Bautista Baños, Silvia; Long, Peter G.; Ganesh, S.
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Revista Mexicana de Fitopatología, vol. 18, núm. 2, julio-diciembre, 2000, pp. 92-96
Sociedad Mexicana de Fitopatología, A.C.
Texcoco, México

Available in: http://www.redalyc.org/articulo.oa?id=61218204
The Role of Relative Humidity During Early Storage on *Botrytis cinerea* Incidence of Kiwifruit

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(Received: August 23, 1999 Accepted: August 23, 2000)


The effect of three ranges of relative humidity (RH) (40-59%, 65-80% and 92-97%) during a week storage period at 0°C was studied on the physiological changes and infection levels by *Botrytis cinerea* of kiwifruit. Fruit was harvested from Massey University, Fruit Crops Unit, Palmerston North, New Zealand, on three different dates to obtain a range of fruit maturities. Before storage, fruit was artificially inoculated with ca. 25,000 *B. cinerea* spores in a 17 µl droplet of water. Weight loss, firmness and soluble solids content (SSC) were measured at the end of this period. Subsequently, fruits were kept at 0°C to evaluate *B. cinerea* infection levels after six or 12 weeks. At the three harvest dates, weight loss increased as RH decreased and firmness was significantly higher at the highest RH range than the lowest for all harvest dates. Compared with the first and second harvest, SSC was significantly higher at the late harvest. Infection levels fluctuated with respect to the RH and to the storage period. The overall general pattern showed that with an increase in RH infection levels decreased. Percentage infection of the non-treated fruit from the three harvest dates, was lower as compared with the treated one. Further studies are necessary to relate this work with specific environmental effects during the postharvest chain of kiwifruit and with infection levels during cool storage.

Additional keywords: *Actinidia delicosa*, Botrytis infection.

Resumen. Durante una semana de almacenamiento a 0°C bajo tres rangos de humedad relativa (HR) (40-59%, 65-80% y 92-97%), se evaluaron los cambios fisiológicos en el kiwifruit y los niveles de infección por *Botrytis cinerea*. La fruta se cosechó en tres diferentes épocas para obtener un rango de madurez de la fruta, en La Unidad de Frutales de la Universidad de Massey, Palmerston Norte, Nueva Zelanda. Antes del almacenamiento, la fruta se inoculó artificialmente con una suspensión de aproximadamente 25,000 esporas en 17 µl de agua. Se evaluó la pérdida de peso, el contenido de sólidos solubles totales (SST), y la firmeza al finalizar este periodo. Posteriormente, la fruta se conservó a 0°C para evaluar los niveles de infección por *Botrytis cinerea* después de seis o doce semanas. En las tres fechas de cosecha, la pérdida de peso se incrementó a medida que la HR disminuyó, mientras que los valores de firmeza fueron significativamente mayores en el rango de HR más alto que en el menor en todas las fechas de cosecha. El contenido de SST fue significativamente mayor en la fruta de la última cosecha en comparación con la primera y segunda. Los niveles de infección fluctuaron en relación a la HR y al tiempo de almacenamiento. En general, los niveles de infección disminuyeron a medida que la HR aumentó. En las tres épocas de cosecha, la fruta no tratada presentó menor porcentaje de infección en comparación con la tratada. Se necesitan estudios adicionales para relacionar este trabajo con el medio ambiental específico durante la cadena postcosecha del kiwi y los niveles de infección durante el almacenamiento frío.

Palabras clave adicionales: *Actinidia delicosa*, infección por *Botrytis*.

As recently as 1994, there were several reports highlighting the high levels of *Botrytis* infection observed in kiwifruit [*Actinidia delicosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa*] sold overseas markets (Pennycook, 1990; Manning and Pak, 1993; Tapper, 1994). It has been widely reported that *B. cinerea* gains entrance into various fruits, including kiwifruit through the stem-end wound produced during the snapping of the fruit from the pedicel at harvest (Poole and McLeod, 1991). Studies have shown that primary *Botrytis* rot in kiwifruit appears after 4-5 weeks, but before 3 months cool storage at 0°C, spreading the disease across the trays from rotten fruit to adjacent healthy fruit (Beever, 1991). One of the main features of postharvest storage...
systems for most commodities, is an adequate storage capability for short or long-term storage. The storage system should be able to reduce or minimize postharvest losses due to the normal physiological ageing processes of the fruit and infection by microorganisms. Davis (1980), considered that for long term storage, the produce should be kept at lower temperatures than those used for shorter periods. Low temperatures will help to extend storage life of the commodity by suppressing pathogen development and prolonging host resistance (Sommer et al., 1992). Likewise, several physiological parameters such as loss of fruit weight and firmness can be positively influenced by low temperature during storage. Development of postharvest pathogens can be prevented as well by means of using low temperatures during fruit storage. In peaches, postharvest rots originated by Rhizopus stolonifer or Mucor piriformis were suppressed when fruit were stored at 0°C (Sommer et al., 1992). In contrast to the uniformity of an optimum requirement for low temperature for long term storage, there is not universal agreement about the best humidity range in which fruit and vegetable crops should be maintained to reduce spoilage by microorganisms. Thompson (1992), considered that for most perishable commodities, the RH should be maintained at 90-95%, and that higher levels of RH in the storage room would encourage high levels of decay. This range of RH (90-95%) has also been reported for storage of potatoes, sugar beets, carrots and cabbages (Ryall and Lipton, 1979). However, other studies carried out on carrots showed that lower or equal levels of infection occurred when they were stored at RH ranges of 98-100% compared with storage at 92-96% (Van den Berg and Lentz, 1973; 1974). Further research (Van den Berg and Lentz, 1977; 1978) with parsnips, rutabagas, carrots, celery and other vegetable crops also showed that RH between 98-100% reduced decay levels during storage compared with lower RH of 90-95%. Studies on kiwifruit (McDonald, 1990; Lallu et al., 1992) have shown that temperatures at 0°C and RH of 95% or above are the most suitable ambient conditions to store this commodity. Sale (1990), recommended a storage RH of 95% only for bin-stored fruit and a RH at 85% for packed fruit. In general, these RH ranges have been proposed in order to avoid infection incidence and high moisture loss. However, under these RH ambient conditions B. cinerea infection of kiwifruit, still remains a problem during cool storage. Therefore the objective of this research was to evaluate three different relative humidities ranges during one week storage period at 0°C on fruit quality and Botrytis incidence after normal cool storage.

MATERIALS AND METHODS

**Fruit Harvesting.** Fruit ‘Hayward’ variety was harvested from the Massey University Growth Unit, Palmerston North, New Zealand in 1993. Orchard temperature and RH at each harvest were 15, 10, and 14°C and 85, 76 and 87%, respectively. The experiment was repeated on three different harvest dates. Fruit from the first and second harvests were taken from one block of the orchard, while fruit from the third one was harvested from the neighbouring block within the same orchard. The harvested fruit was taken to the laboratory and the sepals removed with a soft nailbrush to provide a more uniform environment at the picking scar. The initial SSC and firmness from the early, middle and late harvest were 6.6%, 11.2% and 12.8%, and 88.2, 93.1 and 66.6 N, respectively.

**Inoculum.** The inoculum applied to each stem scar was ca. 25,000 spores/17 µl droplet of water (equivalent to 1.5x10^6 spores/ml). After the droplet had dried (approximately 4 h) fruit was placed either in the RH chambers or in plastic trays in commercial cardboard kiwifruit trays with a solid polyethylene liner and stored at Massey University Plant Unit coolstores.

**Defined RH.** Two series of vertical connected plastic tubes (1m long x 53 mm wide) were used as a salt containers. Air was continuously moved with an aquarium air pump (model Eterna II, 3.5 l/min) into the solution containers (to saturate air) and through the RH system. Analytical grade calcium chloride (CaCl₂) was used to obtain the lowest RH range, sodium chloride (NaCl) for the intermediate range and water for the highest. The RH treatment ranges were 40-59%, 65-80% and 92-97%, corresponding to vapour pressure deficits (VPD) of 0.36-0.25, 0.21-0.12 and 0.04-0.01 kPa, respectively. Inoculated control fruit were placed only in plastic trays in commercial cardboard kiwifruit trays with solid polyethylene liner and stored at 0°C, 95-100 RH.

**RH system.** The relative humidity system was designed following the method of Bautista-Baños et al. (1997). A continuous RH system was designed to carry out the experiments. Polyethylene pipes (2.20 m long x 15 cm wide) were used as a RH chambers. To ensure that fruit was within the desired humidity limits, RH was monitored (Squirrel meter/logger device Model Grant 1200) with humidity probes (Manufacturer: Grant Instrument England, Model GRASLS/233). The probe was inserted in an input and output jar in six equidistant holes in the pipe. Each hole was blocked with a rubber stopper that was removed and replaced with the RH probe to determine humidity along the length of the pipe. Eighteen fruits were introduced inside the RH chamber and placed inclined with scar uppermost. Fruit was placed in plastic kiwifruit trays attached end to end and inoculated. After fruit stem scars dried, kiwifruit trays were pulled into the pipes using two long wires. The ends of the pipe were covered with polyethylene lids connected to the RH solution containers at one end and to the output jar at the other end.

**Physiological parameters.** Firmness was measured with a penetrometer (tip 8mm) (R. Bryce Model FT327) at the mid point of each side of each fruit after removing a 2-3 cm disc of peel. SSC was measured by placing some drops of squeezed kiwifruit juice on a hand-held Atago N-20 refractometer (Model N. McCormick Fruit Tech., brix range from 0-30%).

**Assessments.** At each harvest, the initial incubation at 0°C
was for a period of seven days. Weight loss, firmness and SSC were measured at the end of this initial incubation period. Then, fruit was packed as per normal commercial practice and transferred to standard storage at 0°C and RH of 95-100%, to evaluate infection levels after six and 12 weeks cool storage.

**Statistical Analysis.** At each harvest, the three RH levels were repeated seven times to evaluate percentage infection. Fruit from three additional RH treatments were used to evaluate the physiological parameters. The SAS system program (SAS 1988) was used to carry out Duncan’s t test (P < 0.05) for each harvest. To satisfy normality assumptions, log transformation was carried out for percentage weight loss at each harvest, and for firmness at the third harvest date. Square root transformation was carried out for firmness and SSC in data from the first and second harvest, respectively.

**RESULTS**

**Fruit quality after initial RH cool storage period.** At the first harvest (Fig. 1), the weight loss of fruit under the lowest RH treatment was greater (P < 0.05) than fruit from the medium humidity, which in turn was greater than fruit incubated at high RH. This pattern of weight loss was repeated at both the second and third harvests. Fruit firmness at the first and third harvests was greater (P < 0.05) for fruit from the high RH treatment than from the low RH treatment. The contrary was found at the second harvest for fruit where the lowest firmness was found at the medium and high RH (Fig. 1). SSC was not affected by treatment except at the third harvest, where the SSC in fruit from the medium and high RH treatments were lower than in fruit under low RH.

![Figure 1](image1.png)

**Figure 1.** Weight loss, firmness and soluble solids concentration of kiwifruit held for seven days at 0°C and one of three relative humidities. Letters a, b and c refer to Duncan’s test (P < 0.05). Vertical bars indicate SEM.

![Figure 2](image2.png)

**Figure 2.** Percentage infection of inoculated kiwifruit held for seven weeks at 0°C and one of three relative humidities after six or 12 weeks of cool storage. Letters a, b and c refer to Duncan’s test (P < 0.05). Vertical bars indicate SEM.
The SSC increased with each successive harvest.

**Infection levels during cool storage.** The percentage *Botrytis* infection of fruit was similar after six and 12 weeks of cool storage, although overall, there were more infections after twelve weeks (Fig. 2). Fruit from the first harvest incubated in high humidity had fewer infection after six weeks cool storage than fruit incubated at low relative humidity. A similar pattern emerged at the second and third harvests. Fruit from the second harvest under the medium range of RH had more infection than fruit kept in the other two RH’s. In general, kiwifruit infection levels of fruit kept at various RH’s were higher compared with the inoculated control kept at 95-100% RH.

**DISCUSSION**

**Fruit quality and physiological changes during incubation time.** In this study, weight loss from the third harvest was not different between the medium and low RH ranges, but in general, moisture loss measured during the initial cool storage period increased with decreased relative humidity. A similar tendency among weight loss/RH/storage time has been shown for various vegetables and fruit commodities. For example, in grapes stored at 0°C at RH’s of 95%, 90% and 85% for six and 13 days incubation, weight loss from the lowest RH was almost three times greater at the end of the first incubation period, and three and a half times greater after the second incubation period compared with that at the highest RH (Allen and Pentzer, 1935). Similarly, in vegetables such as carrots, rutabagas, brussels sprouts, celery, chinese cabbage, leeks and to some extent parsnips, weight loss decreased when ambient storage RH was almost saturated (98-100%) at 0 to 3°C for a period of several months, compared with a higher moisture loss when RH range was between 90-95% (Van den Berg and Lentz, 1974; 1978). In general, there was no effect of relative humidities on the SSC. Changes in soluble solids levels during the incubation time and at each harvest can be explained as the normal increase in maturity. Results of SSC content agree with numerous investigations carried out on several fruit crops including kiwifruit (Crisosto et al., 1984), mangoes (Seymour et al., 1990) and apples (Chvyl and Tugwell, 1993). The highest SSC was present in kiwifruit held at the lowest RH and late harvested. This could be related to the stressful conditions given by the low RH. In avocados, it has been demonstrated increase in ripening when they were stored under water-deficit conditions (Adato and Gazzit, 1974). Although differences were detected in kiwifruit firmness after the seven day incubation period for all RH’s and the three harvest dates, the recorded values were maintained in levels similar to those of fruit commercially stored at 0°C (>73.5 N) (McDonald, 1990). Our initial, defined RH produced a rapid loss of firmness of the fruit. However, the firmness values remained within the limits accepted by The New Zealand Kiwifruit Marketing Board. In other studies (Whitelock et al., 1994) fruit were not incubated at 0°C, but a similar pattern of results was reported on four different cultivars of peaches incubated at temperature ranges of 6 to 4.3°C and RH’s of 97% to 75% and two different air flow rates (0.7 to 4.0 and 0.2 to 1.5 m/s). In that study, firmness was reported to be reduced at the lowest relative humidity at both air flows, and overall firmness increased as the VPD decreased.

**Infection levels during cool storage.** There was a more marked increase of infection levels with a low RH. Probably at a low RH the stem scar tissue could desiccate and die before active defense mechanisms were established, thereby, providing dead tissue for colonization by necrotrophic pathogens such as *B. cinerea*, in addition to weakening or inactivating the host defenses. This argument is supported by the work of Van den Berg and Lentz (1974) who observed that decay in some vegetables was reduced when stored at 0-1°C at a RH between 98-100%, compared to decay when stored at RH’s of 90-95%. In the current study, infection levels at the highest RH’s were less compared with those at the lower RH. However, non-treated fruit (just inoculated, packed and stored at 0°C, RH 95-100%) compared with the treated, had less infection levels. In this experiment two variables were different from the normal fruit storage: relative humidity and a continuous air flow. Apparently, these two combined variables affected the levels of resistance of the fruit stem scar. The static air contained in boxes with fruit immediately stored, could have approached the saturation resulting in less weight loss. On the contrary, in fruits held for seven days at different RH’s ranges, the moving air led to greater transpiration of moisture out of the cool stored fruit. An additional explanation for this differences on infection levels could be related with a modified atmosphere effects on the non-inoculated fruit. Our study also showed that percentage infection varied according to harvest maturity. High infection levels were recorded in fruit from the first and third harvest. In epidemiological studies of *B. cinerea*, kiwifruit patterns of infection levels developing in cool storage, varied on a day-to-day basis of harvest (Brook, 1990). He related these daily and seasonal variations to weather conditions, to differences in the overall population of *B. cinerea* in the orchard and to numbers of mobile spores at the time of harvest. Data reported by others (Hopkirk et al., 1990) showed that increase in harvest date reduced *B. cinerea* rots from 14.9% to 1.3% which agrees with our data collected at the first and second harvest date. However, Brook (1990) also reviewed the contradictions in evidence for the concept that kiwifruit stem-end rot susceptibility is reduced with kiwifruit maturity. An important step in this study, was the evaluation of the effect of initial storage (0°C) RH on fruit quality and infection levels during the incubation period. Under practical conditions, even though *Botrytis* develops on kiwifruit stored at RH of 95% (the recommended storage RH), higher RH should achieve the dual objectives of maintaining overall fruit quality as well as minimizing storage rots. Further study is necessary to relate this work to...
specific environmental conditions, such as different rates of air flow. Moreover, it would be important to maintain similar places of harvesting. Studies that could include environmental effects found during postharvest management of fruit such as temperature and RH in bins, during transportation, and particularly during fruit packing, would be of great help to elucidate the pathosystem botrytis-kiwifruit.

Acknowledgements
We thank the National Council for Science and Technology (Mexico), and the Commission of Operation and Development of Academic Activities from the National Polytechnic Institute (Mexico).

LITERATURE CITED