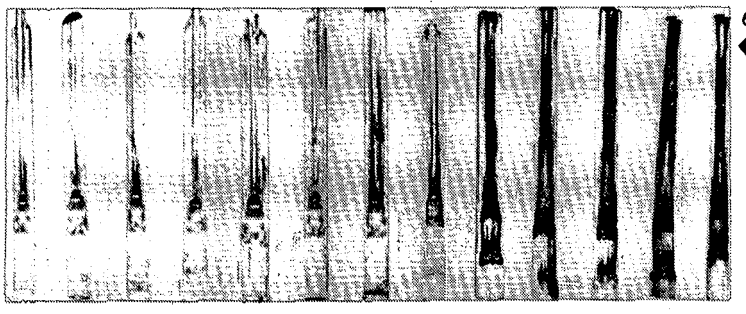


Fig. 1. Longitudinal section through growing points of cane where the canopy alone has been slightly damaged by frost.

Fig. 2. The spindle showing the innermost rolled leaves slightly damaged by frost.

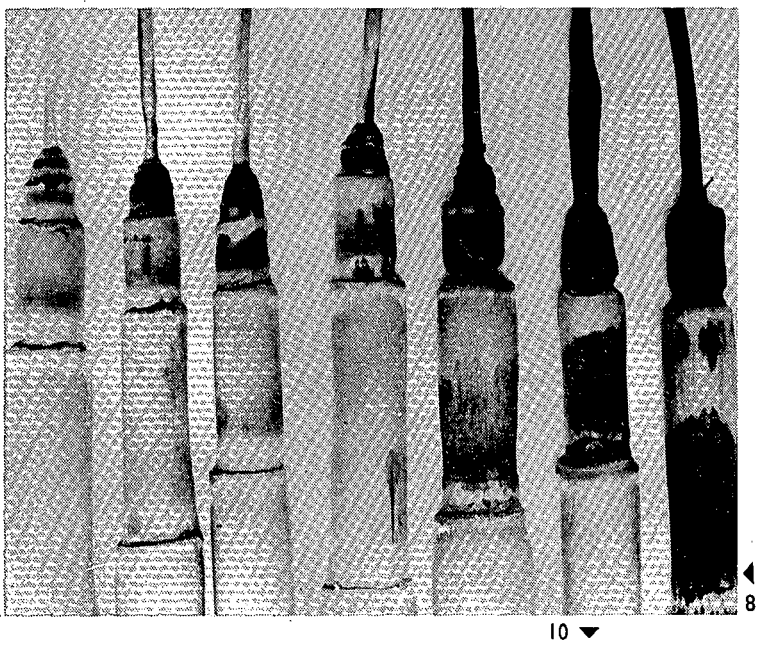
Fig. 3. Symptoms on the leaves a few months after the spindle was damaged by frost.

Fig. 4. Different degrees of regeneration with development of the side shoots.

Fig. 5. Tops of sugarcane exposed to a temperature of -2°C. in increments of half an hour. Left = control, right = 6 hr.

Fig. 6. Longitudinal sections of the sugarcane tops, illustrated in Fig. 5.

Fig. 7. Bundles of sugarcane which have been stood in fresh water for two weeks following treatment. Each bundle contains 11 sticks of cane each of a different variety. Treatments: Left = control. Right = 6 hr. at -2°C.



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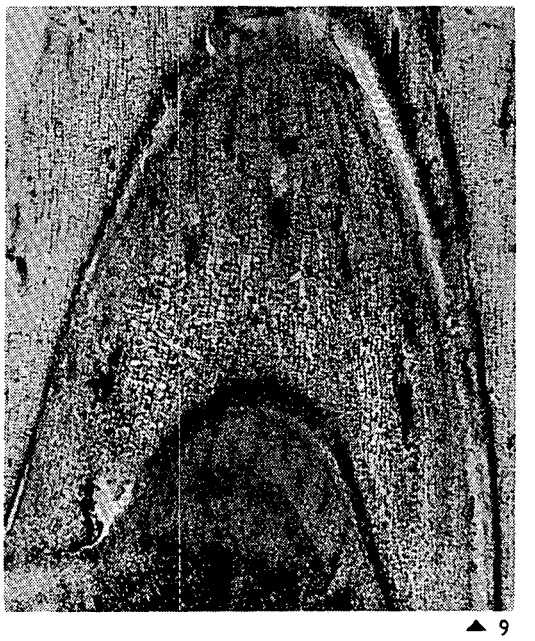

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Fig. 8. Frost damage at –2°C. Left to right (a) to (g). (*a*) Control, (*b*) 2 hr., (*c*) 3 hr., (*d*) 4 hr., (e) 6 hr., (*f*) 8 hr. and (*g*) 10 hr.

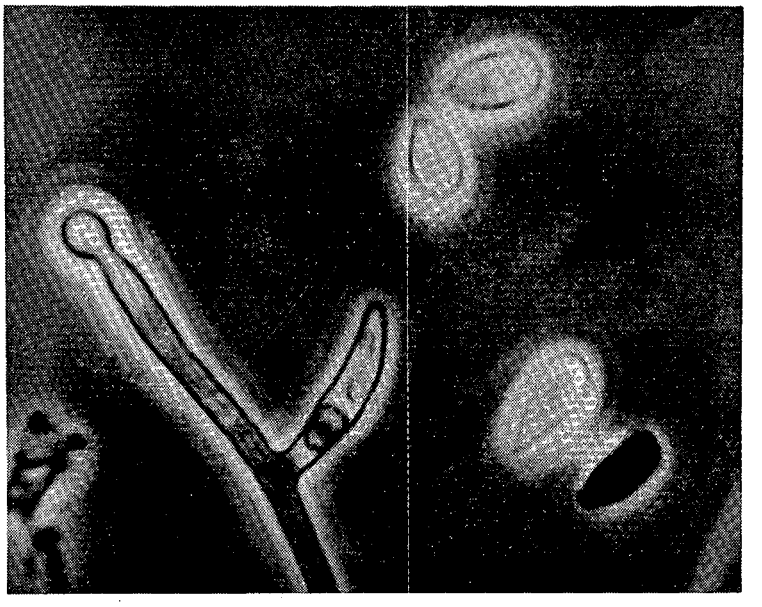
Fig. 9. Photomicrograph of a slightly damaged growing point.

Fig. 10. Hyphae and conidia of *Fusarium poae*, the most important fungus associated with heart rot of slightly frosted sugarcane.

Fig. 11. Yeast species on deteriorating sugarcane. Unidentified.

Fig. 12. Yeast species on deteriorating sugarcane. Unidentified.

Fig. 13. Bacteria on deteriorating sugarcane. Unidentified species.



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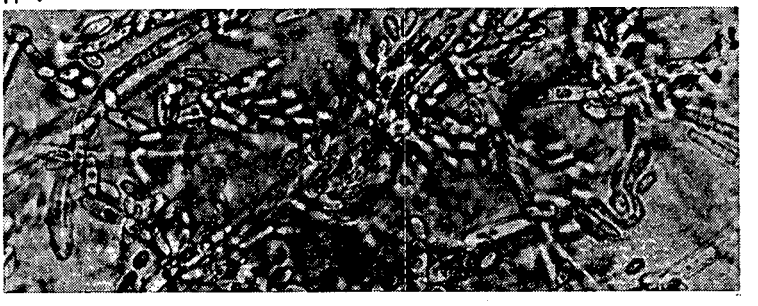

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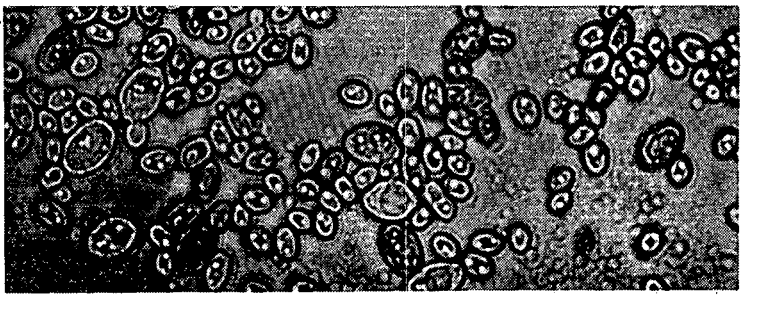

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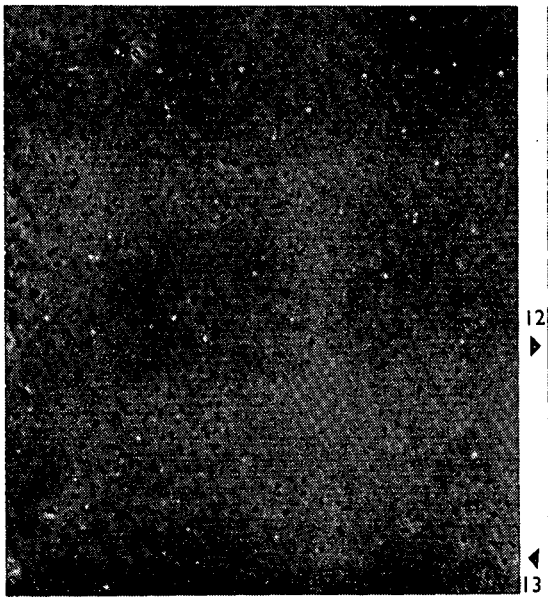

The damage caused by frost can usually be seen on cane for several months after it has been affected. Tissues in the injured lesions decompose and dry up, and then healthy tissue regenerates around these lesions (Fig. 3). Where the terminal growing point has been so badly damaged that it dies, then side shoots will develop (Fig. 4). However, where living cells remain and the growing point recovers, typical black rings or bands will be seen around the upper nodes for many weeks after the frost.

Cane does not normally deteriorate when the weather remains dry, even when the terminal point has been killed. However, when high humidity or rain, accompanied by warm weather follow frosting, the damaged cane will deteriorate rapidly. It has been found that where the damage extends downwards from the growing point for more than 4 in., most of the lateral buds will also have been injured and cane will have reached the category (*d*) defined by Wilson. At this stage, serious deterioration will occur when conditions favour growth of micro-organisms. Various categories of frost damage are illustrated in Fig. 5, 6 and 8.

Studies of Frost Damage

Field Cane

Frosted cane in fields in the Midlands and on the North Coast of Natal was examined during the winter of 1964. Samples of this cane were taken at intervals of four weeks and analysed to determine their microbe content. The samples used consisted of whole sticks taken from randomly selected sites. These sticks were examined in detail from the lowest bud to the terminal growing point. Well known micro-biological techniques were used to isolate and cultivate micro-organisms, the media utilised being:

(1) for bacteria: 1/3-D agar, pH 7.0,
(2) for bacteria and fungi: potato dextrose agar, pH 5.8 and 7.5,
(3) for yeasts: Sucrose-malt extract agar, pH 6.0; and yeast morphology agar B 393 (see DIFCO Manual, 9th Edition) pH 4.5.

A large number of micro-organisms have been isolated, a few of which were found in most of the samples examined, while the others were found only occasionally and have therefore been classified as casual infections. Samples were taken from the growing points of 50 sticks of cane collected in different fields. These revealed that more than 45 genera of bacteria were present, including at least 8 genera of yeasts and 11 genera of other fungi (Fig. 10-13). Longitudinal sections of the cane tops and growing points revealed a pinkish coloured, cotton-wool-like mycelium and the presence of a characteristic odour, symptoms which are typical of infection by *Fusarium poae*. It was this fungus, together with *Fusarium moniliforme* which proved to be the only organisms closely associated with spindle rot in the field (Fig. 10) and it is they which harm the frost damaged growing point. All other fungi found on frosted cane behave as saprophytes, and have been ignored in this study.

Artificial Frosting

Experiments were carried out to study the effect of frost on eleven varieties of cane, namely Co.331; N:Co.293; N:Co.310; N:Co.376; N.50/211; N.50/805; N.51/168; N.51/539; N.53/216; C.B.28/22; and C.B. 36/14. The cane was subjected to low temperatures in a freezing chamber, which measured 6 ft. by 11 ft. by 15 ft. 3 in. Temperature treatments were: - 2° C. Individual cane samples exposed to this temperature for periods varying from 1 to 10 hours, in increments of 1 hour; to -4° C. for periods varying from half an hour to 6 hours, in increments of half an hour; and to -6° C. for periods varying from half an hour to four hours, in intervals of half an hour.

The cane used in these experiments was cut not more than 4 hours before it was treated. The sticks were severed at the surface of the soil, using shears made for this purpose, and after cutting they were packed in bundles for treatment.

Assessing Frost Damage

To assess the damage caused by frost, the five uppermost nodes, including the terminal growing point, were sectioned longitudinally. Four categories of damage were defined and this classification was used to record frost injury. The germination capacity of the frosted cane was also compared with unfrosted samples. Two methods were used to establish germination capacity. In the first, sticks were cut to provide single bud setts, which were put into plastic tubes of 2.5 in. diameter and then kept in the dark for four weeks. The second method employed, involved placing whole sticks in water, which was kept fresh and shaded for a period of four weeks.

Studies of Microbes

Analysis of the microbe populations in cane were carried out on material treated, or to be treated, in the freezing chamber. These analyses were restricted to the variety N:Co.376 and they were carried out both immediately before subjecting cane to low temperatures — but about four hours after cutting — and one week and four weeks after treatment. The treatments involved in this case were:

Cane kept for 2 hr., 5 hr. and 10 hr. at a temperature of - 2° C.
Cane kept for 4 hr. at a temperature of - 4° C.
Cane kept for 4 hr. at - 6° C.

Samples were taken from the terminal growing point and the penultimate node at the base of each harvested stick, and these were analysed. A sterile cork borer was used for sampling and this yielded cores which were identical in size. In each case the core was 0.5 by 1.0 cm. in size, and before it was used it was first homogenized in 10 cc. of 0.7 sterile salt solution and then gradually diluted using a standard dilution technique. The number of micro-organisms present was, in each case, determined by averaging the counts from 4 samples, using the agar plate technique. The data obtained is shown in Table II and they indicate that the greater the damage caused by frost, the more quickly the various micro-organisms responsible for cane deterioration develop.

Table II

The number of micro-organisms per core sample (0.2 cub. cm.) of the variety N:Co.376, when this has been exposed for varying periods to different intensities of sub-zero temperature.

Frost treatment / Analysis	Control		The number of micro-organisms in cane subjected to differing intensities and duration of frost									
			— 2° C.						— 4° C.		— 6° C.	
			2 hr.		5 hr.		10 hr.		4 hr.		4 hr.	
	G.P.*	node	G.P.	node	G.P.	node	G.P.	node	G.P.	node	G.P.	node
4 hr. after cutting												
Bacteria . . .	2.4×10^2	5.9×10^3	—	—	—	—	—	—	—	—	—	—
Yeasts . . .	3.2×10^1	1.8×10^1	—	—	—	—	—	—	—	—	—	—
Fungi . . .	1.7×10^1	4.1×10^1	—	—	—	—	—	—	—	—	—	—
1 week after cutting												
Bacteria . . .	9.7×10^2	6.5×10^3	7.8×10^3	7.4×10^3	8.9×10^3	1.3×10^4	9.4×10^4	6.5×10^4	7.8×10^4	6.3×10^4	1.2×10^5	9.6×10^4
Yeasts . . .	2.2×10^2	3.7×10^2	4.9×10^2	$5\,8 \times 10^2$	4.1×10^2	5.6×10^2	3.7×10^3	2.9×10^3	4.5×10^3	2.8×10^3	6.6×10^3	5.2×10^3
Fungi . . .	7.9×10^1	6.8×10^1	1.9×10^2	5.7×10^1	5.0×10^2	8.4×10^1	9.5×10^2	3.1×10^2	1.7×10^3	5.5×10^2	1.8×10^3	4.7×10^2
4 weeks after cutting												
Bacteria . . .	9.6×10^3	4.3×10^4	2.8×10^4	7.1×10^4	6.3×10^4	7.7×10^4	1.4×10^6	9.7×10^5	2.7×10^6	5.9×10^5	4.2×10^6	1.2×10^6
Yeasts . . .	5.2×10^2	8.1×10^3	8.5×10^2	9.2×10^3	5.3×10^3	9.6×10^3	6.5×10^4	2.3×10^5	7.8×10^4	4.2×10^5	5.9×10^4	1.9×10^5
Fungi . . .	2.5×10^3	1.2×10^2	1.9×10^3	3.9×10^2	2.9×10^3	6.7×10^2	7.2×10^3	3.6×10^2	6.9×10^3	7.9×10^2	8.9×10^3	1.7×10^3

* G.P. = Growing point.

Experiments

Two detailed experiments were put down to determine the effect on individual tissues, of exposure to frosts of different severity for varying lengths of time. In the first of these, eleven varieties of sugarcane were exposed to artificially created temperatures of - 2° C., - 4° C. and - 6° C. Injury to the uppermost 5 nodes including the growing point, and to buds, starting with the 6th and extending to the bottom of the stick, was recorded by scoring from 0 to 5, 0 being interpreted as representing undamaged cane or cane with only the faintest trace of damage, while 5 represents a complete kill. The results of treatments at - 2° C. and - 4° C. are summarised in Fig. 14A.

It can be seen from Fig. 14A that both nodes and buds are damaged within half an hour, when the temperature is kept down to - 4° C., and that this damage increases with the length of time of exposure. After 3 hours at this temperature, damage to the nodes and death of the buds exceeds an estimated 80 per cent. When placed in plastic tubes, single bud setts of cane which had been subjected to this low temperature were, after 4 weeks, so inundated with micro-organisms that the frost-damaged buds were completely water-soaked.

Cane in one replicate in this experiment was kept for 4 weeks with its lower nodes immersed in fresh water to a depth of 9 in. — the water being changed every second day. Four sticks of each of the eleven varieties were used for juice quality tests and the data obtained are summarised in Table I.

Table I

Sucrose percentage, brix and purity of cane, 4 weeks after harvest and exposure to sub-zero temperatures.

(Mean of 11 varieties)

Temperature	*Hours*	*Sucrose*	*Brix*	*Purity*
— 2° C.	Control	9.42	15.2	77.5
	1 to 2 hr.	7.76	13.6	71.1
	3 to 4 hr.	7.85	14.0	69.9
	7 to 8 hr.	6.78	13.9	60.6
	9 to 10 hr.	5.33	12.7	51.6
— 4° C.	½ to 1½ hr.	7.18	13.8	64.4
	2 to 2½ hr.	6.97	13.8	62.5
— 6° C.	1½ to 2 hr.	4.27	12.6	40.7

No significant differences in the frost resistance of different varieties was found in the first experiment. Indeed, differences between individual sticks of the same variety were greater than those found between varieties. A second trial was therefore put down, involving only seven varieties of sugarcane and exposure to only - 2° C. for periods of 1 to 8 hours, in increments of 1 hour. Once again, variations in frost hardiness between sticks of the same variety was so great that no statistically significant varietal differences were found. The results from this trial are shown graphically in Fig. 14B.

Results and Conclusions

(1) Damage caused by frost increases as the temperature drops, and as the period of exposure lengthens. Thus, a light frost in which the temperature does not fall below - 2° C. and does not continue longer than 1 hour, causes only superficial damage to the canopy, or slight injury to the leaves of the spindle. However, where frost of a similar intensity lasts for more than 1 hour, but less than 2 hours, it may cause minor damage to the canopy and the spindle, and eventually to the growing point. The extent of this injury will vary according to the conditions under which cane has been grown and its age. Young cane is more susceptible to frost than older cane.

(2) At temperatures below - 2° C. cane suffers more noticeable damage. Severe damage is caused by exposure to temperatures of - 4° C. and - 6° C. for half an hour.

(3) The terminal growing point is more easily damaged by frost than any other part of the plant. Greatest frost resistance is found at the internodes, and at the nodes and the buds towards the bottom of the stick.

(4) Frost injury starts where cells have been separated by rifts along the middle lamellae, the cells becoming isolated from the neighbouring tissues and then dying. Complete recovery may occur when only a relatively small peninsula of uninjured cells remains in the growing point or in the axillary buds, provided that these are not isolated from uninjured sections of the vascular bundles.

(5) Deterioration of tissue, both at the growing point and at the nodes following damage by frost, is associated with a tremendous increase in the population of microbes in these tissues.

(6) The most harmful fungi attacking, the frost damaged growing point are *Fusarium poae* and *F. moniliforme*. These are responsible for "heart rot" of cane.

(7) Juice quality of harvested cane, which has been stored standing in fresh water in the shade, is not affected by frost when the temperatures at harvest drop to a mere - 2° C., even when this low temperature is maintained for up to 8 hours. However, when cane has been seriously damaged by frost, as is the case when it is exposed to temperatures of - 4° C. and - 6° C., then there is a very marked drop in sucrose percentage and purity. This drop is associated with an increase in the population of micro-organisms during storage.

(8) Eleven varieties were examined for their resistance to frost, but no sound indications of resistance were found.

(9) The data obtained in these studies should help the cane grower to determine the extent of frost damage suffered by his crop. It should also enable him to decide whether to cut his cane to avoid

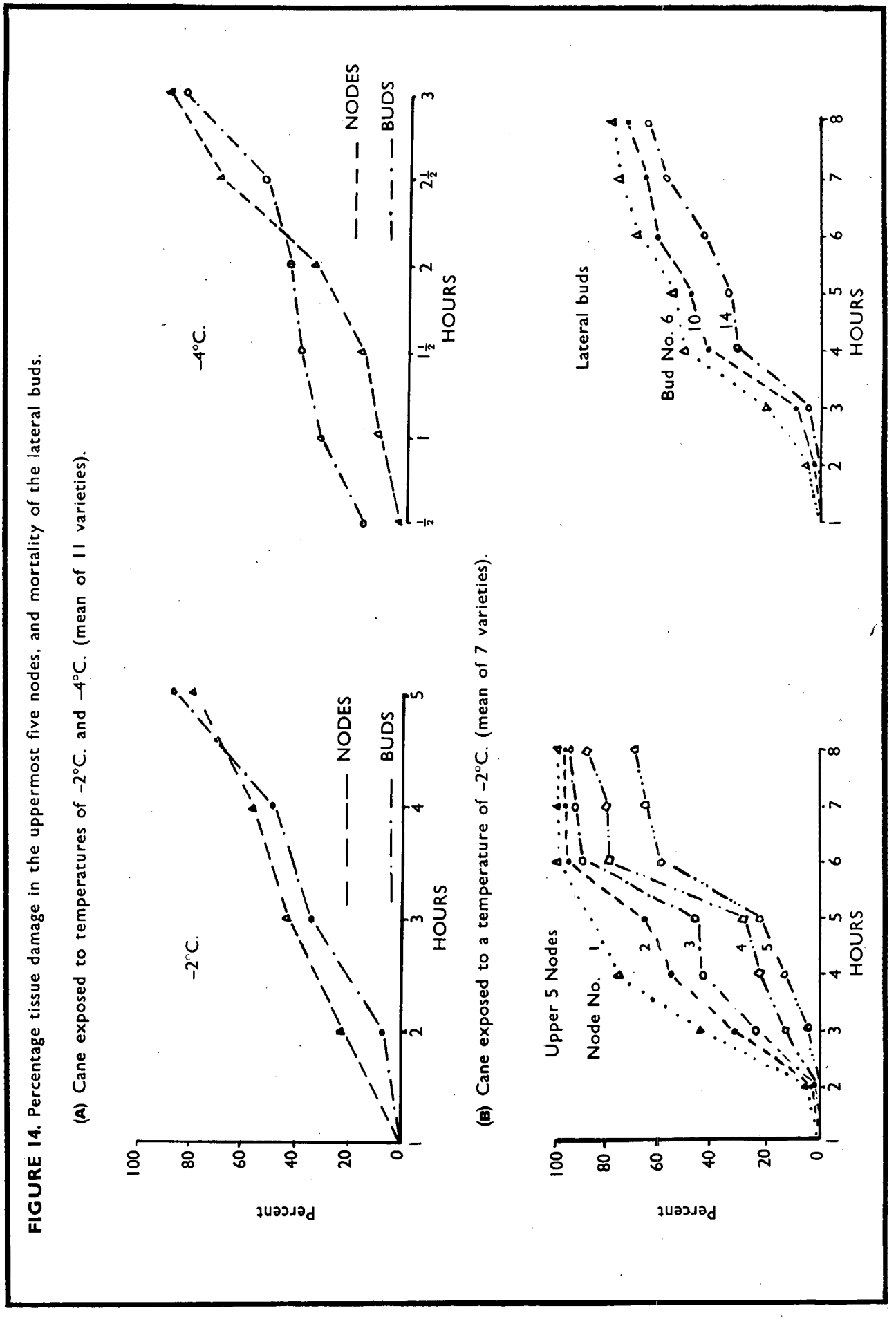

FIGURE 14. Percentage tissue damage in the uppermost five nodes, and mortality of the lateral buds.

loss or if he should allow it to regenerate. Alternatively, he will be able to judge how quickly frost-damaged cane will deteriorate.

Summary

In the winter of 1964, sugarcane in several parts of South Africa suffered serious damage as a result of unusually harsh frosts. Studies of the effects of frost damage were carried out and, in this paper, the nature of the damage is described. Investigations include the effect of frost on the quality of cane juice, the relative susceptibility of different varieties, the identification of micro-organisms associated with damaged tissue and their influence on purity, brix and sucrose percentage.

To enable this investigation to be carried out, it was necessary to supplement studies of frost damage in the field, by inducing frost damage in cooling chambers. Details are given of these studies. Damage to cane was negligible at temperatures down to – 2° C., provided the exposure period did not exceed 1 hour. Longer periods of exposure, or a further lowering of temperature, have a steadily greater impact. The terminal growing point has proved to be the tissue which is most susceptible to frost damage. The uppermost nodes are also liable to suffer damage quite easily, but the lower down the stem they are located, the more frost hardy they are.

The most important micro-organisms affecting cane are two semi-parasitic fungi *Fusarium poae* and *F. moniliforme*. No differences could be found in the susceptibility of varieties to frost damage. The data obtained from this investigation should, however, help the grower to decide on the action he should take following a frost.

Acknowledgment

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