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# POSTHARVEST ENVIRONMENTAL FACTORS AFFECTING INFECTION OF KIWIFRUIT BY *BOTRYTIS CINEREA*.

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science at Massey University Palmerston North New Zealand.

> Silvia Bautista Baños May 1995

To my husband J. Raúl: With my deepest gratitude and love, forever.

# ABSTRACT

*In vitro* germination of *B. cinerea* isolates from diseased blueberry, camellia, grapes, kiwifruit and strawberry were compared at Massey University (Palmerston North) and pathogenicity on kiwifruit at both Massey University and at the New Zealand Institute for Crop and Food Research (Levin). An average of 74.4% of spores germinated on agar when the concentration was  $5.9 \times 10^4$  but only 62.8% when it was  $1.5 \times 10^6$ . Percentage spore germination on agar did not indicate relative pathogenicity on kiwifruit and there was no significant difference in disease incidence of fruit inoculated with different isolates.

The effect of culture medium and of age of culture from which conidial inoculum was prepared were evaluated by growing *B. cinerea* on malt agar, potato dextrose agar and autoclaved kiwifruit leaves for seven, 18 or 28 days. Each fruit was inoculated with one drop of a 0.05% Tween 20 suspension containing 1,000, 5,000, 25,000 or (Levin only) 125,000 spores per drop. Disease incidence was proportional to inoculum concentration. There was no significant effect of colony age. The highest disease incidence at Massey University was with inoculum produced on malt agar whereas at Crop and Food Research it was with inoculum produced on autoclaved kiwifruit leaves. All further inoculation work was carried out using the K3 isolate from Massey University grown on Malt agar for 10-14 days.

The ability of *B. cinerea* conidia to survive temperature/humidity regimes that could be used for curing kiwifruit was tested by exposing conidia on glass slides to combinations of 0, 10, 15, 25 or 30°C with low (<50%), medium-low (64-80%), medium-high (80-90%) or high (>90%) relative humidities for two, four, six or eight days. Both the percentage germination and the speed of germination decreased at the higher temperatures and with longer exposure times.

The effect of temperature during curing on subsequent infection levels was investigated in 1992, of humidity in 1993 and of both temperature and humidity in 1994. After harvest, each fruit was inoculated with 125,000 spores (1992) or 25,000 spores (1993 and 1994). In 1994 dry conidial application using a paintbrush was also included. The greatest curing effect was obtained at 10°C. Disease incidence increased at 0°C and the curing effect diminished at temperatures above 10°C. Fruit cured at 20°C and at 30°C softened rapidly and developed a high incidence of disease. In 1994 a three day curing period was used and 10°C again gave the lowest subsequent disease incidence. After twelve weeks coolstorage (1993) there was less disease in fruit cured at 89-95% relative humidity than at lower humidities. In 1994 comparable results were obtained.

The effect of curing regimes on fruit physiology showed that ethylene production increased and rate of respiration decreased with higher curing temperature but both increased with incubation time. There was no consistent pattern of treatment effect on ethylene production or on rate of respiration during subsequent coolstorage. Fruit firmness decreased with higher curing temperatures and as the curing period was extended. Firmness fluctuated with harvest and in general decreased with storage although a satisfactory firmness was maintained throughout coolstorage There was no consistent relationship between from all treatments. temperature/time of incubation and total soluble solids content during curing and during storage. As the period of storage increased glucose and fructose content of fruit increased. pH remained constant in fruit from all treatments and there was no consistent relationship between acid buffering capacity measured citric acid equivalent and curing as temperature/incubation times during subsequent coolstorage. For all experiments, weight loss increased with increased curing temperature or with decreased relative humidity.

Kiwifruit stem scars consisted of two main tissue systems: Ground and

vascular. Parenchyma, collenchyma and idioblasts containing raphides were the main components of the ground tissue. The vascular system consisted of xylem vessels, phloem and cambium. There was no evidence of anatomical structures blocking the xylem vessels in *Botrytis* infected fruit cured at 0°C or at 10°C. Samples from both showed some evidence of thickening of the parenchyma cell walls in contact with conidial hyphae. Positive reactions to lignin, suberin and reducing compounds were observed in all treatments. Suberin development in xylem and parenchyma scar tissue was found at 10°C but not at 0, 20 or 30°C.

Initial relative humidity ranges of 34-80%, 75-90% and 100% were tested during coolstorage at 0°C in 1992 and 40-59%, 65-80% and 92-97% in 1994. Inoculum levels applied to the stem scar were 5000 and 25000 spores/ml respectively and infection levels were evaluated after 12 weeks coolstorage. There was no definite pattern in ethylene production and rate of respiration during the incubation period. In both, 1992 and 1994 experiments weight loss increased as relative humidity decreased. TSS increased during incubation for all treatments. Firmness decreased with incubation time and after three months coolstorage for all treatments. In the second experiment of 1994 there was a more marked effect of relative humidity on firmness. Fruit firmness decreased as incubation time increased and in 1994, infection levels decreased as relative humidity increased.

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   sclereids (s) and collenchyma (q) cells. Bar indicates
   0.2 mm.
- 6-10 Longitudinal section of a kiwifruit stem scar stained 143 with methyl violet eosin showing idioblast containing calcium oxalate crystals (raphides) (r) and parenchyma (p) cells. Bar indicates 0.2 mm.
- 6-11 Longitudinal section of a kiwifruit stem scar stained 143 with methyl violet eosin showing xylem vessels (x) with helicoidal secondary wall thickening and parenchyma (p) cells. Bar indicates 0.02 mm.
- 6-12 Longitudinal section of a kiwifruit stem scar stained 144 with methyl violet eosin after two days curing at 20 or 30°C. Most of the spores (s) scattered on the surface of the xylem vessels (x) have germinated. Bar indicates 0.2 mm.

- 6-13 Longitudinal section of a kiwifruit stem scar stained 144 with methyl violet eosin after two days curing at 10°C. Most of the spores (s) scattered on the surface of the xylem vessels (x) have not germinated. Bar indicates 0.2 mm.
- 6-14 Longitudinal section of a kiwifruit stem scar cured at 145 either 0 or 10°C for two days stained with methyl violet eosin. Parenchyma cell walls (p) in contact with hyphae (h) are thicker than normal. Bar indicates 0.2 mm.