New technique predicts gray mold in stored kiwifruit

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Sampling fruit from vineyards 4 months after fruit set and recording the incidence of Botrytis colonization in sepals or stem ends was used as a field-monitoring method to predict the incidence of kiwifruit Botrytis gray mold after 3 or 5 months in cold storage. Spraying the fungicide vinclozolin one or two weeks before harvest significantly reduced postharvest gray mold after 5 months storage only in vineyards with more than 6% gray mold. Preharvest sprays were not needed when expected gray mold was below 6%. The method reported here can be used successfully by growers to make decisions about preharvest sprays, sorting, repacking and when to market and ship fruit.

Approximately 6,300 acres of kiwifruit (Actinidia deliciosa) are grown in California each year, producing 10 million to 11 million boxes of fruit valued at $100 million to $110 million. Although kiwifruit initially was thought to be a “disease-free” crop in California, increasing acreage and expanding exports over the last 10 years have increased the quantity of fruit in cold storage. Postharvest rotting due to Botrytis gray mold has become a potentially serious problem.

This postharvest decay is a direct result of Botrytis cinerea infections that occur in the field but remain latent in the senescent floral parts (sepals and stamens), stem-end scars, or small wounds inflicted during fruit harvest. During long-term cold storage, the kiwifruit become physiologically susceptible to the pathogen, which usually invades the fruit tissues from the stem end.

Research in 1983 (California Agriculture January-February 1983) showed that bloom and preharvest spraying with vinclozolin (Ronilan 50WP) — which is registered for control of Botrytis rot in kiwifruit — significantly decreased the incidence of postharvest gray mold. Best control of Botrytis rot was achieved with four fungicide applications (two at bloom and two at preharvest), but this may not be economical when the cost of the fungicide applications exceeds loss from rot. In addition, four applications per year of vinclozolin increase the risk of developing resistant strains of the pathogen. As a result of these problems, along with environmental issues and public concern regarding pesticide usage, efforts are being made to discover alternatives or to reduce usage.
Decay during transit

Controlled atmosphere (CA) technology has extended the storage life of kiwifruit from a few weeks to 5 or 6 months. Although near-optimum conditions can be achieved in commercial storage, conditions during transport to distant export markets are often less than optimum. With transit times of up to 30 days, there is potential for considerable gray mold decay and loss of fruit quality during shipment from California to European markets. Fruit loss and the costs for re-sorting and re-packing can add considerably to the final price of the product. Unfortunately, the incidence of Botrytis gray mold is unpredictable since it is highly variable from year to year and from vineyard to vineyard. Up to 20% incidence of Botrytis stem-end rot has been recorded in California, with a range of 32% to more than 50% for kiwifruit from Italy and New Zealand. In California kiwifruits, decay is limited to fruit in storage or in marketing channels, with environmental conditions and field location influencing the severity of damage. In our trials, vineyards that had the highest levels of infection in floral parts also had the highest fruit rot after 6½ months in storage.

Presently there is no reliable method for predicting the development of gray mold during cold storage so some growers routinely apply one preharvest spray of vinclozolin regardless of the expected levels of disease. Shippers also lack reliable criteria for determining which fruit to sell first and which to keep for longer storage. In the past, shippers generally have relied on historical incidences of Botrytis gray mold in a field. However, if the incidence of kiwifruit sepals or stem ends colonized by B. cinerea is a correct predictor of decay in storage, it is possible to do one or more things: (1) send first to market those fruit expected to have higher incidence of gray mold and hold in storage those expected to have lower incidence of gray mold; (2) only spray fields expected to produce fruit with high potential for disease; (3) reduce the number of fungicide applications to fields with medium disease potential; or (4) avoid spraying fields that have low or nil disease potential.

The purposes of this study were to determine the relationship between colonization of kiwifruit sepals and stem ends by B. cinerea and the incidence of gray mold in cold storage, and to determine when spraying vinclozolin in kiwifruit vineyards is justified.

Field sampling (1993 and 1994)

In 1993 and 1994, three samples of 20 fruit were collected arbitrarily 2, 3 and 4 months after fruit set, and 1 week before and during commercial harvest. In 1994, fruit samples were also collected 1 month after fruit set. Vineyards used in 1993 included three each in Butte County (Sacramento Valley), Kern County (San Joaquin Valley), and San Luis Obispo County (coastal area). Vineyards used in 1994 included two in Butte County, three in Kern County, one in Fresno County, and three near the coast, in San Luis Obispo and Santa Cruz counties. All vines were trained using a standard T-bar system and irrigated by either drip or fan-jet irrigation. Fruits were collected with stems intact and placed in plastic holders in one-layer boxes to prevent discharge of sepals.

We cut fruit tissue bearing sepals and stem ends with a sharp sterile knife, then separated the sepals from the stem ends by hand (fig. 1). Sepals were surface-disinfected in a 0.5% sodium hypochlorite solution for 1 to 2
This cross-sectioned fruit with stem-end rot shows water-soaked tissues, a sign of decay by *Botrytis cinerea*.

This is a natural representation of the text content.
mold), two in Kern County (expected moderate to low incidence), and one in Butte County (expected low incidence). Vinclozolin was applied on October 25 or October 31, or both dates, in the San Luis Obispo vineyard; on September 27 or October 3, or both dates, in both vineyards in Kern County; and on September 28 or October 7, or both dates, in Butte County. The rate and method of application were the same as previous years. At commercial harvest time, five boxes of 39 fruit (6.5° Brix) each were harvested from each replicated vine, packed commercially, and stored in commercial CA storage as described above. Botrytis gray mold was recorded after 3 and 5 months storage for each year’s experiment. To prevent secondary spread of decay, we discarded all decayed fruit recorded after 3-month storage.

**Statistical analyses**

We combined data from two recordings of *B. cinerea* (after 6 days at 44° F [7°C] and 3 days at 73°F [23°C]). The percent of colonization of sepals and stem ends by *B. cinerea* in both 1993 and 1994, and the incidence of gray mold after 3- and 5-month cold storage, were analyzed with regression analysis using the REG procedure of the SAS statistical methods. Results were similar for sepal or stem-end colonization by *B. cinerea* and Botrytis gray mold developed in CA storage; therefore, 1993 and 1994 results were averaged for the 4-month sampling and presented as regressions.

**Botrytis dips after fruit set**

Because similar trends were noted in the levels of colonization of sepals and stem ends by *B. cinerea* in the various vineyards, the data were averaged over all vineyards. In both years of testing, sepal colonization decreased 3 months after fruit set, but increased afterwards until harvest (fig. 2). Colonization of stem ends by *B. cinerea* increased linearly with time as fruit approached maturity, and the data in 1994 were best described with a positive linear equation (fig. 2).

With the exception of the two first samplings in 1993, there was significant positive correlation between the levels of *B. cinerea* colonization of sepals and stem ends and the incidence of Botrytis mold decay on kiwifruit after 3 and 5 months cold storage (table 1). For both years, the strongest relationship was obtained in fruit sampled 4 months after fruit set and after 3 months in cold storage (table 1). Some secondary spread of the fungus may explain the lower correlation coefficients for the 5-month storage of fruit (fig. 3).

On the other hand, the correlation coefficients for colonization of sepals and stem ends sampled at harvest were lower than those at other sampling times, suggesting that sampling at commercial harvest time will underestimate the incidence of gray mold in storage.

For both years of testing, the three levels of colonization of sepals or stem ends by *B. cinerea* 4 months after fruit set corresponded to the majority of vineyards having a low, moderate or high level of gray mold decay at the end of the CA storage period. For example, after 3-month CA storage, the levels of sepal and stem-end colonization by *B. cinerea* predicted correctly the postharvest gray mold decay in 8

**Fig. 3. Postharvest Botrytis gray mold of kiwifruit kept at 31°F (-0.5°C) and 8 parts per billion ethylene for 3 months (square points) and 5 months (circle points).** X-axis shows incidence of *Botrytis cinerea* isolated from sepals (A) and stem ends (B) from fruit sampled 4 months after fruit set. Points are averages from nine vineyards in 1993 and eight in 1994.
out of 9 (89%) and 7 out of 9 (78%) vineyards in 1993, and 7 out of 8 (88%), and 6 out of 8 (75%) vineyards in 1994, respectively (table 2). After 5-month CA storage, the same levels of sepal and stem end colonization predicted gray mold decay in 100% and 78% of vineyards in 1993, and 75% and 63%, of vineyards in 1994, respectively (table 3). These results suggest that both sepal and stem end colonization by \textit{B. cinerea} 4 months after fruit set are reliable methods for predicting the incidence of gray mold decay after 3- or 5-month CA storage of kiwifruit. For both periods, sepal colonization by \textit{B. cinerea} more accurately predicted incidence of gray mold.


In 1991, a preharvest spray of vinclozolin reduced the incidence of gray mold \((P < 0.05)\) in the San Luis Obispo County vineyard (table 4). In 1992, spraying with vinclozolin 7 days before harvest did not significantly reduce gray mold in the Kern County vineyard. In the San Luis Obispo vineyard none of the sprays applied at bloom, and/or preharvest in 1992 significantly reduced the incidence of gray mold after 3 months of fruit storage (table 4). In the 1993 experiments, the level of Botrytis gray mold on fruit from untreated vines in the Kern County vineyard was very low (3.3%) and none of the vinclozolin sprays significantly reduced the incidence of gray mold after 3-month CA storage of fruit (table 4).

In 1994, only the sprays that included vinclozolin applied 1 week before harvest significantly reduced the incidence of Botrytis gray mold after CA storage, and then only in the San Luis Obispo plot where fruit from the unsprayed vines developed 5.6% and 9.1% incidence of gray mold after 3- and 5-month CA storage, respectively (table 5). Vinclozolin applied 2 weeks before harvest did not reduce disease. The levels of disease in fruit harvested from the other three plots ranged from 0.5 to 1.2%; fungicide applications resulted in no significant reductions of postharvest disease incidence (table 5).

**Predicting gray mold**

It is possible to use the incidence of colonization of sepals and stem ends by \textit{B. cinerea} of immature fruit as a criterion for predicting the levels of postharvest gray mold decay of mature kiwifruit in commercial CA storage. In a preliminary study (1990 to 1991), we found that vineyards with fruit having low levels of sepal colonization by \textit{B. cinerea} also showed low incidence of postharvest gray mold in cold storage. In 1992, a preliminary study showed a linear relationship between sepal infestation by \textit{B. cinerea} and incidence of gray mold after 5-month CA storage of fruit. These initial observations prompted the present study.

To implement this new Botrytis monitoring system, growers can collect fruit samples (three samples of 20 fruit throughout the vineyard)
4 months after fruit set and submit them to a laboratory to determine the extent of colonization. By using averages from our 1993 and 1994 data (fig. 3), one can easily calculate the level of gray mold expected to develop after 3 or 5 months of storage. This prediction can also help growers decide whether preharvest vinclozolin applications are necessary.

The higher levels of stem-end colonization by *B. cinerea* than those of sepals (fig. 2) can be attributed to the fact that colonization of stem ends represents a far more advanced infection capable of progressing and invading fruit tissues during storage. In contrast, infected sepals can break off during packing before the fungus has invaded the stem end. But even with this deficiency, plating more than 300 sepals (three 20-fruit samples with 5 or 6 sepals per fruit) resulted in a greater success in predicting postharvest decay (75 to 100% vineyards) than stem ends (63 to 78% vineyards). Plating stem ends may provide an easier way to forecast storage rot (for three 20-fruit samples, it is necessary to plate only 60 stem ends), but it seems to be a less successful method for predicting Botrytis gray mold in storage. It takes only 6 days for initial results on colonization of sepals or stem ends in a diagnostic test, and 8 to 9 days for final results.

By using the forecasting method described here, packing house operators and shippers can arrange to sell fruit lots with expected high incidence of sepal or stem end infection first and hold fruit with low incidence for longer storage. Alternately, if a diagnostic test conducted 4 months after fruit set shows a very low colonization of sepals by *B. cinerea*, re-sorting, re-packing, and preharvest fungicides may not be necessary. If this approach is tested and proves successful in other countries where preharvest fungicide treatments with vinclozolin are used (i.e., New Zealand, Chile, Italy, Spain, Greece), it could eliminate treatment for vineyards showing low (0 to 15%) sepal or stem-end incidence of *B. cinerea* colonization.

Although selective and semi-selective media have been developed for isolating *B. cinerea*, the medium used in this study was a simple recipe (39 g of potato-dextrose agar per liter of medium acidified after autoclaving with lactic acid to pH 3.5), and the technique described here can easily be used by private consultants to predict Botrytis gray mold of kiwifruit in storage. Because of the simplicity and the low cost of the method developed in

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**TABLE 5. Effect of vinclozolin sprays in reducing Botrytis gray mold decay caused by *Botrytis cinerea***  
In four kiwifruit vineyards with different levels of expected disease

<table>
<thead>
<tr>
<th>Vineyard*</th>
<th>Date of harvest</th>
<th>Treatment†</th>
<th>Time of application (weeks before harvest)‡</th>
<th>Total incidence of gray mold after§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>3 months</td>
</tr>
<tr>
<td>1</td>
<td>Nov. 7</td>
<td>Untreated</td>
<td>—</td>
<td>5.6a</td>
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<tr>
<td>(San Luis</td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>4.4a</td>
</tr>
<tr>
<td>Obispo Co.)</td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>2.4b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>1.2a</td>
</tr>
<tr>
<td>2</td>
<td>Oct. 13</td>
<td>Untreated</td>
<td>—</td>
<td>1.2a</td>
</tr>
<tr>
<td>(Kern County)</td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>0.4a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>0.6a</td>
</tr>
<tr>
<td>3</td>
<td>Oct. 13</td>
<td>Untreated</td>
<td>—</td>
<td>0.7a</td>
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<tr>
<td>(Kern County)</td>
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<td>0.0a</td>
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<td></td>
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<td>—</td>
<td>0.0a</td>
</tr>
<tr>
<td>4</td>
<td>Oct. 18</td>
<td>Untreated</td>
<td>—</td>
<td>0.3a</td>
</tr>
<tr>
<td>(Butte County)</td>
<td></td>
<td>Untreated</td>
<td>—</td>
<td>0.5a</td>
</tr>
</tbody>
</table>

*Vineyards were selected based on both previous history of levels of Botrytis gray mold and on isolations of *B. cinerea* in 1993 and 1994.
†Vinclozolin was applied at 2 lb/100 gallons of water (2.4 g a.i/L).‡Time of application was selected based on the time of commercial harvest.
§Fruit was stored in a commercial controlled-atmosphere cold storage (31°F [-0.5°C] and 8 parts per billion ethylene).¶Numbers followed by different letters are significantly different using Fisher’s protected LSD test at P < 0.05.
Most of the fruit in this "kiwifruit cemetery" was discarded because it had gray mold decay caused by Botrytis cinerea. The incidence of B. cinerea in immature fruit can be used to predict the levels of postharvest gray mold decay of mature kiwifruit in commercial, controlled-atmosphere storage.

In this study, growers can implement it immediately.

Furthermore, we found fungicide application to be effective only when disease levels of un sprayed controls were higher than 6% (tables 4 and 5). Our studies also showed that only one spray one week before harvest significantly reduced disease. When disease levels were lower than 3%, spraying was not necessary. Thus, using the 4-month sampling to predict storage decay can be very useful in making decisions for two, one, or no preharvest sprays.

In summary, monitoring incidence of B. cinerea colonization of sepals and stem ends to predict Botrytis gray mold in stored kiwifruit is simple, inexpensive and quick. It provides growers with useful and practical criteria for preharvest spray decisions and informs packing-house operators and shippers to make better marketing and shipping decisions.

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