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# The Influence of Temperature, Inoculum Level and Race of *Fusarium oxysporum* f. sp. *cubense* on the Disease Reaction of Banana cv. Cavendish

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*Abstract*

Cavendish banana plants were grown in soil infested with different levels of microconidia of races 1 and 4 of *Fusarium oxysporum* Schlect. f. sp. *cubense* (E.F. Smith) Snyd. & Hans. The 4-week-old plants were incubated at 20° and 28°C in temperature controlled cabinets subject to natural light fluctuations. Race 1, which does not normally infect Cavendish in the field, infected the plants at both temperatures, as did race 4. Results indicated that temperature was primarily affecting plant growth rather than influencing the aggressiveness of the pathogen. Inoculum levels were also found to influence disease severity with a minimum of $4{\cdot}5 \times 10^4$ microconidia/g dry weight of soil required before macroscopic symptoms were observed. The suitability of month-old tissue culture derived plants for screening for resistance and the evolution of the Cavendish attacking race 4 strains are discussed in the light of these findings.

*Keywords:* Fusarium, wilt, bananas, temperature, inoculum, race 4.

## Introduction

In the subtropical regions of Australia, Taiwan, South Africa, Canary Islands and Brazil, Cavendish cultivars of bananas (*Musa* sp.) have succumbed to the wilt-causing pathogen *Fusarium oxysporum* Schlect. f. sp. *cubense* (E.F. Smith) Snyd. & Hans. (Foc) (Ploetz *et al.* 1990). In the past, environmental factors, particularly edaphic factors such as poor soil drainage or unfavourable chemical or physical conditions, were thought to play a major role in the predisposition of race 1 resistant Cavendish cultivars to Fusarium wilt (Stover and Malo 1972; Waite 1977). However, this was prior to the identification of race 4, which has the ability to attack Cavendish cultivars.

Although present in some tropical regions, race 4 severely affects Cavendish only in the subtropics (Ploetz *et al.* 1990), indicating that temperature may have an important influence on disease development. The resistance of race 1 resistant Lacatan (and susceptible Gros Michel) cultivars is temperature dependent (Beckman *et al.* 1962). However, there has been little research on the effects of temperature on disease development.

Inoculum level is also thought to influence disease development, but has been given little attention. Stover and Waite (1954), Rishbeth (1957) and Stover (1962) all found evidence that inoculum levels in field soil influenced wilt incidence

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and severity. Rishbeth (1957) found that by increasing the inoculum level, the otherwise race 1 resistant Lacatan cultivar became infected. Evidence from other *Fusarium* spp. also indicates that inoculum levels can influence the host's response to infection (Douglas 1970; Martyn and McLaughlin 1983). It is evident that inoculum concentration needs to be considered when screening for resistance to these wilt pathogens.

The aims of the work reported in this paper were to investigate the influences of temperature and inoculum concentration on wilt development in cv. Cavendish challenged with races 1 and 4 of Foc, and to use these findings in developing a routine glasshouse assay to screen for resistance to the disease.

## Materials and Methods

*Plants*

Plants of cv. Cavendish derived from tissue culture (accession C99) were obtained from Gaincover Laboratories, Kenmore, Brisbane, Qld. The plants were removed from the bottles and grown in flats under high humidity for 4 days before being planted out separately into pasteurized University of California (U.C.) Mix (1:1, v/v; sand:peat) in 100 mm diameter pots. They were then grown in a temperature controlled cabinet set at 27±2°C for 4 weeks before being used in the experiment.

*Isolates and Inoculum Preparation*

One isolate of race 1 (702) and one of race 4 (22402) of FOC were used in experiment 1, while five isolates of each race were used in experiment 2. These isolates are listed in Table 1, along with the host and region from which they were obtained.

**Table 1. Accessions of *Fusarium oxysporum* f.sp. *cubense* indicating vegetative compatibility group (Vcg) (Brake *et al.* 1991) and original host**

| Isolate | Vcg | Host | Location |
|---|---|---|---|
| Race 1 | | | |
| 8610 | 0125 | Lady finger (AAB) | New South Wales |
| 8603 | 0125 | Mysore (AAB) | Currumbin, Qld |
| 8624 | 0125 | Lady finger (AAB) | Currumbin, Qld |
| 22479 | 0125 | Lady finger (AAB) | North Queensland |
| 702 | 0125 | Lady finger (AAB) | New South Wales |
| Race 4 | | | |
| 1040 | 0129 | Mons Mari[A] (AAA) | Wamuran, Qld |
| N5331 | 0120 | Mons Mari (AAA) | Yandina, Qld |
| 8620 | 0120 | Mons Mari (AAA) | Landsborough, Qld |
| 8622 | 0120 | Mons Mari (AAA) | Eudlo, Qld |
| 22402 | 0120 | Mons Mari (AAA) | Wamuran, Qld |

[A] Cavendish cultivar.

To obtain sufficient inoculum, isolates were grown in Armstrong's liquid medium (Booth 1977), which markedly increases the production of microconidia. Flasks containing 100 mL of Armstrong's medium were each inoculated with four mycelial plugs (3–4 mm diam.) from the edge of 7-day-old cultures grown on carnation leaf-piece agar (CLA) (Burgess *et al.* 1988). The flasks were incubated at room temperature under a fluorescent light for 7 days and manually shaken twice daily.

Harvesting of inoculum was carried out by filtering the cultures through two layers of muslin to remove the bulk of the mycelium. The filtrate was centrifuged at 5000 rpm for

10 min to concentrate the inoculum. Inoculum was adjusted to the desired concentration using a haemocytometer.

*Experiment 1: Determination of the Critical Inoculum Level*

For each race (race 1: isolate 702; race 2: isolate 22402), a two-fold dilution series was prepared as follows: U.C. Mix for six replicates was weighed (900 g wet weight/pot) and infested with 1/2 of the total inoculum of one of the races. The soil and inoculum were thoroughly mixed by hand before dividing the soil equally by weight into the six pots and planting one 4-week-old cv. Cavendish plant (6 to 7 leaves, 100 to 150 mm high) in each pot. Half of the remaining inoculum was then added to another amount of U.C. Mix sufficient for six pots and mixed thoroughly. In this way six, twofold dilutions (1/1 to 1/32) were prepared for both races 1 and 4 of the fungus. The concentration of inoculum in the 1/1 dilution was $1{\cdot}3 \times 10^5$ microconidia/g dry wt of U.C. Mix. Three replicates of each dilution were randomly selected and placed in one of two controlled temperature glasshouses set at 20/17±2°C and 28/25±2°C. Six non-inoculated plants (three in each temperature regime) served as controls.

*Experiment 2: Influence of Race, Inoculum Level and Temperature on Disease Severity in cv. Cavendish*

For each isolate listed in Table 1, a twofold dilution series was prepared as described for experiment 1. However, in this experiment only two dilutions (1/1 and 1/2) were used. The concentration of inoculum in the 1/1 dilution was approximately $2{\cdot}5 \times 10^5$ microconidia/g dry wt of U.C. Mix for all isolates except 8622 (race 4), where the concentration was only $4{\cdot}5 \times 10^4$ microconidia/g dry weight in the 1/1 dilution owing to a reduced production of microconidia. The six replicates for each isolate-dilution treatment were randomly divided into two groups of three, and each group placed in one of two temperature controlled glasshouses set at 20/17±2°C and 28/25±2°C. Six plants grown in non-inoculated U.C. Mix (three in each temperature regime) served as controls.

All plants were potted in plastic containers without drainage holes and watered to weight daily. The moisture content of the soil was kept at approximately 20% (−0·005 MPa).

**Table 2. Disease severity assessment system for leaf symptoms and rhizome discoloration caused by *Fusarium oxysporum* f. sp. *cubense* on banana plants**

| | |
|---|---|
| **Leaf Symptom Index** | |
| 1 | No streaking or yellowing of leaves. Plant appears healthy. |
| 2 | Slight streaking and/or yellowing of lower leaves. |
| 3 | Streaking and/or yellowing of most of the lower leaves. Discoloration of the younger leaves may be just beginning to appear. |
| 4 | Extensive streaking and/or yellowing on most or all of the leaves. |
| 5 | Dead plant. |
| **Rhizome Discoloration Index** | |
| 1 | No discoloration of tissue of stellar region of rhizome or surrounding tissue. |
| 2 | No discoloration of stellar region of rhizome but discoloration present at junction of root and rhizome. |
| 3 | Trace to 5% of stellar region discoloured. |
| 4 | 6–20% of stellar region discoloured. |
| 5 | 21–50% of stellar region discoloured. |
| 6 | More than 50% of stellar region discoloured. |
| 7 | Discoloration of the entire rhizome stele (dead plant). |

*Disease Severity Assessment*

Plants were assessed for disease severity using a rating system for leaf symptoms and rhizome discoloration (Table 2). Dry weights for both leaf/pseudostem combined and root/rhizome combined were also recorded for the second experiment only. To do this, plants were dissected at the base of the pseudostem where the swelling of the rhizome becomes apparent.

*Soil Plating*

Soil plating of the infested U.C. Mix was carried out at the start of the experiment to monitor the level of viable inoculum in the mix. Five g (dry weight) of soil from the 1/1 dilution for each treatment were saved at the start of each experiment.

A 10-fold dilution series was carried out for each soil sample. Each series consisted of five dilutions with five replicates per dilution. Five 1-mL aliquots were each spread onto a plate of PCNB (pentachloronitrobenzene) agar (Nash and Snyder 1962) and incubated on a laboratory bench at room temperature for seven days. The number of plates with *Fusarium* colonies was then recorded for each dilution, and an estimate of the number of colony forming units (cfu)/gram dry weight was obtained using the Maximum Likelihood Program (Ross 1980).

*Methods of Statistical Analysis*

*Disease severity ratings*

Owing to the discontinuous nature of the disease severity ratings a non-parametric method was needed to analyse the data. However, a non-parametric analysis of variance (ANOVA) for a multi-factorial experiment is not available. Neave and Worthington (1988) have proposed that where one factor (in this case temperature) has only two levels, the differences between these levels can be analysed to assess the interaction between this factor and another. The two factors, isolate and dilution, were combined to represent a single factor with several levels.

For each experiment, the differences in disease ratings between the temperatures were found for each isolate/dilution treatment and the controls. A one-way non-parametric ANOVA using the Kruskal-Wallis technique (Conover 1980) was then carried out on these differences in ratings between the temperatures. The test statistic of this technique is a function of the ranks of the observations and approximates the chi-square distribution. Critical values are therefore given as chi-square (CHSQ) values. Where significance occurred between the differences for the treatment means, the three separate interactions of (i) isolate by temperature, (ii) dilution by temperature and (iii) isolate by dilution by temperature were assessed through pairwise comparisons of the appropriate treatment rank means of the temperature differences (Conover 1980).

For experiment 1, the one way analysis of variance consisted of 5 treatments (2 isolates each with 2 dilutions plus a control), while for experiment 2, the ANOVA consisted of 21 treatments (10 isolates each with two dilutions plus a control). For ease of understanding in relation to the original data, results in the tables are given as the means of the temperature differences for each treatment, rather than the rank means.

Tests for significance between treatments for race, dilution or temperature but not controls were based on the l.s.d. procedure (Conover 1980; Zar 1984) when five or less treatment means were compared, and the Student-Newman-Keuls (SNK) procedure (Zar 1984) when more than five means were compared. Control means were compared to treatment means using Dunnett's procedure (Zar 1984).

*Leaf and Root Dry Weight Analysis—Experiment 2*

Analyses of the weight data involved factorial ANOVAs with 10 isolates (five for each of races 1 and 4) by two dilutions plus a control at each of the two temperatures, i.e. $(10 \times 2+1) \times 2$.

Following the $F$ test for combined treatments and controls, tests for significance between treatment means and the control means were made using Dunnett's procedure. Treatment means were compared using the l.s.d. or SNK procedures, depending on the number to be compared (Zar 1984). All tests for significance were carried out at the $P = 0{\cdot}05$ level. All statistical procedures were carried out using the Statistical Analysis System (SAS) (1987).

## Results

*Experiment 1: Determination of Critical Inoculum Level*

*Disease Severity Ratings*

Means of disease severity ratings for leaf symptoms and rhizome discoloration are shown in Table 3. Disease development did not occur at an inoculum level

of $3{\cdot}2 \times 10^4$ microconidia/g dry weight of soil or lower for either race 1 or race 4. However, both race 1 and race 4 infected cv. Cavendish at both temperatures in the 1/1 and 1/2 inoculum levels. There were significant differences between treatments (race 1, 1/1 and 1/2; race 4, 1/1 and 1/4; control) in the effect of temperature on rhizome discoloration (CHSQ 10·09) and leaf symptoms (CHSQ 9·24)

**Table 3. Mean disease severity ratings for Cavendish infected with races 1 and 4 of Foc—experiment 1**

Leaf symptoms and rhizome discolouration were rated using the rating systems cited in Table 2

| Dilution | Leaf symptoms | | Rhizome discolouration | |
|---|---|---|---|---|
| | 20°C | 28°C | 20°C | 28°C |
| Race 1 (702) 1/1 | 4·3 | 1·7 | 6·3 | 3·7 |
| 1/2 | 2·0 | 1·3 | 2·7 | 1·7 |
| 1/4–1/32 | 1·0 | 1·0 | 1·0 | 1·0 |
| Race 4 (22402) 1/1 | 3·0 | 3·0 | 4·7 | 3·7 |
| 1/2 | 1·0 | 2·3 | 1·0 | 3·7 |
| 1/4–1/32 | 1·0 | 1·0 | 1·0 | 1·0 |
| Controls | 1·0 | 1·0 | 1·0 | 1·0 |

**Table 4. Mean temperature differences for disease severity ratings for Cavendish infected with races 1 and 4 of Foc—experiment 1**

Chi-square (Kruskal-Wallis) values are significant ($P = 0{\cdot}05$). Values are the mean temperature differences (MTDs) (28°C–20°C) in leaf symptoms and rhizome discoloration ratings for Cavendish infected with races 1 and 4 of Foc. Within a column, means followed by the same letter are not significantly different ($P = 0{\cdot}05$)

| Race | Dilution | Mean differences(28°C–20°C) Leaf symptoms | Rhizome discolouration |
|---|---|---|---|
| Race 1 | 1/1 | −2·7a | −2·7a |
| | 1/2 | −0·6ac | −1·0ac |
| Race 4 | 1/1 | 0·0bc | −1·0ac |
| | 1/2 | 1·3b | 2·7b |
| Control | | 0·0bc | 0·0bc |
| Chi-Square* | | 9·24 | 10·09 |

Both dilutions of race 1 caused more leaf disease at 20°C than 28°C, while the 1/1 dilution of race 4 caused the same amount of leaf disease at both temperatures, and the 1/2 dilution of race 4 caused more disease at 28°C than 20°C. Foc was reisolated from all infected plants.

*Experiment 2: Influence of Race, Inoculum Level and Temperature on Disease Development in cv. Cavendish*

*Disease severity ratings*

The mean disease severity ratings presented in Table 5 show that cv. Cavendish was infected by all isolates of both races 1 and 4 of Foc at both temperatures.

At 20°C the control plants were chlorotic and stunted compared to the controls at 28°C which were dark green and 50 to 70 mm taller. All inoculated plants at 20°C also appeared chlorotic and stunted, while the more severely infected plants showed the characteristic discoloration of the veins typical of Fusarium wilt. At 28°C, the inoculated plants were generally a darker green than those at 20°C, although the more severely infected the plants, the more leaf chlorosis and vein discoloration. Foc was reisolated from all infected plants.

**Table 5. Mean disease severity ratings for Cavendish infected with isolates of races 1 and 4 of Foc—experiment 2**

Leaf symptoms and rhizome discoloration were rated using the rating systems cited in Table 2

| | Dilution | Leaf symptoms | | Rhizome discolouration | |
|---|---|---|---|---|---|
| | | 20°C | 28°C | 20°C | 28°C |
| Race 1 isolates | | | | | |
| 8610 | 1/1 | 2·0 | 1·3 | 3·7 | 3·3 |
| | 1/2 | 1·0 | 1·3 | 2·3 | 3·3 |
| 8603 | 1/1 | 1·0 | 1·7 | 2·3 | 2·7 |
| | 1/2 | 1·3 | 2·0 | 2·3 | 3·0 |
| 8624 | 1/1 | 1·0 | 1·7 | 2·0 | 3·3 |
| | 1/2 | 1·0 | 1·3 | 1·6 | 3·7 |
| 22479 | 1/1 | 1·7 | 2·0 | 4·3 | 4·0 |
| | 1/2 | 1·7 | 1·5 | 3·0 | 3·7 |
| 702 | 1/1 | 1·7 | 1·2 | 3·3 | 2·3 |
| | 1/2 | 1·7 | 1·3 | 3·0 | 2·0 |
| Race 4 isolates | | | | | |
| 1040 | 1/1 | 3·0 | 2·0 | 2·3 | 4·3 |
| | 1/2 | 4·3 | 1·7 | 5·0 | 3·3 |
| N5331 | 1/1 | 1·5 | 2·0 | 2·8 | 3·3 |
| | 1/2 | 1·5 | 2·0 | 2·3 | 3·3 |
| 8620 | 1/1 | 1·8 | 2·0 | 4·3 | 3·7 |
| | 1/2 | 1·7 | 1·3 | 3·3 | 1·7 |
| 8622 | 1/1 | 1·3 | 1·3 | 2·0 | 3·3 |
| | 1/2 | 1·2 | 1·2 | 1·7 | 2·0 |
| 22402 | 1/1 | 2·8 | 3·3 | 4·7 | 3·0 |
| | 1/2 | 1·7 | 3·3 | 3·3 | 5·7 |
| Control | | 1·0 | 1·0 | 1·0 | 1·0 |

Only leaf symptoms showed any significant difference ($P = 0{\cdot}05$) between the temperatures (Table 6). The mean temperature differences (MTDs) for both dilutions of isolate 1040 (race 4) differed significantly from that of the 1/2 dilution of isolate 22402 (race 4). Infection by isolate 1040 produced more severe leaf symptoms at 20°C than 28°C (as indicated by the negative mean temperature differences of $-1{\cdot}00$ and $-2{\cdot}67$), while isolate 22402 at the lower inoculum level produced more severe leaf symptoms at 28°C (Table 6).

*Leaf and Root Weights*

There were no significant interactions between temperature and dilution, or between temperature, dilution and race in the effect on leaf and root weight.

**Table 6. Mean temperature differences for leaf symptoms for Cavendish infected with different isolates of races 1 and 4 of Foc - experiment 2**

* Chi-Square (Kruskal-Wallis) value significant at $P = 0{\cdot}05$. Values are the mean temperature difference (MTD) (28°C–20°C) in leaf symptom ratings for plants infected with a particular isolate. Means within the column, followed by the same letter are not significantly different ($P = 0{\cdot}05$)

| | Isolate | Dilution | Mean difference in leaf symptoms (28°C–20°C) |
|---|---|---|---|
| Race 1 | 8610 | 1/1 | −0·67ab |
| | | 1/2 | 0·33ab |
| | 8603 | 1/1 | 0·67ab |
| | | 1/2 | 0·67ab |
| | 8624 | 1/1 | 0·67ab |
| | | 1/2 | 0·33ab |
| | 22479 | 1/1 | 0·33ab |
| | | 1/2 | −0·20ab |
| | 702 | 1/1 | −0·50ab |
| | | 1/2 | −0·33ab |
| Race 4 | 1040 | 1/1 | −1·00a |
| | | 1/2 | −2·67a |
| | N5331 | 1/1 | 0·50ab |
| | | 1/2 | 0·50ab |
| | 8620 | 1/1 | 0·17ab |
| | | 1/2 | −0·33ab |
| | 8622 | 1/1 | 0·00ab |
| | | 1/2 | 0·00ab |
| | 22402 | 1/1 | 0·50ab |
| | | 1/2 | 1·67b |
| | Control | | 0·00ab |
| Chi-Square* | | | 33·05 |

The mean leaf and root weights were both significantly greater ($P = 0{\cdot}05$) at 28°C than at 20°C in controls and inoculated plants (Table 7).

Root weight was significantly less ($P = 0{\cdot}05$) in the 1/1 than the 1/2 dilution (Table 7). Root weight for all treatments combined (0·73±0·03 g) was also significantly less ($P = 0{\cdot}05$) than the control root weight (1·07±0·14 g). Significant differences for root weight ($P = 0{\cdot}05$) were also present between isolates, with infection by isolate N5331 (race 4) resulting in a significantly lower root weight than that of isolate 22479 (race 1) (Table 8).

*Comparisons of Mean Temperature Differences (MTDs) between Controls and Isolates for Leaf and Root Weights*

A comparison was made between the control MTDs and the isolate MTDs to determine whether the effect of temperature on leaf and root weights of infected plants was due to an effect of temperature on the severity of the infection or was simply a reflection of the basic effect of temperature on plant growth. However,

**Table 7. Effect of temperature and inoculum dilution on leaf and root weight (mean ± s.e.) of Cavendish infected with different isolates of races 1 and 4 of Foc–experiment 2**

Means within columns for each criterion followed by the same letter are not significantly different ($P = 0{\cdot}05$)

| Temp. | Controls | Inoculated plants | All plants |
|---|---|---|---|
| | | Leaf weight (g) | |
| 20°C | 0·85±0·11a[A] | 0·64±0·02a | 0·65±0·02a |
| 28°C | 1·66±0·16b | 1·40±0·05b | 1·42±0·05b |
| | | Root weight (g) | |
| 20°C | 0·90±0·18a | 0·52±0·03a | 0·53±0·03a |
| 28°C | 1·24±0·18b | 0·93±0·05b | 0·95±0·05b |

| Dilution | Leaf wt (g) | Root wt (g) |
|---|---|---|
| 1/1 | 1·00±0·06a[B] | 0·66±0·05a |
| 1/2 | 1·05±0·06a | 0·79±0·05b |

[A] Mean leaf weight and standard error for the control plants grown at 20°C.
[B] Mean leaf weight and standard error for all plants in the 1/1 dilution.

**Table 8. Effect of isolate of Foc on mean root weights (±s.e.) of Cavendish plants inoculated with different isolates of races 1 and 4 of Foc averaged over both temperatures (20°C and 28°C) and both dilutions—experiment 2**

Within the column, means followed by the same letter are not significantly different ($P = 0{\cdot}05$)

| | | Mean root weight (g) |
|---|---|---|
| Race 1 | 8610 | 0·63±0·10ab |
| | 8603 | 0·78±0·08ab |
| | 8624 | 0·86±0·12ab |
| | 22479 | 0·93±0·14a |
| | 702 | 0·70±0·13ab |
| Race 4 | 1040 | 0·63±0·09ab |
| | N5331 | 0·54±0·07b |
| | 8620 | 0·72±0·11ab |
| | 8622 | 0·86±0·11ab |
| | 22402 | 0·61±0·11ab |

none of the MTDs for plants inoculated with any isolates differed significantly from that of the controls for either leaf or root weight.

Therefore, the differences present between the temperatures for all infected plants were due mainly to the expected effect of temperature on growth of these plants.

*Soil Plating Results*

The viability of the microconidia for each of the isolates at the start of the experiments, as determined 12 h after plating onto water agar, ranged from 91 to 98%.

The original concentration of inoculum with which the U.C. Mix was infested was $1 \cdot 3 \times 10^5$ microconidia/g dry weight of soil for experiment 1 and $2 \cdot 5 \times 10^5$ microconidia/g dry weight of soil for experiment 2 (except for isolate 8622 for which only $4 \cdot 5 \times 10^4$/g microconidia were used). As can be seen from Tables 9 and 10, the amount of inoculum surviving in the mix at the start of the experiments, as determined by soil plating, was considerably less than that with which it was infested, but within the range normally encountered in the field.

**Table 9. Most probable number of colony forming units of Foc at the start of experiment 1**

| | MPN[A] (cfu/g dry wt) | 95% Fiducial limits |
|---|---|---|
| Start | | |
| Race 1 (702) | 637 | 196–1629 |
| Race 4 (22402) | 245 | 78–710 |

[A] Most Probable Number of colony forming units per gram dry weight (cfu/g) obtained from the Maximum Likelihood Program (MLP) (Ross 1980). For both races the U.C. Mix was originally infested with approximately $1 \cdot 3 \times 10^5$ microconidia per gram dry weight in the 1/1 dilution. All samples were taken from the 1/1 dilution.

**Table 10. Most probable number of colony forming units of Foc at the start of experiment 2**

| | MPN[A] (cfu/g dry wt) | 95% Fiducial limits |
|---|---|---|
| Race 1 | | |
| 22479 | 338 | 115–811 |
| 8624 | 423 | 157–967 |
| 8603 | 912 | 300–2571 |
| 8610 | 893 | 144–5548 |
| 702 | 658 | 214–1950 |
| Race 4 | | |
| 8622 | 25 | 8–65 |
| 8620 | 462 | 145–1438 |
| 1040 | 658 | 214–1951 |
| N5331 | 156 | 47–420 |
| 22402 | 255 | 79–652 |

[A] MPN is the most probable number of propagules as colony forming units (cfu) per gram dry weight of U.C. mix obtained from the MLP program (Ross 1980). U.C. mix was originally infested with approximately $2 \cdot 5 \times 10^5$ microconidia/gram dry weight for all isolates except isolate 8622 in which $4 \cdot 5 \times 10^4$ microconidia/g dry weight was used. All samples were taken from the 1/1 dilution.

## Discussion

It is evident from these results that both races 1 and 4 of Foc can infect month-old tissue culture derived Cavendish plants at both 20° and 28°C, and at spore concentrations of $4 \cdot 5 \times 10^4$/g dry weight of potting mix or greater. Although infected by race 1 at 28°C, the Cavendish plants showed very few leaf

symptoms and were as vigorous as the controls. While the resistance mechanism/s of the Cavendish plants restricted the spread of race 1 in the tissues, these mechanisms appeared to be less effective at 20°C than 28°C, based on disease severity assessments.

The occurrence of race 4 mainly in the subtropics would seem to indicate that temperature is a critical factor in wilt development. Being a tropical species, banana plants may be predisposed to infection in areas where they are subject to low temperatures. It has been suggested that race 4 was derived from race 1 in areas where Cavendish are subject to seasonally low temperatures (Stover and Buddenhagen 1986). This seems unlikely in light of the results of the vegetative compatibility work (Brake *et al.* 1990) and random amplified polymorphic DNA studies (Sorensen 1993), which indicate that races 1 and 4 are genetically distinct populations. The results presented in this paper make it difficult to generalize about the effect of temperature on the different races of Foc. While isolate 702 (race 1) was more aggressive at 20°C than 28°C in both experiments, several of the race 1 isolates in the second experiment showed a tendency to be slightly more aggressive at 28°C (based on leaf symptoms and rhizome discoloration). Beckman *et al.* (1962) have shown, however, that the defence mechanisms in banana plants are affected by temperature. Working with the susceptible cultivar Gros Michel, they found that this cultivar could resist infection at only 34°C, at which temperature gel and tylose formation were permanent and occluded the fungus from spreading through the plant. At 21° and 27°C, gel and tylose formation were scattered and delayed, allowing systemic invasion by the fungus and wilt development. Plants of cv. Lacatan, however, were able to resist infection at all three temperatures.

In the present study, race 1, which does not normally infect Cavendish in the field, infected this cultivar at both 20° and 28°C. However, at the higher temperature, symptoms appeared only to a limited extent on the oldest leaves, and the plants appeared no less vigorous than the control plants despite infection of the rhizome. At this temperature the host defences may have been stimulated early enough to prevent extensive spread of the pathogen, resulting in the generally healthy appearance of the plants. At 20°C, however, plants inoculated with race 1 were pale and stunted and appeared similar to the controls, making it difficult to distinguish symptoms of the disease. Since MTDs for the inoculated plants did not differ significantly from that of the controls for leaf and root weight in experiment 2, the differences present between the temperatures for all infected plants were due mainly to the expected effect of temperature on the growth of these plants.

There were no apparent differences in the aggressiveness (ie. the amount of disease caused) of the isolates of the two races, although inoculation with isolate N5331 (race 4) did result in a significantly lower root weight than inoculation with isolate 22479 (race 1).

While there is some evidence in the present study that Cavendish banana plants are predisposed to infection by race 1 of Foc at 20°C, infection by this race also occurred at 28°C. Moore (1994) has found that while low winter temperatures result in a decrease in chlorophyll concentration and rate of $CO_2$ assimilation in both race 4 resistant and susceptible Cavendish varieties, the reduction in the susceptible variety, Williams, was greater than in the resistant Dwarf Parfitt. It

was also found that Dwarf Parfitt was able to recover more rapidly from cold stress than Williams once temperatures began to increase. As the resistance response of bananas is reliant on photoassimilates for gel and tylose production, any reduction in carbon assimilation may result in the inability of the host plant to successfully block the spread of the pathogen. If Cavendish are predisposed to infection by race 1 under low temperatures in the field, we would expect, at some time, to isolate race 1 (VCG 0124, 0125) from diseased Cavendish plants. This has not yet been reported with any certainty. This lack of isolation of race 1 may be due to the ability of older field-grown plants to limit the spread of the fungus in the roots before the pathogen can reach the rhizome from which isolations are normally made.

Inoculum concentration also influenced disease severity as indicated by the significantly lower root weight in the 1/1 dilution than the 1/2 dilution for experiment 2. Results from the first experiment indicated that a concentration of at least $6 \times 10^4$ microconidia/g soil was required for infection to occur. However, infection did occur in those plants inoculated with isolate 8622 (race 4), in which only $4{\cdot}5 \times 10^4$ microconidia/g soil were used.

Although the soil plating at the start of the experiment indicated that inoculum levels were either within the range of inoculum densities found in the field or slightly higher (Trujillo and Snyder 1963; Rishbeth 1955, 1957; Sun and Su 1984; Brake 1991), these results must be considered as giving only estimates of the number of viable propagules in the soil. Given the range of the colony forming units of some of the isolates, the fact that inoculum levels were higher than those that occur naturally may have contributed to the infection of cv. Cavendish by race 1. Evidence that inoculum levels alter the resistance response and ranking of watermelon and muskmelon cultivars to infection by formae speciales of *Fusarium oxysporum* has been reported by Martyn and McLaughlin (1983) and Douglas (1970), respectively.

Perhaps a more important factor affecting the results of the experiment was the nature of the plants themselves. At 4 weeks of age, differentiation and maturation of the plant tissues may not have been complete due to the method of propagation. The immature nature of the plants may have allowed infection to occur, but with increasing physiological maturity, the plants at 28°C may have been able to limit the spread of race 1.

While the results reported in this paper reflect to some degree the virulence of races 1 and 4 of Foc, absolute correlation with field results was not obtained. Although the use of month-old tissue cultured plants is desirable in screening tests because of their ease of handling, it would appear that a glasshouse screening test in which temperature and inoculum levels are strictly controlled will not produce reliable results using such young plants. Further investigations of the effect of plant age (and other factors such as light intensity) on disease development are required before a glasshouse screening assay that correlates with field results can be developed.

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## References

Beckman, C. H., Halmos S., and Mace, M. E. (1962). The interaction of host, pathogen and soil temperature in relation to susceptibility to Fusarium wilt of bananas. *Phytopathology* **52**,134–40.

Brake, V. M., Pegg, K. G., Irwin, J. A. G., and Langdon, P. W. (1990). Vegetative compatibility groups within Australian populations of *Fusarium oxysporum* f. sp. *cubense* the cause of fusarium wilt of bananas. Australian Journal of Agricultural Research. **41**, 863–71.

Brake, V. M. (1991). *Fusarium wilt of bananas.* Ph.D. Thesis. University of Queensland. 357 pp.

Booth, C. (1977). Fusarium-laboratory guide to the identification of the major species. Commonwealth Mycological Institute., Kew, Surrey, England. p. 10.

Burgess, L. W., Liddell, C. M., and Summerell, B. A. (1988). Laboratory manual for Fusarium research. 2nd Edn Fusarium Research Laboratory, Department of Plant Pathology and Agricultural Entomology. Univ. Sydney. 156 pp.

Conover, W. J. (1980). Practical Non-parametric Statistics. 2nd Edn pp. 229–37. (John Wiley and Sons: New York.)

Douglas, D. R. (1970). The effect of inoculum concentration on the apparent resistance of muskmelons to *Fusarium oxysporum* f. sp. *melonis*. *Canadian Journal of Botany*. **48**,687–93.

Martyn, R. D., and McLaughlin, R. J. (1983). Effects of inoculum concentration on the apparent resistance of watermelons to *Fusarium oxysporum* f. sp. *niveum*. *Plant Disease* **67**,493–95.

Moore, N. (1994). Fusarium wilt of bananas; pathogen variability and host-pathogen interactions. Ph.D. Thesis. University of Queensland. (in press).

Nash, S. M., and Snyder, W. C. (1962). Quantitative estimation by plate counts of propagules of the bean root rot Fusarium in field soils. *Phytopathology* **52**, 567–72.

Neave, H. R., and Worthington, P. L. (1988). Distribution-Free tests. pp. 272–84. (Unwin, Hyman Ltd: London.)

Ploetz, R. C. (1989). Population biology of *Fusarium oxysporum* f. sp. *cubense*. In 'Fusarium Wilt of Banana' (Ed. R. C. Ploetz.) Proceedings of an International Conference on Fusarium wilt of Banana, pp. 63–76. (APS Press: Miami, Florida.)

Ploetz, R.C., and Correll, J.C. (1988). Vegetative compatibility among races of *Fusarium oxysporum* f. sp. *cubense*. *Plant Disease* **72**, 325–28.

Ploetz, R. C., Herbert, J., Sebasigari, K., Hernandez, J. H., Pegg, K. G., Ventura, J. A., and Mayato, L. S. (1990). Importance of fusarial wilt in different banana-growing regions. In 'Fusarium Wilt of Banana' (Ed. R. C. Ploetz.) Proceedings of an International Conference on Fusarium wilt of Banana. pp. 9-26. (APS Press: Miami, Florida.)

Rishbeth, J. (1955). Fusarium wilt of bananas in Jamaica. I. Some observations on the epidemiology of the disease. *Annals of Botany* n.s. **21**, 293–329.

Rishbeth, J. (1957). Fusarium wilt of bananas in Jamaica. II Some aspects of host-parasite relationships. *Annals of Botany* n.s. **21**, 215–45.

Ross, G. J. S. (1980). MLP. Maximum Likelihood Program. Section 7·3. Rothamsted Experimental Station, Harpenden.

SAS Institute Inc. (1987). SAS/STAT Guide for Personal Computers, Version 6 Edition, Cary, North Carolina. 378 pp.

Sorensen, S. (1993). Genetic variation within *Fusarium oxysporum* f. sp. *cubense* in banana. Ph.D. Thesis. Queensland University of Technology. 222 pp.

Stover, R. H. (1962). Fusarial wilt (Panama disease) of bananas and other *Musa* species. Phytopathology Paper No. 4. Commonwealth Mycological Institute. 117 pp.

Stover, R. H., and Buddenhagen, I. W. (1986). Banana breeding: polyploidy, disease resistance and productivity. *Fruits* **41**, 175–91.

Stover, R. H., and Malo, S. E. (1972). The occurrence of fusarial wilt in normally resistant 'Dwarf Cavendish' banana. *Plant Disease Reporter* **56**, 1000–3.

Stover, R. H., and Waite, B. H. (1954). Colonisation of banana roots by *Fusarium oxysporum* f. *cubense* and other soil fungi. *Phytopathology* **44**, 689–93.

Sun, E. J., and Su, H. J. (1984). Rapid method for determining differential pathogenicity of *Fusarium oxysporum* f. sp. *cubense* using banana plantlets. *Tropical Agriculture* (*Trinidad*) **61**, 7–8.

Trujillo, E. E., and Snyder, W. C. (1963). Uneven distribution of *Fusarium oxysporum* f. sp. *cubense* in Honduras soils. *Phytopathology* **53**, 167–70.
Waite, B. H. (1977). Inoculation studies and natural infection of banana varieties with races 1 and 2 of *Fusarium oxysporum* f. sp. *cubense*. *Plant Disease Reporter* **61**, 15–19.
Zar, J. H. (1984). Biostatistical analysis 2nd Edn pp. 185–205. (Prentice-Hall International Editions.)

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