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Pyrrolnitrin, Captan + Benomyl, and High CO_2 Enhance Raspberry Shelf Life at 0 or 18C

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Abstract. **The effects of preharvest applications of pyrrolnitrin (a biologically derived fungicide) on postharvest longevity of 'Bristol' black raspberry** *(Rubus occidentals* **L.) and 'Heritage' red raspberry [R.** *idaeus* **L. var. strigosus (Michx.) Maxim] were evaluated at two storage temperatures. Preharvest fungicide treatments were 200 mg pyrrolnitrin/liter, a standard fungicide treatment (captan + benomyl or iprodione) or a distilled water control applied 1 day before first harvest. Black raspberries were stored at 18 or 0 ± lC in air or 20% CO_2. Red raspberries were stored at the same temperatures in air only. Pyrrolnitrin-treated berries often had less gray mold** *(Botrytis cinerea* **Pers. ex Fr.) in storage than the control but more than berries treated with the standard fungicides. Storage in a modified atmosphere of 20% CO_2 greatly improved postharvest quality of black raspberries at both storage temperatures by reducing gray mold development. The combination of standard fungicide or pyrrolnitrin, high CO_2, and low temperature resulted in more than 2 weeks of storage with less than 5% disease on black raspberries; however, discoloration limited marketability after≈ 8 days under these conditions. Chemical names used: 3-chloro-4-(2'-nitro-3'-chlorophenyl) -pyrrole (pyrrolnitrin); N-trichloromethylthio-4-cyclohexene-l12-dicarboximide (captan); methyl 1-(butylcarbamoyl) -2-benzimidazolecarbamate) (benomyl); 3-(3,5 -dichlorophenyl) -N-(l-methylethyl -2,4-dioxo-l-imidazolidinecarboxamide (Rovral, iprodione).**

Raspberry production for fresh-market consumption is severely limited by the rapid deterioration of the fruit. While the raspberry fruit has morphological and physiological characteristics that account for a portion of its short postharvest life, specifically loosely connected drupelets, an open cavity that predisposes it to crushing, and a high postharvest rate of respiration; the most common cause of postharvest decline is gray mold fruit rot (Janisiewicz, 1988).

While the benefits of modified atmosphere storage are well documented on strawberries (Couey and Wells, 1970; Harvey, 1982a, 1982b), research is sparse and less conclusive on raspberries. Winter et al. (1939) reported increased shelf life of raspberries at 10 to 15C using an initial CO_2 concentration of 30%. Smith (1958) found that modified atmospheres with 20% to 25% CO_2 slowed ripening and reduced decay.

Preharvest applications of fungicides have been shown to control postharvest gray mold occurrence (Freeman and Pepin, 1976). However, the development of fungicide-resistant fungal populations and increasingly stringent governmental regulation limit the use of currently available fungicides. Biological control agents and naturally produced compounds may offer alternative means of controlling fungal infections in raspberries. While little biological control research has been conducted on raspberries, Tronsmo and Dennis (1977) worked with strawberry and found that several microbial organisms, including *Trichodenna* spp., *Gliocladium virens*, and *Hypocrea semiorbitis*, reduced gray mold spoilage in fruit in both the field and storage when applied from early flowering until 2 weeks before harvest. However, postharvest incidence of fruit rot caused by *Mucor* spp. increased (Janisiewicz and Roitman, 1988).

Pyrrolnitrin, isolated from *Pseudomonas cepacia,* a gram-negative bacterium, has broad-spectrum antibiotic properties (Imanaka et al., 1965a, 1965b; Janisiewicz and Roitman, 1988). It inhibits growth of fungi, yeasts, and gram-positive bacteria and has low mammalian toxicity (Arima et al., 1965). Takeda et al. (1990) reported inhibition of fungal growth on strawberries treated with a postharvest dip of 250 ppm pyrrolnitrin and stored at 18C, or at 0C followed by 18C. Unlike some other biocontrol agents, pyrrolnitrin did not increase the incidence of fruit decay caused by *Mucor.*

The objective of this research was to evaluate the effect of preharvest applications of pyrrolnitrin on the postharvest longevity of raspberries under various postharvest conditions.

Materials and Methods

Experiments were conducted in 1989 and 1990 at the Russell E. Larson Research Center in Rock Springs, Pa. For Expts. 1 and 2 (1989), a mature 'Bristol' black raspberry planting grown in a hedgerow system according to commercial recommendations was sprayed with recommended rates of captan + benomyl weekly for fungal control until 2 weeks before harvest. At this time, field treatments consisting of 200 ppm pyrrolnitrin (pure crystals were dissolved in 5 ml of methanol, and distilled water was added for a total volume of 1 liter) plus 0.5 ml Tween 20 wetting agent; captan + benomyl at 1.8 + 0.45 g a.i./liter; or distilled water (control) were applied until drip. Two spray applications were made, on 6 and 13 July 1989. In 1990, 'Heritage' red raspberries were harvested from a 3-year-old planting. Fungicide treatments were applied on 13 Sept. For all experiments, berries were harvested 1, 4, and 6 days after treatment application, cooled to 5C immediately after harvest in a forced-air cooler, and kept cold during transport and handling. Each postharvest experimental unit consisted of a 235 x 150 mm plastic tray (a candy box insert) with 24 separate compartments. Individual berries were placed in tray compartments, so that each berry was clearly visible and did not touch the surrounding berries.

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Both experimental seasons were wet, with exceptionally high rainfall during harvest; rainfall in July 1989 and Sept. 1990 was 51 mm and 17 mm above the 25-year means, respectively.

Field plot and postharvest treatments were arranged in a randomized complete block with three replications. Data collection for both experiments included initial and final weights and daily evaluations of percent marketable berries, percent unmarketable due to gray mold, and percent unmarketable for other reasons (other diseases and discoloration). Percentages were calculated by counting the number of berries diseased or deteriorated and dividing by the total number of berries in each treatment plot. To analyze data, the curve integral for percent gray mold-infected fruit or percent unmarketable fruit vs. time for each experimental unit was calculated using the trapezoid method of integral calculation. Integrals were calculated for areas under the curve up to the number of days after harvest when 100% of the berries were infected or unmarketable for all treatments. Integrals were analyzed using an analysis of variance for repeated measurements. Means were separated using Tukey's studentized range (HSD) test, $P(F) < 0.05$.

Pyrrolnitrin effects on black raspberry (Expt. 1). The effects of field applications of pyrrolnitrin and of captan + benomyl on postharvest longevity of black raspberry fruit were evaluated when stored at 0 or 18 ± lC. Field treatments were arranged in a randomized complete-block design. Fruit stored at 0C was removed after 7 to 11 days and kept at 18C for further evaluation to simulate removal from storage for transport or marketing. After each harvest, three trays that contained berries were placed in a polystyrene box with water covering its bottom to maintain high humidity. Each box held one tray from each treatment, and thereby constituted a replication. Trays were stacked in the boxes and separated by hardware cloth to prevent crushing.

Pyrrolnitrin and modified atmosphere effects on black raspberry (Expt. 2). The effects of preharvest applications of pyrrolnitrin and captan + benomyl on black raspberry postharvest longevity under modified atmosphere conditions were evaluated at 0 or 18 ± lC in a complete factorial experiment (two atmospheres x three fungicides). Treatments were arranged in a split plot, with fungicide treatments as main plots, and atmosphere as subplots. The inlet gas mixtures (treatments) were air or 20% CO_2 in air. Berries were harvested on 7 and 14 July, 1 day following field treatment application. Individual trays were placed in large 4-mil polyethylene self-sealing bags fitted with flow-through gas mixtures in rooms at 0 or 18C. The test atmospheres were humidified and distributed at a flow rate of 1 liter·h^{-1} using capillary flow meters.

Pyrrolnitrin effects on red raspberry (Expt. 3). In *1990,* the effects of preharvest applications of pyrrolnitrin on 'Heritage' red raspberry postharvest longevity were evaluated when stored at 0 or 18 ± lC. Methods were the same as Expt. 1 above, with the following exceptions: 1) Iprodione (Rovral) fungicide at 0.6 g a.i./liter was used instead of captan + benomyl for the standard fungicide treatment. This was necessary because new regulatory restrictions prevent reentry into a captan-treated plot for 4 days. Use of benomyl alone has historically resulted in resistant fungal populations. 2) Berries were inoculated with a suspension of 10,000 B. *cinerea* conidia/ml distilled water to ensure uniform disease pressure just before the berries were placed into the postharvest environments. The *B. cinerea* isolate was obtained the previous year from a raspberry planting. 3) The surfactant Triton B-1956, a modified phthalic glycerol alkyd resin (Rohm and Haas, Philadelphia), was added at 5 ml·$liter^{-1}$ to the pyrrolnitrin instead of the Tween 20. Triton B-1956 provides greater adhesion of chemicals to the fruit surface (Crop Protection Chem. Ref., 1991).

Results and Discussion

Pyrrolnitrin effects on black raspberry

Berries harvested 1 day after application. Fungicide-treated fruit stored at 0C had little gray mold mycelium development (Fig. 1A). However, as soon as berries were moved to 18C, gray mold developed rapidly. The amount of gray mold development was highest for the control, followed by pyrrolnitrin, and was lowest for berries treated with preharvest applications of captan + benomyl (Fig. 1A, Table 1). Analysis of the integrals supported these observations (Table 1). When the experiment was repeated (fungicide applied on 13 July), pyrrolnitrin and captan + benomyl both resulted in less gray mold than the control (Table 1). We attribute this improved control of gray mold by pyrrolnitrin to lack of rain that would have washed off the pyrrolnitrin after the second application. Pyrrolnitrin is not a commercial formulation but is used as a pure product; thus, it lacks the spreaders and stickers in commercial formulations of fungicides.

Following gray mold, the most frequent cause of deterioration was discoloration (Table 2). Black raspberries discolored by becoming paler, and individual drupelets became pink where they touched adjacent drupelets. Other fungal infections were rare. Although there was less deterioration at 0C than at 18C, preharvest treatments did not affect deterioration at 0C (Fig. 1 B, D, Table 2). When berries were transferred from 0 to 18C, discoloration occurred more rapidly than disease progression, masking treatment effects on gray mold (Fig. 1 A, B).

When berries were stored at 18C, the rate of disease development did not differ between pyrrolnitrin-treated berries and

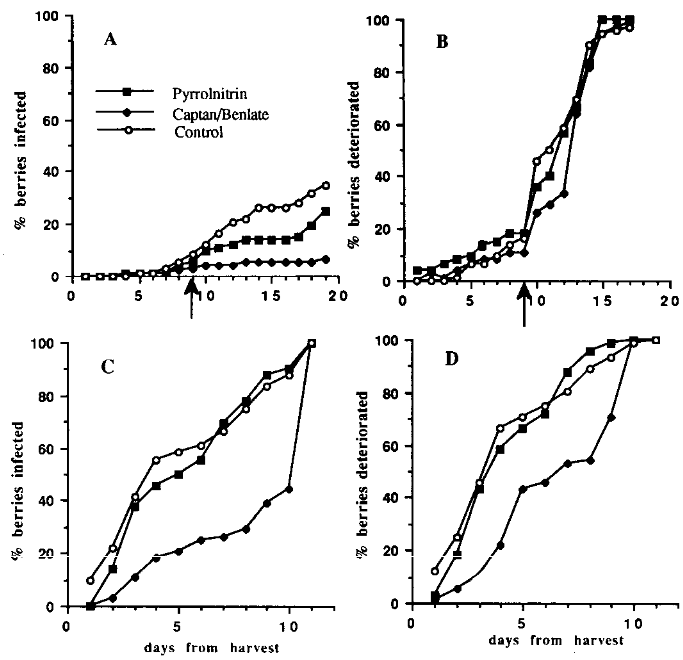

Fig. 1. Efficacy of preharvest fungicide treatment in controlling postharvest gray mold and deterioration on 'Bristol' black raspberries harvested 1 day after 29 June 1989 application. (**A**) 0C, gray mold (arrow indicates date berries were moved from 0 to 18C); (**B**) 0C, deterioration (arrow indicates date berries were moved from 0 to 18C); (**C**) 18C, gray mold; and (**D**) 18C, deterioration (Expt. 1).

Table 1. Effect of preharvest fungicide applications on gray mold development on 'Bristol' black raspberries harvested 1, 4, or 6 days after the sprays and stored at 18 or 0C followed by 18C. Data shown are integrals of gray mold vs. time (Expt. 1).

Harvest date (days after spray)	Storage temp(°C)	Integral days[z]	Preharvest spray treatment: Pyrrolnitrin	Captan + benomyl	Control	P(F)[y]
First application: 6 July 1989				*Integrals*		
1	0[x]	17	36.3 ab	14.8 b	59.5 a	0.03
1	18	8	116 b	46.7 a	124 b	0.02
4	0	15	15.8	4.0	10.8	0.21
4	18	10	79.3	16.7	26.0	0.09
6	0	18	64.3 a	12.7 b	62.5 a	0.05
6	18	9	56.8	12.5	46.3	0.07
Second application: 13 July 1989						
1	0[x]	21	36.3 b	36.3 b	161 a	<0.001
1	18	7	69.0 a	14.7 b	72.8 a	<0.001
4	0	17	61.5	15.8	55.3	0.15
4	18	6	61.3 a	21.8 b	68.2 a	<0.001
6[w]	18	6	62.7 ab	29.7 b	74.0 a	0.03

[z]Days used to calculate integrals varied according to harvest date and temperature. Integrals were calculated for areas under the curve up to the number of days after harvest when 100% berries were infected or unmarketable. Because of this, values should not be compared within columns.

[y]$P(F)$ values indicate level of significance of the ANOVA type III sum of squares. Mean separation in rows by Tukey's studentized range (HSD) test, $P < 0.05$.

[x]Berries stored at 0C were moved to 18C after 8 to 12 days to simulate transfer from storage to transport or sale.

[w]Due to a shortage of berries, the 0C treatment was not evaluated for the third harvest of the second application.

Table 2. Effect of preharvest fungicide applications on deterioration of 'Bristol' black raspberries harvested 1, 4, or 6 days after the sprays and stored at 18 or 0C followed by 18C. Data shown are integrals of deteriorated berries vs. time (Expt. 1).

Harvest date (days after spray)	Storage temp(°C)	Integral days[z]	Preharvest spray treatment: Pyrrolnitrin	Captan + benomyl	Control	P(F)[y]
First application: 6 July 1989				*Integrals*		
1	0[x]	17	152	127	146	0.68
1	18	8	310 a	254 b	314 a	0.03
4	0	15	137	96.0	95.0	0.20
4	18	10	99.3	81.5	79.2	0.38
6	0	18	333	322	329	0.92
6	18	9	80.7 a	35.2 b	63.2 ab	0.04
Second application: 13 July 1989						
1	0[x]	21	370	372	376	0.90
1	18	7	87.0	53.2	77.5	0.06
4	0	17	236	248	230	0.09
4	18	6	65.7 a	38.5 b	77.5 a	0.02
6[w]	18	9	66.3 ab	48.7 b	84.3 a	0.04

[z]Days used to calculate integrals varied according to harvest date and temperature. Integrals were calculated for areas under the curve up to the number of days after harvest when 100% berries were infected or unmarketable. Because of this, values should not be compared within columns.

[y]$P(F)$ values indicate level of significance of the ANOVA type III sum of squares. Mean separation in rows by Tukey's studentized range (HSD) test, $P < 0.05$.

[x]Berries stored at 0C were moved to 18C after 8 to 12 days to simulate transfer from storage to transport or sale.

[w]Due to a shortage of berries, the 0C treatment was not evaluated for the third harvest of the second application.

the control, but captan + benomyl-treated berries had lower gray mold integrals than either pyrrolnitrin-treated or control berries (Fig. 1C, Table 1). The pattern of deterioration was similar to that for gray mold development (Fig. 1 C, D), suggesting that the primary effects of the preharvest treatments were on gray mold development. Low temperature inhibited gray mold and discoloration (Fig. 1).

The low level of gray mold infection at 0C was encouraging, particularly when berries were treated with captan + benomyl. At 18C, 20% of the berries from the captan + benomyl treatment had mycelial growth after 4 days (Fig. 1 C), while this level of infection was never reached by berries stored in 0C (Fig. 1A). When berries were stored at 0C, deterioration, primarily discoloration, was more prominent than gray mold development (Fig. 1). When berries were moved from 0 to 18C, deterioration was the overwhelming cause of berry unmarketability (Fig. 1).

Berries harvested 4 and 6 days after application. Captan +

benomyl continued to provide good control of gray mold in berries harvested up to 6 days after treatment (Table 1). Pyrrolnitrin did not significantly reduce gray mold or deterioration in either of these harvests. Heavy rain a few hours after the 6 July application may have washed off the pyrrolnitrin.

When the experiment was repeated with spray application on 13 July 1989, results for the 4- and 6-day harvests were similar to those discussed above (Tables 1, 2).

Pyrrolnitrin/modified atmosphere effects on black raspberry

The addition of 20% CO_2 consistently decreased the gray mold integral, increasing shelf life on both harvest dates (Table 3, Fig. 2). Pyrrolnitrin was no better than the control in preventing gray mold development after the 6 July treatment, but after the 13 July treatment it reduced gray mold only in berries stored at 0C. This pattern is consistent with that in Expt. 1 and is probably due to heavy rain a few hours after the 6 July application. At 0C and high CO_2, both the pyrrolnitrin- and captan + benomyl-treated berries had no gray mold development after 16 days (Fig. 2A). The captan + benomyl-treated berries stored in air also had a low incidence of gray mold infection. Again, low temperature dramatically reduced berry gray mold integrals (Fig. 2C, Table 3).

Storage in 20% $C0_2$ in combination with either pyrrolnitrin or captan + benomyl resulted in extremely low rates of berry gray mold infection at either temperature (Fig. 2A, C). At 0C, however, berry deterioration, primarily discoloration, became the limiting factor, with 50% deterioration occurring on all treatments by 12 days postharvest (Fig. 2B). There was a dramatic change in rates of deterioration in all treatments after 9 to 10 days of storage at 0C (Fig. 2B), even though there was no change in storage conditions. In fruit harvested 1 day after the 13 July field treatment, the relationship among treatments was similar to that following the first harvest, but the change in rate of deterioration was less abrupt and occurred earlier, so that 50% deterioration had occurred in all treatments by 8 days in storage at 0C (data not shown). While high CO_2 combined with 0C controlled gray mold extremely well, it did not substantially reduce discoloration after the first (Fig. 2B) or second (data not shown) field treatment. At 18C, gray mold and deterioration curves were very similar (Fig. 2C,D), indicating that gray mold was the primary cause of deterioration at 18C.

Table 3. Effect of modified atmosphere and preharvest fungicide sprays on 'Bristol' black raspberry gray mold development. Data shown are integrals of gray mold or deteriorated berries vs. time (Expt. 2).

	6 July 1989 (°C)		13 July 1989 (°C)	
Variable	18	0	18	0
	Gray mold integral[z]			
Atmosphere[y]				
Air	97.4	47.0	66.0	77.5
20% CO_2	50.8	5.1	31.1	10.7
P(F)	0.003	<0.001	0.002	<0.001
Preharvest fungicide				
Pyrrolnitrin	93.3 a	26.8 a	55.0 a	29.4 b
Captan + benomyl	26.3 b	5.6 b	26.5 b	17.0 b
Distilled water	87.6 a	30.6 a	64.2 a	85.8 a
P(F)	0.002	0.002	0.007	0.001
	Deterioration integral			
Atmosphere[y]				
Air	118	199	80.6	424
20% CO_2	72.2	181	63.3	436
P(F)	0.006	0.08	0.004	0.10
Preharvest fungicide				
Pyrrolnitrin	114 a	196	71.8	429
Captan + benomyl	57.5 b	182	59.5	427
Distilled water	113 a	192	84.5	433
P(F)	0.004	0.80	0.24	0.77

[z]Integrals for 0C berries calculated based on 19 days in storage, while those for 18C berries calculated based on 11 days in storage, so values should not be compared between columns.

[y]*P*(F) values indicate level of significance of main effects, ANOVA type III sum of squares. No significant interactions occurred. Mean separation in columns by Tukey's studentized range (HSD) test, $P < 0.05$.

Pyrrolnitrin effects on red raspberries

In this experiment, we were able to reduce variability in postharvest disease development in control berries by inoculating the berries with *B. cinerea* conidia before storage. Despite this, there were no significant effects of preharvest fungicide treatments on gray mold of berries stored at 0C (Table 4, Fig. 3 A, C). However, iprodione consistently reduced gray mold at 18C (Table 4, Fig. 3 B, D). Pyrrolnitrin significantly reduced gray mold in the first harvest after the field spray (Table 4).

All 'Heritage' berries stored at 18C darkened, making them unmarketable after 4 days (data not shown). At 0C, darkening did not occur on all berries until 7 to 8 days after harvest. While color change was not quantified in this experiment, the rates of change are in agreement with other findings on red raspberry (Robbins and Moore, 1990). There were no treatment effects on darkening, which, except for gray mold, was the primary cause of deterioration (data not shown). Although the physiological basis for discoloration is not certain, it was not reduced as much by low temperature and high CO_2 as gray mold disease.

There were no statistical interactions between preharvest fungicide treatment and postharvest environment. High CO_2, low temperatures, and fungicides significantly reduced gray mold development in storage; however, once gray mold was controlled (in some cases, for up to 20 days), discoloration became the factor that limited marketability.

In all three experiments, pyrrolnitrin-treated raspberries consistently developed more gray mold in storage than those treated with commercial fungicides but often had less gray mold than the control. The inferior performance of pyrrolnitrin may be due to the low volubility of the compound, which restricted treatments to rates $\leq$ 250 mg·liter^{-1}. The absence of an appropriate sticker or spreader agent in the pyrrolnitrin solution probably contributed to lower retention, particularly when heavy rains occurred soon after fungicide application. Although Triton-B was added to the pyrrolnitrin, it may not be as effective as those stickers that are chemically formulated for a specific pesticide. Field applications of pyrrolnitrin on strawberries also failed to lower gray mold to levels obtained with captan + benomyl applications (Takeda and Janisiewicz, 1991).

As expected, red raspberries decayed more quickly after transfer from 0 to 18C (Fig. 3 A,C) than black raspberries (Fig. 1A), since red raspberries are reported to be more susceptible to gray mold than black raspberries (Keep, 1988); also, the red raspberries were intentionally inoculated before storage. However, at 18C, the development of gray mold was more rapid on black (Fig, lC) than on red raspberry (Fig. 3 B, D). This pattern also occurred on the second black raspberry harvest when the experiment was repeated (data not shown).

Pyrrolnitrin has performed extremely well in controlling postharvest diseases in the laboratory (Janisiewicz and Roitman,

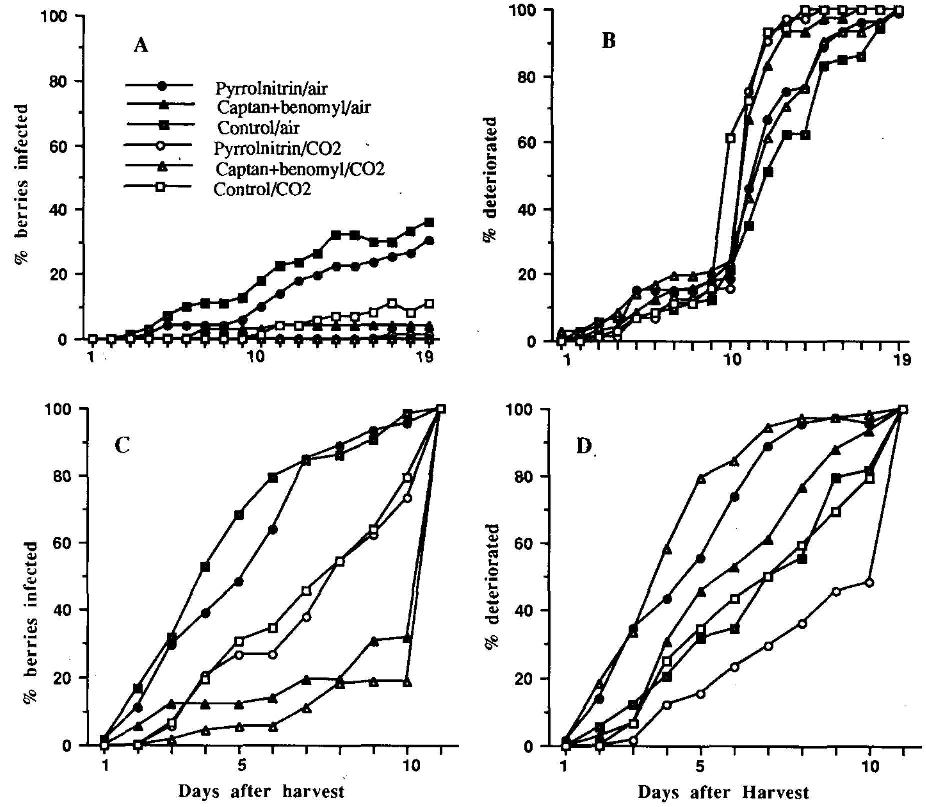

Fig. 2. Efficacy of preharvest fungicide treatment and 20% CO_2 in controlling postharvest infections of gray mold and berry deterioration on 'Bristol' black raspberry. Fruit were harvested 1 day after 29 June 1989 application. (**A**) 0C, gray mold; (**B**) 0C, deterioration; (**C**) 18C, gray mold; and (**D**) 18C, deterioration (Expt. 2).

Table 4. Effect of preharvest sprays of pyrrolnitrin, iprodione, or water (control) on gray mold development on 'Heritage' red raspberries harvested 1, 4, or 6 days after the sprays and stored at 18 or 0C followed by 18C. Data shown are integrals of gray mold vs. time (Expt. 3).

Day harvested after spray	Storage temp (°C)	Integral days[z]	Preharvest spray treatment Pyrrolnitrin	Iprodione	Control	p(F)[y]
			Integrals			
1	0[x]	15	33.8	40.6	43.5	0.51
1	18	10	72.9 b	45.9 c	98.9 a	0.001
4	0	16	45.5	39.9	55.0	0.09
4	18	11	111 a	91.5 b	118 a	0.008
6	0	15	47.2	38.5	51.1	0.08
6	18	10	98.9 a b	82.7 b	107 a	0.02

[z]Days used to calculate integrals varied according to harvest date and temperature. Integrals were calculated for areas under the curve up to the number of days after harvest when 100% berries were infected or unmarketable. Because of this, values should not be compared within columns.

[y]P(F) values indicate level of significance of ANOVA type III sum of squares. Mean separation in rows by Tukey's studentized range (HSD) test, $P < 0.05$.

[x]Berries stored at 0C were moved to 18C after 8 to 12 days to simulate transfer from storage to transport or sale.

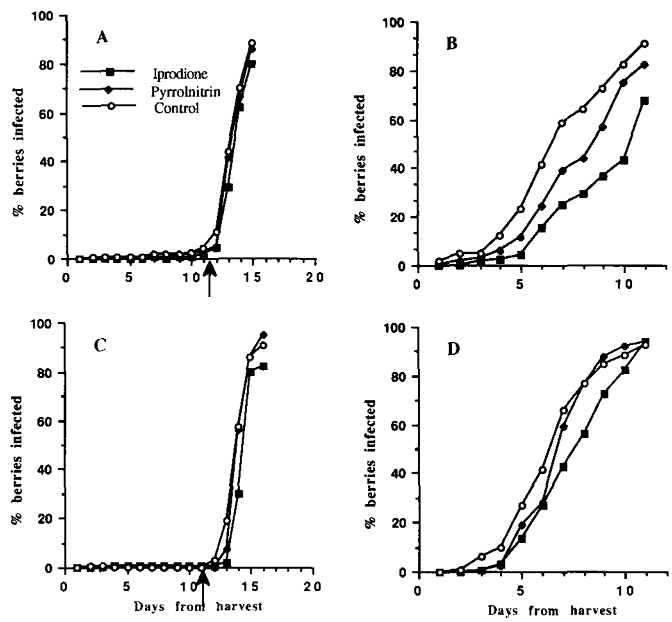

Fig. 3. Efficacy of preharvest fungicide treatment in controlling gray mold on 'Heritage' red raspberry. (**A**) 0C, 1 day after field treatment application (arrow indicates date berries were moved from 0 to 18C); (**B**) 18C, 1 day after field treatment application; (**C**) 0C, 6 days after field treatment application; and (**D**) 18C, 6 days after field treatment application (Expt. 3).

1988; Takeda and Janisiewicz, 1991; Takeda et al., 1990); therefore, it does offer potential as a fungicide, despite its intermediate performance in these experiments. Its efficacy was superior to the control 1 day after application; however, it may have degraded quickly or washed easily from the berries in its present form. Additional research needs to be conducted to determine whether pyrrolnitrin efficacy can be improved through chemical formulation.

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