# A Fungicide-Wax Treatment to Suppress Botrytis cinerea and Protect Fresh-Market Tomatoes

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## ABSTRACT

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The percentage of postharvest decay caused by *Botrytis cinerea* (Y) and the percentage of *B. cinerea* decay at harvest (X) was correlated positively ($r = 0.87$). The equation $Y = 2.47577 + 2.36729\ X - 0.02944\ X^2$ generated a curve that best fits the data. Apparently healthy mature-green tomato fruits harvested from diseased fields were treated with Botran 75W (DCNA, 3,000–30,000 μg AI/ml) in wax. *B. cinerea* was controlled for 14 days at 20 C when DCNA residues were 3.3 μg/g of fruit or more. Residues of 2.2–4.9 μg provided good protection against infection by conidia of *B. cinerea*, with higher residues providing better protection. Lesion diameters (X) on fruits inoculated with mycelial plugs of *B. cinerea* were correlated negatively ($r = -0.81$) with the amount of DCNA residues (Y). From the regression equation $Y = 31.94799 - 2.91065\ X$, a residue of 11.0 μg of DCNA per gram of fruit should prevent lesion development. Residues of DCNA between 3.3 and 4.6 μg/g of fruit suppressed the development of established infections, and the number of visible fruit infections was reduced further by higher residues (5.6–12.9 μg).

*Additional key words*: Botran 75W, DCNA, 2,6-dichloro-4-nitroaniline, gray mold.

Gray mold of tomatoes (*Lycopersicon esculentum* Mill.), caused by *Botrytis cinerea* Pers., is the most important preharvest and postharvest fruit-decay problem for growers and packers of fresh-market tomatoes in California. It is common throughout the growing season in the coastal tomato districts and becomes a serious problem in the northern San Joaquin Valley in the late summer and early fall harvest periods. Losses in the field can reach as high as 35%, a level at which apparently healthy fruits cannot be packed because they rot during shipment and storage. Preharvest chemical control is not effective because about half of the infections originate at points where the fruit is in contact with the soil (1).

Present postharvest wax treatments containing *o*-phenylphenol do not reduce decay caused by *B. cinerea*. It is impossible to detect all infected fruits before packing. When infected tomatoes are packed, *B. cinerea* can "nest" and spread within the box, causing severe losses.

The tomato industry currently uses two paste-type waxes in tomato treaters: (i) Decco Food Grade Tomato Wax WT-3 (Decco Division, Pennwalt Corp., PO Box 120, Monrovia, CA 91016), which is an emulsion of wax and oils and contains 0.1% *o*-phenylphenol, and (ii) Decco Food Grade Tomato Wax WT-22, which is the same as WT-3 except that the *o*-phenylphenol content is 2.5%. These waxes are dripped onto an overhead rotating horsehair brush in the tomato treater. A typical wax application rate for commercial tomato treaters is 0.5 ml/kg of fruit. As the fruits move through the treater, the wax is distributed evenly over their surfaces by a series of additional overhead brushes that are not treated with wax.

Botran (DCNA, 2,6-dichloro-4-nitroaniline; Tuco, Division of the Upjohn Co., Kalamazoo, MI 49001), currently used as a postharvest treatment on a number of crops, has an established tolerance of 5 μg/g of tissue for preharvest application on fresh-market tomatoes (9). This paper reports tests of DCNA-wax mixtures as a postharvest treatment on tomatoes to control decay caused by *B. cinerea*.

## MATERIALS AND METHODS

**Correlation between preharvest and postharvest decay.**—To relate postharvest decay in storage to preharvest decay, tests were made from 1973 to 1975 on apparently healthy fruits from 16 field plots: three plots at Davis, California, in 1973; and 13 plots near Tracy, California, in 1974 and 1975. The tomato cultivars were 6718, Pearson A-1, and Cal Ace at Davis; and Pearson A-1, VFN Bush, and Cal Ace at Tracy. All plots were maintained under commercial conditions in the early fall growing season. Mature green tomato fruits were harvested between 2 October and 2 November in all three years. The incidence of fruit decay at harvest was determined in three to five replicated 3-m lengths of row. Apparently healthy fruits were sorted, surface-sterilized by immersion in sodium hypochlorite (100 μg/ml for 1 min), dried, and packed in boxes for storage. The incidence of postharvest decay was determined after 11–16 days of storage at 15 ± 2 C. Relations between preharvest and postharvest decay were determined by regression analysis (3).

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**Wax mixtures containing DCNA.**—Various concentrations of DCNA were mixed with a water-soluble wax (WT-52 or Aqueous High Shine Wax, Decco Division, Pennwalt Corp., Monrovia, CA 91016). These aqueous waxes were used so that the DCNA-wax mixtures could be applied by a spray system to the overhead brush in the tomato treater; DCNA was suspended in water and then diluted 1:1 (v/v) with wax. The DCNA-wax mixtures were placed in a Namco Sprayer (Neil A. MacLean Co., Milpitas, CA 95035) fitted with a two-stage pressure regulator. Fruits were treated in a 90-cm-wide commercial tomato treater (Decco Division, Pennwalt Corp., Monrovia, CA 91016) located in the Plant Pathology field area at Davis, California. The DCNA-wax mixtures were applied to the overhead brush through two spray nozzles (800067 Tee Jet with 149-$\mu$m screen openings) 35 cm apart. The mixtures, continually agitated, were applied under 1.3 bars of pressure.

Mature green tomato fruits were immersed for 1 min in chlorinated water followed by two chlorinated rinses (100 $\mu$g of sodium hypochlorite per milliliter of water) for surface-sterilization before the DCNA-wax treatments. After excess moisture was removed with a series of sponge doughnut rollers, the fruits were treated with mixtures of DCNA ranging from 3,000–30,000 $\mu$g/ml of wax.

**Determination of DCNA residues.**—Residues of DCNA on treated fruits, extracted in reagent-grade benzene, were determined with a gas chromatograph equipped with a $^{63}$Ni electron-capture detector (2). The detector, injector, and oven temperatures were 200 C, with a nitrogen flow of 40 ml/min through a 1,830 × 2-mm glass column packed with 10% OV 101 AW-DCMS treated on Chromosorb W-100-200-mesh.

**Decay index.**—The effectiveness of treatments in controlling decay in storage was determined with a decay index (DI) based on visual inspection of each fruit for infection. Infected fruits were placed in one of five categories; 1 = superficial fleck (no soft decay); 2 = 1–24% of the surface decayed; 3 = 25–49% of the surface decayed; 4 = 50–74% of the surface decayed; and 5 = 75% or more of the surface decayed. The DI for each replication was obtained as follows: DI = $\Sigma$ (number of fruit per category × category number)/total number of fruit infected.

**Persistence and range of DCNA residues.**—Persistence of DCNA residues in storage was obtained from cultivar Cal Ace tomato fruits treated with 10,000 $\mu$g of DCNA per milliliter of wax and stored at 12.5 C. Fruits were removed after 0, 3, and 6 days of storage for residue analyses determinations (six to eight fruits per sample). The mean residue, after treatment of fruits with 3,000, 10,000, and 30,000 $\mu$g of DCNA per milliliter of wax, was determined on 17–19 residue analyses per application rate over a 2-yr period.

**Effect of DCNA residue on development of natural decay.**—Cultivar Cal Ace tomato fruits were harvested from a commercial field in late November. Apparently healthy fruits were given DCNA-wax treatments. Each treatment (50 fruits) was replicated three times. Treated fruits were placed in boxes and held at 20 C for 15 days before decay incidence was determined.

**Protection from infection by conidia.**—To determine the effects of DCNA residues on protecting fruits, treated Cal Ace and Royal Flush treated fruits were inoculated by spraying them with a spore suspension containing $2.5 \times 10^5$ conidia of *B. cinerea* per milliliter of water. Each treatment consisted of five replications of 35 fruit. The 35 fruits were placed in a plastic bag and sprayed with 10 ml of the spore suspension. Inoculated fruits were stored 7 days at 12.5 C and then 7 days at 20 C before incidence and severity of decay were determined.

**Protection from infection by mycelium.**—The relation of lesion size to DCNA residue was determined in a series of three experiments in 1976 and 1977. Twenty-two samples of 5–10 fruits given DCNA-wax treatment were inoculated with a 1-cm-diameter mycelial plug of *B. cinerea* grown on potato-dextrose agar. Inoculated fruits were placed on wire racks over water in plastic crispers at 20 C. After 7 days of incubation, average diameters of lesions were calculated. Lesion diameters for each of the 22 samples of fruits were then compared with the DCNA residues by regression analysis (3).

**Suppression of infections.**—To test for suppression of infections from DCNA residues, Royal Flush fruits were sprayed with a conidial suspension containing $2.5 \times 10^5$ conidia of *B. cinerea* per milliliter of water, or a drop of the conidial suspension was placed on a wound made at the blossom end of each fruit with the point of a sterile 2-mm-diameter glass rod. For the spray treatment, five replications of 35 fruits per treatment were inoculated 0, 12, 24, 36, 48, and 60 hr before being surface-sterilized by immersions for 1 min in 400 $\mu$g of sodium hypochlorite per milliliter of water and then treated with DCNA wax. Incidence and severity of decay were determined after 14 days of incubation at 20 C. For wound inoculations three replications of five surface-sterilized fruit were inoculated 0, 24, 48, and 72 hr before DCNA treatments. Fruits were incubated for 7 days on wire racks over water in plastic crispers at 20 C before lesion diameters were measured.

## RESULTS

**Correlation between preharvest and postharvest decay.**—The amount of postharvest decay on apparently healthy fruits correlated positively (r = 0.87) with the amount of fruit decay at harvest. The curve generated from the equation $Y = 2.47577 + 2.36729\,X - 0.02944\,X^2$ best fits the data for the percent postharvest decay (Y) that resulted from a specific amount of fruit decay at harvest (X) (Fig. 1).

**Persistence and range of DCNA residues.**—Residues of DCNA on tomato fruits did not decrease during storage at 12.5 C for 6 days. The DCNA residues after 0, 3, and 6 days of storage were respectively 5.9, 5.8, and 5.8 $\mu$g/g of fruit. The mean residues, plus or minus one standard deviation, on fruits treated with 3,000, 10,000, and 30,000 $\mu$g of DCNA per milliliter of wax were respectively 3.82 ± 0.94, 6.41 ± 1.75, and 12.81 ± 5.58 $\mu$g/g of fruit.

**Effect of DCNA residue on development of natural decay.**—The DCNA residues of 3.3 $\mu$g/g of fruit or greater reduced decay whereas 1.0 $\mu$g/g did not (Table 1).

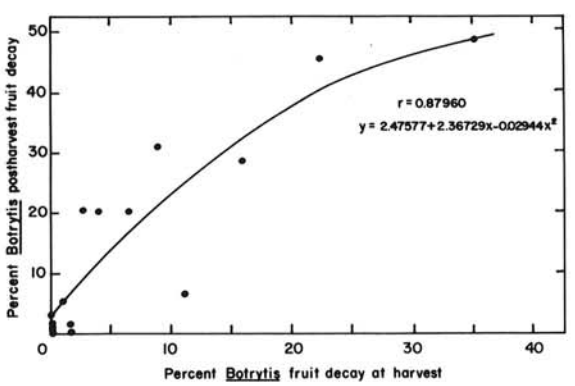


**Fig. 1.** Correlation between percent of postharvest decay caused by *Botrytis cinerea* (Y) and percent decay caused by *B. cinerea* at harvest (X).

**Protection from infection by conidia.**—Residues of DCNA sufficient to reduce the incidence of decay following inoculation with conidia were 2.2 and 5.6 μg/g, respectively, for fruits of cultivars Cal Ace and Royal Flush. Decay was reduced on all fruits having a DCNA residue, but higher residues reduced decay severity more than did lower residues (Table 2).

**Protection from infection by mycelium.**—There was a negative correlation (r = −0.81) between DCNA residues on fruits and lesion diameters after inoculation with mycelial plugs (Fig. 2). Residues of 11.0 μg/g of fruit prevented lesions completely. Although these high residues prevented lesions beyond the edge of the mycelial plugs, they did not prevent infection beneath the plugs. These infections were localized and did not spread. *Botrytis cinerea* was readily isolated from these necrotic areas after surface sterilization of the fruits.

**Suppression of infections.**—A residue of 3.3 μg of DCNA per gram of fruit was sufficient to suppress the development of established infections in fruits. Residues of 6.3 μg of DCNA per gram of fruit were needed to consistently reduce the severity of decay on fruits inoculated with conidia up to 60 hr before treatment (Table 3). At these higher residues, established lesions were darker than on fruits without a DCNA residue and did not spread as rapidly (Fig. 3).

When fruits were wound-inoculated before treatment, residues of 4.6 μg/g of fruit or more reduced the development of already established lesions on fruits inoculated 72 hr before treatment. A residue of 5.6 μg/g, however, resulted in better disease control than did 4.6 μg/g (Table 4).

TABLE 1. Effect of DCNA (2,6-dichloro-4-nitroaniline) residue on the natural incidence of decay caused by *Botrytis cinerea* on tomato fruits stored 14 days at 20 C

| DCNA residue (μg/g) | Percentage of fruits decayed[a] |
|---|---|
| 0.0 | 33.4 x |
| 1.0 | 27.4 x |
| 3.3 | 4.0 y |
| 7.6 | 1.4 y |
| 14.3 | 0.0 y |

[a]Average of three replications of 50 Cal Ace tomato fruits per treatment. Numbers in vertical column followed by the same letter are not significantly different ($P$=0.05, Duncan's multiple-range test).

TABLE 2. Protection of tomato fruit from infection by conidia of *Botrytis cinerea* following DCNA (2,6-dichloro-4-nitroaniline) in wax treatments

| DCNA residue (μg/g) | Incidence and severity of decay[a] | |
|---|---|---|
| | Fruit infected (no.) | Decay index |
| Experiment 1 (cultivar Cal Ace) | | |
| 0.0 | 34.0 x | 3.5 x |
| 1.9 | 33.4 x | 2.7 y |
| 2.2 | 21.8 y | 2.5 y |
| 3.7 | 13.2 z | 2.2 yz |
| 4.9 | 8.0 z | 1.7 z |
| Experiment 2 (cultivar Royal Flush) | | |
| 0.0 | 33.6 w | 2.3 x |
| 4.6 | 33.8 w | 1.7 y |
| 5.6 | 25.8 x | 1.6 y |
| 8.3 | 20.0 y | 1.4 z |
| 12.9 | 9.2 z | 1.4 z |

[a]Average of five replications of 35 tomato fruit per treatment. Fruits were inoculated with a spore suspension containing 2.5 × $10^5$ conidia of *B. cinerea* by spraying treated fruits before incubation for 7 days at 12.5 C followed by 7 days at 20 C. Decay index is as follows: 1 = superficial fleck (no soft decay); 2 = 1–24% decay; 3 = 25–49%; 4 = 50–74%; and 5 = 75% or more of fruit surface decayed. Numbers in vertical columns followed by the same letter are not significantly different ($P$=0.05, Duncan's multiple-range test).

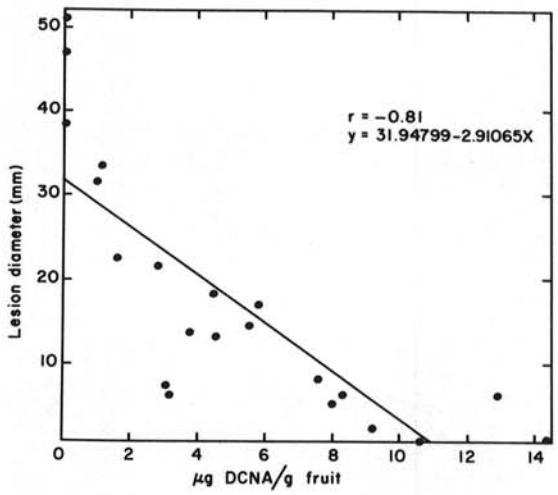


**Fig. 2.** Correlation between the amount of DCNA (2,6-dichloro-4-nitroaniline) residues (Y) on fruits and the lesion diameters in mm (X) on fruits inoculated with mycelial plugs of *Botrytis cinerea* and incubated for 7 days at 20 C.

TABLE 3. Suppression of infection and decay severity on tomato fruits inoculated with conidia of *Botrytis cinerea* 0–60 hr before treatment with DCNA (2,6-dichloro-4-nitroaniline) in wax after 14 days storage at 20 C[a]

| DCNA residue (μg/g) | Fruit infected | | | | Decay index | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 hr (no.) | 12 hr (no.) | 36 hr (no.) | 60 hr (no.) | 0 hr | 12 hr | 36 hr | 60 hr |
| 0.0 | 8.2 x | 7.4 x | 21.4 x | 29.8 x | 4.0 x | 4.3 x | 3.8 x | 4.3 w |
| 3.3 | 3.8 y | 4.2 xy | 6.2 y | 12.6 y | 3.0 x | 3.4 x | 3.7 x | 3.5 x |
| 6.2 | 1.8 y | 1.8 y | 4.6 yz | 5.4 z | 3.0 x | 1.7 y | 2.9 y | 2.7 y |
| 12.6 | 0.6 y | 2.2 y | 2.2 z | 3.2 z | 1.2 y | 1.8 y | 2.3 z | 2.1 z |

[a]Average of five replications of 35 fruits per treatment. Fruits were inoculated with a spore suspension containing 2.5 × $10^5$ conidia of *B. cinerea* per milliliter by spraying fruits 0, 12, 36, and 60 hr before they were surface-sterilized and treated with DCNA in wax. None of the fruits showed symptoms of infection at treatment. Decay index is as follows: 1 = superficial fleck (no soft decay); 2 = 1–24% decay; 3 = 25–49%; 4 = 50–74%; and 5 = 75% or more of fruit surface decayed. Numbers in vertical columns followed by the same letter are not significantly different ($P$ = 0.05, Duncan's multiple-range test).

TABLE 4. Suppression of lesion development on tomato fruit wound-inoculated with conidia of *Botrytis cinerea* 0–72 hr before treatment with DCNA (2,6-dichloro-4-nitroaniline) in wax

| DCNA residue (μg/g) | Lesion diameter (mm) after 7 days at 20 C[a] | | | |
|---|---|---|---|---|
| | 0 hr | 24 hr | 48 hr | 72 hr |
| 0.0 | 10.6 x | 20.2 x | 44.0 x | 92.3 x |
| 4.6 | 9.9 x | 10.4 y | 11.1 y | 53.0 y |
| 5.6 | 9.2 x | 9.2 y | 10.3 y | 28.1 z |
| 8.3 | 10.4 x | 8.3 y | 8.3 y | 25.5 z |
| 12.9 | 7.3 x | 7.0 y | 8.3 y | 16.6 z |

[a]Average of three replications of five Royal Flush tomato fruit per treatment. Fruits were wound-inoculated by placing a drop containing spores of *B. cinerea*, $2.5 \times 10^5$ conidia per milliliter of water, on an injury made with the tip of a sterile 2-mm-diameter glass rod 0, 24, 48, and 72 hr before treatment with DCNA in wax. All fruits were surface-sterilized before treatment with DCNA in wax. The increase in lesion diameter from the time of treatment with DCNA was measured. Average lesion diameters at treatment were respectively 0.0, 3.4, 5.8, and 8.4 mm for fruits inoculated 0, 24, 48, and 72 hr before treatment. Numbers in vertical columns followed by the same letter are not significantly different ($P = 0.05$, Duncan's multiple-range test).

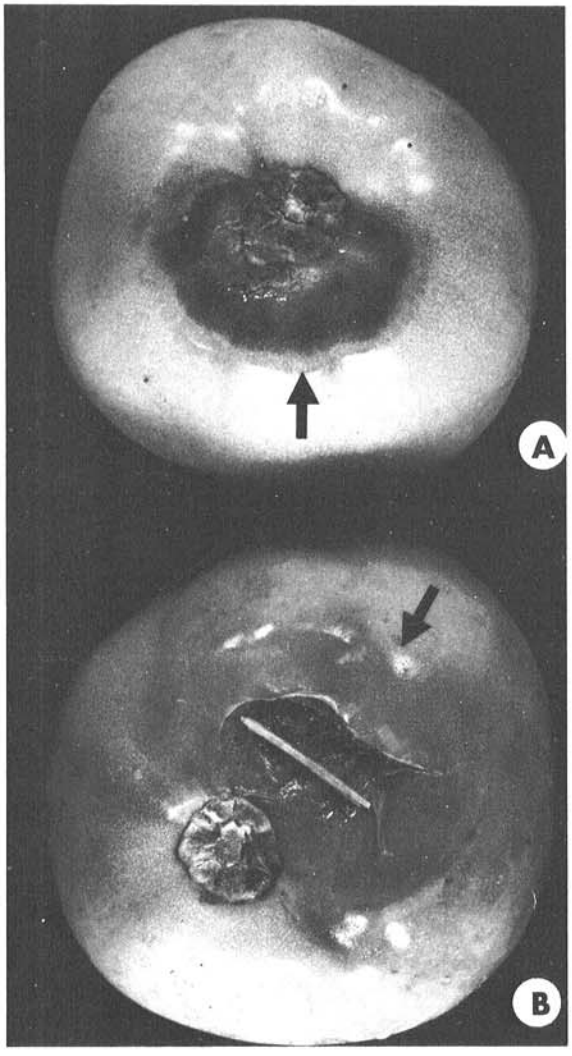


**Fig. 3-(A,B).** Comparison of lesions on fruits inoculated with conidia of *Botrytis cinerea*. Darkened lesion on fruit with a DCNA (2,6-dichloro-4-nitroaniline) residue of 6.2 μg/g (**A**) compared with lesion on fruit with no DCNA residue (**B**). Arrows indicate the lesion margins.

## DISCUSSION

Postharvest application of DCNA in wax with rotating brushes to fresh-market tomato fruits controlled decay caused by *B. cinerea* during storage and shipment. Infections by conidia of *B. cinerea* and severity of decay development related inversely to residues of DCNA on healthy fruits (Table 2).

The DCNA residues required to obtain a level of control differed between cultivars Cal Ace and Royal Flush by 8 μg/g (Table 2). The difference may be due in part to the fact that the Cal Ace fruits were larger (average diameter, 7.5 cm) than the Royal Flush fruits (average diameter, 6.4 cm). In any case, inoculated fruits needed higher DCNA residues than did naturally infected fruits to achieve the same level of control (Table 1). Thus, 3–4 μg of DCNA per gram of fruit was sufficient to control decay in some cases of field infections or contamination.

Because fruits infected in the field often are packed along with healthy fruits, postharvest treatments must suppress decay on infected fruits besides protecting other fruits from infection. Lesion development was limited by residues of 4.6–6.2 μg of DCNA per gram of fruit (Fig. 2, Table 4). Residues in this range also suppressed decay on fruits infected but not showing symptoms when treated (Table 4).

Residues of DCNA have similarly suppressed decay caused by *Rhizopus stolonifer* (Fr.) Lind on peaches (4,6), nectarines (4), sweet cherries (5), and apricots (7). The fungistatic effect of DCNA on mycelium in tomato fruits inoculated with *B. cinerea* also has been found for *R. stolonifer* on sweet cherries treated with DCNA (5). Ravetto and Ogawa (8) reported that DCNA applied postharvest penetrated the mesocarp tissues of peach fruits in sufficient quantities to reduce decay caused by *R. stolonifer*. We have not determined whether DCNA also penetrated tomato fruits.

Because suppression required DCNA residues of 4–6 μg/g of fruit, an increase in the established DCNA tolerance from 5 to 10 μg/g of fruit seems advisable if DCNA is to be an effective postharvest treatment. Mixtures of 10,000 μg of DCNA per milliliter of wax applied to tomatoes by rotating brushes should provide residues of DCNA that would satisfactorily control decay caused by *B. cinerea*.

These treatments reduce decay development both by preventing infection of healthy fruits from germinating conidia of nesting mycelium and by suppressing the development of established infections.

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