

GROWTH RATES OF RIPE ROT FUNGI AT DIFFERENT TEMPERATURES

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ABSTRACT

Fruit storage and ripening studies have shown that the severity and incidence of ripe rots is affected by temperature and the ripeness of the fruit. High temperatures and long storage times favour the development of ripe rots while low temperatures and fast ripening times generally inhibit the growth of ripe rots. There is the potential for some fungi to grow faster or more slowly relative to one another depending on storage conditions. Differences in the growth rate at different temperatures of each type of fungi may explain, in part, why there are different incidences and severity of ripe rots in fruit from different orchards. To determine the growth rate of fungi, fungal isolates taken from ripe rots were grown *in vitro* on agar or *in vivo* on ripening fruit at a range of temperatures. Overall the fungi grew faster the warmer the temperature but each fungi species grew at different rates across the temperatures. The fungal growth on avocado flesh was exponential compared to a linear growth on agar suggesting it is not a good indication of the growth on avocado flesh. The growth of fungi could be linked to the rate of avocado flesh ripening, implying the riper the avocado flesh, the faster the fungal growth irrespective of temperature. In this study it was not possible to separately identify the effect of temperature or growth media on the fungal growth rates. Having prior knowledge of the predominant fungi in different lines of fruit could potentially aid in improving storage management.

Keywords: *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Phomopsis* sp., *Botryosphaeria dothidea* or *Botryosphaeria parva*, ripening

INTRODUCTION

Since the 1996/97 harvest season the New Zealand avocado industry has exported between 47 to 67% of the total crop each year to distant markets in Australia, the USA and Asia (Annual Report, 2008). The price received for the fruit in the export markets is determined by the amount of fruit available in the market and the quality of the fruit when it arrives and is ripened. When avocado fruit ripen they become edible but can also develop ripe rots. The development of ripe rots is seen as a reducing the quality of the fruit. Five species of fungi, *Colletotrichum acutatum* (C.a.), *Colletotrichum gloeosporioides* (C.g.), *Phomopsis* sp. (P. sp.), *Botryosphaeria dothidea* (B.d.) or *Botryosphaeria parva* (B.p.), have been shown to be the most common cause of ripe rots in New Zealand avocados (Everett, 2002). Fruit storage and ripening studies have shown that the severity and incidence of ripe rots is affected by temperature and the ripeness of the fruit (Dixon *et al.*, 2003a; Dixon *et al.*, 2004). High temperatures and long storage times tend to favour the development of ripe rots while low temperatures and fast ripening times generally inhibit the growth of ripe rots (Dixon *et al.*, 2003a). In a series of experiments looking at storage temperatures, storage duration and fruit maturity it was determined that the incidence and severity of ripe rots was greatest in early and late season fruit that were more than 31 to 33 days old after harvest (Dixon *et al.*, 2003b). Once the fruit age increased beyond 33 days the incidence and severity of ripe rots increased exponentially (Dixon *et al.*, 2003b).

The rots on avocado fruit represent a complex of different fungi that may have different temperature optima for growth as they do for spore germination (Everett and Pak, 2002). Temperatures change throughout the postharvest handling chain with the potential for some fungi to grow faster or more slowly depending on storage conditions. A difference in growth rate at different temperatures may, in part, explain why fruit from different orchards can have different incidence and severity of ripe rots. If fruit from one orchard has

predominantly fungal infections of one type of fungi that grows more slowly than other fungi then the fruit quality will appear better even though the fruit will have received similar postharvest handling and have similar fungal inoculum loading. Identifying which species of fungi are fast or slow growing would therefore be useful to determine if one or two types of fungi are responsible for most of the rots on ripe fruit. To determine the growth rate of fungi, fungal isolates taken from ripe rots were grown *in vitro* on agar or *in vivo* on ripening fruit at a range of temperatures.

MATERIALS AND METHODS

Identified isolates of the five most common ripe rot fungi found on New Zealand avocados were provided by Dr Kerry Everett of Plant and Food Research Ltd.

In vitro growth rates

A 5mm diameter agar plug containing inoculum from one of the following fungi: C. a., C.g., P. sp., B. d. or B. p. was placed onto the edge of a fresh potato dextrose agar plate under aseptic conditions. Each agar plate only received inoculum from one fungus. Following inoculation, agar plates were placed into temperature controlled cabinets set at 25°C, 15°C, 10°C or 5°C. Each cabinet had a temperature tolerance of $\pm 0.5^\circ\text{C}$ from the set point. Agar plates were also maintained in a room at $20^\circ\text{C} \pm 1^\circ\text{C}$. Five agar plates for each fungus were placed either on the top, middle or bottom shelves of each cabinet, giving a total of 15 agar plates per fungus at each temperature. Within 24 hours of inoculation and then once a day after commencement of the experiment the agar plates were assessed for growth development. Fungal growth was measured from the point of inoculation to the outer edge of the infection zone down a plate. The measurements were taken at two longitudinal positions on each of the agar plates to the nearest mm.

In vivo growth rates on ripening fruit flesh

Fruit were harvested at three times during 2003 from the Whangarei region of New Zealand. The

average dry matter content of a twenty fruit sample from five orchards was on 6 August 23.99%, 20 August 24.00% and 2 September 25.46%.

Avocado flesh was prepared for inoculation by cutting individual 'Hass' avocado fruit in half longitudinally and then removing the seed. Each fruit half was dipped in a 1% solution of sodium hypochloride for 2 minutes then drained and allowed to dry. To inoculate the fruit flesh a slice of flesh through the equator, approximately 10mm in thickness, was removed. Fungal material as spores (C.a., C.g., *Phomopsis* sp) or mycelium (B.d., B.p.) was placed onto the flesh under the skin of the slice of fruit. The slice was then placed back onto the fruit half. For each fungus five fruit halves per temperature were inoculated. Each group of five fruit halves were then carefully placed into moistened unsealed plastic bags on the middle shelf of a temperature controlled cabinet or on the bench in the temperature controlled room set at 20°C. The flesh under the slice for each fruit half were assessed for fungal lesion development in the flesh every 2 to 3 days for the first 7 days after inoculation and then daily until no further growth could be observed. Lesion size was measured from the point of inoculation to the outer edge of the infection zone. Two measurements were taken: one from the inner side of the slice and one from the inner side of the fruit half. Lesion size was measured to the nearest mm. To measure ripeness each fruit half was assessed for colour development as described in the AIC Fruit Assessment Manual 2003 (Dixon, 2003).

Non-stored fruit

Experiment 1

Fruit harvested on 6/8/2003 were prepared and inoculated on the 8/8/2003 then ripened at 25°C, 20°C, 15°C, 10°C and 5°C for up to 42 days. Five fruit halves per fungus at each temperature for a total of 125 fruit halves inoculated.

Experiment 2

Fruit harvested on 1/9/2003 were prepared and inoculated on 2/9/2003 then ripened at 25°C and 20°C. There were five fruit halves per fungus at

each temperature for a total of 50 fruit halves inoculated.

Stored fruit

Experiment 3

Fruit harvested on 19/8/2003 were prepared on the 20/8/2003 then stored at 5°C for one week before inoculation on 27/8/2003 then ripened at 25°C or 20°C. Five fruit halves per fungus at each temperature for a total of 50 fruit halves inoculated.

Experiment 4

Fruit harvested on 1/9/2003 were prepared on 2/9/2003 then stored at 5°C for one week before inoculation on 9/9/2003 then ripened at 15°C, 10°C, 5°C. Five fruit halves per fungus at each temperature for a total of 75 fruit halves inoculated.

Data analysis

The growth rate of each fungal species at each temperature was calculated for each replicate as the slope of a line as determined by linear regression using MINITAB version 13.31. The fungi grown on agar grew in a constant linear manner; growth was expressed as a rate of mm.day⁻¹. For fungi grown on avocado tissue the growth data was natural log transformed from an exponential form to a linear form. The rate constant of growth was calculated as the slope of a linear regression of the transformed data. The data presented in the tables represents the average growth rate or rate constant of growth.

RESULTS

In vitro growth rates

Fungal colonies grew at a constant rate with growth faster the warmer the temperature (Figure 1). Results are presented for the growth of fungal colonies of C.g. in Figure 1 as representative of the growth pattern observed for all the fungi studied. B.p. and B.d. had the fastest growth rate on agar at 25°C and was about 3 to 4 times faster than the growth of C.g, C.a. and Phomopsis (Table 1). Over all the temperatures the fastest growing fungi were B.p followed by B.d (Table 1). The slowest growing fungus at each temperature was C.a. (Table 1).

Phomopsis failed to grow at 5°C. The fungi C.g and Phomopsis grew at similar rates at temperatures from 25°C to 10°C (Table 1). At 5°C the growth of B.p. and B.d. was similar to C.g.

In vivo growth rates on ripening fruit flesh

Fungal growth on avocado flesh was curvilinear and best described by an exponential function (Figure 2, upper panel). A natural log

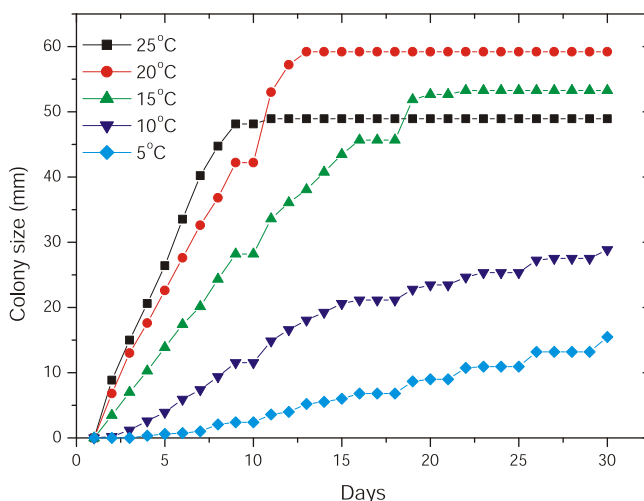


Figure 1. Average growth of colonies of Colletotrichum gloeosporioides on potato dextrose agar at different temperatures.

Table 1. Growth rates (mm.day⁻¹) of five different fungi on potato dextrose agar at temperatures from 25°C to 5°C.

Fungi	Temperature (°C)				
	25	20	15	10	5
C.g.	6.3	5.0	3.4	1.6	0.6
C.a.	4.6	2.4	1.2	0.8	0.3
B.d.	21.2	8.5	6.5	1.5	0.8
B.p.	25.6	12.0	10.5	4.1	0.7
Phomopsis sp.	7.1	4.4	3.4	1.4	0.0

transformation of the data allows the growth of the fungal colony to be described as a rate constant or the constant proportional increase in colony size. Fungal colony growth on avocado flesh was also

curvilinear in relation to the ripeness of the fruit and best described by an exponential function (Figure 3). This relationship means that as the fruit halves ripened the growth of the fungi gets faster. Therefore, to describe the growth of the fungal lesion the rate constant for the change in lesion size on the flesh was used.

Growth of fungi at different temperatures in vitro and in vivo

In general, over all the temperatures the fastest growing fungi on ripening avocado flesh was Phomopsis followed by B.p. and B.d. (Table 2). The slowest growing fungi were C.g. and C.a. (Table 2). The increase in rate constant with temperature was linear for C.g. and Phomopsis while there was a sigmoid pattern of the rate constant with

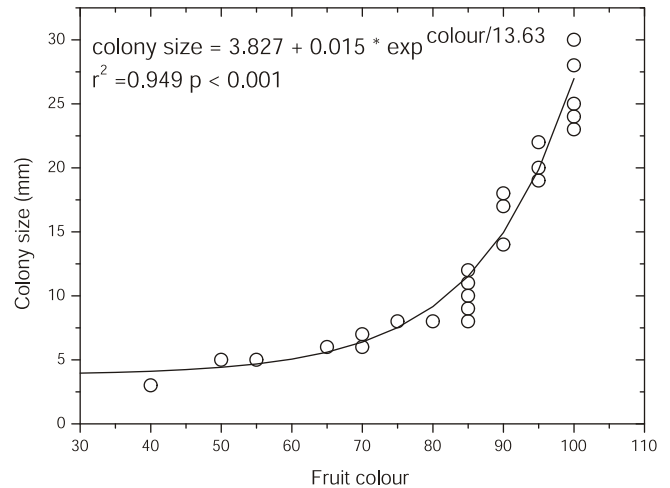


Figure 3. Relationship between colony size and fruit colour of *Colletotrichum gloeosporioides* on avocado tissue stored one week at 5°C then ripened at 5°C.

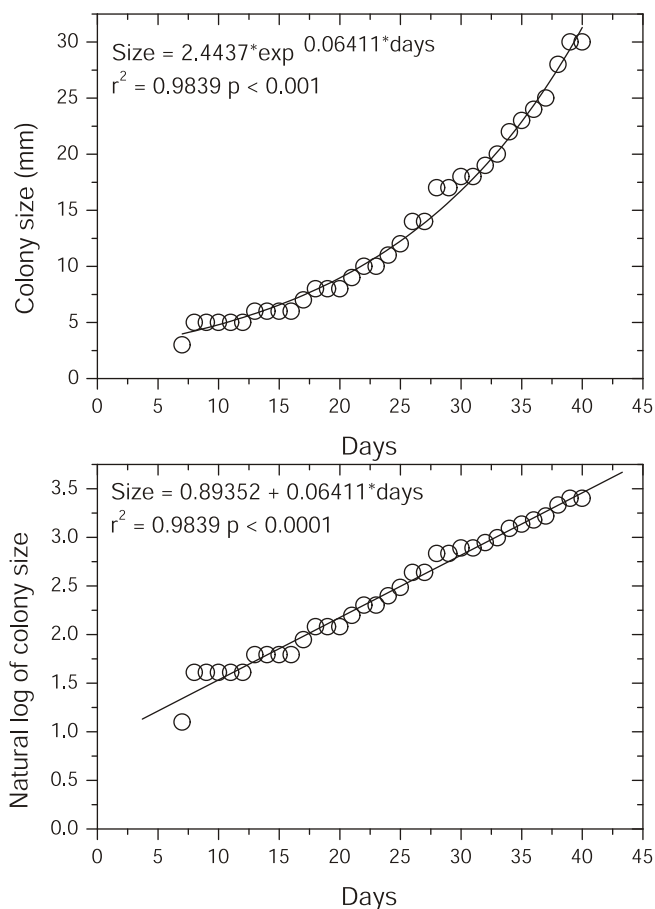


Figure 2. Growth of *Colletotrichum gloeosporioides* on avocado tissue stored one week at 5°C then ripened at 5°C. Upper panel: exponential growth pattern, lower panel: natural log transformed colony size.

temperature for C.a., B.d. and B.p. where the growth at 25°C was less than or similar to the growth at 20°C. At 5°C the greatest rate constant was for B.d.

Growth of fungi in vivo on non-stored and stored fruit

In general, the growth of fungi on one week old avocado flesh was faster than non-stored avocado flesh maintained at 20°C (Table 3). The growth of fungi on avocado flesh maintained at 25°C for one week did not follow the same pattern as the growth at 20°C. At 25°C, for C.g. the rate constants were

Table 2. Rate constant of growth (per day) of five different fungi on ripening non-stored avocado tissue at temperatures from 25°C to 5°C, fruit were harvested on 6/8/2003 and inoculated 8/8/2003.

Fungi	Temperature (°C)				
	25	20	15	10	5
C.g.	0.23	0.18	0.13	0.07	0.04
C.a.	0.24	0.23	0.13	0.07	0.04
B.d.	0.29	0.32	0.24	0.11	0.11
B.p.	0.38	0.39	0.33	0.09	0.02
Phomopsis sp.	0.56	0.43	0.21	0.14	0.00

Table 3. Rate constant of growth (per day) of five different fungi growing on non-stored avocado flesh or avocado flesh after one week storage at 5°C then ripened at 25°C or 20°C.

Ripening temperature Storage Harvest date Inoculated	25°C		20°C	
	Non-stored	Stored	Non-stored	Stored
	1/9/2003	19/8/2003	1/9/2003	19/8/2003
	2/9/2003	27/8/2003	2/9/2003	27/8/2003
Fungi				
C.g.	0.36	0.34	0.32	0.28
C.a.	0.38	0.56	0.39	0.59
B.d.	0.74	0.54	0.48	0.50
B.p.	0.94	0.71	0.49	0.65
Phomopsis sp.	0.26	0.43	0.68	1.09

similar between non-stored and stored avocado flesh. At 20°C, C.a., B.d., B.p and Phomopsis sp. the rate constants were greater on stored fruit than non-stored fruit (Table 3). The rate constant of C.g. was less on the stored fruit compared to the non-stored fruit. For non-stored fruit, the rate constant of growth for B.p. and B.d at 25°C and 20°C was about 2.5 times greater on ripening avocado flesh at the beginning of September than it was at the beginning of August (Tables 2 and 3). The growth of C.g. and C.a. at 25°C and 20°C was also greater than one month earlier. For Phomopsis the growth at 25°C was less in ripening avocado flesh harvested in September than on ripening avocado flesh harvested in August but was greater at 20°C than any other fungi.

For avocado flesh maintained at temperatures below 20°C when ripening after storage for one week at 5°C the rate constants of the fungi growth were lower than at 20°C and 25°C (Tables 3 and 4). For the C.g. and C.a fungi growth on avocado flesh was dependant on temperature with an approximate doubling of the rate constant with each 5°C increase (Table 4). For the fungi B.p. and B.d. the rate constant of growth was similar at 10°C and 5°C. For Phomopsis the growth at 10°C had the greatest rate constant.

DISCUSSION

Growth rates of the five most common fungi that cause ripe rots in New Zealand avocados were

determined both *in vitro* and *in vivo* at a range of temperatures. Overall B. d., B. p., C.a., C.g. and Phomopsis all grew faster the warmer the temperature when grown on agar. Each of the different fungi species grew at different rates

Table 4. Rate constant of growth (per day) of five different fungi on ripening avocado tissue at 15°C, 10°C or 5°C after one week storage at 5°C, fruit harvested on 1/9/2003 then cut in two on 2/9/2003 and inoculated 9/9/2003.

Fungi	Temperature (°C)		
	15	10	5
C.g.	0.18	0.10	0.06
C.a.	0.24	0.12	0.05
B.d.	0.27	0.10	0.11
B.p.	0.42	0.16	0.17
Phomopsis sp.	0.28	0.35	0.09

across the temperatures used in this study. At the storage temperature of 5°C, B.p., B.d., and C.g. grew the fastest. C.a. grew more slowly and Phomopsis failed to grow. When incubated at the avocado ripening temperature of 20°C, B.p. grew faster than any other fungi. Phomopsis fungi grew slightly more slowly than C.a. The growth of C.a. continued to be slow. The growth rates of the fungi on agar may not reflect the true growth of the fungi on avocado flesh. Avocado flesh is a different

medium to agar that could promote or inhibit fungal growth. Therefore fungal growth on avocado flesh at different temperatures was compared to the growth on agar.

The fungal growth on avocado flesh was exponential requiring comparisons between temperatures to be made using the rate constant of growth (the constant percentage increase in fungal lesion size). On avocado flesh incubated at 5°C the fastest growing fungi was B.d. but in contrast to agar the B.p. fungus was very slow growing. C.a. and C.g. grew at the same rate and *Phomopsis* failed to grow. In contrast to agar at 20°C *Phomopsis* grew the fastest on avocado flesh and C.a. and C.g. the slowest. B.p. was the second fastest to grow. These results indicate that the growth on agar is not necessarily a good indication of the growth on avocado flesh for *Phomopsis* but could be a suitable indicator for B.p., B.d., C.a. and C.g.

Avocado fruit that take a long time to ripen have the greatest incidence and severity of ripe rots (Dixon *et al.*, 2005). Therefore having fruit that ripen quickly could be considered beneficial in that the growth of the fungi will be slower than the ripening of the fruit. To ship avocado fruit long distances the fruit is maintained at cold temperatures to inhibit the growth of ripe rot fungi and to delay the ripening of the fruit. When ripening avocado flesh softens in an exponential manner. This pattern of softening is similar to the growth pattern of the fungal lesions observed here. Therefore the growth of fungi could be linked to the rate of avocado flesh ripening, implying the riper the avocado flesh, the faster the fungal growth irrespective of temperature.

As the fruit halves ripened the growth rate of the fungi increased. In the experiments where avocado fruit stored at 5°C for as little as a week the fungi grew at a faster rate when the fruit were ripening than the unstored fruit maintained at 20°C. B.p. and B.d. grow well at all temperatures whereas *Phomopsis* grows poorly in cold conditions. Some fungi are more suited to cooler conditions than others thus storage at cool temperatures promotes

the growth of certain fungi species over others. As the time in storage gets longer the ripeness of the fruit when removed from storage also increases. Both situations make conditions ideal for rapid ripe rot expression at ripening temperatures after long coolstorage periods. Maintaining a good cool chain using low temperatures is therefore a key component to managing the expression of post harvest rots.

Having knowledge of the growth rates of the different ripe rot fungi could be one possible way to identify the most common fungi within a line of fruit but other information on factors like time of harvest, climatic conditions, host wellbeing and fungicide response would also be needed to ensure correct identification. Having prior knowledge of the predominant fungi in different lines of fruit could potentially aid in the storage management of fruit if characteristics of these fungi were better known.

CONCLUSIONS

The different ripe rot fungi grow faster in warm temperatures than cold temperatures but each type of fungi has a different rate of growth compared to one another. The fungi grew differently on agar compared to avocado flesh. The growth of the fungi on avocado flesh was related to the ripening of the avocado flesh and it was not possible to separately identify the effect of temperature or growth media on the fungal growth rates.

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