Effect of Snail (*Helix aspersa*) Damage on Botrytis Gray Mold Caused by *Botrytis cinerea* in Kiwifruit

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ABSTRACT

Michailides, T. J., and Morgan, D. P. 1996. Effect of snail (*Helix aspersa*) damage on Botrytis gray mold caused by *Botrytis cinerea* in kiwifruit. Plant Dis. 80:1141-1146.

In three (1994) and two (1995) kiwifruit vineyards in the coastal area of California (San Luis Obispo County), the incidence of fruit with all sepals removed by the common brown garden snail (*Helix aspersa*) ranged from 11 to 35% in 1994 and 2 to 15% in 1995. Partially damaged fruit with fewer than three sepals per fruit ranged from 7 to 20% in 1994 and 3 to 5% in 1995. Swollen tissues associated with healing developed around the receptacle (stem end) from which sepals had been removed by snails. In a 3-year study, kiwifruit with and without characteristic damage (full removal of sepals) by snails were harvested from two vineyards and stored in controlled-atmosphere (CA) cold (–0.5°C) storage. After 3- to 5-month storage, fruit with snail damage consistently had more Botrytis gray mold than fruit not damaged by snails ($P < 0.01$). In separate studies, kiwifruit caged with snails developed more gray mold than fruit caged without snails after 3 to 5 months in CA storage in 1994 but not in 1993. In two vineyards, removal of sepals by hand did not consistently increase gray mold in CA storage. More viable propagules of *Botrytis cinerea* and other mycoflora were recovered from fruit that had snail slime than fruit without slime ($P < 0.05$). Although snail slime did not affect the germination of *B. cinerea* conidia on a nutrient-rich medium (acidified potato-dextrose agar), snail slime increased germination of *B. cinerea* conidia on a nutrient-poor medium (acidified water agarose) to more than 50% compared with 1 to 2% germination without slime after incubation at 21°C for 22 h. These results suggest that wounds caused by snails eating the sepals around the receptacle area of kiwifruit and (or) stimulation of conidial germination by snail slime may lead to more infections by *B. cinerea*.

Additional keywords: *Actinidia deliciosa*, herbivores, stem-end rot, storage rot

Botrytis gray mold, also called stem-end rot or storage rot, caused by *Botrytis cinerea* Pers.:Fr., is the most important disease of California kiwifruit (*Actinidia deliciosa*) in storage (6,31). In addition to the direct losses caused by *B. cinerea*, Botrytis-infected fruit produce ethylene in cold storage, thereby accelerating softening in nearby healthy fruits and increasing production costs (2).

B. cinerea is a necrotrophic pathogen capable of colonizing senescent, wounded, and dead tissues of flowers, leaves, and canes in kiwifruit vineyards (2,7). Infection of California kiwifruit occurs through the stem end of stored fruit under conventional and controlled-atmosphere (CA) cold storage (–0.5°C and ethylene at 8 ng/g) (20,32). *B. cinerea* can also infect kiwifruit through surface wounds created during harvest (3). To avoid wounding the fruit, those who harvest and repack it wear cotton or cotton-and-rubber gloves, respectively, and handle fruit gently. In addition to losses due to decay of fruit, losses are incurred when fruit infected by Botrytis gray mold produces ethylene in cold storage; the presence of only a few fruit with gray mold accelerates softening of nearby healthy fruit and increases sorting costs (2).

In New Zealand, infection of kiwifruit occurs during harvest as a result of *Botrytis* contamination of the picking wound ("button") made when the fruit is snapped from the pedicel (8,25). In contrast, Michailides and Morgan (20) found that in California *B. cinerea* infects sepals and stem ends of fruit throughout the year, with the highest incidence close to harvest. Sepal infections have also been observed in New Zealand, but their role in the epidemiology of *B. cinerea* has not been clearly defined (N. B. Pyke and P. A. G. Elmer, *personal communications*). Differences evidently exist in the epidemiology of *B. cinerea* in kiwifruit between California and New Zealand conditions (32).

Several factors affect the incidence of gray mold. Rainfall before and during harvest may affect the level of colonization of sepals and receptacles by *B. cinerea* (20). Other factors include inoculum load in vines and on fruit surface (10), the frequency and size of wounds created on the fruit during harvest (3,32), and infestation of kiwifruit flowers by thrips (*Thrips obscuratus*) (11). Michailides and Morgan (20) showed that the incidence of gray mold in storage is directly related to the levels of colonization of fruit receptacles or sepals by *B. cinerea* 3 to 4 months after fruit set. Based on this knowledge, a monitoring system has been developed for use in California by kiwifruit growers to determine the need for fungicide preharvest spray(s), and by fruit packers and (or) shippers to assist with decisions on which fruit to send to market first.

The common brown garden snail, *Helix aspersa* Müeller, has recently caused problems in several ornamental crops, as well as in commercial kiwifruit fields. Although the brown garden snail is edible (17), in California it is now considered a pest, particularly in the cooler coastal areas and in the Sacramento Valley. *H. aspersa* is characterized as a generalist moluscan herbivore, but it exhibits discriminatory chemotaxis (18), which allows it to find and feed consistently on certain plants while avoiding others. In the spring, high snail densities result in substantial damage to kiwifruit flowers. Later in the growing season, the snails feed on the fruit sepals without damaging the developing or fully developed fruit (Fig. 1). Growers place copper bands around the trunks of vines to prevent snails from moving from sheltered areas (dry, rolled leaves, etc.) into the canopy via the trunk (29).

Snails and slugs have been reported in close associations with fungi. Water snails enhanced the rate of germination of resting spores of *Synchytrium endobioticum* (15) and oospores of *Phytophthora* and *Pythium* spp. (14,28,33). Both snails and slugs have been reported to transmit plant diseases (16,34,35). In kiwifruit vineyards, snails and slugs commonly feed on fruits on the ground and in the canopy. Furthermore, garden slugs have been photographed eating the pilei of *Coprinus* spp. mushrooms (21). However, there is no research suggesting that garden snails contribute to postharvest fruit decays.

The symptom of snail damage on well-developed kiwifruit is the complete removal of sepals around the stem end, and signs that indicate snail visitation are the

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This project was partially supported by the California Kiwifruit Commission, Sacramento.

Accepted for publication 25 June 1996.

Publication no. D-1996-0719-05R

slime and excrement deposited on the fruit surface. Because higher sepal colonization by *B. cinerea* resulted in greater incidence of gray mold (20,22), removal of sepals by snails should reduce gray mold in storage. The objective of our study was to determine the effect of sepal removal on incidence of gray mold on kiwifruit in storage.

MATERIALS AND METHODS

Snail damage evaluation. In three (1994) and two (1995) commercial vineyards where snails were present, five (on 2 November 1994) and 10 (on 6 November 1995) vines were inspected for removal of sepals by snails on 50 arbitrarily selected fruit per vine. Sepal condition was recorded as follows: (i) complete, all sepals were removed; (ii) partial, with some of the sepals absent and snail slime and excrement present; and (iii) sepals present. Fruit with conditions (i) and (ii) were considered "damaged."

Incidence of *B. cinerea* in kiwifruit with and without snail damage. From two vineyards (designated B and C) where snail damage was severe in 1991, 1992, and 1993, 5, 11, and 14 boxes, respectively, of 33 to 38 fruit each with obvious snail damage (sepals were completely removed) and identical numbers of boxes with fruit having all their sepals attached (control fruit) were harvested commercially. Fruit were transported to the laboratory, placed in commercial plastic holders, wrapped with a perforated plastic bag, packed into standard wooden, single-layer trays, and stored in a CA cold facility (–0.5°C and ethylene at 8 ng/ml). Gray mold incidence was determined after 3 months, and fruit with gray mold were discarded. Remaining fruit were placed back in CA storage for 2 months, and the incidence of gray mold was determined. For the 1992 harvest, fruit were discarded after the 3-month disease evaluation because the incidence of gray mold was too high to continue storage for 5 months.

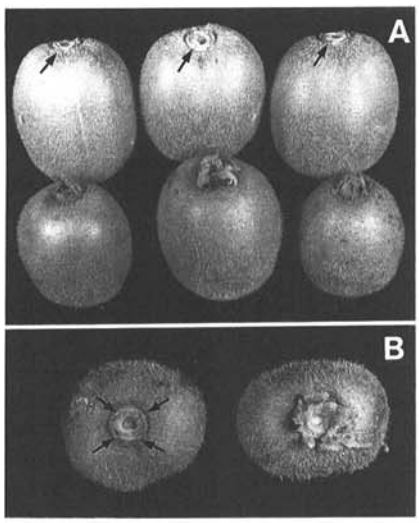

Fig. 1. Kiwifruit showing **(A)** snail (*Helix aspersa*) damage (sepals removed, upper row) in comparison with undamaged fruit (sepals intact, lower row), and **(B)** close-up of healed tissues (arrows) along the stem end after wounding during sepal consumption by snails.

Effect of snail slime on fruit surface microflora. At commercial harvest time, replicated samples of 15 mature kiwifruit with deposited snail slime and without slime or any apparent snail damage were collected arbitrarily throughout a vineyard and transported to the laboratory in a cooler. Five 1-cm-diameter disks from the surface of each fruit, including the snail slime, were cut with a cork borer, and the disks (2 to 3 mm thick) were placed in a test tube (1.5 × 18 cm) containing 2 ml of sterile deionized water plus 5 µl of Triton-100. Similar disks removed from fruit without slime served as controls. Five replicated tubes were used per treatment. The test tubes and their contents were vortexed (high setting) for 15 s to wash the microflora from the disks. Aliquots of 100 µl were plated onto each of three petri dishes (9 cm diameter) (a total of 15 per treatment) containing acidified (2.5 ml of 25% [vol/vol] lactic acid per liter) potato-dextrose agar (APDA). The dishes were incubated at 7°C for 6 days, and all *B. cinerea* colonies were counted. The dishes were incubated for an additional 2 to 3 days at 21 to 23°C, and any new *B. cinerea* colonies were added to the original count. The use of this incubation method was necessary to reduce the incidence of contamination of dishes by fast-growing *Rhizopus* spp. (20). Colonies of other fungi and yeasts were also counted. The results were expressed as propagule numbers per cm^2. The experiment was repeated twice.

Effect of snail slime on germination of *B. cinerea* conidia. To determine the effects of snail slime on conidial germination of *B. cinerea*, slime was removed from salivating brown garden snails with a sterile transfer loop. The surfaces of two petri dishes (9 cm diameter) containing acidified water agarose (AWA) (0.8% agarose [SEAKEM HGT (P), FMC, Bioproducts, Rockland, ME], 2.5 ml of 25% [vol/vol] lactic acid per liter) was streaked with a loop onto four different areas (4 cm^2) marked on each petri dish. One 50-µl droplet of 10^5 conidia per ml suspension of a 10-day-old culture of *B. cinerea* derived from APDA was placed in the center of each slime-streaked area. The droplet suspension was evenly spread over the slime-streaked area with a 2 × 2 cm cover slip laid gently on the droplet and immediately removed. Dishes of AWA without any slime were inoculated similarly and used as controls. Germination of 50 conidia arbitrarily selected per inoculated area was determined in each of two dishes after 7, 10, and 22 h of incubation at 20°C in absence of light. The same procedure was used with dishes containing APDA with and without slime. Because conidial germination in the APDA dishes after 7 h was as high as 95%, no additional recordings were made. Both experiments were repeated once.

Bagging experiments. To induce snail damage, 35 canes bearing 15 to 20 fruit (1993) and 20 canes bearing 14 to 30 fruit (1994) were caged individually in a nylon organdy (mesh) sleeve (0.5 × 1.0 m, 0.5 × 0.5 mm opening, 180 threads per cm^2) after pollination. One to two adult *H. aspersa* snails were added to each of the bagged canes on 12 June, 30 July, and 6 September 1993 and six snails per cane on 20 June 1994. Bagged canes without snails served as controls. The bags with and without snails were left in the canopy until fruit were mature (solid solids 6.5%). The fruit were evaluated for snail damage and stored in CA storage as described in previous sections. Snail mortality was also recorded at the time of fruit harvest. After 3 months of CA storage, fruit with gray mold were recorded and discarded. Apparently healthy fruit were returned to CA storage for 2 months before a final assessment of gray mold incidence was made.

Effects of artificial sepal removal on gray mold. To determine if the removal of sepals by hand would affect the incidence of gray mold, experiments were performed in two commercial kiwifruit vineyards. Vineyard A was located in Nipomo (San Luis Obispo County, coastal California) and vineyard D was located in Delano (Kern County, Central Valley). Plots chosen arbitrarily consisted of two adjacent female vines located between two male vines, and there were five replicate plots per treatment. All the sepals from 80 fruit per plot (40 per vine) were removed on 20 June, 1 August, 12 September, 26 October, and 2 November in vineyard A in Nipomo, and on 2 and 29 July, 1 and 28 September, and 5 October in vineyard D in Delano. Sepals were surface-sterilized in 0.5% sodium hypochlorite solution plus 0.001% Triton X-100 (two drops per liter), washed in sterile distilled water, dried under a laminar flow hood, and plated in petri dishes (9 cm diameter) containing APDA. Five to six sepals per fruit were evenly placed in each petri dish, and the dishes were incubated at 7°C for 6 days under 12 h dark/12 h diffuse light. The number of colonies of *B. cinerea* growing from the sepals was recorded. The dishes were incubated for 3 additional days at 20°C, and additional colonies of *B. cinerea* were added to the original count. All the fruit from which sepals had been removed and control fruit (sepals not removed) were harvested on 5 October (Vineyard D) and 2 November (Vineyard A) and placed in CA storage as previously described. Numbers of fruit showing symptoms of gray mold were recorded after 3 and 5 months storage.

Statistical analyses. Experimental data were subjected to ANOVA with SAS soft-

ware procedures (SAS Institute, Cary, NC), and treatment means were compared with Fisher's protected least significant difference (LSD) test. The incidences of *B. cinerea* infection in the 1993 bagging experiment were transformed to arcsine square root values because the variances failed the *F*-max test for homogeneity of variances (4).

RESULTS AND DISCUSSION

Snail damage evaluation. In 1994, 11 to 29% of kiwifruit had all their sepals removed by snails; 7 to 20% were partially damaged by snails (Table 1). In 1995, the incidence of snail damage was lower. The higher damage in 1994 could be explained by higher rainfall during the growing season (May to November) in that area in that year (Table 2). For the most part, snails have not been a problem for a number of years in California orchards and vineyards due to low precipitation (29). Because snails lose a large percentage of water due to muscle contractions of their "foot" and secretion of slime, survival is enhanced in shaded places and high humidity conditions (29). We also observed that brown garden snails usually started grazing after the end of rainfall. In 1995, growers with snail problems in their vineyards reported to us 15 to 25 (Central Valley) and 25 to 35 (Sacramento Valley) snails per vine. Macroscopic examination of stem ends of fruit with sepals removed by snails (arrows, Fig. 1A) showed healed tissues on the area of the attachment of sepals (arrows, Fig. 1B), suggesting that tissue wounding and healing had occurred. Snails maintained in the laboratory as stock colonies chewed paper towels and ice cream containers, reflecting their ability to consume lignified tissues such as those of kiwifruit sepals.

Incidence of *B. cinerea* in kiwifruit with and without snail damage. In 1991, 1992, and 1993, fruit with sepals removed by snails during the growing season had more gray mold than fruit with intact sepals (control fruit) after 3 months of storage ($P < 0.05$, Table 3). This pattern was consistent for the 5-month assessment. Because of the excessive incidence of gray mold after 3 months, fruit from the 1992 sample were not kept for the 5-month disease evaluation. There was a higher incidence of gray mold in 1992, which may be explained by the relatively high amount of rainfall and frequency of rain events during the growing season (May to October) prior to harvest (Table 2).

The higher incidence of Botrytis gray mold in kiwifruit that had their sepals removed by snails is surprising because Sommer et al. (31) showed that colonization of floral parts by *B. cinerea* contributed to high levels of gray mold and suggested that removal of floral parts (including sepals) might reduce the disease. In contrast, Pyke et al. (27) proposed that sepals were a source of several potential *Botrytis* antagonists and suggested that sepal removal prior to packing may increase storage rots. The active removal of sepals by snails may differ from natural loss; for example, snails may create microwounds around the receptacle using their radula (a sclerotized jaw) (24). Because *B. cinerea* spores are present on the surface of kiwifruit throughout development and during harvest (7,8) and because sepals are continuously colonized by *B. cinerea* (19,20,22), inoculum for infection of wounded receptacle tissue is available after snail damage.

The incidence of gray mold in 1993 was lower than in 1992, but sepal removal by snails resulted in more gray mold than was present in fruit with intact sepals after 3 or 5 months in CA storage ($P < 0.01$, Table 3). The percentage of kiwifruit sepals colonized by *B. cinerea* from this vineyard was 55% in 1991, 84% in 1992, and 67% in 1993. High sepal colonization was likely to have occurred in 1992 as a result of repeated rainfall events. Weather records indicated that no rains occurred in 1991 during the bloom of kiwifruit plants in the Nipomo area, compared with 8 days with measurable rains during the same time in 1992 (Table 2). Baudry et al. (1), using artificial inoculations, showed that the bloom to fruit set stage is one of the phases

Table 1. Incidence of kiwifruit damaged by snails (*Helix aspersa*) in commercial vineyards in Nipomo (San Luis Obispo County, California)

		Sepal removal by snails (%)[x]		
Year	Vineyard	Complete[y]	Partial[z]	Sepals intact
1994	A	11.2	6.6	82.2
	B	34.7	19.8	45.5
	C	28.9	14.8	56.3
1995	A	1.8	3.0	95.2
	B	15.4	4.8	79.8

[x] Fifty fruit per vine from five (1994) to 10 (1995) vines selected arbitrarily per vineyard were recorded on 2 November 1994 and 6 November 1995 (commercial harvest).
[y] All sepals missing and slime present.
[z] Two to four of the sepals missing and slime present.

Table 2. Rainfall in San Luis Obispo area from May to November (1991 to 1995)

	Number of rains (total rainfall in mm)[w]				
Month	1991	1992	1993	1994	1995
May	0 (0)[x]	4 (32)[y]	1 (5)	5 (20)	5 (15)
June	0 (0)	4 (47)	2 (5)	0 (0)	2 (20)
July	0 (0)	2 (9)	0 (0)	0 (0)	0 (0)
Aug.	1 (1)	1 (1)	2 (7)	1 (5)	0 (0)
Sep.	0 (0)	0 (0)	0 (0)	2 (79)	1 (2)
Oct.	3 (15)	3 (33)	2 (6)	3 (23)	0 (0)
Nov.	2 (19)	0 (0)	5 (51)[z]	6 (66)	0 (0)
Total rainfall (May to Nov.)	6 (35)	14 (122)	10 (74)	17 (193)	8 (37)

[w] Rainfall data were retrieved from California Irrigation Management Information System (CIMIS) Station 52.
[x] Numbers in parentheses represent total rainfall in each month.
[y] One 14-mm rain occurred after full bloom. Kiwifruit harvests in 1991, 1992, and 1993 were done on 21 and 2 November, and 18 October, respectively.
[z] One 1-mm rain occurred on 3 November, two rains (26 mm total) on 10 and 11 November, and two rains (24 mm total) on 28 and 29 November.

Table 3. Effects of removal of kiwifruit sepals by brown garden snails (*Helix aspersa*) on incidence of Botrytis gray mold after 3 and 5 months in commercial controlled-atmosphere storage (–0.5°C and ethylene at 8 ng/g)

	Gray mold (%)				
	1991[w]		1992[y]	1993[z]	
Type of fruit[v]	3 mo[x]	5 mo	3 mo	3 mo[x]	5 mo
Sepals removed by snails	27.7	31.8	40.2	11.6	19.5
Sepals intact	5.0	7.9	20.4	2.0	3.5
$LSD_{0.05}$	9.0	12.1	6.0	3.4	5.7

[v] All fruit were from a vineyard in Nipomo, California
[w] Averages from five boxes with 39 fruit each; harvested on 21 November.
[x] Fruit showing gray mold were discarded to reduce secondary spread of the fungus in storage.
[y] Averages from 11 boxes with 39 fruit each; harvested on 2 November. Only the 3-month storage data are presented (decay too high to continue storage for 5 months).
[z] Averages from 18 boxes of 33 fruit each; harvested on 19 October.

of susceptibility to *B. cinerea* for kiwifruit grown in France. Although the stage of susceptibility of sepals and receptacles to *B. cinerea* was not identified for California-grown kiwifruit, other studies indicated that the sepal and receptacles were colonized by *B. cinerea* continuously from fruit set until harvest (19,20,22).

Because Sommer et al. (31) showed that the removal of flower parts just before storage of fruits reduced the incidence of gray mold in storage, we expected less disease in fruit from which sepals had been removed by snails; however, fruit with sepals removed consistently had more gray mold than fruit with sepals attached ($P < 0.01$, Table 3). Although macroscopic examinations of stem ends damaged by snails showed healing all around the area of sepal attachment (arrows, Fig. 1B), either the healing was insufficient to overcome infections by *B. cinerea*, or wounds healed after *B. cinerea* infection locked the inoculum in the receptacle. To avoid confounding the Botrytis gray mold prediction method that was developed recently in our laboratory (20), we recommend that growers avoid including fruit damaged by snails in the samples.

Effect of snail slime on fruit surface microflora. Washings of fruit skin disks with and without slime contained *Cladosporium, Epicoccum, Alternaria, Fusarium, Mucor, Paecilomyces, Penicillium, Phoma,* and *Phomopsis* spp. Some of these fungi are considered surface contaminants, and others such as *Phomopsis* are reported as postharvest pathogens (2,26,30–32). *Epicoccum nigrum, Cladosporium* spp., and various yeasts were consistently recovered in all three experiments. More *B. cinerea* and *Cladosporium* spp. propagules were recorded per cm^2 of fruit surface when snail slime was present ($P < 0.05$, Table 4). In addition, more propagules of *E. nigrum* and yeasts were recorded on fruit with slime than on fruit without slime ($P < 0.05$, Table 4). The larger numbers of fungal propagules on kiwifruit surfaces with slime may be explained by the stickiness of the slime, which may trap airborne spores on the fruit surface. Alternatively, chemical properties of the slime may assist survival of certain fungi. Although differences were consistent in the three experiments for the propagules of *B. cinerea* and *Cladosporium* spp., only in the third experiment were the numbers of propagules of *E. nigrum* and yeasts higher in kiwifruit with slime than without slime ($P < 0.05$, Table 4). Viable conidia of the downy mildew of lima beans were present on the slime that covers slugs (35). The presence of *E. nigrum* on fruit surfaces is potentially important, because *E. nigrum* has been reported to suppress *B. cinerea* on kiwifruit (9) and other fruit (23) and white mold caused by *Sclerotinia sclerotiorum* in bean flowers (36).

Effect of snail slime on germination of *B. cinerea* conidia. Snail slime did not influence conidial germination on APDA, a nutrient-rich medium. Approximately 95% of the conidia germinated after 7 h incubation at 23°C. In contrast, snail slime affected spore germination on AWA, a nutrient-poor medium (Fig. 2). These results suggest that slime of the brown garden snail has enough nutrients and (or) other germination stimulants to support germination of *B. cinerea* conidia and receptacle infection after snail feeding. To the best of our knowledge, this is the first report that slime from a snail can stimulate germination of fungal conidia. Enhanced germination of resting spores of *S. endobioticum* (15) and oospores of *Phytophthora* (28) and *Pythium* (33) by the digestive system of water snails have been reported previously. In addition, ingestion of oospores of *Phytophthora erythroseptica* and *P. cactorum* by *H. aspersa* stimulated oospore germination (14). Other biota, such as insect larvae, have been reported to increase incidence of *B. cinerea* infection (12) or transmit *B. cinerea* and induce infection in fruit of grapes grown in France (13) and fruit rot of faya (*Myrica faya*), a weedy tree, in Hawaii (5).

Bagging experiments. In 1993, caged snails removed the sepals of 80 to 84% of fruit in the cage, whereas all the fruit in the control canes had intact sepals (Table 5). Fruit from canes caged with snails in June and July showed 9 to 12.5% Botrytis gray mold after 3 months of storage, and fruit in the absence of snails had 2.5% gray mold (Fig. 3). After 5 months of storage, gray

Table 4. Effect of brown garden snail (*Helix aspersa*) slime on the surface microflora of kiwifruit harvested from Nipomo, California (1995)

Experiment	Presence of slime	Viable propagules per cm^2 surface[y,z]			
		Botrytis cinerea	*Cladosporium* spp.	*Epicoccum nigrum*	Yeasts
1	+	41	853	6	628
	–	6	249	2	297
$LSD_{0.05}$		15	420	4	583
2	+	39	1,295	6	351
	–	4	332	2	206
$LSD_{0.05}$		20	353	5	220
3	+	24	2,301	5	1,314
	–	1	243	1	208
$LSD_{0.05}$		13	1,364	3	825

[y] Washings from five 1-cm-diameter by 2- to 3-mm-thick skin disks per kiwifruit were plated on three replicated dishes.
[z] Data are means of five three-dish replicates per treatment.

Table 5. Removal of kiwifruit sepals by the brown garden snail (*Helix aspersa*) and viability of snails in a kiwifruit vineyard (Nipomo, California)

Year	Treatment Date[x]	Reps.	Sepals (%)		Snails (%)		Total snails
			Removed[y]	Intact	Dead	Alive[z]	
1993	Snails						
	12 June	9	84.1	15.9	28	72	18
	30 July	9	80.4	19.6	31	69	16
	6 Sep.	8	83.1	16.9	29	71	17
	No snails (control)	9	0.0	100.0	...	...	...
1994	Snails						
	20 June	10	100.0	0.0	41	59	59
	No snails (control)	10	0.0	100.0	...	...	...

[x] One to two adult snails were enclosed in nylon bags caging one or two canes with 15 to 20 (1993) and 14 to 30 (1994) kiwifruit per bag.
[y] Fruit with all sepals removed by snails.
[z] By harvest, 3 November 1993 and 7 November 1994.

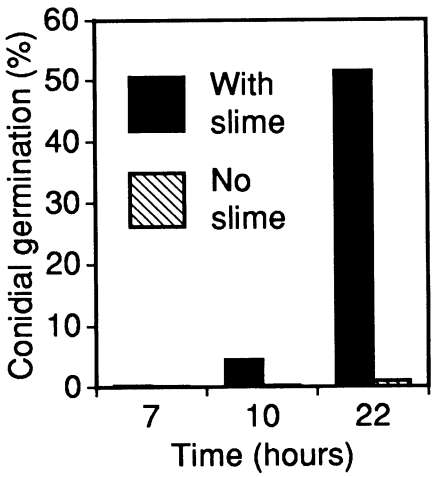

Fig. 2. Effect of brown garden snail (*Helix aspersa*) slime on the germination of conidia of *Botrytis cinerea* in petri dishes containing 0.8% acidified water agarose (a nutrient-poor medium) solidified media incubated at 23°C for 22 h.

mold increased to 5.2 to 17% for fruit that had been caged with snails and to 7% for fruit from the control canes (Fig. 3); however, these differences were not significant ($P = 0.675$) because of the high variability among the replications. A reason for this variability could be because, in some of the replicated caged canes, both snails had died by the end of the season. In 1994, six snails were caged per cane and all fruit in the cage had their sepals removed. After 3 months of CA storage, 1.9% of fruit that had been caged with snails developed gray mold and none of the fruit caged with no snails was diseased ($P = 0.086$). After 5 months of CA storage, however, fruit that had been caged with snails showed more (9%) gray mold than did the control (0%) ($P = 0.002$, Fig. 3). Variability among replications was reduced in 1994 when six snails were placed in each bag, probably because there were always at least three snails still alive by harvest.

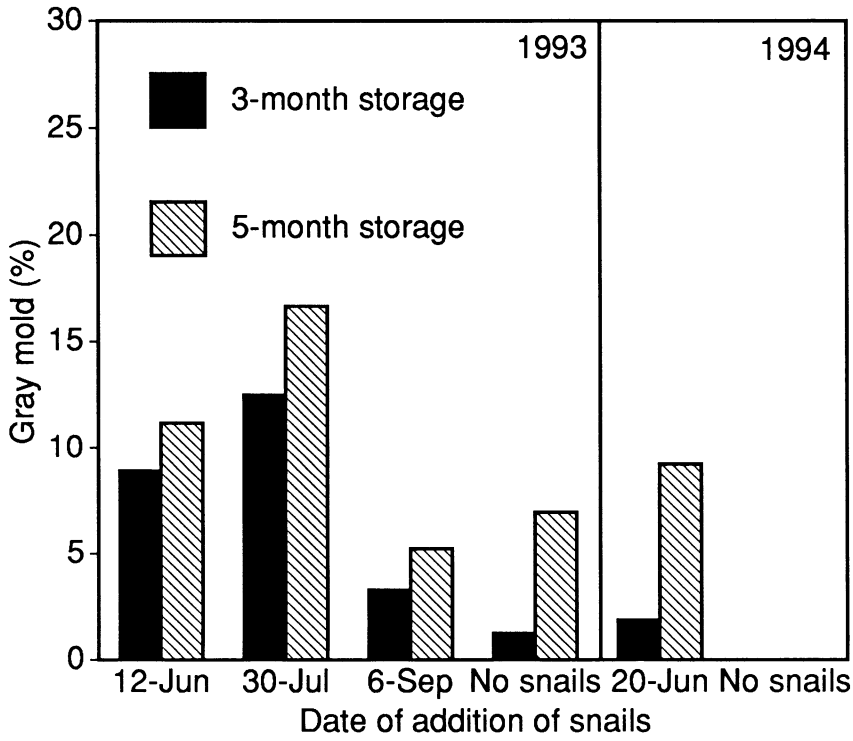

Fig. 3. Effect of caging one to two snails (*Helix aspersa*) per kiwifruit cane on 12 or 30 July, or 6 September 1993, and six snails on 20 June 1994, on the incidence of gray mold caused by *Botrytis cinerea* after 3 and 5 months in controlled-atmosphere (–0.5°C and ethylene at 8 ng/g) storage. Differences between treatments and control (no snails) were not significant for the 1993 data, but they were significant ($P < 0.01$) for the 5 months of storage 1994 data. (Eight to 10 replicated canes bearing 14 to 30 fruit were used for each date.)

Table 6. Effect of removal of sepals by hand during the growing season on Botrytis gray mold after controlled-atmosphere (–0.5°C and ethylene at 8 ng/g) storage of fruit from two commercial kiwifruit vineyards (1992)

Date of sepal removal[w]	Colonization of sepals by *B. cinerea*	Gray mold (%) after storage	
		3 months	5 months
Vineyard A (Nipomo, CA)			
20 June	90.4 a[x]	13.0 a	17.0 a
1 Aug.	50.0 d	18.7 a	18.7 a
12 Sep.	64.8 c	15.4 a	16.2 a
26 Oct.	...	14.0 a	15.0 a
2 Nov.	76.0 b	17.1 a	18.7 a
Control[y]	...[z]	19.9 a	20.7 a
Vineyard D (Delano, CA)			
2 July	46.0 a	0.8 bc	2.1 c
29 July	26.4 b	2.6 ab	4.9 ab
1 Sep.	43.8 a	2.3 a-c	3.9 a-c
28 Sep.	42.6 a	3.3 a	6.4 a
5 Oct.	46.0 a	0.3 c	1.5 c
Control[y]	...	1.0 bc	2.6 bc

[w] Sepals were removed by hand from 80 fruit per each of the five pairs of replicated vines on each date.
[x] Numbers followed by the same letters are not significantly different according to Fisher's protected least significant difference test at $P < 0.05$.
[y] Sepals were left on the fruit until commercial harvest.
[z] These sepals were not plated but left attached to the fruit.

Effect of artificial sepal removal on gray mold. In vineyard A in Nipomo, where the disease incidence was high, removal of sepals at different times of the season did not affect the overall levels of gray mold after 3 or 5 months of cold storage (Table 6). Colonization of sepals by *B. cinerea* ranged from 50 to 90%. In contrast, in vineyard D in Delano, where disease incidence was low and colonization of sepals by *B. cinerea* was lower than in vineyard A in Nipomo (Table 6), only the removal of sepals on 28 September resulted in significantly greater levels of gray mold after 3 and 5 months of cold storage (Table 6). The reason for the increased levels of gray mold on fruit from which sepals had been removed on 28 September is unknown. Therefore, removing sepals by hand did not consistently decrease or increase the incidence of *B. cinerea* infection, but sepals removed by snails consistently increased Botrytis gray mold in CA storage.

The vines in the majority of kiwifruit vineyards in California are trained on pergola or T-bar trellis systems, which can provide a shady and cool habitat with abundant weeds growing between and (or) under the vine rows, suitable for successful development and reproduction of the common brown garden snail. This study provides incentives to kiwifruit growers with vineyards where snails are present to use management methods for keeping the snail populations down and thus reducing the risk of gray mold losses of fruit in storage.

ACKNOWLEDGMENTS

We thank Gary L. Suthers (Ag Associates, Inc.), Mark Shurtleff (KiwiWest, Inc.), George Tanimoto (Tanimoto Brothers, Inc.), Carl Buxman (SunnyCal Packing Co.), and Jean Clough (Rancho de Oro), who provided sites for field experiments and donated boxes of fruit for disease evaluation. We also thank L. Boeckler, V. Bijman, J.-P. Burdet, L. Hou, R. Gonzalez, R. Kölliker, and K. Tsuda for technical assistance, P. A. G. Elmer (Hort Research, New Zealand) for reviewing this manuscript and making helpful suggestions, and N. Sakovich for identifying the species of the snail involved in these studies.

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