Efficacy of Ethanol in Postharvest Benomyl-DCNA Treatments for Control of Brown Rot of Peach

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ABSTRACT

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Ethanol at concentrations ≥30% improved the efficacy of benomyl-DCNA mixtures in 1-min dip treatments for control of postharvest brown rot in peach. The model of the regression analyses was significant ($P \leq 0.01$) and linear ($r^2 = 0.97$). The slope of the equation for the incidence of brown rot and the concentration of ethanol with fungicide was similar, but the Y intercept and midpoint values were lower than when ethanol was used alone. Control of brown rot was best with 70% ethanol, but shriveling of fruit epidermis from dehydration was greatest at this concentration. Addition of 30% ethanol to a benomyl-DCNA mixture reduced disease incidence and fungal sporulation after 6 days of incubation. Fruit were commercially acceptable, with only limited dehydration. Slopes of each linear equation were similar ($P \geq 0.05$) for 0.5-, 1.0-, and 2.0-min dipping periods as related to the incidence of brown rot and the concentration of ethanol (0, 30, 50, or 70%) in a benomyl-DCNA mixture. The incidence of brown rot vs. dipping time (5, 10, 20, and 30 sec) in a benomyl-DCNA mixture suspended in 30% ethanol was significant ($P \leq 0.05$) and linear ($r^2 = 0.92$). Less disease accompanied longer dipping periods. When fruit were inoculated with conidia of *Monilinia fructicola* and incubated for 4–48 hr before being dipped in a benomyl-DCNA mixture suspended in 30% ethanol, lesions after 6 days were smallest in fruit treated ≤12 hr after inoculation. The regression of lesion diameter vs. delay of treatment after inoculation was linear ($r^2 = 0.91$). In canned peach flesh, residues between treatments with or without ethanol differed by ≤0.54 μg/ml for benomyl and ≤0.29 μg/ml for DCNA; in the peach syrup, differences between treatments were ≤0.36 μg/ml for benomyl and ≤0.11 μg/ml for DCNA residues.

Additional keywords: disease control, postharvest disease

The primary postharvest decay pathogens of peaches (*Prunus persica* (L.) Batsch) in Brazil are *Monilinia fructicola* (G. Wint.) Honey and *Rhizopus stolonifer* (Ehrenb.:Fr.) Viull. *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. may also cause postharvest decay when rains occur during harvest. Most peach orchards in Brazil are distant from canneries, and fruit are transported in nonrefrigerated trucks on unpaved roads for 70 km or more. Bruising and injuries sustained during harvest and transport may provide additional sites for infection (7). Postharvest treatments with benomyl-DCNA after the fruit are delivered to canneries are ineffective for the control of brown rot on fruit excessively damaged during shipment (A. Feliciano, *unpublished*). Without fungicides, losses from *M. fructicola* alone can be severe during cold storage. Dip treatment of fruit using mixtures of benomyl and DCNA is recommended both in the field and in canneries to minimize postharvest decay (4).

The toxic effects of ethanol on spores of *M. fructicola* and its possible use in postharvest disease control have been reported but not commercially explored (3,5,6). The purpose of this research was to determine whether a benomyl-DCNA mixture suspended in ethanol controls postharvest brown rot under commercial conditions. Concentration of ethanol, exposure time, rinsing methods, and the potential for controlling established infections of *M. fructicola* with an ethanol-fungicide combination were evaluated.

MATERIALS AND METHODS

Source of fruit. Experiments were conducted from 1982 to 1983 at the National Center for Research on Temperate Fruit Crops in Pelotas, Rio Grande do Sul, Brazil. Peach cultivars Diamante, Capdeboscq, and Taruma were harvested from commercial orchards with a high incidence of brown rot in Cangucu, a city approximately 70 km north of Pelotas, and transported in nonrefrigerated trucks to the research station. All treatments were within 6–8 hr of harvest.

Ethanol and fungicide preparations. Concentrations of ethanol between 30 and 70% were prepared by diluting 96% commercial-grade ethanol in tap water (ethanol-water combinations). The 50% wettable-powder fungicides benomyl and DCNA were used in combination at rates of 300 and 900 μg a.i./ml, respectively.

Incubation and evaluation methods. Treated fruit were placed in cardboard boxes and incubated under ambient conditions (23–25 C, 80–82% RH) for 6 days, unless otherwise stated. Treatments were evaluated by determining the percentage of infected fruit, degree of sporulation, and size of lesions. Sporulation was rated on a scale of 0–3 based on area of lesion covered with spores: 0 = none, 1 = 0–25%, 2 = 26–50%, 3 = >50% of the lesion surface. Data were analyzed according to regression, analysis of variance (ANOVA), and Duncan's multiple range test for mean separation procedures of SAS (Statistical Analysis Systems, SAS Institute Inc., Cary, NC).

Ethanol concentration in fungicide mixtures. Commercially harvested Capdeboscq fruit were dipped for 1.0 min in solutions of 0, 30, 50, or 70% ethanol with or without a mixture of benomyl-DCNA. Taruma fruit were dipped in a conidial suspension (1×10^2 conidia per milliliter) of *M. fructicola*, air-dried, and then dipped for 0.5 min in a benomyl-DCNA mixture suspended in water or a benomyl-DCNA mixture suspended in 30% ethanol. Treatments with noninoculated fruit had four replications of 40 fruit and were analyzed using regression analysis procedures. Treatments with inoculated fruit had five replications of 100 fruit and were analyzed using ANOVA and Duncan's multiple range test procedures. Both experiments were repeated once.

Dipping time. Commercially harvested Capdeboscq fruit were dipped in a benomyl-DCNA mixture suspended in 0, 30, 50, or 70% ethanol for 0.5, 1.0, or 2.0 min, incubated, and evaluated as

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described previously. Taruma fruit were inoculated, air-dried as described above, and dipped in benomyl-DCNA suspended in 30% ethanol for 5, 10, 20, or 30 sec, incubated, and evaluated after 3 and 6 days for the incidence of brown rot. Each treatment using Taruma fruit had four replicates of 100 fruit. Replications (except where noted) and experiments were performed as described above, and data were analyzed using regression analysis procedures.

Posttreatment rinse. The following posttreatment rinsing procedures were evaluated to reduce shriveling in treatments with high concentrations of ethanol: 1) no rinse, 2) water rinse, and 3) benomyl-DCNA rinse. Capdeboscq fruit that had been dipped for 0.5 min in a benomyl-DCNA mixture suspended in either 0, 30, 50, or 70% ethanol were air-dried and incubated (no rinse treatment) or rinsed in water or benomyl-DCNA (volumes equal to those of the fungicide treatment) for 0.5 min, then air-dried and incubated. Each treatment had five replications of 50 fruit, and the experiment was repeated once. Data were analyzed using ANOVA and Duncan's multiple range test procedures.

Fruit quality and residue determination. After evaluation for decay, samples of healthy fruit from selected treatments were canned at a pilot cannery of the Center for Research using standard processing procedures. Treatments selected were: 1) 1-min dip in benomyl-DCNA with no rinse, 2) 0.5-min dip in benomyl-DCNA in 30% ethanol with no rinse, and 3) 0.5-min dip in benomyl-DCNA in 30% ethanol with a benomyl-DCNA rinse. Three cans per treatment were shipped to the Department of Environmental Toxicology at the University of California at Davis for residue analyses, and two cans were stored at ambient conditions for quality evaluation after 6 mo. Evaluations of fruit from each treatment were based on standard methods of measuring canned fruit quality, including wholeness, firmness, color, taste, aroma, and syrup turbidity.

Suppression of decay. Diamante fruit were injured by making a 0.5 × 1.0 mm puncture into the epidermis with a glass rod and then were inoculated by depositing 10 μl of a spore suspension (1 × 10^2 conidia per milliliter) of *M. fructicola* in the wounded area. Fruit were incubated for 4, 8, 12, 24, 36, or 48 hr before application of the postharvest treatment (30-sec dip in benomyl-DCNA in 30% ethanol), air-dried, and incubated as previously described. Lesion diameter was evaluated for four replications of 40 fruit for each treatment, the experiment was repeated once, and data were analyzed using regression analysis procedures.

RESULTS

Effect of ethanol concentration in fungicide mixtures on incidence of brown rot and sporulation of *M. fructicola*. Ethanol at concentrations of 30–70% with or without the addition of benomyl and DCNA fungicide mixture decreased the incidence of brown rot in noninoculated fruit of the cultivar Capdeboscq (Fig. 1). In general, nontreated fruit had a higher incidence of brown rot. The models of regression for ethanol concentration (0–70%) and for ethanol with or without benomyl and DCNA were significant ($P \leq 0.05$) and linear, with r^2 values of 0.77 and 0.97, respectively. The Y intercept and midpoint values were significantly lower ($P \leq 0.05$) (Fig. 1), but the slopes of the two regression lines did not differ significantly ($P > 0.05$). Ethanol without benomyl and DCNA reduced the average frequency of brown rot from 34.0 to 27.4% in 0–70% concentrations of ethanol. In contrast, average incidence of brown rot decreased from 16.5 to 1.9% in 0–70% concentrations of ethanol in benomyl-DCNA mixtures.

When fruit were inoculated, ethanol improved the efficacy of benomyl-DCNA treatments by reducing the incidence of brown rot infections and sporulation of *M. fructicola* (Table 1). In treatments using benomyl-DCNA or benomyl-DCNA in 30% ethanol, disease incidence and fungal sporulation were significantly lower than in nontreated control fruit (Table 1).

Effect of dipping time on brown rot incidence. The incidence of brown rot in noninoculated Capdeboscq fruit decreased as concentrations of ethanol increased (Fig. 2). The probabilities of significance for the models of regression of disease incidence on ethanol concentration for the 0.5-, 1.0-, and 2.0-min dipping times were 0.10, 0.08, and 0.05, respectively, and the r^2 values were 0.77, 0.84, and 0.90, respectively. Slopes, Y intercepts, and midpoints of each regression did not differ significantly ($P > 0.05$).

Incidence of brown rot in inoculated fruit of the cultivar Taruma dipped in benomyl-DCNA in 30% ethanol decreased as duration of dipping increased from 5 to 30 sec (Fig. 3). After 6 days, average incidence of brown rot ranged from 14 to 4.8% for 5- to 30-sec dipping times. The regression model of the incidence of brown rot on dip time was

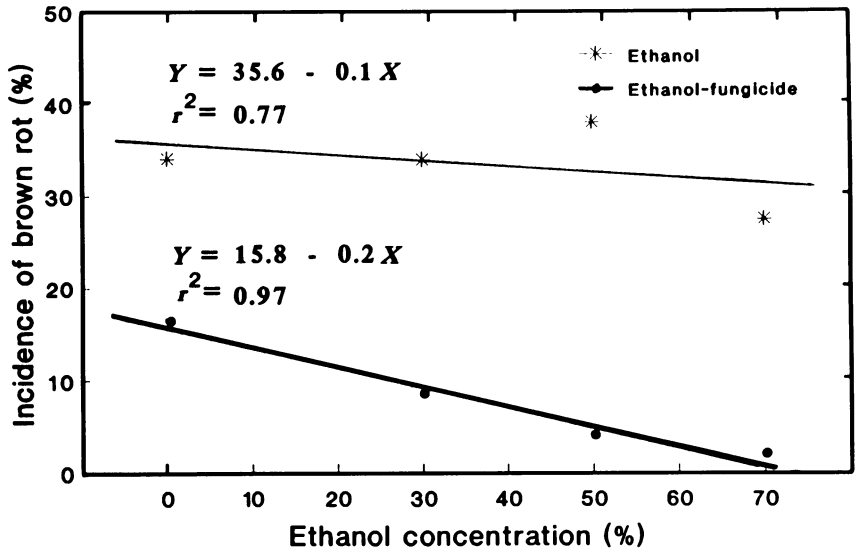

Fig. 1. Regression of the average incidence of brown rot (%) per four replicates of 40 Capdeboscq peaches on the concentration of ethanol in an ethanol-water or an ethanol-benomyl-DCNA mixture. For each treatment, fruit were dipped for 1 min, air-dried, incubated (23–25 C, 80–82% RH), and evaluated after 6 days. Concentrations of benomyl and DCNA were 300 and 900 μg a.i./ml, respectively. Data are the results of one experiment. Two experiments gave similar results.

Table 1. Effect of benomyl-DCNA in water or 30% ethanol on incidence of brown rot and sporulation of *Monilinia fructicola* on inoculated peach fruit (cv. Taruma) after 6 days of incubation at 23–25 C

Treatment[w]	Infected fruit[x] (%)	Sporulation index[y]
Inoculated control	100.0 a[z]	3.0 a
Benomyl-DCNA	26.0 b	2.0 b
Benomyl-DCNA in 30% ethanol	12.4 c	1.0 c

[w] Fruit were dip-inoculated in a spore suspension of *M. fructicola* containing 1 × 10^4 conidia per milliliter. Inoculated fruit were dipped for 30 sec in each fungicide treatment and air-dried before incubation. Rates of benomyl and DCNA were 300 and 900 μg a.i./ml, respectively.

[x] Average of five replications of 100 fruit each.

[y] Based on area of lesion covered with spores, with 0 = none, 1 = 0–25%, 2 = 26–50%, and 3 = ≥50% of lesion surface.

[z] Values in a column followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's multiple range test.

significant ($P \leq 0.05$) and linear, with an r^2 value of 0.92 (Fig. 3).

Effect of posttreatment rinse on brown rot incidence. A water rinse after a benomyl-DCNA or benomyl-DCNA in 30% ethanol treatment significantly increased disease incidence in commercially harvested Capdeboscq fruit compared with no rinse or with benomyl-DCNA rinse treatment (Table 2). When benomyl-DCNA mixtures in 50 or 70% ethanol were used as postharvest treatments, disease incidence was low for all treatments and the treatments did not differ significantly ($P > 0.05$). Shriveling of fruit, however, occurred at ethanol concentrations of 50 and 70%, regardless of the rinse treatment.

Effect of postharvest treatment on fruit quality and amount of residue. Evaluation of external fruit quality 6 days after treatment showed that peaches dipped in benomyl-DCNA suspended in ethanol were firmer and greener than those dipped in benomyl-DCNA suspended in water. Dehydration by ethanol, manifested as shriveling of the epidermis, was observed in treatments using high concentrations of ethanol and was minimal in treatments using 30% ethanol. Furthermore, dehydration by high concentrations of ethanol (50 and 70%) was more severe in treatments with no rinse than in those with water or benomyl-DCNA rinse. Six months after canning, fruit treated with benomyl-DCNA in 30% ethanol and either rinsed or not rinsed showed no loss in quality compared with fruit treated with benomyl-DCNA in water.

For all treatments, benomyl residues in the flesh of canned peaches ranged from 1.32 to 0.78 μg/ml and DCNA residues ranged from 1.35 to 1.20 μg/ml. In the canned syrup, benomyl residues ranged from 0.86 to 0.32 μg/ml and DCNA residues ranged from 0.41 to 0.26 μg/ml. In general, fruit treated with fungicide suspended in ethanol had higher concentrations of benomyl and DCNA residues than those treated with fungicide in water, regardless of rinsing treatment. Differences, however, in the residues of benomyl in the flesh of fruit treated either with benomyl-DCNA in water or with benomyl-DCNA in ethanol, regardless of rinsing treatments, were ≤0.54 μg/ml. Similarly, differences between treatments in DCNA residues were ≤0.29 μg/ml. In peach syrup, differences between treatments were ≤0.36 μg/ml for benomyl and ≤0.11 μg/ml for DCNA.

Suppression of decay. After 6 days, lesions were smaller when postharvest treatment was applied within 4–12 hr after inoculation, with lesion diameters of 0.1 and 1.8 cm for the 4- and 48-hr applications, respectively. The model for regression was significant ($P \leq 0.05$) and had an r^2 value of 0.91 (Fig. 4).

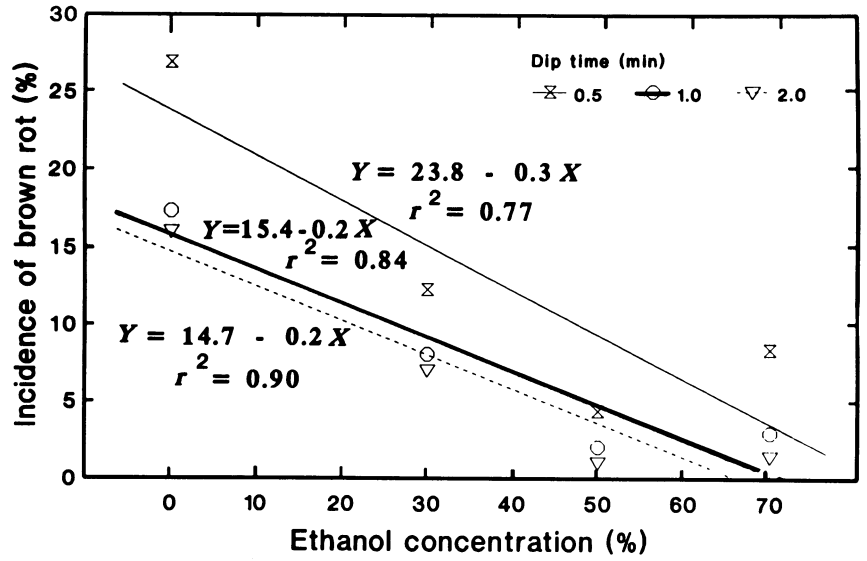

Fig. 2. Regression of the average incidence of brown rot (%) per four replicates of 40 Capdeboscq peaches on the concentration of ethanol in a benomyl-DCNA mixture for a 0.5-, 1.0-, or 2.0-min dip treatment. After each treatment, fruit were air-dried, incubated (23–25 C, 80–82% RH), and evaluated after 6 days. Concentrations of benomyl and DCNA were 300 and 900 μg a.i./ml, respectively. Data are the results of one experiment. Two experiments gave similar results.

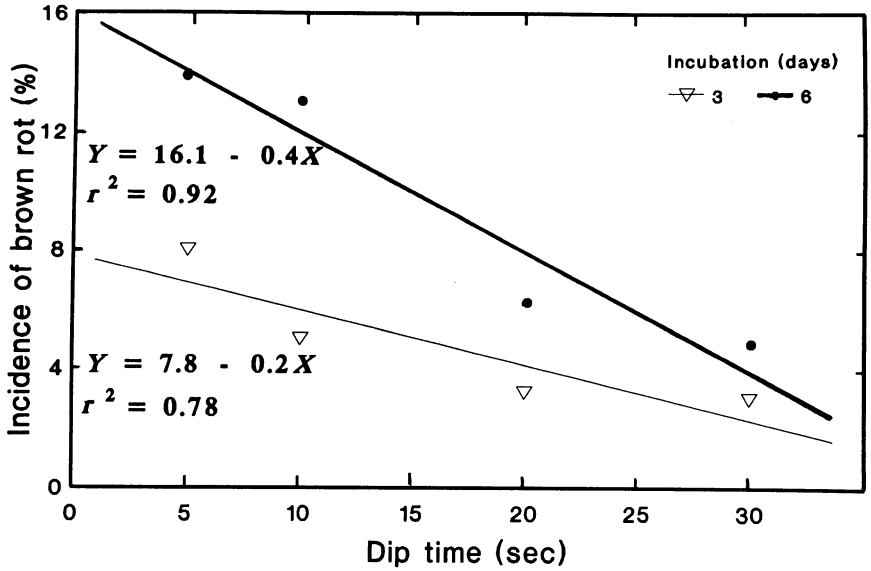

Fig. 3. Regression of the average incidence of brown rot (%) per four replicates of 100 Taruma peaches inoculated with conidia of *Monilinia fructicola* (1×10^2 conidia per milliliter) on the dip time of peaches in a benomyl-DCNA mixture suspended in 30% ethanol after 3 and 6 days of incubation. After each treatment, fruit were air-dried and incubated at 23–25 C, 80–82% RH. Concentrations of benomyl and DCNA were 300 and 900 μg a.i./ml, respectively. Data are the results of one experiment. Two experiments gave similar results.

DISCUSSION

Ethanol improved the efficacy of a benomyl-DCNA mixture for control of postharvest decay caused by *M. fructicola*. Ethanol-fungicide combinations

Table 2. Effect of rinsing method on incidence of brown rot of peach fruit after postharvest treatment with benomyl-DCNA suspended in various concentrations of ethanol

	Incidence of brown rot (%) per ethanol concentration[z]			
Rinse[y]	0%	30%	50%	70%
Water	37.1 a	16.1 a	2.0 a	6.9 a
None	13.9 b	7.2 b	2.9 a	2.9 a
Benomyl-DCNA	9.2 b	2.9 b	1.0 a	2.9 a

[y] Fruit were 1) dipped for 30 sec in a solution of benomyl-DCNA in water or ethanol; 2) air-dried (no rinse), rinsed in water, or rinsed in a benomyl-DCNA mixture and then air-dried; and 3) incubated for 6 days at 23–25 C. Rates of benomyl and DCNA were 300 and 900 μg a.i./ml, respectively.

[z] Values are averages of five replicates of 50 fruit taken 6 days after treatment. Values for each concentration of alcohol followed by the same letter are not significantly different ($P > 0.05$) using Duncan's multiple range test.

reduced the incidence of brown rot and fungal sporulation in inoculated fruit. High concentrations of ethanol were more effective in reducing decay, although dehydration was severe with the 50 and 70% concentrations of ethanol. Addition of 30% ethanol to a benomyl-DCNA mixture reduced disease incidence and fungal sporulation after 6 days of incubation. Fruit from this treatment showed limited dehydration and were commercially acceptable.

Possible explanations for the increased efficacy of benomyl-DCNA in ethanol include the fungitoxic effect of ethanol (3,5,6), increased solubility of benomyl in ethanol (5), reduced surface tensions and improved wettability of fruit tissue, and improved penetration of benomyl. Since the slopes of the two regression lines (ethanol alone and benomyl-DCNA in ethanol) did not differ significantly, the fungitoxic activity of ethanol (5) may solely account for the increased efficacy of postharvest treatments using higher concentrations of ethanol. Residue analyses indicated that the flesh of canned fruit treated with benomyl-DCNA in ethanol contained approximately 0.5 μg/ml more benomyl and 0.2 μg/ml more DCNA than the flesh of fruit treated with benomyl-DCNA in water. Thus, at these concentrations of benomyl and DCNA in the fruit tissue, the improved efficacy of treatments with higher concentrations of ethanol in benomyl-DCNA mixtures may not be explained by more residual benomyl and DCNA in processed peach fruit.

Calmon and Sayag (2) and E. I. du Pont de Nemours and Co. (1) indicated a greater solubility of benomyl in organic solvents including ethanol. Preliminary analyses using UV spectrophotometry indicated over three times more benomyl in solution when benomyl was suspended in 70% ethanol than when suspended in water (J. E. Adaskaveg, *unpublished*). The mechanism by which ethanol increased the efficacy of the benomyl-DCNA mixture requires further study.

No significant differences were observed in the slopes, midpoints, or Y intercepts of the regression of incidence of brown rot on ethanol concentration for three dipping times, i.e., 0.5, 1.0, and 2.0 min. On the basis of this information and the minimal dehydration caused by the 30% concentration of ethanol, a 0.5-min dip in a benomyl-DCNA mixture suspended in 30% ethanol was used in subsequent experiments. A regression of the incidence of brown rot on dip time, i.e., 5, 10, 20, and 30 sec, indicated that the incidence of brown rot in treated fruit was lowest with the 30-sec dip. An additional regression of brown rot lesion diameter on the time of treatment after inoculation indicated that postharvest treatments should be applied within 12 hr of harvest and postharvest handling when the possibility of injury to the fruit exists.

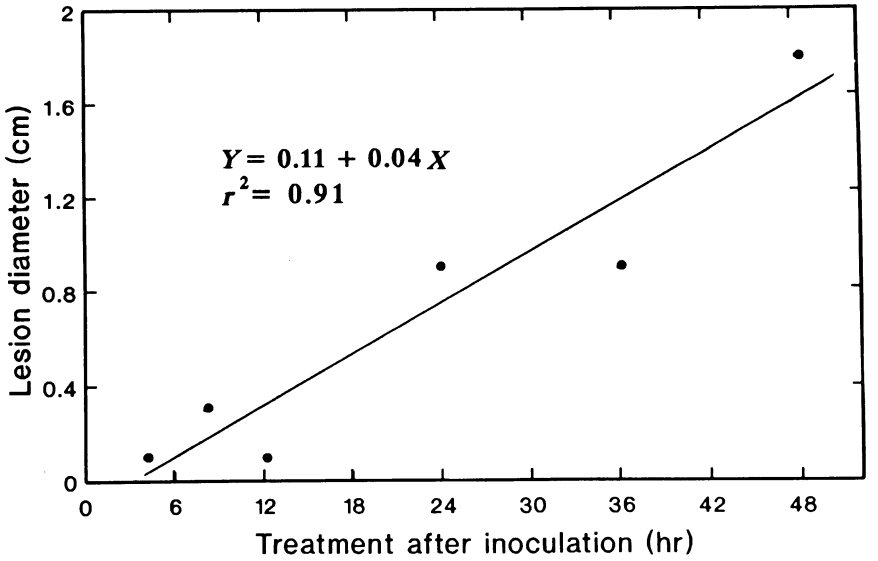

Fig. 4. Regression of the diameter of brown rot lesions per four replicates of 40 Diamante peaches on time of postharvest treatment after inoculation of peaches with conidia of *Monilinia fructicola* (1×10^2 conidia per milliliter). For each postharvest treatment, fruit were dipped for 0.5 min in a benomyl-DCNA mixture suspended in 30% ethanol, air-dried, and incubated for 6 days at 23–25 C, 80–82% RH. Concentrations of benomyl and DCNA were 300 and 900 μg a.i./ml, respectively. Data are the results of one experiment. Two experiments gave similar results.

A water rinse after a postharvest treatment using benomyl-DCNA in 30% ethanol increased the incidence of brown rot and did not prevent shriveling of fruit treated with benomyl-DCNA in 50 or 70% ethanol. A benomyl-DCNA rinse after any benomyl-DCNA-ethanol treatment did not significantly decrease disease incidence compared with a no-rinse treatment. Furthermore, as indicated above, benomyl residues in all treatments (with or without ethanol or rinses) differed by ≤0.54 μg/ml. Thus, rinsing with water or benomyl-DCNA did not provide any additional advantage in disease control in treatments with 50 or 70% ethanol, did not prevent fruit dehydration (with high concentrations of ethanol), and did not cause substantial reductions of fungicide residues (with or without the addition of ethanol). Some processors in Brazil effectively use a benomyl-DCNA mixture suspended in 30% ethanol in a postharvest dip treatment of peaches for control of postharvest decay by *M. fructicola, R. stolonifer*, and *C. gloeosporioides*. In Brazil, ethanol is readily available and the addition of 30% ethanol to the fungicide mixture is economically feasible.

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