# Using Incidence of *Botrytis cinerea* in Kiwifruit Sepals and Receptacles to Predict Gray Mold Decay in Storage

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**ABSTRACT**

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A field-monitoring system has been developed to predict the incidence in storage of gray mold decay of kiwifruit caused by *Botrytis cinerea.* Kiwifruits were harvested from nine vineyards in 1993 and 1994, and their sepals and receptacles (stem ends) were surface sterilized and placed on acidified potato dextrose agar. The incidence of *B. cinerea* was determined after incubating dishes at 6 to 7°C for 6 days followed by 3 days at 23°C. At commercial harvest time, kiwifruits were harvested from these vineyards and stored at a controlled-atmosphere, cold (–0.5°C) facility. Postharvest gray mold was recorded after 3 and 5 months of storage. In both years, the incidence of sepal colonization decreased 3 months after fruit set and then increased until harvest time. In contrast, the incidence of receptacle colonization increased continuously from 4 and 1 months after fruit set until harvest in 1993 and 1994, respectively. In 1993, the relationship between incidence of *B. cinerea* in fruit sepals or receptacles and incidence of gray mold after 3 and 5 months of fruit storage was significant, as determined by linear regression, for most sampling dates from 4 months after fruit set until harvest. In 1994, all regressions were significant ($R^2$ = 0.55 – 0.96, $P < 0.05$ or <0.01) and for both years the best correlation was obtained with the samplings done 4 months after fruit set. Furthermore, for both years, low (<15%), medium (16 to 50%), and high (>50%) incidence of sepal or receptacle colonization by *B. cinerea* distinguished (predicted) the majority of the vineyards as having low (<2%), moderate (2 to 6%), and high (>6%) incidence of postharvest gray mold decay after 5 months of storage, respectively. In vineyards where the incidence of gray mold decay was low to moderate (<2% and up to 3.3%), one or two sprays of vinclozolin (at bloom and/or preharvest) did not significantly reduce the incidence of gray mold. However, preharvest spray(s) of vinclozolin applied 1 and/or 2 weeks before harvest in vineyards with high (>6%) incidence of gray mold significantly reduced fruit decay in storage.

Additional keywords: *Actinidia deliciosa*, stem-end scars

Approximately 2,900 ha of kiwifruit (*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson) are presently grown in California (1). Initially, kiwifruit was regarded as a "disease-free" crop in New Zealand (11) and also in California (25). However, because acreage increased as exports expanded during the past 10 years, the quantity of fruit in cold storage has increased and postharvest rotting of kiwifruit has become a potentially serious problem. Kiwifruit are kept in storage for long periods, particularly since controlled-atmosphere (CA) storage has extended the postharvest life of kiwifruit from a few weeks to several (usually 5 or 6) months (8,16). Extended storage helps coordinate orderly marketing of fruit but results in losses due to gray mold decay caused by *Botrytis cinerea* Pers.:Fr.

Gray mold storage decay is the most important disease of kiwifruit (10,19,21,25, 26,29). Even though the disease does not occur in California vineyards (25,27), postharvest decay is a direct result of *B. cinerea* infections that occur in the vineyard but remain latent in the senescent floral parts (sepals and stamens) or stem-end scars (receptacles). In addition, small wounds created during fruit harvest can serve as infection entries by the pathogen (25). During long-term cold storage, kiwifruit become physiologically susceptible to the pathogen, which then invades the fruit tissues (10), occasionally resulting in large losses.

Because transit times are frequently as long as 30 days and conditions during transport to distant export markets can be poor, there is a potential for considerable loss of fruit quality and for gray mold decay during shipment (9). Beraha (3) reported an average of 20% stem-end decay in New Zealand–grown fruit reaching the Chicago markets. Incidences as high as 32, 50, and 20% gray mold decay have been reported from New Zealand (21), Italy (4), and California (5,6,17,24,25), respectively. Similarly, inspection of California kiwifruit arriving in European markets indicates that the principal causes of loss are excessive desiccation and decay caused by *B. cinerea*. Additional costs result from resorting and re-packing, which increase considerably the price of the final, marketable product. Unfortunately, the incidence of gray mold storage decay is unpredictable because it is highly variable from year to year and from vineyard to vineyard.

Environmental conditions during the growing season influence the incidence and severity of gray mold storage decay. For example, more significant postharvest losses occurred when cool and wet spring conditions prevailed during and after full bloom of kiwifruit vines (27). Sommer and Fortlage (24) showed that fruit from plots having the highest levels of blossom infection also had the highest fruit rot after 6.5 months of storage. Furthermore, in a preliminary study in 1992, we found that vineyards with fruit that had low levels of sepal colonization by *B. cinerea* also showed low incidence of postharvest gray mold (T. J. Michailides and D. P. Morgan, unpublished data).

Bloom and preharvest sprays of vinclozolin [3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione] (Ronilan 50DF, BASF Corp., Research Triangle Park, NC) have been shown to decrease the incidence of postharvest gray mold. Although excellent control has been achieved with two bloom and two preharvest sprays (10,24), there are still problems. Disease reduction achieved by four fungicide applications may not be economical because the cost of materials and application may exceed the loss from rot (27). In addition, frequent applications of vinclozolin increase the risk of selecting resistant strains of the pathogen (15). As a result of these problems, along with environmental issues and a great and ever-growing public concern that many pesticides may be carcinogens, efforts are being made to discover alternatives to the use of pesticides or, at the least, ways to reduce their use.

To develop more efficient methods for managing gray mold, it is necessary to determine the relationship of *B. cinerea* latent infections in the vineyard to incidence of gray mold decay in storage. Cur-

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rently, some growers routinely apply bloom and preharvest vinclozolin sprays, regardless of the expected levels of storage gray mold decay. Furthermore, when kiwifruit are removed from storage, shippers lack criteria to select which fruit to sell first. An indirect criterion used by shippers is the historical background of fields in relationship to gray mold. In a preliminary study in 1992, we found that vineyards with fruit that had low levels of sepal colonization by *B. cinerea* also showed low incidence of postharvest gray mold (T. J. Michailides and D. P. Morgan, unpublished data). Therefore, if the incidence of kiwifruit sepals or stem ends colonized by *B. cinerea* is an accurate predictor of anticipated decay in storage, then shippers could send to market first those fruit that are expected to have higher incidence of gray mold. Conversely, shippers could store longer those lots expected to have lower incidence of gray mold, and growers could spray with vinclozolin only those fields that are expected to produce fruit with moderate or high potential for disease. The purpose of this study was to determine (i) the relationship between colonization of kiwifruit sepals and receptacles by *B. cinerea* and the incidence of gray mold in storage and (ii) when the applications of vinclozolin sprays in kiwifruit vineyards are justified.

## MATERIALS AND METHODS

**Incidence of colonization of kiwifruit sepals and receptacles by *B. cinerea*.** *1993 sampling.* Three samples of 20 fruit each were collected arbitrarily 2, 3, and 4 months after fruit set, and 1 week before and again during commercial harvest from nine kiwifruit vineyards. These vineyards were in three distinct growing areas with three each in Butte County (Sacramento Valley), Kern County (San Joaquin Valley), and San Luis Obispo County (coastal area). All vineyards had either a T-bar or pergola training system and irrigated by drip or microsprinkler irrigation. The kiwifruits were collected with pedicels (stems) intact and placed in plastic holders in one-layer boxes to prevent discharge of sepals. Fruit tissue bearing the sepals and the receptacle was cut with a sharp knife, the sepals were separated from the receptacle by hand (Fig. 1), surface disinfected in a 0.1% sodium hypochlorite (NaOCl) solution plus 0.001% Triton X-100 (2 drops per liter) for 1 min, washed in sterile distilled water, allowed to dry under a laminar flow hood, and plated in petri dishes (90 mm in diameter) containing acidified (2.5 ml of 25%, vol/vol, lactic acid per liter of medium) potato dextrose agar (APDA). This method had been used successfully in preliminary experiments in 1992. Five to six sepals and one receptacle per fruit were placed in each petri dish and the dishes were incubated at 7°C for 6 days under 12 h dark/12 h diffuse light. Colonies of *B. cinerea* growing from the sepals and receptacles were recorded and the dishes were incubated at 23°C for 3 additional days and a final recording of *B. cinerea* colonies was completed. The data from the two recordings of *B. cinerea* were combined. The use of this incubation method was necessary to reduce the incidence of contamination of dishes by fast-growing *Rhizopus* spp. Whenever colonies of *Rhizopus* spp. were evident during incubation of dishes at 23°C, they were sprinkled with dicloran (2,6-Dichloro-4-nitroaniline) (Botran 75WP, Gowan Chem. Co., Yuma, AZ) to prevent their growth.

*1994 sampling.* Three samples of 20 kiwifruit with their pedicels were collected with sepals intact as described above. Sampling was done from nine vineyards at 1, 2, 3, and 4 months after fruit set, 1 week before, and once during commercial harvest. Three of these vineyards were located near the coast (San Luis Obispo and Santa Cruz counties), three in Kern County, one in Fresno County, and two in Butte County. After cutting the receptacle portion of fruit (Fig. 1) with a sterile knife and removing the sepals, the sepals and receptacles were surface disinfested as above, allowed to dry under a laminar flow hood, and five or six sepals and one receptacle of each fruit were placed in each petri dish with the receptacle always positioned in the center of each dish. Incubation of dishes and recording of *B. cinerea* colonies were completed as described for the 1993 sampling.

**Fruit harvest, storage, and evaluation of postharvest gray mold decay.** At commercial harvest in both 1993 and 1994, 38 one-layer boxes with 33 fruit per layer per vineyard were harvested and stored in a commercial, CA cold storage facility (–0.5°C and 8 ng of ethylene per ml) in Kingsburg, CA. Because of prescheduled harvest by the grower of one of the vineyards in Butte Co., fruit from only eight vineyards were harvested in 1994. Fruit were packed 16 to 24 h after picking. The incidence of infected fruit with gray mold in storage was recorded after 3 and 5 months. To minimize secondary spread of the pathogen in storage, all infected fruit were discarded after the first recording.

**Effects of vinclozolin sprays in controlling gray mold (1991 to 1994).** In 1991, in a commercial vineyard in San Luis Obispo County, five vines were sprayed 1 week before commercial harvest (14 November) with 1.2 g a.i. vinclozolin (Ronilan 50DF) per liter of water (label recommended dosage, 1 lb a.i./acre) using a knapsack-type power duster and mist blower (model DM-9, Echo Inc., Lake Zurich, IL). In 1992, in the same commercial vineyard, 10 vines were sprayed with vinclozolin either during full bloom (25 May) or 1 week before harvest (26 October) or during full bloom and 1 week before harvest (two sprays) with the same rate of fungicide using a knapsack-type power duster and mist blower as described above. In another vineyard in Kern County only the 1-week preharvest spray was applied on 28 September 1992. Ten vines not sprayed with vinclozolin served as controls for the above experiments. Six boxes of 33 fruits per replicated vine were harvested on 21 November (1991) and 2 November (1992) from the vineyard in San Luis Obispo County, and five boxes of 39 fruits per replication on 5 October from the plot in Kern County. All fruit were stored in the same CA storage facility at –0.5°C and 8 ng of ethylene per ml for 5 months.

In 1993, in a commercial vineyard in Kern County, 10 vines each were sprayed during full bloom (5 May) or 1 week before harvest (12 October), or both at full bloom and 1 week before harvest. The rate and the method of fungicide application were similar to those described for the previous years. Ten vines not sprayed with vinclozolin served as controls in each fungicide trial. Two boxes each of 33 fruit per replicated vine were harvested 1 day before commercial harvest (18 October) and stored at –0.5°C and 8 ng of ethylene per ml for 5 months.

In 1994, five vines each in four commercial kiwifruit vineyards were sprayed with vinclozolin either 2 or 1 weeks or 2 and 1 weeks before commercial harvest. The dates of fungicide applications 2 and/or 1 weeks before commercial harvest varied for the four vineyards, depending on the different maturation dates and harvest times. The four trials were conducted in a vineyard in San Luis Obispo (expected high incidence of gray mold), two in Kern County (moderate to low incidence), and one in Butte County (low incidence). Sprays with vinclozolin were applied either 25 or 31, or 25 and 31 October in the vineyard in San Luis Obispo; 27 September or 3 October or both dates in both vineyards in Kern County; and 28 September or 7 October or both dates in the vineyard in Butte County. The rate and method of application were as described for the previous years. At commercial harvest time five boxes of 33 fruit each were

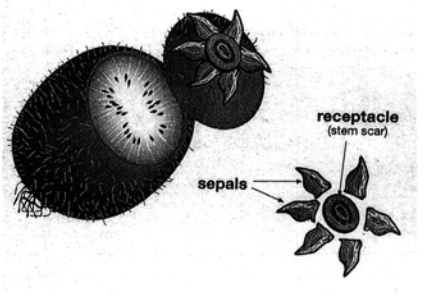


**Fig 1.** Schematic of kiwifruit as dissected for plating of sepals and receptacles to determine colonization by *Botrytis cinerea*.

harvested from each replicated vine, packed commercially, and stored in a commercial CA storage as described above. For each year's experiments, gray mold evaluation was done after 3 and 5 months of storage. To prevent secondary spread of the pathogen, all decayed fruit recorded after 3 months of storage were discarded. The experimental design for each of the above fungicide spray experiments was a randomized complete-block design.

**Statistical analyses.** For both years, data on percent colonization of sepals and receptacles by *B. cinerea* in relationship to sampling time or in relationship to the incidence of gray mold after 3 and 5 months of cold storage were subjected to regression analysis (SAS Institute Inc., Cary, NC, release 6.08). Data from the fungicide trials were subjected to analysis of variance, and treatment means were separated using Fisher's protected least significant difference test at $P < 0.05$.

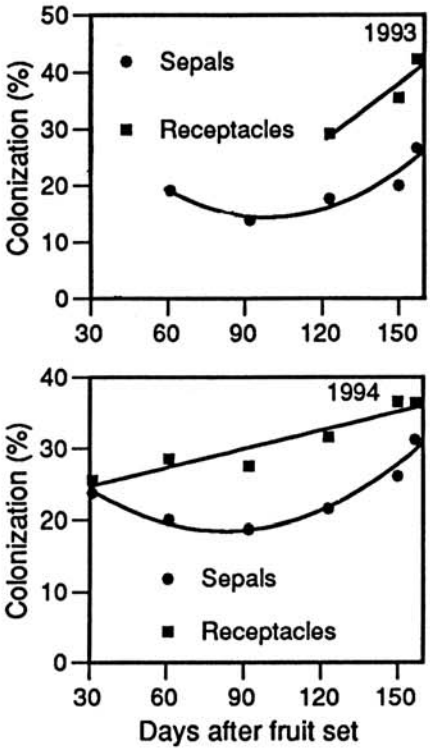


**Fig. 2.** Progress of colonization by *Botrytis cinerea* of sepals and receptacles of kiwifruit in nine vineyards each in 1993 and 1994 in California. Data points represent the means of nine vineyards per year with >300 sepals and 60 receptacles per vineyard in each sampling date.

## RESULTS

**Incidence of colonization of kiwifruit sepals and receptacles by *B. cinerea* (1993 and 1994).** Because similar trends were noted in the levels of colonization of sepals and receptacles by *B. cinerea* in the various vineyards, the data were averaged over all vineyards. In both years of testing, sepal colonization was lowest 3 months after fruit set but increased afterward until harvest (Fig. 2). Sepal colonization was best described with a second-degree polynomial; for 1993, $y = 45.5 - 0.63x + 0.0031x^2$ ($R^2 = 0.86$, $P < 0.01$), and for 1994, $y = 32.7 - 0.34x + 0.0021x^2$ ($R^2 = 0.96$, $P < 0.01$). Colonization of receptacles by *B. cinerea* increased with time and the 1994 data were best described with a positive linear equation, $y = 22.1 + 0.087x$ ($R^2 = 0.88$, $P < 0.01$) (Fig. 2).

**Fruit harvest, storage, and relationship of colonization of kiwifruit sepals and receptacles by *B. cinerea* and gray mold decay.** *1993 sampling.* Fruit samplings 2 and 3 months after fruit set showed no significant relationship between the incidence of colonization of sepals by *B. cinerea* and gray mold in storage determined 3 or 5 months after harvest (Table 1). In contrast, there was a significant positive correlation between levels of sepals or receptacles colonized by *B. cinerea* for all succeeding samplings and gray mold decay after 3 months of storage (Table 1). Specifically, the higher the levels of sepals or receptacles colonized by *B. cinerea* the more gray mold in storage 3 months after harvest (Table 1). Similar significant ($P < 0.05$) correlations were obtained between levels of receptacle colonization by *B. cinerea* sampled 4 months after fruit set, or on the day of commercial harvest, and gray mold levels recorded after 5 months of storage of fruit (Table 1). The best correlation was obtained with the incidence of *B. cinerea* in sepals or receptacles of fruit sampled 4 months after fruit set and gray mold decay after 3 months of storage. In addition, there was a highly significant correlation of incidence of *B. cinerea* in the receptacles of fruit harvested on the day of commercial harvest and incidence of gray mold in storage 3 months after harvest (Table 1).

*1994 sampling.* In 1994, regardless of the sampling date, all regressions were significant at $P < 0.01$ or $P < 0.05$ (Figs. 3 and 4). For both sepals and receptacles the strongest correlations were determined for the 3- or 4-month samplings (Fig. 3E–H). The coefficients of determination ($R^2$) for the relation of colonization of receptacles sampled 2 months after fruit set were similar to those for samplings done 3 and 4 months after fruit set (Fig. 3D). The coefficients of determination for relationships of colonization of both sepals and receptacles sampled at commercial harvest (Fig. 4C and D) were lower than those at other sampling times (Figs. 3A–H; 4A and B).

For both years of testing, the three levels, low (<15%), medium (16 to 50%), and high (>50%), of colonization of sepals and receptacles sampled 4 months after fruit set corresponded well to the majority of the vineyards having low (<2%), moderate (2 to 6%), and high (>6%) gray mold decay, respectively, after 5 months of storage (Table 2).

**Effects of vinclozolin sprays in controlling gray mold (1991 to 1994).** In 1991, in the vineyard in San Luis Obispo County with high level of gray mold decay, a preharvest spray with vinclozolin reduced the incidence of gray mold decay after 3 months of cold storage ($P < 0.04$) (Table 3). In 1992, in the vineyard in Kern County, a spray with vinclozolin 7 days before harvest did not significantly reduce gray mold (fruit from untreated control vines had only 0.6% gray mold decay) after 3 months of storage. In addition, in the vineyard in San Luis Obispo County,

**Table 1.** Regression equations, coefficients of determination, and significance of sepal and receptacle of kiwifruit colonized by *Botrytis cinerea* and incidence of gray mold decay after 3 and 5 months of storage at –0.5°C and 8 ng of ethylene per ml (from nine kiwifruit vineyards, 1993)

| | | 3 months of storage | | | 5 months of storage | | |
|---|---|---|---|---|---|---|---|
| Time of sampling[w] | Sample | Regression equation[x] | $R^2$[y] | $P$[y] | Regression equation | $R^2$ | $P$ |
| 2 months | Sepal | $-0.32 + 0.06x$ | 0.16 | NS[z] | $-0.095 + 0.22x$ | 0.22 | NS |
| 3 months | Sepal | $-0.98 + 0.12x$ | 0.24 | NS | $-2.40 + 0.40x$ | 0.24 | NS |
| 4 months | Sepal | $-1.30 + 0.13x$ | 0.92 | <0.01 | $-3.5 + 0.39x$ | 0.85 | <0.01 |
| | Receptacle | $-1.1 + 0.07x$ | 0.90 | <0.01 | $-2.90 + 0.20x$ | 0.83 | <0.01 |
| 7 days before harvest | Sepal | $-0.41 + 0.07x$ | 0.64 | <0.01 | $-0.04 + 0.18x$ | 0.48 | <0.01 |
| | Receptacle | $-0.79 + 0.05x$ | 0.49 | <0.05 | $-0.82 + 0.13x$ | 0.32 | NS |
| On day of harvest | Sepal | $-0.71 + 0.06x$ | 0.71 | <0.01 | $-1.00 + 0.17x$ | 0.50 | <0.05 |
| | Receptacle | $-1.20 + 0.05x$ | 0.83 | <0.01 | $-2.8 + 0.16x$ | 0.69 | <0.01 |

[w] Three 20-fruit samples each were collected 2, 3, and 4 months after fruit set and 7 days before and on the day of harvest from each vineyard.
[x] Regression equations were based on incidence of *B. cinerea* from >300 sepals or 60 receptacles (60 petri dishes) per vineyard and incidence of gray mold decay in storage from 38 one-layer boxes containing 33 fruit per vineyard.
[y] $R^2$ = coefficient of determination and $P$ = probability.
[z] Nonsignificant.

none of the sprays with vinclozolin applied at bloom and/or 1 week preharvest reduced significantly the incidence of gray mold after 3 months of fruit storage (Table 3).

In 1993 experiments, the level of gray mold decay on fruit from untreated vines was 3.3% (moderate) and none of the vinclozolin sprays reduced significantly the incidence of gray mold after 3 months of cold storage of fruit (Table 3). In 1994, significant differences between treatments were observed only in the plot with the high level of gray mold decay established in San Luis Obispo County (Table 4). The sprays that included the application of vinclozolin 1 week before harvest were effective in significantly reducing the incidence of gray mold decay after 3 months of storage. Vinclozolin applied 2 weeks before harvest did not result in significant reduction of disease from the control. However, after 5 months of storage, only fruit from vines sprayed both 2 and 1

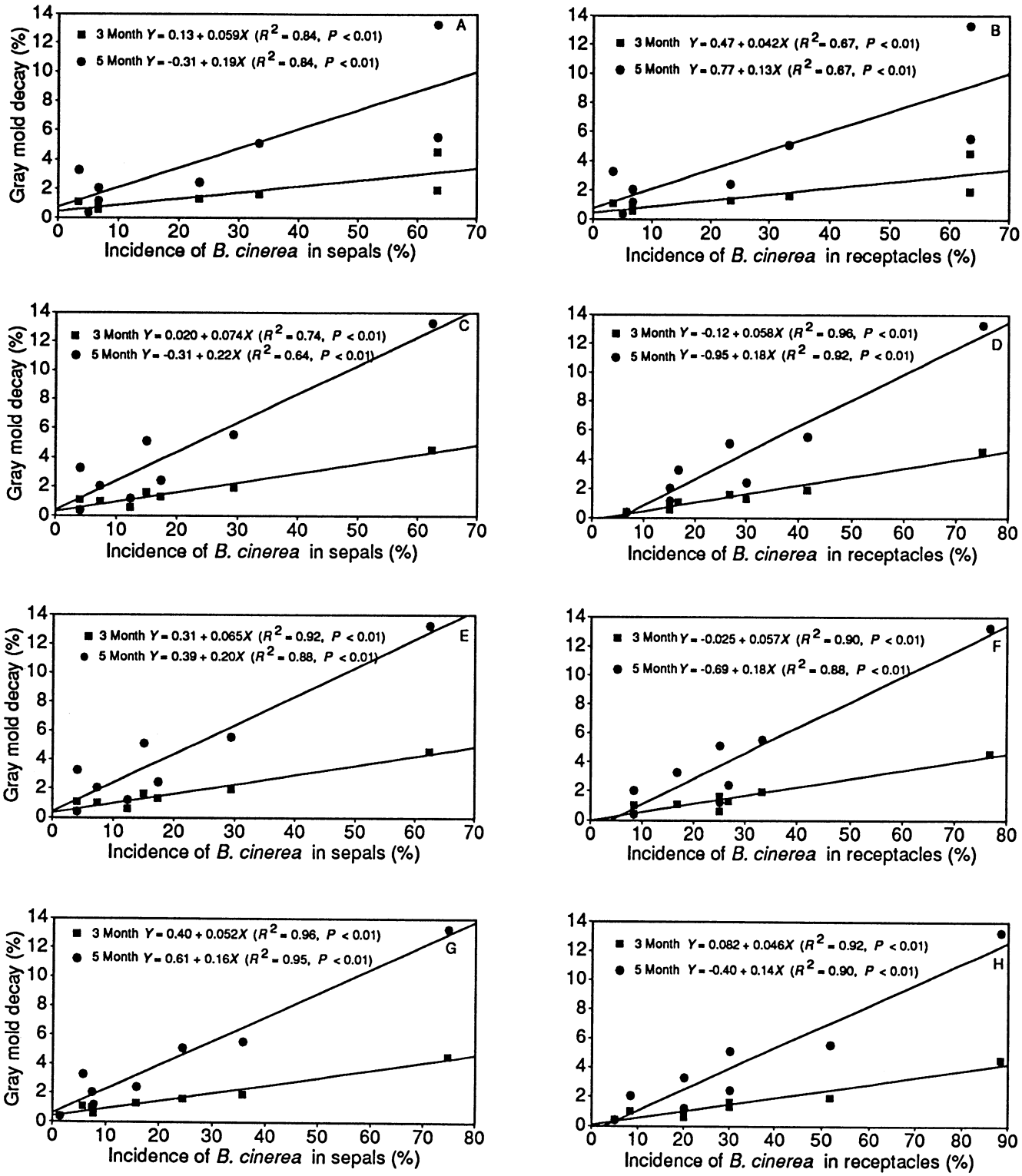


**Fig. 3 (A–G).** Relationship of *Botrytis cinerea* isolated from **(A), (C), (E), (G)** sepals and **(B), (D), (F), (H)** receptacles (stem ends) 1 (**A** and **B**), 2 (**C** and **D**), 3 (**E** and **F**), and 4 (**G** and **H**) months after fruit set with postharvest gray mold decay of kiwifruits kept at –0.5°C and 8 ng of ethylene per ml for 3 (square points) and 5 (circle points) months in storage. Incidence of sepal and receptacle colonization was determined from three samples of 20 dishes with 5 to 6 sepals and one central receptacle per dish and incidence of postharvest gray mold decay from 38 boxes of 33 fruit per box for each vineyard.

weeks before harvest showed significantly lower levels of decay (Table 4). The levels of disease in fruit harvested from the other three vineyards ranged from 1.0 to 1.9% after 5 months of storage and the fungicide applications resulted in no significant reductions of the disease incidence (Table 4).

## DISCUSSION

This study showed that it is possible to predict the levels of postharvest gray mold decay of kiwifruit in commercial storage by using the incidence of colonization of sepals or receptacles by *B. cinerea*. For both years, the best correlations were obtained with data from sepals and receptacles collected 4 months after fruit set. The results from 1993 and 1994 differed in the values of sampling sepals earlier than 4 months (Table 1 and Fig. 3). It is not clear why this difference occurred, but a possible explanation could be differences in environmental conditions during that period. In general, 1994 was a wetter year than 1993. Baudry et al. (2), using artificial inoculations, showed for kiwifruit grown in France that there are two periods of susceptibility to *B. cinerea,* (i) the bloom to fruit set phase and (ii) the period during harvest. Although our results cannot pinpoint the stages of susceptibility to *B. cinerea* of kiwifruit grown under California environmental conditions, they at least showed that sepals and receptacles of kiwifruit continuously have infections by *B. cinerea* with high levels occurring early after fruit set and close to harvest (Fig. 2) (18). *Botrytis cinerea* infections are favored under cool and wet conditions (12). The high levels of colonization of sepals early and late in the season can be explained by the cooler and wetter weather conditions usually prevailing during these periods, suggesting that some of these infections cannot survive the hot California summers. In contrast, infections of receptacles, which are in close contact with the fruit flesh tissue, may survive independent of the environmental conditions. The sterilization technique was effective in killing conidia of *B. cinerea* from the surface of sepals and receptacles because washings from surface sterilized sepals and receptacles revealed no viable colonies of *B. cinerea* after plating on APDA (T. J. Michailides and D. P. Morgan, unpublished data).

In general, the correlations of sepal or receptacle colonization by *B. cinerea* were better for the 3-month than the 5-month postharvest gray mold (Table 1; Figs. 3 and 4). Even though infected fruit were discarded after 3 months of storage, some secondary spread may have occurred since we noticed mycelia of *B. cinerea* that had spread on the surface of the plastic holders reaching some of the surrounding fruit. Although limited, the secondary spread of Botrytis gray mold that occurred during the 5 months of storage period may account for the lower $R^2$ values of the data for the 5 months than the 3 months of storage (Table 1; Figs. 3 and 4).

There are several advantages to using receptacles rather than sepals for sampling. There was a better relationship (greater $R^2$ values) for receptacle colonization by *B. cinerea* than for sepals collected at harvest time (Table 1; Fig. 4). Because infected sepals frequently fall off before or during harvest and packing of fruit, many infections might not progress from the sepals to the fruit. Because receptacles are in close contact with the fruit tissues, colonization of the receptacle almost assures infection of kiwifruit. Pennycook (21) reported that *Botrytis* infections in kiwifruit growing in New Zealand occur via the picking wound on the receptacle created as the fruit is detached from its pedicel during harvest. In contrast, our results showed that receptacles become infected throughout the growing season (Table 1; Figs. 2 to 4).

Packinghouse operators and shippers can now plan to use fruit lots with high incidence of sepal or receptacle infection by *B. cinerea* for early sales and hold lots with low incidence of sepal or receptacle infection for longer storage. Alternatively, if a diagnostic test conducted before harvest (4 months after fruit set) showed a very low colonization of sepals by *B. cinerea*, it would indicate that re-sorting and re-packing would not be necessary. Also, additional handling of fruit during repacking may create other storage or shipping problems by facilitating fruit deterioration and reducing fruit storage life. Furthermore, in those countries where postharvest treatments are registered and used, these treatments will not be necessary for fruit harvested from vineyards showing

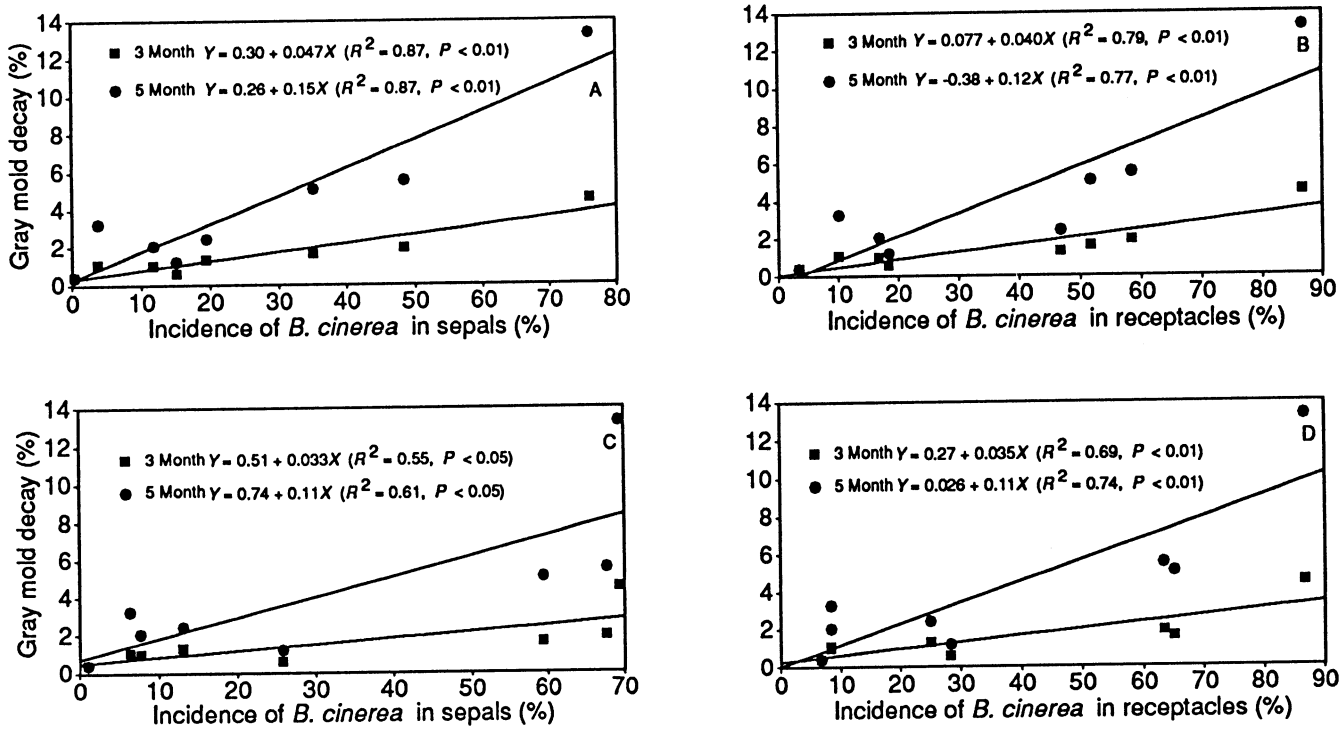


**Fig. 4 (A–D).** Relationship of *Botrytis cinerea* isolated from **(A), (C)** sepals and **(B), (D)** receptacles (stem ends) 7 days before harvest (**A** and **B**) and on the day of harvest (**C** and **D**) with postharvest gray mold decay of kiwifruits kept at –0.5°C and 8 ng of ethylene per ml for 3 (square points) and 5 (circle points) months in storage. Incidence of sepal and receptacle colonization was determined from three samples of 20 dishes with 5 to 6 sepals and one central receptacle per dish and incidence of postharvest gray mold decay from 38 boxes of 33 fruit per box for each vineyard.

low incidence of sepal or receptacle colonization by *B. cinerea*.

Unnecessary preharvest application of fungicides can be very costly and significantly limit growers' profits. Growers can collect samples of kiwifruits (with sepals and stem attached) 4 months after fruit set, send them to a laboratory to determine colonization by *B. cinerea,* and, based on infection levels (low, medium, or high) predict the expected levels (low, moderate, or high) of gray mold decay (Table 2) and decide on preharvest spray(s) of vinclozolin. It takes 6 days for the diagnostic test to provide initial results on incidence of colonization of sepals or receptacles by *B. cinerea* and 8 to 9 days for final results, which allows sufficient time for making decisions on whether to spray before harvest. Obviously, field diagnostic tests can lower the use of pesticides in the environment because unnecessary preharvest vinclozolin applications currently done by some kiwifruit growers could be avoided.

Although selective (14) or semi-selective (13) media have been developed for isolating *B. cinerea*, the APDA used in this study in combination with the specific incubation temperatures worked successfully in revealing colonization of sepals and receptacles by *B. cinerea* with limited contamination by *Rhizopus* spp. In addition, this medium, which is simple and easy to prepare, allowed development of most of the other mycoflora present in sepals or receptacles along with *B. cinerea.* These fungi might be of interest because some of these microorganisms might act as biocontrol agents. For instance, in one of the vineyards, plates for isolating sepals and receptacles had a high (75%) incidence of *Epicoccum purpurascens* Ehrenb. (18). Although the effects of this fungus on *B. cinerea* infecting kiwifruit are not known, *E. purpurascens* has been reported to suppress the incidence of *B. cinerea* on stamens and fruits of strawberries (20) and to reduce the incidence of white mold caused by *Sclerotinia sclerotiorum* in bean flowers (30).

Monoclonal antibodies have been developed for detection of *B. cinerea* in cut flowers (23) and bunch rot of grapes (22). However, the technique described here is the first simple method of detecting *B. cinerea* in sepals and receptacles of kiwifruit, and it can be used easily by private consultants to predict gray mold decay of fruit in storage. An analogous method using petal infestation has been developed for forecasting Sclerotinia stem rot of canola in Canada (28). Initially, Gugel and Morrall (7) demonstrated a positive linear relationship between the percentage of canola petals infested with *Sclerotinia sclerotiorum* and disease incidence. Based on these relationships and additional studies over a wide range of geographic, environmental, and agronomic conditions, Turkington and Morrall (28) successfully

**Table 2.** Categories of kiwifruit sepal and receptacle colonization by *Botrytis cinerea* 4 months after fruit set and vineyards with fruit in low, moderate, and high levels of postharvest gray mold decay after 5 months in storage (–0.5°C and 8 ng of ethylene per ml) in 1993 and 1994

| | | | No. of vineyards with fruit showing different levels of gray mold decay[z] | | |
|---|---|---|---|---|---|
| Sampled plant part | Colonization level | Colonization (%)[y] | Low (<2%) | Moderate (2 to 6%) | High (>6%) |
| Sepals | Low | 0 to 15 | 6, 2 | 0, 2 | 0, 0 |
| | Medium | 16 to 50 | 0, 0 | 3, 3 | 0, 0 |
| | High | >50 | 0, 0 | 0, 0 | 0, 1 |
| Receptacles | Low | 0 to 15 | 4, 1 | 0, 1 | 0, 0 |
| | Medium | 16 to 50 | 2, 1 | 2, 3 | 0, 0 |
| | High | >50 | 0, 0 | 0, 1 | 1, 1 |

[y] Percentage of colonization was determined by plating >300 sepals and 60 receptacles per sampling in each orchard.

[z] Paired numbers represent nine vineyards in 1993 and eight vineyards in 1994, respectively; fruit from one vineyard in Butte County was not sampled because of pre-scheduled grower's harvest.

**Table 3.** Effect of one or two sprays with vinclozolin in the field to reduce gray mold decay caused by *Botrytis cinerea* after 3 months of cold storage at –0.5°C and 8 ng of ethylene per ml of kiwifruit in 1991 to 1993

| | Incidence of gray mold decay (%)[y] | | | |
|---|---|---|---|---|
| | San Luis Obispo County | | Kern County | |
| Treatment[x] | 1991 | 1992 | 1992 | 1993 |
| Unsprayed control | 8.2 a[z] | 12.1 a | 0.6 a | 3.3 a |
| Vinclozolin (bloom) | ... | 9.2 a | ... | 0.8 a |
| Vinclozolin (bloom + preharvest) | ... | 9.4 a | ... | 1.5 a |
| Vinclozolin (preharvest) | 0.7 b | 7.4 a | 0.4 a | 1.8 a |

[x] The rate of application was 1.2 g a.i. per liter (2 lbs product per 100 gallons of water). Applications made at full bloom and/or 1 week preharvest.

[y] Two to six boxes each containing 33 to 39 fruit were harvested at commercial harvest time from each experimental vine. Means of disease incidence are the average of 5 to 10 single-vine replications.

[z] Numbers followed by the same letter are not significantly different according to Fisher's protected least significant difference test at $P < 0.05$.

**Table 4.** Effect of vinclozolin sprays in reducing gray mold decay of kiwifruit caused by *Botrytis cinerea* in four vineyards with different levels of expected disease in 1994

| | | | Total incidence of gray mold decay (%)[y] | |
|---|---|---|---|---|
| Vineyard/(date of harvest)[v] | Treatment[w] | Time of application (weeks before harvest)[x] | 3 months | 5 months |
| 1 (San Luis Obispo County) | Untreated | ... | 5.6 a[z] | 9.1 a |
| 7 November | Vinclozolin | 2 | 4.4 a | 6.5 a |
| | Vinclozolin | 1 | 2.4 b | 6.3 a |
| | Vinclozolin | 2 and 1 | 1.2 b | 1.7 b |
| 2 (Kern County) | Untreated | ... | 1.2 a | 1.9 a |
| 13 October | Vinclozolin | 2 | 0.4 a | 0.7 a |
| | Vinclozolin | 1 | 0.1 a | 0.8 a |
| | Vinclozolin | 2 and 1 | 0.6 a | 1.3 a |
| 3 (Kern County) | Untreated | ... | 0.7 a | 1.3 a |
| 13 October | Vinclozolin | 2 | 0.0 a | 0.2 a |
| | Vinclozolin | 1 | 0.0 a | 0.5 a |
| | Vinclozolin | 2 and 1 | 0.3 a | 0.8 a |
| 4 (Butte County) | Untreated | ... | 0.5 a | 1.0 a |
| 18 October | Vinclozolin | 2 | 0.1 a | 0.2 a |
| | Vinclozolin | 1 | 0.1 a | 1.0 a |
| | Vinclozolin | 2 and 1 | 0.0 a | 0.1 a |

[v] Orchards were selected based on both previous history of levels of gray mold decay and on isolations of *B. cinerea* in 1993 and 1994.

[w] Vinclozolin was applied at 1.2 g a.i per liter (2 lbs product per 100 gallons of water).

[x] Time of application was selected based on the time of commercial harvest.

[y] Fruit was stored in a commercial controlled-atmosphere cold storage (–0.5°C and 8 ng of ethylene per ml) for 5 months.

[z] Numbers followed by different letters are significantly different using Fisher's protected least significant difference test at $P < 0.05$.

used petal infestation by *S. sclerotiorum* to forecast Sclerotinia stem rot of canola. In a similar way, our studies showed that there is a positive linear relationship between the percentage of kiwifruit sepals or receptacles infected with *B. cinerea* and the incidence of gray mold decay after 3 or 5 months of storage of kiwifruits. Additionally, in most of the cases, the low, medium, and high levels of colonization corresponded well to the low, moderate, and high levels of incidence of gray mold decay in storage (Table 2).

Vinclozolin is registered for control of Botrytis rot of kiwifruit (24). Fungicide trials in seven locations in California showed a significant decrease in the amount of gray mold decay of kiwifruit in storage following four applications of vinclozolin, two of which were made at or near bloom and two near harvest (25). The bloom applications alone did not reduce storage rot incidence (27). In fact, sprays applied near harvest were more effective than were the bloom sprays. Our results agree with these studies and also showed that only one spray (1 week before harvest) was needed and was most effective when the disease potential was high (>6% storage decay) but a spray was not needed when the disease levels were <2% (Tables 3 and 4). In 1992, although the incidence of gray mold decay was 12% for fruit harvested from the vineyard in San Luis Obispo, none of the fungicide treatments significantly reduced the postharvest decay (Table 3). Three rainfalls totaling 33 mm occurred in October with one rain following 1 to 3 days after each fungicide application. Therefore, these rains might have removed the chemical residues from the fruit surfaces. In contrast, in 1991 the total rainfall in October was only 15 mm, and no rain occurred within the week following the fungicide sprays.

To implement this new Botrytis monitoring system, growers could collect a sample of 60 fruit (three subsamples each of 20 fruit per 2 ha field) 4 months after fruit set and submit them to a laboratory to determine the colonization of sepals or receptacles by *B. cinerea.* Plating only receptacles may provide an easier method for forecasting gray mold storage decay because it is necessary to plate more than five times more sepals than receptacles (at least five sepals per fruit). Private laboratories are already available and have been instructed on the procedures. The predictive scheme developed in this study is already being adopted by some growers. In 1995, several kiwifruit growers in California submitted samples of kiwifruit to private laboratories, obtained the exact figures on sepal and receptacle colonization by *B. cinerea* of the fruit from their vineyards, and made decisions on the need for preharvest vinclozolin spray(s). Similarly, some packinghouse operators and shippers now have the necessary criteria to decide on the need for fruit sorting and repacking to minimize secondary spread of disease in storage and plan on the timing for marketing these fruit in early 1996.


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## LITERATURE CITED


1. Anonymous. 1992. California Fruit and Nut Acreage. California Agricultural Statistics Service, Sacramento.
2. Baudry, A., Kenrinck, P., and Morzieres, J. P. 1991. *Botrytis* susceptibility of different stages of flower and fruit development of *Actinidia deliciosa* (Planch). Proc. Int. Symp. Kiwifruit, 2nd.
3. Beraha, L. 1970. Stem-end rot of Chinese gooseberry (*Actinidia chinensis*) on the market. Plant Dis. Rep. 54:422-423.
4. Bisiach, M., Minervini, G., Vercesi, A. 1984. Biological and epidemiological aspects of the kiwi fruit (*Actinidia chinensis* Planchon) rot, caused by *Botrytis cinerea* Pers. Riv. Patol. Veg. 20:38-55.
5. Duncan, R. 1991. Biological control of *Botrytis cinerea* on kiwifruit through field applications of antagonistic microorganisms. M.S. thesis. California State University, Fresno.
6. Duncan, R., and Michailides, T. J. 1990. Epidemiology and biological control of gray mold of kiwifruit caused by *Botrytis cinerea.* Annu. Res. Rep. Calif. Kiwifruit Comm., Sacramento.
7. Gugel, R. K., and Morrall, R. A. A. 1986. Inoculum-disease relationships in Sclerotinia stem rot of rapeseed in Saskatchewan. Can J. Plant Pathol. 8:89-96.
8. Harman, J. E., McDonald, B., and Fletcher, B. 1982. Controlled atmosphere storage of kiwifruit: Effects on storage life and fruit quality. (Abstr. No. 1229) Int. Hortic. Congr., 21st.
9. Harvey, J. M., Harris, C. M., and Marousky, F. J. 1983. Transit temperatures and quality maintenance in export shipments of kiwifruit (*Actinidia chinensis* Planch.). Int. J. Refrig. 6:219-224.
10. Hasbun, J. E. S. 1985. Pathological and physiological interactions between *Actinidia deliciosa* (kiwifruit) and *Botrytis cinerea* in low temperature environments. Ph.D. thesis. University of California, Davis.
11. Hawthorn, H. T., Rees-George, I., and Samuels, G. J. 1982. Fungi associated with leaf spots and post-harvest fruit rots of kiwifruit (*Actinidia chinensis*) in New Zealand. N.Z. J. Bot. 20:143-150.
12. Jarvis, W. R. 1977. *Botryotinia* and *Botrytis* species: Taxonomy, physiology, and pathogenicity. Can. Dep. of Agric. Monogr. 15. Harrow, Ontario.
13. Johnson, K. B., and Powelson, M. L. 1983. Influence of prebloom disease establishment by *Botrytis cinerea* and environmental and host factors on gray mold pod rot of snap bean. Plant Dis. 67:1198-1202.
14. Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore-trap. Neth. J. Plant Pathol. 96:247-250.
15. Lorenz, G., and Pommer, E. H. 1985. Morphological and physiological characteristics of dicarboximide-sensitive resistant isolates of *Botrytis cinerea.* Bull. OEPP/EPPO 15: 353-360.
16. McDonald, B., and Harman, J. E. 1982. Controlled-atmosphere storage of kiwifruit. I. Effect on fruit firmness and storage life. Sci. Hortic. 17:113-123.
17. Michailides, T. J., Morgan, D. P., and Duncan, R. 1992. Further studies on the biological control of gray mold of kiwifruit caused by *Botrytis cinerea.* Annu. Res. Rep. Crop Year 1991. Calif. Kiwifruit Comm., Sacramento.
18. Morgan, D. P., and Michailides, T. J. 1993. Infection of kiwifruit by *Botrytis cinerea* and its control by sepal removal and one or two vinclozolin sprays. Annu. Res. Rep. Crop Year 1992. Calif. Kiwifruit Comm., Sacramento.
19. Opgenorth, D. C. 1983. Storage rot of California-growing kiwifruit. Plant Dis. 67: 382-383.
20. Peng, G., and Sutton, J. C. 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. Can J. Plant Pathol. 13: 247-257.
21. Pennycook, S. R. 1985. Fungal fruit rots of *Actinidia deliciosa* (kiwifruit). N.Z. J. Exp. Agric. 13:289-299.
22. Ricker, R. W., Marois, J. J., Dlott, J. W., Bostock, R. M., and Morrison, J. C. 1991. Immunodetection and quantification of *Botrytis cinerea* on harvested wine grapes. Phytopathology 81:404-411.
23. Salinas, J., and Schots, A. 1994. Monoclonal antibodies-based immunofluorescence test for detection of conidia of *Botrytis cinerea* on cut flowers. Phytopathology 84:351-356.
24. Sommer, N. F., and Fortlage, R. J. 1985. Ronilan orchard sprays to control Botrytis rot of kiwifruit in storage: Research update. Annu. Res. Rep. Calif. Kiwifruit Comm., Sacramento.
25. Sommer, N. F., Fortlage, R. J., and Edwards, D. C. 1983. Minimizing postharvest diseases of kiwifruit. Calif. Agric. 37(1-2):16-18.
26. Sommer, N. F., Suadi, J. E., and Fortlage, R. J. 1994. Postharvest storage diseases. Pages 116-122 in: Kiwifruit Growing and Handling. Pub. 3349. J. Hasey, R. S. Johnson, J. A. Grant, and W. O. Reid, eds. University of California. Oakland.
27. Teviotdale, B. L., and Sommer, N. F. 1988. Botrytis fruit rot of kiwifruit. Calif. Plant Pathol. 80 (June):2.
28. Turkington, T. K., and Morall, R. A. A. 1993. Use of petal infestation to forecast Sclerotinia stem rot of canola: The influence of inoculum variation over the flowering period and canopy density. Phytopathology 83:682-689.
29. Warrington, I. J., and Weston, G. C. 1990. Kiwifruit Science and Management. R. Richards Publisher, Auckland, N.Z.
30. Zhou, T., Reeleder, R. D., and Sparace, S. A. 1991. Interactions between *Sclerotinia sclerotiorum* and *Epicoccum purpurascens.* Can J. Bot. 69:2503-2510.