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REDUCTION OF BANANA ANTHRACNOSE FOLLOWING HOT-WATER TREATMENT OF THE GREEN FRUIT

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SUMMARY

The tolerance of banana fruit to hot-water dipping was established. Complete control of artificially induced latent infections of *Colletotrichum musae* was achieved in the laboratory by dipping green fruit in hot water at 55°C for 2 min before ripening. This treatment also effectively destroyed conidia of the fungus.

In larger semi-commercial scale experiments using naturally infected fruit, significant but not complete control of anthracnose and crown rot was obtained.

Some delay in ripening was caused by the hot-water treatment, but this was variable. The rate of pulp ripening was not affected by the treatment.

I. INTRODUCTION

In Queensland-grown bananas, most losses from ripe fruit rots are due to infections by *Colletotrichum musae* (Berk. and Curt.) Arx (*Gloeosporium musarum* Cke. and Mass.) (Hoette 1935; Simmonds and Mitchell 1940; Simmonds 1941, 1963).

In Australia it is customary to consider *C. musae* infections as falling into two categories: lesions occurring on the body of the fruit, mainly latent infections, have been termed anthracnose, while infections of the finger stalk have been referred to as "black-end" (Young *et al.* 1932; Hoette 1935). The latter form of infection was associated with the local custom of marketing bananas in the form of single fruit, removed from the hand by the grower and packed into wooden cases (Young *et al.* 1932). In south-eastern Queensland, however, most of the fruit now coming onto the market is in the form of hands packed

in fibreboard cartons. As a consequence, black-end has given place to comparable infections of the cut surface of the cushion-like stalk which attaches the hand of fruit to the main bunch stalk. Up to date this form of anthracnose has been referred to in Queensland as "cushion rot", but since it is already known in the U.S.A. as "crown rot" (Green and Goos 1963) this term will be adopted so as to avoid confusion. This disease commences to develop during the ripening process, and on removal of the fruit from the ripening room the rot progresses rapidly from the crown into the individual fruit stalks, causing the fruit to drop from the hand. As with black-end, these crown rots are mostly due to *C. musae*.

It has been demonstrated that crown rot is caused by conidial infections of the cut surface (Green and Goos 1963) but there is an alternative possibility, namely that the rots in this region may develop from already established latent infections which are stimulated into activity following the wounding inflicted at dehanding (Simmonds 1963).

A wide range of fungicides and other chemicals has been used experimentally in attempts to find a practical means of reducing the losses due to *C. musae*. The work in this field was summarized by Meredith (1961). None of the chemical treatments examined up to that time gave consistent control of the disease at economic levels. The use of field sprays for the protection of bunches before harvesting is not economic.

Simmonds and Mitchell (1940) found that anthracnose and black-end infections were reduced by dipping green fruit in a hot borax solution at 50–54°C for 5 min. They did not establish at that time, however, whether the reduction in the disease was due to the temperature of the solution, to the presence of the borax, or to both. In recent years the effectiveness of thermotherapy has been demonstrated in the control of ripe fruit rots or storage rots of papaws (Akamine and Arisumi 1953), peaches (Smith 1962; Smith and Bassett 1964), nectarines (Smith and Bassett 1964), mangoes (Pinnock and Maldonaldo 1962; Smoot and Segall 1962), apples (Burchill 1964) and oranges (Smoot and Melvin 1964). Since latent anthracnose infection in the banana presents a similar problem to the fruit rots referred to above, and since Simmonds and Mitchell (1940) showed that green bananas survived immersion at temperatures of up to 50°C for 5 min without any obvious effect on subsequent ripening, it was apparent that heat treatment might provide a means of minimizing the effect of infections by *C. musae*.

II. MATERIALS AND METHODS

Fruit.—The fruit used in these experiments was of the Cavendish type. Bunches of green fruit were selected at the light full three-quarters to full three-quarters stage of maturity (von Loesecke 1949). In each experiment, the green fruit from the hands or bunches was distributed as evenly as possible over the various treatments so as to reduce variability in reaction and in infection.

Inoculation.—In experiments carried out between April and December, when the natural incidence of anthracnose is low, the experimental fruit were artificially inoculated using a suspension in sterile water of *C. musae* spores

obtained from actively sporulating cultures of the fungus growing on potato dextrose agar. The concentration of spores in the suspension was adjusted to approximately 40,000 per ml and the suspension was sprayed onto the fruit by means of a hand atomizer.

Inoculated fruit were held for 24 hr after inoculation in 0·015-in. thick clear polythene bags. As much air as possible was trapped in the bags when they were closed by means of rubber bands, leaving them partially inflated. At the end of this time the inoculated fruit were transferred to brown paper wraps for a further 24 hr before being treated. This 48-hr incubation period at room temperature was adopted to ensure that a reasonable number of latent infections would be established on the fruit (Simmonds and Mitchell 1940), and transfer from the polythene bags was to avoid skin damage which occurred if the fruit were left in the bags for the full 48 hr.

Inoculation of fruit in marked areas for laboratory experiments was carried out as described by Simmonds and Mitchell (1940) and Simmonds (1963). Fruit inoculated in this way were incubated under belljars with cotton-wool plugs for 48 hr.

Hot-water treatments.—Hot-water dipping experiments in the laboratory were carried out in an unlagged metal dish containing 2 gal of water heated by means of a Braun Thermomix. There was no appreciable drop in temperature when up to 12 single fruit were immersed at any one time, and the temperature was maintained to within ±0·2°C throughout the dipping period.

Up to 100 single fruit or the equivalent in part or full hands were dipped in a lagged 25-gal water-bath heated by a 3-kW heater supplemented by two Braun Thermomixes. This kept the fluctuations in temperature within the same range as the laboratory experiments.

In large semi-commercial scale experiments involving several cartons of hands of bananas, dipping was carried out in either a 300-gal steam-jacketed pan, in which the water was agitated by means of an electrically driven propellor type agitator, or in a 100-gal stainless-steel tank heated by means of three 3-kW immersion heaters, the water being recirculated rapidly by means of a centrifugal pump. Temperature readings in various positions within these two tanks showed that the temperature was uniform throughout. Because of the bulk of water, a drop of only 0·1°C occurred when up to 12 hands of fruit were immersed in either tank. Fluctuation of temperature was restricted without difficulty to ±0·2°C by manual control of the heating equipment.

In all except the laboratory experiments, the fruit were placed in a 1-in. mesh, 8-gauge galvanised wire cage for dipping.

Ripening.—All the fruit was ripened under normal commercial conditions. Where single fruit were used they were allowed to cool after dipping and packed in half-bushel wooden cases lined with paper and closed with nailed wooden

lids. Where a number of treatments were replicated within the cases, the various treatments were separated by vertical corrugated cardboard partitions. Hands of fruit used in larger scale experiments were packed in the current commercial manner in fibreboard cartons without lids.

Experimental fruit were placed in ripening rooms with normal commercial consignments and given the same ripening conditions. Air temperatures in these rooms is maintained between 18 and 19°C for the first 24 hr, and is dropped to 15·5°C on the second day. From the third day onwards the temperature is varied between 10 and 13°C according to the progress of ripening in relation to the current demand for fruit. Normal ripening time is between 5 and 7 days. Two applications of 100 p.p.m. ethylene gas are made during the first 2 days, with an interval of 24 hr between applications.

Disease rating.—After removal from the ripening room, the fruit were retained for a further 4 or 5 days at room temperature to simulate the degree of ripeness which would normally have been reached at the time of consumption. Ripeness was assessed in accordance with the coloured chart of von Loesecke (1949). Ratings were made when control fruit had reached stage 8 of ripeness according to this chart.

Anthracnose ratings were divided into 5 categories—0, no infection; 1, up to 25% of surface area of fruit affected; 2, between 25 and 50% of surface area affected; 3, between 50 and 75% of surface area affected; 4, 75% to total surface area infected. These categories were applied to both single fruit and hands.

Black-end ratings were made on single fruit. Fruit stalks into which the rot had spread 5 mm or more at the time of rating were classified as having black-end. No gradings as to degree of infection were made. Results are expressed as percentage of fruit infected.

Crown rot was rated according to the depth of penetration of the rot into the crown or beyond, the ratings being: 0, no infection; 1, rot penetrating up to 5 mm into crown; 2, penetration to junction of crown and finger stalks; 3, penetration up to 5 mm into finger stalks; and 4, penetration of fruit stalk sufficient to cause fruit drop.

III. EXPERIMENTAL AND RESULTS

The following experiments were carried out between April 1963 and April 1965.

(a) Effect of Hot-water Treatment on Fruit Ripening

Before examining the value of hot-water treatment in controlling the development of disease in ripening bananas, it was necessary to determine the tolerance of bananas to such treatment and the subsequent effect on ripening.

Experiment 1.—In this experiment, 4 lots of 12 fruit each were dipped for 15 min in water at temperatures of 31, 50, 52·5 and 55°C respectively. Treated fruit were ripened for 7 days.

When removed from the ripening room, the fruit heated at 52·5 and 55°C were split and their skins were blackened. Those treated at 31 and 50°C were at stages 7 and 5 of ripeness respectively. The delay in ripening caused by the 50°C treatment affected only skin coloration; pulp ripening proceeded normally as in the 31°C treatment.

Experiment 2.—Since the fruit heated at 50°C in experiment 1 survived the 15-min treatment without appreciable skin damage, further treatments were carried out at this temperature for varying times. Four lots of 24 fruit each were treated in water at 50°C for 0, 5, 10 and 15 min respectively. The fruit were ripened for 6 days.

The time taken for fruit to reach stage 8 ripeness was recorded for the various treatments. The delays in ripening time shown by the 5, 10 and 15 min treatments were 3, 4 and 7 days respectively as compared with control fruit.

Experiment 3.—Owing to the delay in ripening caused by hot-water treatment at 50°C for 5 min and longer, the effect of a higher temperature for a shorter time was examined. Four lots of 24 fruit each were treated for 2 min at room temperature, 55, 60 and 65°C respectively. Fruit were ripened for 7 days.

The fruit treated at 60 and 65°C suffered skin blackening and splitting during the period of treatment and were at once discarded. The delay in ripening shown by the bananas heated for 2 min at 55°C was 2 days as compared with control fruit. The appearance of the heat-treated fruit after ripening was very satisfactory.

(b) Effect of Heat Treatment on Spores of *C. musae*

In order for hot-water dipping of bananas to be effective as a means of disease control, it would be necessary for any spores which may be present on the surface of fruit to be destroyed by the treatment.

In experiment 4, the survival of *C. musae* spores following exposure to hot water at 50 and 55°C for 0, ½, 1, 1½ and 2 min was determined.

Test-tubes each containing 10 ml sterile tap water were heated in a constant-temperature water-bath. When the water in the tubes reached the required temperature, three drops of a concentrated spore suspension were added to the hot water in each test-tube. The tubes were maintained at the treatment temperature for the appropriate time after the addition of the spores, and were then rapidly cooled in iced water. Two tubes were used in each treatment.

When the tubes were cool, 1 ml of spore suspension was added to 9 ml melted sterile potato dextrose agar held at 45°C. The mixture was shaken well and poured into a sterile petri dish. The petri dishes were incubated for 3 days

at 28°C. Four plates were prepared for each treatment. At the end of the incubation period the plates were examined for germination of the *C. musae* spores.

The results of this experiment are shown in Table 1. They indicate that spore germination was inhibited by treatment at 55°C but not by treatment at 50°C.

TABLE 1

GERMINATION OF *C. musae* SPORES FOLLOWING HOT-WATER TREATMENT

Time (min)	Temperature	
	50°C	55°C
Nil	+	+
½	+	−
1	+	−
1½	+	−
2	+	−

+ = Germination

− = No germination

(c) Effect of Hot-water Treatment on Latent Infections

Three experiments were carried out to determine the time and temperature of hot-water dipping required to destroy latent infections.

Single green fruit were used. The fruit were inoculated in marked areas, with five marked circles on each banana. They were incubated to allow infections to develop and then treated.

In experiment 5, eight fruit were used in each of five treatments. These treatments consisted of dipping the fruit in hot water at 55°C for 0, ½, 1, 1½ and 2 min. In experiment 6, four fruit were used in each of five treatments which involved dipping fruit for 2 min in water at 50, 51, 52, 53 and 54°C. In experiment 7, 15 fruit were used in each of the two treatments employed, namely: control fruit which were not dipped, and fruit dipped in hot water at 55°C for 2 min. This experiment sought confirmation of the results of experiments 5 and 6.

In all three experiments, isolations were made onto potato dextrose agar from each of the inoculation sites after the fruit had cooled. One isolation was made from each marked area and all isolations from any one fruit were placed in the same petri dish. Standard-size isolations were made by punching a 4-mm diam. circle within the inoculation area, using a sterile cork borer, and removing the disc of skin about 1 mm thick with a sterile scalpel. The plates were incubated for 5 days at 25°C, after which counts of *C. musae* colonies were made.

The results shown in Table 2 suggest that the threshold treatment for controlling latent infection may be 2 min at 52°C.

TABLE 2

EFFECT OF HOT-WATER TREATMENT ON LATENT INFECTIONS OF *C. musae* ARISING FROM LOCALIZED INOCULATION

Treatment	Experiment 5			Experiment 6			Experiment 7		
	Temp. (°C)	Time (min)	Colonies *C. musae**	Temp. (°C)	Time (min)	Colonies *C. musae***	Temp. (°C)	Time (min)	Colonies *C. musae****
1	55	0	25	room temp.		11	room temp.		47
2	55	½	15	50	2	7	55	2	0
3	55	1	8	51	2	6			
4	55	1½	1	52	2	0			
5	55	2	0	53	2	0			
6				54	2	0			

* 40 isolations. ** 20 isolations. *** 75 isolations.

(d) Effect of Hot-water Treatment on Semi-commercial Batches of Fruit

These experiments were carried out to compare the effects of hot-water treatment on a semi-commercial scale with the results obtained in laboratory experiments.

In experiment 8, four 30-lb lots of bananas were used in each treatment. Fruit were treated for 2 min at 52, 53, 54 and 55°C, with untreated control fruit for comparison. The fruit used were not inoculated.

The bunches of bananas used were dehanded and the hands were washed until the sap flow ceased, as in normal commercial practice. The hands were then treated, allowed to cool and packed into cartons for ripening.

Results are shown in Table 3. They indicate that on a commercial scale a temperature of at least 54°C for 2 min would be preferable for disease control.

TABLE 3

AVERAGE ANTHRACNOSE AND CROWN ROT RATINGS PER HAND IN EXPERIMENT 8

Treatment			Anthracnose	Crown Rot
Temperature (°C)		Time (min)	Mean	Mean
A	control		0·688	2·69
B	52	2	0·758	2·56
C	53	2	0·258	2·22
D	54	2	0·267	1·15
E	55	2	0·300	1·53
S. E. difference			±0·018	±0·30
Necessary differences for significance		5% level	0·252	0·63
		1% level	0·349	0·87
			A, B ⩾ C, D, E	A, B, C ⩾ D A, B ⩾ E C > E

Three further experiments were conducted in which fruit treated at 55°C for 2 min were compared with untreated control fruit. Dehanding, washing, treatment and ripening were carried out as already described. The fruit used in these experiments were not inoculated.

Details of the various experiments and the results are included in Table 4. The results show that on a semi-commercial scale, heat treatment gives significant control of anthracnose and crown rot.

TABLE 4

AVERAGE ANTHRACNOSE AND CROWN ROT RATINGS PER HAND IN EXPERIMENTS 9, 10 AND 11

Experiment No.	9*		10*		11*	
Type of Infection	Anthracnose	Crown Rot	Anthracnose	Crown Rot	Anthracnose	Crown Rot
Treatment	mean	mean	mean	mean	mean	mean
Control (0)	no anthracnose incidence	0·43	3·31	3·52	3·47	3·74
55°C for 2 min. (1) ..		0·29	3·17	3·38	3·31	3·57
Standard error of mean ..		±0·01	±0·01	±0·01	±0·01	±0·01
Least significant difference—						
5% level		0·04	0·04	0·03	0·05	0·07
1% level		0·05	0·05	0·04	0·06	0·10
		1 ≪ 0	1 ≪ 0	1 ≪ 0	1 ≪ 0	1 ≪ 0

* Number of hands of fruit used

Experiment ..	9	10	11
Control ..	75	96	68
55°C for 2 min	113	96	60

In these semi-commercial scale experiments, there was some variation in the rate of ripening. In some instances the heat-treated fruit showed no delay in the development of skin colour, while in others there was a delay of up to 2 days in skin coloration, the degree of ripeness displayed by the heat-treated fruit varying between stages 4 and 7 when control fruit were at stage 7.

IV. DISCUSSION

Hot-water treatment of bananas shows some promise as a means of control for anthracnose and crown rot. However, the expense of applying such a treatment commercially is not warranted at this stage since the degree of control achieved in the semi-commercial scale trials was less than in laboratory experiments.

It is probable that where hands of fruit are used the circulation of water through the fruit may be less effective than when using single fruit in the

laboratory. Failure of circulation through the hands may lead to localized cooling of trapped water, or even to trapped air pockets, with consequent failure to destroy infections due to insufficient heating.

The variation in ripening rate shown by heat-treated fruit cannot be accounted for at this stage.

Further investigations are warranted in an attempt to reduce variability and to improve the general degree of control.

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