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 (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% CO₂ greatly improved postharvest quality of black raspberries at both storage temperatures by reducing gray mold development. The combination of standard fungicide or pyrrolnitrin, high CO₂, 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-1,2-dicarboximide (captan); methyl 1-(butylcarbamoyl)-2-benzimidazolcarbamate (benomyl); 3-(3,5-dichlorophenyl)-N-(1-methylethyl-2,4-dioxo-l-imidazolidinecarboxamide (Rovral, iprodione).

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 18°C, or at 0°C followed by 18°C. 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 5°C 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.
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 percent marketable 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 ± 1°C. Field treatments were arranged in a randomized complete-block design. Fruit stored at 0°C was removed after 7 to 11 days and kept at 18°C 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 of captan + benomyl on black raspberry postharvest longevity under modified atmosphere conditions were evaluated at 0 or 18 ± 1°C 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% CO₂ 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 18°C. 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 ± 1°C. 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 promotes 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 0°C had little gray mold mycelium development (Fig. 1A). However, as soon as berries were moved to 18°C, 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 0°C than at 18°C, preharvest treatments did not affect deterioration at 0°C (Fig. 1B, D, Table 2). When berries were transferred from 0 to 18°C, discoloration occurred more rapidly than disease progression, masking treatment effects on gray mold (Fig. 1A, B).

When berries were stored at 18°C, the rate of disease development did not differ between pyrrolnitrin-treated berries and...
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. 1C, 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. 1C), 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).

**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 | Preharvest spray treatment | P(F) | Integrals |
|-------------------------------|-----------------|---------------|---------------------------|------|-----------|
| First application: 6 July 1989 |                 |               |                           |      |           |
| 1                             | 0              | 17            | Pyrrolnitrin              | 0.03 | 36.3 ab   |
| 1                             | 18             | 8             | Captan + benomyl          | 0.02 | 116 b     |
| 4                             | 0              | 15            | Control                   |       | 14.8 b    |
| 4                             | 18             | 10            |                           |      | 36.3 b    |
| 6                             | 0              | 18            |                           |      | 15.8     |
| 6                             | 18             | 9             |                           |      | 64.3 a    |
| Second application: 13 July 1989 |                 |               |                           |      |           |
| 1                             | 0              | 21            | Pyrrolnitrin              | <0.001 | 161 b     |
| 1                             | 18             | 7             | Captan + benomyl          | 0.15 | 69.0 a    |
| 4                             | 0              | 17            | Control                   |       | 14.7 b    |
| 4                             | 18             | 6             |                           |      | 61.5     |
| 6                             | 0              | 6             |                           |      | 61.3 a    |
| 6                             | 18             | 6             |                           |      | 62.7 b    |

*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.

*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.*

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

*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 | Preharvest spray treatment | P(F) | Integrals |
|-------------------------------|-----------------|---------------|---------------------------|------|-----------|
| First application: 6 July 1989 |                 |               |                           |      |           |
| 1                             | 0              | 17            | Pyrrolnitrin              | 0.68 | 152       |
| 1                             | 18             | 8             | Captan + benomyl          | 0.03 | 310 a     |
| 4                             | 0              | 15            | Control                   |       | 137       |
| 4                             | 18             | 10            |                           |      | 99.3      |
| 6                             | 0              | 18            |                           |      | 333       |
| 6                             | 18             | 9             |                           |      | 80.7 a    |
| Second application: 13 July 1989 |                 |               |                           |      |           |
| 1                             | 0              | 21            | Pyrrolnitrin              | 0.90 | 370       |
| 1                             | 18             | 7             | Captan + benomyl          | 0.06 | 87.0      |
| 4                             | 0              | 17            | Control                   |       | 236       |
| 4                             | 18             | 6             |                           |      | 65.7 a    |
| 6                             | 18             | 9             |                           |      | 66.3 ab   |

*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.

*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.*

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

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

Berries harvested 4 and 6 days after application. Captan +
vent gray mold development after the 6 July treatment, but change in rates of deterioration in all treatments after 9 to 10 gray mold infection at either temperature (Fig. 2A, C). At 0°C, an application may have washed off the pyrrolnitrin. In air also had a low incidence of gray mold infection. Again, pyrrolnitrin was no better than the control in pre-

Storage in 20% CO$_2$ 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 0°C, however, berry deterioration, primarily discoloration, became the limiting factor, with 50% deterioration occurring on all treatments by 12 days postharvest (Fig. 2B). 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).

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 0°C. 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 0°C 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).

Pyrrrolnitrin/modified atmosphere effects on black raspberry

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).

Pyrrrolnitrin 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 0°C (Table 4, Fig. 3 A, C). However, iprodione consistently reduced gray mold at 18°C (Table 4, Fig. 3 B, D). Pyrrolnitrin significantly reduced gray mold in the first harvest after the field spray (Table 4).

Pyrrrolnitrin has performed extremely well in controlling postharvest diseases in the laboratory (Janisiewicz and Roitman, 1991). As expected, red raspberries decayed more quickly after transfer from 0 to 18°C (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 18°C, the development of gray mold was more rapid on black (Fig. 1C) 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).

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).

| Variable | 6 July 1989 (°C) | 13 July 1989 (°C) |
|----------|-----------------|-------------------|
| Atmosphere | 18 | 18 |
| 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 | 26.8 | 55.0 | 29.4 |
| Captan + benomyl | 26.3 | 5.6 | 26.5 | 17.0 |
| Distilled water | 87.6 | 30.6 | 64.2 | 85.8 |
| P(F) | 0.002 | 0.002 | 0.007 | 0.001 |

| Atmosphere | 18 | 18 |
| 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 | 196 | 71.8 | 429 |
| Captan + benomyl | 57.5 | 182 | 59.5 | 427 |
| Distilled water | 113 | 192 | 84.5 | 433 |
| P(F) | 0.004 | 0.80 | 0.24 | 0.77 |

4Integrals for 0°C berries calculated based on 19 days in storage, while those for 18°C berries calculated based on 11 days in storage, so values should not be compared between columns.

5P(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.
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.

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.

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 0°C followed by 18°C. Data shown are integrals of gray mold vs. time (Expt. 3).

| Day harvested after spray | Storage temp (°C) | Integral | Preharvest spray treatment | p(F) |
|---------------------------|------------------|----------|-----------------------------|------|
|                           |                  |          | Pyrrolnitrin | Iprodione | Control |      |
| 1                         | 0*               | 15       | 33.8          | 40.6      | 43.5    | 0.51 |
| 1                         | 18               | 15       | 72.9 b        | 45.9 c    | 38.5 a  | 0.001|
| 4                         | 0                | 16       | 45.5          | 39.9      | 51.1    | 0.99 |
| 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        | 82.7 b    | 107 a   | 0.02 |

*aDays 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.

*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, \( P < 0.05 \).

*Berries stored at 0°C were moved to 18°C 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) 0°C, 1 day after field treatment application (arrow indicates date berries were moved from 0 to 18°C); (B) 18°C, 1 day after field treatment application; (C) 0°C, 6 days after field treatment application; and (D) 18°C, 6 days after field treatment application (Expt. 3).
Literature Cited
Arima, K., H. Imanaka, M. Kousaka, A. Fukuda, and G. Tamura. 1965. Studies on pyrrolnitrin, a new antibiotic. I. Isolation and properties of pyrrolnitrin. J. Antibiotics Ser. A. 18(5):201-204.
Couey, H.M. and J.M. Wells. 1970. Low-oxygen or high-carbon dioxide atmospheres to control postharvest decay of strawberries. Phytopathology 60:47-49.
Crop Protection Chemicals Reference. 1991. 7th ed. Wiley, New York, Chemical and Pharmaceutical Press, Paris. p. 2001.
Freeman, J.A. and H.S. Pepin. 1976. Control of pre- and postharvest fruit rot of raspberries by field sprays. Acts Hort. 60:73-80.
Harvey, J.M. 1982a. CO₂ atmosphere for truck shipments of strawberries, p. 359-366. In: D.G. Richardson and M. Meheriuk (eds.). Controlled atmosphere for storage and transport of perishable agricultural commodities. Oregon State Univ. Symp. Section 1.
Harvey, J.M. 1982b. Quality and decay of California strawberries stored in CO₂-enriched atmospheres. Plant Dis. Rptr. 57(1):44-46.
Imanaka, H., M. Kousaka, G. Tamura, and K. Arima. 1965a. Studies on pyrrolnitrin, a new antibiotic. II. Taxonomic studies on pyrrolnitrin-producing strain. J. Antibiotics Ser. A. 18(5):205-206.
Imanaka, H., M. Kousaka, G. Tamura, and K. Arima. 1965b. Studies on pyrrolnitrin, a new antibiotic. 111. Structure of pyrrolnitrin. J. Antibiotics Ser. A. 18(5):207-210.
Janisiewicz, W. 1988. Biological control of diseases of fruits, p. 153-165. In: K.G. Mukerji and K.L. Garg (eds.). Biocontrol of plant diseases, vol. 2. CRC, Boca Raton, Fla.
Janisiewicz, W.J. and J. Roitman. 1988. Biological control of blue mold and gray mold on apples and pears with Pseudomonas cepacia. Phytopathology 78:1697-1700.
Keep, E. 1988. Breeding red raspberry for resistance to diseases and pests. Plant Breeding Rev. 6:245-321.
Robbins, J.A. and P.P. Moore. 1990. Color change in fresh red raspberry fruit stored at O, 4.5 or 20C. HortScience 25(12):1623-1624.
Smith, W.H. 1958. The harvesting, precooking, transport, and storage of strawberries and raspberries. Food Invest. Board Misc. Paper 1058.
Takeda, F. and W.J. Janisiewicz. 1991. Extending strawberry fruit shelf life with pyrrolnitrin, p. 174-176. In: A. Dale and J. Luby (eds.). The strawberry into the 21st century. Timber Press, Portland, Ore.
Takeda, F., .W.J. Janisiewicz, J. Roitman, N. Mahoney, and F.B. Abeles. 1990. Pyrrolnitrin delays postharvest fruit rot in strawberries. HortScience 25(3):320-322.
Tronsmo, A. and C. Dennis. 1977. The use of Trichoderma species to control strawberry fruit rots. Neth. J. Plant Pathol. 83 (Suppl. 1):449-455.
Winter, J. D., R.H. Landon, and W.H. Alderman. 1939. Use of carbon dioxide to retard the development of decay in strawberries and raspberries. Proc. Amer. Soc. Hort. Sci. 37:583-587.