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EFFECT OF COLLETOTRICHUM MUSAE INFECTION ON THE PRECLIMACTERIC LIFE OF BANANAS

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SUMMARY

Development of *Colletotrichum musae* infection of the stem end of green bananas has been shown to reduce the green-life of the fruit. The amount of green-life lost was found to be dependent on the concentration of inoculum used to initiate infection and on the stage of maturation of the fruit. Disease development, measured as millimetres of rotted stem end tissue 6 days after inoculation, was also shown to vary with these two variables. Diseased fruit have been shown to have greatly increased ethylene production rates.

I. INTRODUCTION

The organism *Colletotrichum musae* (Berk. & Curt.) Arx has been recorded in most banana-growing regions of the world. The various diseases it initiates have been estimated to cause considerable wastage within the banana industry (Wardlaw and McGuire 1931; Young *et al.* 1932; Simmonds and Mitchell 1940; Meredith 1961). Diseases caused by this organism originate from both latent and wound (non-latent) infections. In bananas, latent infections generally do not develop until fruit commence to ripen (Simmonds 1963), while with wound infections the damage tissue can be rapidly invaded even though the fruit may be immature—that is, still in the preclimacteric state (Meredith 1961). These diseases cause most wastage through spoiling the appearance of fruit, thus lowering its market value, rather than rendering the fruit inedible.

There is some evidence that wound infections on preclimacteric fruit have yet another detrimental effect. Agati (1922) reported that, in the Philippines, wound infection by *C. musae* was common within plantations, and that infected fruit ripened prematurely. Tomkins (1931) observed that rots due to *C. musae* may affect the "rate of ripening" of bananas, but stated there was no good evidence for this. It is not clear whether, by "rate of ripening", this author meant the duration of the preclimacteric phase (green-life—Peacock and Blake 1970) or the rate at which ripening proceeds once it has commenced.

It thus appears that wound infections by this organism may reduce green-life. Such an effect may be of commercial significance, since even though the symptoms may not develop sufficiently to disfigure a fruit, green-life may still be so shortened that the fruit may ripen *en route* to a market.

This investigation was undertaken to ascertain whether infection by *C. musae* would shorten the green-life of Queensland-grown bananas. When an initial trial demonstrated that this was so, a second trial was conducted to determine if the results could perhaps be explained by an effect on the ethylene production rate of infected tissue.

II. MATERIALS AND METHODS

Bananas (cv. Giant Cavendish) used in this study were obtained locally.

Trial 1

Fifteen bunches were chosen, as judged by the degree of filling of the fingers, to be at different stages of maturation, that is, having different green-lives. On the day of harvest, fruit from two adjacent hands of each bunch were randomly distributed amongst six treatments, providing six fruits per treatment.

In each treatment fruit were dipped for 10-15 sec in the following solutions.

Treatment 1: 400 p.p.m. thiabendazole (TBZ).
Treatment 2: Water.
Treatment 3: *C. musae* spore suspension, 520 spores/ml.
Treatment 4: *C. musae* spore suspension, 5,200 spores/ml.
Treatment 5: *C. musae* spore suspension, 52,000 spores/ml.
Treatment 6: *C. musae* spore suspension, 520,000 spores/ml.

The thiabendazole treatment was included in an attempt to prevent infection from natural spore loads, thus providing a control treatment in which no disease would develop (Scott and Roberts 1967).

All solutions contained sufficient "Agral 60", a commercial wetting agent, to ensure fruit were wet uniformly. After dipping, fruit were held in a specially designed cabinet which was ventilated with humidified fresh air at a rate in excess of 100 ml/min/fruit. The cabinet was temperature controlled and was maintained at 20°C. Green-life was measured as the time that elapsed from harvest until the first detectable change in skin colour occurred.

In almost all instances, disease development commenced only at the proximal end of the fingers where they had been damaged by dehanding. The distance along the finger that rotting had progressed by the sixth day after dipping was measured (Scott and Roberts 1967).

Trial 2

Fruit from one bunch of bananas were randomly divided between two treatments:

Treatment 1: Fruit dipped in TBZ.
Treatment 2: Fruit dipped in *C. musae* spore suspension containing 42,000 spores/ml.

Because disease was not prevented with 400 p.p.m. TBZ in the previous trial, the level was increased to 1,600 p.p.m. Both treatments again included sufficient Agral 60 to ensure wetting of the fruit.

Five fruit per treatment were then held in individual containers at 20°C and ventilated with humidified air. The respiration rates of these fruit were monitored daily using an infrared gas analyser. The data obtained with infected fruit necessarily reflected the carbon dioxide output of the fungus also. Since the contribution from this source was only small and could not mask the onset of the respiratory climacteric of the fruit, the data will still be referred to simply as respiratory data. The remainder of the fruit were held in the cabinet described earlier. At 0, 4, 6, 11 and 13 days after dipping, ethylene production rates were determined on each of five fruit from each treatment. These fruit were then placed back in the cabinet and observed for a further 4 days. If a fruit showed colour and firmness changes during this period, it was assumed that it had entered the respiratory climacteric by the time its ethylene production rate was determined (Peacock 1966) and hence that determination was discarded.

To determine ethylene production rates, fruit were sealed for approximately 24 hr in glass jars. Each jar contained potassium hydroxide, to prevent carbon dioxide accumulation which would have affected the readings, a sampling port sealed with a rubber septum and a side arm. The arm consisted of a U-tube containing a small quantity of water which acted as a block to free diffusion of gas into or out of the jar, but allowed ingress of air as soon as the pressure in the jar decreased slightly, and another section containing "Purafil" (alumina pellets infiltrated with potassium permanganate). This section ensured that air drawn into the jar remained free of ethylene. Bananas were supported over the potassium hydroxide by standing in porcelain crucibles. Intercellular space of banana tissue is reported to amount to about 10% by volume (Palmer and McGlasson 1969), and so this factor was included when assessing free air space in a jar for the purpose of calculating ethylene production rates from measured ethylene concentration. Ethylene concentrations within jars were determined by gas chromatography, using an aluminium oxide column and flame ionization detector.

This trial was repeated, the following alterations to procedures being made:

(*a*) Control fruit were treated with 800 p.p.m. benomyl instead of TBZ, in an attempt to reduce disease development still further (Burden 1969).

(*b*) The spore suspension contained 1,650,000 spores /ml.

(*c*) Ethylene determinations were made at 0, 4, 6, 10, 12 and 14 days after dipping.

III. RESULTS

Green-life data and measurements of disease development obtained in trial 1 are shown in Tables 1 and 2 respectively. At all inoculum levels used, green-lives of fruit were reduced below that of the controls, the amount lost being dependent on the level of inoculum (Table 1). All differences are highly significant ($P<0\cdot01$).

Regression analysis shows that there is a highly significant relationship between the amount of green-life lost and the green-life fruit had at harvest (treatment 1)—that is, their stage of maturation. Data were significant ($P<0\cdot01$) for all treatments except treatment 2.

Disease development was found to vary with the level of inoculum used to initiate infection (Table 2), differences between all treatments being significant ($P<0\cdot05$); a similar result was reported by Meredith (1960). Also regression analysis showed that disease development varied inversely with the green-life fruit had at harvest. Differences were significant for treatments 4 ($P<0\cdot05$), 5 and 6 ($P<0\cdot01$) but not for the remainder, probably due to the experimental error involved in measuring the very small amounts of disease that developed in these treatments.

Respiratory rates and ethylene production rates for trial 2 are shown in Table 3. For simplicity, only the respiration rates measured at the times ethylene production rates were determined are shown. As stated earlier, however, respiration rates were monitored daily. Infection was found to significantly increase both respiratory rate ($P<0\cdot05$) and ethylene production rate ($P<0\cdot01$). Fruit remained preclimacteric throughout the period over which ethylene production rates were determined.

TABLE 1

CHANGES IN GREEN-LIFE AS A RESULT OF *C. musae* INFECTION*

Sample No.	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15
Treatment 1 TBZ															
Mean green-life ..	9·5	11·2	11·3	11·7	11·8	11·8	13·0	13·5	13·8	14·2	16·3	17·0	18·3	2·17	23·2
S.D.	0·837	1·472	1·211	1·211	0·983	1·169	2·098	1·517	1·169	1·602	3·204	2·098	2·733	1·033	3·189
Treatment 2 Water															
Mean green-life ..	7·0	10·5	10·8	9·8	11·0	10·8	10·7	12·5	12·7	9·8	15·5	14·0	13·2	18·2	22·0
Mean green-life lost	2·5	0·7	0·5	1·9	0·8	1·0	2·3	1·0	1·1	4·4	0·8	3·0	5·1	3·5	1·2
S.D.	0·633	1·049	1·941	1·722	1·095	1·472	2·251	0·548	1·366	2·137	2·168	3·347	3·061	2·927	4·690
Treatment 3 520 spores/ml															
Mean green-life ..	7·3	8·5	7·7	7·8	8·8	7·3	7·5	11·0	10·0	9·2	10·3	13·3	10·2	15·3	16·3
Mean green-life lost	2·2	2·7	3·6	3·9	3·0	4·5	5·5	2·5	3·8	5·0	6·0	3·7	8·1	6·4	6·9
S.D.	0·516	0·837	0·817	1·169	0·983	0·516	1·643	1·265	1·789	3·125	2·503	2·658	0·983	2·582	2·160
Treatment 4 5,200 spores/ml															
Mean green-life ..	6·5	7·3	7·2	6·2	7·7	6·8	6·5	7·8	8·5	7·0	8·2	8·8	9·0	12·2	13·2
Mean green-life lost	3·0	3·9	4·1	5·5	4·1	5·0	6·5	5·7	5·3	7·2	8·1	8·2	9·3	9·5	10·0
S.D.	0·548	0·516	0·408	0·408	0·516	0·408	0·548	0·753	1·871	0·000	1·602	2·229	2·608	2·639	2·787
Treatment 5 52,000 spores/ml															
Mean green-life ..	6·0	6·5	6·7	6·0	6·3	6·2	6·2	7·0	6·3	6·2	7·2	7·5	8·2	9·3	7·3
Mean green-life lost	3·5	4·7	4·6	5·7	5·5	5·6	6·8	6·5	7·5	8·0	9·1	9·5	10·1	12·4	15·9
S.D.	0·000	0·548	0·516	0·000	0·516	0·408	0·408	0·000	0·516	0·408	0·408	1·517	1·169	2·503	0·817
Treatment 6 520,000 spores/ml															
Mean green-life ..	4·8	6·2	5·2	5·3	6·0	6·5	5·7	6·3	5·3	5·2	5·7	6·7	7·2	8·3	8·2
Mean green-life lost	4·7	5·0	6·1	6·4	5·8	5·3	7·3	7·2	8·5	9·0	10·6	10·3	11·1	13·4	15·0
S.D.	0·983	0·408	0·408	0·817	0·633	0·837	0·817	0·516	0·516	0·408	0·516	0·817	0·753	1·033	1·941

* For ease of reference, data are expressed both as mean green-life (days) and as mean green-life lost (days). Standard deviations are also shown.

TABLE 2

DISEASE DEVELOPMENT, EXPRESSED IN mm OF ROTTED TISSUE 6 DAYS AFTER TREATMENT, IN RELATION TO STAGE OF MATURATION (GREEN-LIFE, TREATMENT 1)

Sample No.	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15
Stage of maturation (green-life in days) ..	9·5	11·2	11·3	11·7	11·8	11·8	13·0	13·5	13·8	14·2	16·3	17·0	18·3	21·7	23·2
Treatment 1 TBZ															
Mean	0·3	0·1	0·5	0·0	0·0	0·3	0·1	0·0	0·2	0·1	0·1	0·1	0·1	0·0	0·0
S.D.	0·606	0·204	0·000	0·000	0·000	0·274	0·204	0·000	0·258	0·204	0·204	0·204	0·204	0·000	0·000
Treatment 2 Water															
Mean	4·0	0·4	0·8	0·3	0·4	1·0	2·8	0·5	0·0	0·6	0·2	1·6	0·6	0·1	0·1
S.D.	3·162	0·801	0·606	0·418	0·801	0·837	4·568	0·316	0·000	0·376	0·258	2·010	0·204	0·204	0·204
Treatment 3 520 spores/ml															
Mean	1·3	2·7	2·3	2·2	1·6	4·5	6·0	0·9	0·9	2·8	1·2	0·8	5·2	0·3	0·2
S.D.	0·516	3·266	0·816	1·722	1·114	3·391	3·795	0·585	1·068	2·859	1·438	0·689	3·869	0·258	0·408
Treatment 4 5,200 spores/ml															
Mean	9·5	2·9	3·3	6·8	3·1	7·3	8·8	3·9	3·0	8·1	3·7	6·7	7·0	1·7	0·5
S.D.	4·183	1·563	1·862	2·994	3·499	4·227	2·787	2·940	1·789	5·142	2·658	3·327	6·197	2·137	0·447
Treatment 5 52,000 spores/ml															
Mean	11·5	6·7	6·5	11·7	8·5	13·0	11·5	4·5	7·0	12·2	5·0	7·2	10·3	3·0	3·0
S.D.	1·378	2·251	4·183	2·066	8·573	2·530	3·937	1·871	3·286	1·329	2·280	2·563	3·830	1·897	2·000
Treatment 6 520,000 spores/ml															
Mean	16·0	14·5	17·5	17·5	17·0	15·0	18·0	12·8	15·3	17·0	14·2	19·5	15·5	10·5	7·8
S.D.	2·530	1·378	2·588	4·037	3·899	2·828	1·549	1·941	2·582	1·673	1·169	3·450	3·016	1·643	2·994

TABLE 3

EFFECT OF *C. musae* INFECTION ON ETHYLENE PRODUCTION RATE (μl/kg/hr) AND RESPIRATION (mg/kg/hr) OF BANANAS

	Time elapsed since treatment (days) ..	0	4	6	11	13	
Trial 2a, Treatment 1 (TBZ)	Mean ethylene production rate	0·024	0·009	0·009	0·014	0·019	
	S.D.	0·0050	0·0032	0·0023	0·0020	0·0037	
	Mean respiration rate	24·9	25·0	25·5	31·3	32·1	
	S.D.	1·905	1·889	2·993	9·783	11·615	
Trial 2a, Treatment 2 (42,000 spore/ml) ..	Mean ethylene production rate	0·030	0·041	0·041	0·042	0·030	
	S.D.	0·0123	0·0305	0·0176	0·0116	0·0045	
	Mean respiration rate	26·0	27·6	31·8	39·5	44·0	
	S.D.	1·306	2·006	6·532	8·823	12·053	
	Time elapsed since treatment (days) ..	0	4	6	10	12	14
Trial 2b, Treatment 1 (Benomyl)	Mean ethylene production rate	0·018	0·011	0·009	0·008	0·011	0·013
	S.D.	0·0046	0·0033	0·0008	0·0013	0·0013	0·0056
	Mean respiration rate	..	23·7	29·6	20·8	24·0	24·7
	S.D.	..	2·417	4·886	2·835	1·787	1·436
Trial 2b, Treatment 2 (16,500 spore/ml) ..	Mean ethylene production rate	0·016	0·092	0·059	0·099	0·053	0·043
	S.D.	0·0019	0·0453	0·0242	0·0374	0·0230	0·0099
	Mean respiration rate	..	34·5	45·4	33·9	36·4	38·6
	S.D.	..	3·688	7·097	3·754	2·187	3·742

Significance levels—
Differences in ethylene production rate—
Trial 2a $P < 0{\cdot}01$
Trial 2b $P < 0{\cdot}05$
Differences in respiration rate—
Trial 2a $P < 0{\cdot}05$
Trial 2b $P < 0{\cdot}01$

IV. DISCUSSION

The results obtained in trial 1 clearly show that disease due to *C. musae* infection of the stem end of bananas reduced green-life. That the amount lost varied with the level of inoculum used and the stage of maturation of the fruit (green-life when TBZ treated) is apparently due to the variation in disease development that occurred in relation to these two parameters (Table 2). The slightly higher standard deviations obtained in treatment 2 (Table 1) are probably due to the variable infection obtained under the influence of natural spore loads.

Increasing infection with increasing spore loads is not a result to be unexpected, nor is the variation in infection with stage of maturation (Meredith 1960). The existence of a natural antifungal agent in the skin of green bananas has been reported (Simmonds 1963), and several compounds having such activity have recently been identified (Mulvena, Webb and Zerner 1969). The variation in disease development which occurs in fruit at different stages of maturation may well reflect changes in the concentration of these substances, changes which occur even before ripening commences.

The increased production of ethylene (trial 2—Table 3) by diseased fruit would appear to be the cause of the reduced green-life, since evidence has been presented (Peacock 1971) that the ethylene produced by fruit throughout the preclimacteric period is functional in determining when the climacteric rise will commence. Diseased fruit showed increased respiratory activity (Table 3). This is a characteristic response of plant material to the attack of a parasite (Uritani and Akazawa 1959). In this instance, it is undoubtedly partly due to the respiratory output of the fungus itself, but also may be a result of the increased ethylene production rate (Hansen 1967).

The results obtained in this investigation have demonstrated that the infection of wounds by *C. musae* stimulates the ripening of fruit. In this investigation, infection mainly occurred through the stem end; however, it is probable that a similar effect would occur should the fungus gain entry through a wound elsewhere on the fruit. This effect further emphasizes the importance of treating fruit after harvest with a fungicide that is effective against this organism.

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