Combination Heat and 2,6-Dichloro-4-Nitroaniline Treatments for Control of Rhizopus and Brown Rot of Peaches, Plums, and Nectarines

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ABSTRACT

Combined hot water and 2,6-dichloro-4-nitroaniline (DCNA) treatments of peaches, plums, and nectarines were more effective in the control of postharvest decays than either hot water or DCNA alone. Naturally-occurring decay was reduced from a mean of 65% in untreated peaches and nectarines to 7.8% in lots immersed for 1.5 min in water at 51.5°C with 225 ppm DCNA. DCNA residues on fruit treated for 1.5 min in hot water with 225 ppm DCNA were equivalent to residues from treatments with 900 ppm DCNA in unheated water. Lesion diam on fruit inoculated with *Rhizopus* or *Monilinia*, and treated with hot water, decreased linearly as exposure time increased from 0.5 to 3 min. Regression coefficients of lesion diam on exposure times increased with increasing water temperatures. When 225 or 450 ppm DCNA was added to the hot water, equivalent inhibition of lesion development was possible with treatments of half the exposure times necessary with hot water alone. Phytopathology 60: 116-120.

The principal postharvest diseases of peaches (*Prunus persica* [L.] Batsch), plums (*P. salicina* Lindl.), and nectarines (*P. persica* [L.] Batsch var. *nectarina* [Ait.] Maxim.) are brown rot, caused by *Monilinia fructicola* (Wint.) Honey and *M. laxa* (Aderh. & Ruhl.) Honey, and Rhizopus rot, caused by *Rhizopus stolonifer* (Fr.) Lind. Of less importance, but frequently occurring, are black mold rot (*Aspergillus niger* v. Teigh.) and blue mold rot (*Penicillium* sp.) (6). Losses from these diseases during transporting and marketing in the United States have been estimated at 9% for peaches and 3% for plums (9). In 1968, postharvest rots of peaches through the consumer level caused 15% and 24% losses in the New York and Chicago markets, respectively (10).

Brown rot of peaches, plum, and nectarines has become a serious problem in California, particularly with late-maturing varieties. Although past occurrences of this disease have been associated with rainfall or unusually humid growing seasons, the disease now appears to be endemic. Most postharvest decays develop after storage and transit, when the fruit is transferred to ripening temperatures. Thus, fruit that is of acceptable quality at shipping point may develop rot when ripened at destination.

Postharvest rots of peaches and nectarines have been reduced with fungicidal dips and with fungicide-impregnated wrappers (3, 4, 5). Hot-water treatments also are effective against brown rot and Rhizopus rot of eastern-grown cultivars of peaches and nectarines (7). Fruit treated in hot water for 7 min at 49°C, or for 3 min at 54.5°C, developed about 70% less decay than untreated controls. A 2- to 3-min treatment at 51.5°C is recommended at present (8).

This report summarizes research that demonstrates (i) that hot-water treatments reduce decay on western-grown cultivars of peaches, plums, and nectarines, and (ii) that combined heat and 2,6-dichloro-4-nitroaniline treatments are more effective than either used alone. Also, the combined treatments permit shorter exposure times and lower fungicide dosages than separate treatments.

MATERIALS AND METHODS.—Freshly harvested fruits, without apparent injury or decay and of uniform maturity, were selected from local orchards or packing sheds. Peaches were brushed prior to selection.

Early cultivars of fruit were inoculated prior to treatment, because of the low incidence of naturally-occurring decay. Each fruit was punctured twice with an instrument 2 mm long, and dipped into a spore suspension of *Monilinia fructicola* or of *Rhizopus stolonifer*. Inoculated fruit were then incubated 16-20 hr at 21°C in polyethylene bags.

A total of 48 treatments were arranged factorially with all combinations of 4 temperatures, 4 exposure times, and 3 concentrations of Botran 75W [75% 2,6-dichloro-4-nitroaniline (DCNA)]. Water temperatures were 24°C, 49°C, 51.5°C, and 54.5°C. Exposure times were 0.5, 1.5, 3, and 6 min. DCNA concentrations were 0, 225, and 450 ppm active toxicant (zero, one-fourth, and one-half lb., respectively, of Botran 75W/100 gal).

Ten fruits constituted a treatment lot, and treatments were repeated six times, once each with Red Top and Suncrest cultivars of peach, with Sungrand, Redgrand, and Late Legrand cultivars of nectarine, and with the Casselman cultivar of plum.

Fruits were placed in wire baskets and immersed in an insulated, stainless steel tank of 100-gal capacity. Water was circulated constantly by pump at 100 gal/min, and temperatures were maintained at ±0.1°C of set point with electric heating coils activated by a temperature controller with a thermister probe.

Treated fruits were stored in polyethylene bags for 5 days at 4.5°C to simulate transit, and were ripened

for 2 days at 21°C. Decay development was determined by measuring lesion diam and calculating an average for each test lot. Fruit was rated for possible injury from the treatments, on a scale of 1 to 4 corresponding to no injury, slight, medium, and severe injury, respectively. Fruits with no evidence of decay on the 1st day of examination were held up to 10 days to determine shelf life.

Late cultivars of fruit, harvested when the incidence of naturally-occurring decay was significant, were not inoculated and were tested in larger lots. Treatments for this fruit were 3 min in water at 49°C, 0.5 and 1.5 min at 51.5°C, or 0.5 min at 54.5°C, with 0, 225, or 450 ppm DCNA. Treatment lots consisted of 100 peaches or nectarines, or of 264 plums. The cultivars Gold King, Regal Grand, and September Grand nectarine, Fiesta and Halloween peach, and Swall Rosa and Casselman plums were treated. Treated fruit were packed into commercial lugs with polyethylene liners, stored for simulated transit and holding times of 15-21 days at 2°C, ripened at 21°C for 5-7 days, and examined for decay and injury. Fruit was considered decayed if *Monilinia, Rhizopus, Penicillium,* or *Aspergillus* infections were present at any stage of development.

Residue analyses of peaches, plums, and nectarines were made by the Upjohn Co., using the method of Kilgore et al. (2). Each analysis was based on a 1-kg sample of treated fruit.

Data were evaluated by analysis of variance and by Duncan's Multiple Range Test. Results from the inoculated tests also were analyzed with a computer (IBM 1130 Computing System). The analysis was based on a split-split plot experimental design, with cultivars used as replications. Differences were considered significant at the 99% level in the inoculated tests, and at the 95% level in the noninoculated tests. Regression coefficients were calculated by linear regression analysis.

RESULTS.—*Decay (lesion diam) of inoculated fruit treated with hot water and DCNA.*—The diameters of lesions of inoculated fruit treated with hot water decreased linearly as exposure time increased from 0.5 to 3 min (Fig. 1-A, B). Linear regressions of lesion size on exposure time at 49, 51.5, and 54.5°C were significant at the 1% level. The regression coefficients, or slopes, were a function of treatment temperatures. Coefficients decreased linearly as water temperatures increased from 49° to 54.5°C.

Hot-water treatments with sufficiently long exposure times controlled lesion development until the first examination. Lesions on *Rhizopus*-inoculated fruit were controlled with 3-min treatments at 51.5 or at 54.5°C (Fig. 1-A). Lesions on *Monilinia*-inoculated fruit were significantly reduced with 3-min treatments (Fig. 1-B), but control required 6-min treatments at 51.5° or 54.5°C (Table 1).

Hot-water treatments, at all exposure times tested, significantly reduced the diameters of lesions on inoculated fruit, compared to the checks. Differences among the high-temperature treatments, however, were generally not significant. With *Rhizopus*-inoculated fruit, water temperatures of 51.5° and 54.5°C were significantly more effective than 49°C at 1.5 min, but not at other exposure times. With *Monilinia*-inoculated fruit, 54.5°C was significantly more effective than 49° or 51.5°C only at the 3-min exposure time.

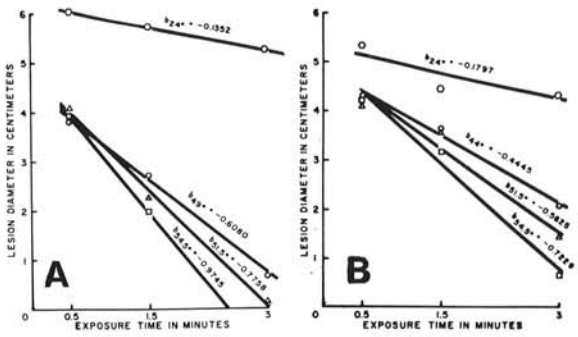

Fig. 1. Linear regression curves of lesion diam as a function of exposure times in water at 24°, 49°, 51.5°, and 54.5°C for fruit inoculated with **A)** *Rhizopus stolonifer* and **B)** *Monilinia fructicola.* Treated fruits were stored for 5 days at 4.5°C and for 2 days at 21°C prior to examination. Linear regressions are significant at the 1% level, and coefficients of regression (b) for heat treatments decrease linearly with increasing temperatures. Each point represents a mean value for six experiments, which include two with peaches, three with nectarines, and one with plums. The regression for *Rhizopus* lesion diam on exposure times at 54.5°C was estimated from two points; all others were calculated from three points.

The fungicidal effects of heat treatments were improved by the addition of DCNA to the water. Heat treatments with 225 or 450 ppm DCNA were significantly more effective in controlling decay than treatments with hot water alone or with DCNA in unheated water. Lesions on inoculated fruit treated in hot water plus DCNA were controlled in half the exposure time necessary with hot-water treatments alone (Fig. 2-A, B).

Lesions on *Rhizopus*-inoculated fruit were controlled with hot DCNA treatments in 1.5 min (Fig. 2-A). At 0.5 and 1.5 min, there were no significant differences due to treatment temperature, between 49° and 54.5°C, or between the use of 225 or 450 ppm DCNA. Hot DCNA treatments were significantly more effective at 1.5 min than at 0.5 min.

Lesions on *Monilinia*-inoculated fruit were controlled with 3-min treatments at 51.5° or at 54.5°C with either 225 ppm DCNA or 450 ppm DCNA (Fig. 2-B). At any exposure time, and at any temperature tested, there were no statistical differences in the lesion diam between 225 or 450 ppm DCNA treatments. However, a 1.5-min treatment at 54.5° with 450 DCNA was significantly better than 49°C with 225 ppm DCNA.

Shelf life of inoculated fruit treated with hot water and DCNA.—Hot-water treatments only retarded the development of lesions on inoculated fruit. Continued incubation of treated fruit resulted in the eventual development of normal lesions. Inoculated fruits treated for 6 min at 51.5°C were free of lesions at the first examination, but lesions began developing after approximately 6 additional days at ripening temperatures (Fig. 3-A, B).

The shelf life of inoculated fruit was significantly

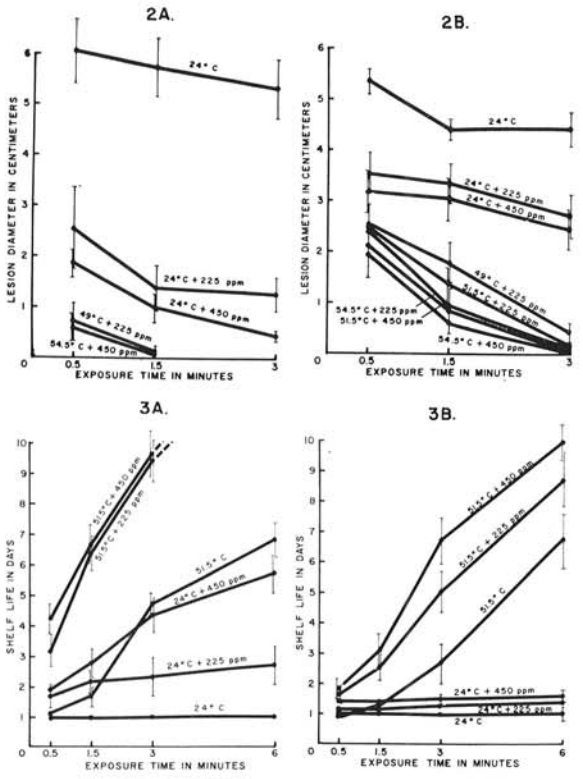

Fig. 2-3. **2)** Lesion diam on fruit inoculated with **A)** *Rhizopus stolonifer,* and **B)** *Monilinia fructicola* as influenced by treatment exposure times in water at selected temperatures and concentrations of 2,6-dichloro-4-nitroaniline (DCNA). Treated fruit were stored for 5 days at 4.5°C and for 2 days at 21°C prior to examination. Each point represents a mean value for six experiments which included two with peaches, three with nectarines, and one with plums. The brackets define the standard error of the means. **3)** Shelf life with respect to the appearance of decay on fruit inoculated with **A)** *Rhizopus stolonifer,* and **B)** *Monilinia fructicola,* and treated in water at 24° and 51.5°C with 0, 225, and 450 ppm DCNA at different exposure times. Treated fruit were stored for 5 days at 4.5°C and for 2 days at 21°C prior to examination. Each point represents a mean value for six experiments which included two with peaches, three with nectarines, and one with plums. The brackets define the standard error of the means. Slight-to-medium injury resulted from the 6-min hot DCNA treatments.

greater when treated with hot water plus DCNA than with hot water alone. *Rhizopus*-inoculated fruit treated at 51.5°C with 225 or 450 ppm DCNA had a shelf life approximately double that of fruit treated at the same temperature but without DCNA, or of fruit treated with 450 ppm DCNA in unheated water (Fig. 3-A). At any one exposure time, there were no significant differences due to treatment temperature, within the range of 49° to 52.5°C, or to the use of 225 or 450 ppm DCNA.

The shelf life of fruit inoculated with *Monilinia* and treated in hot water plus DCNA was significantly greater than that of fruit treated with heat alone or with DCNA in unheated water (Fig. 3-B). Differences between 225 and 450 ppm DCNA were significant only if exposure times were as long as 3 or 6 min. DCNA at 225 or at 450 ppm in unheated water had no significant effect on shelf life.

TABLE 1. Lesion diameters and injury level on fruit inoculated with *Rhizopus* or *Monilinia* and treated for 6 min in water at different temperatures

Water temp	Lesion diam		Injury level
	Rhizopus	*Monilinia*	
C	*cm*	*cm*	
24	5.7[a]	4.3	None
49	0	0.5	None
51.5	0	0	Slight
54.5	0	0	Medium[b]

[a] Mean values for 10 inoculated fruit in 6 tests with peaches, plums, and nectarines. Fruit were held for 5 days at 4.5°C, and for 2 days at 21°C under polyethylene liners.
[b] Considered to be detracting from the market quality of the fruit.

Injury to inoculated fruit treated with hot water and DCNA.—Hot water controlled decay without affecting the market quality of treated fruit; however, treatments of 6 min at 54.5°C caused significant injury (scalding) on the peach and nectarine cultivars tested (Table 1). In general, the use of heated DCNA solutions increased the level of injury, as compared to hot water treatments alone. Slight to medium injury resulted from treatments with 225 or 450 ppm DCNA for 6 min at 49 or 51.5°, or for 3 min at 54.5°C. Plums were more tolerant of heat-DCNA treatments than were peaches or nectarines, and were not injured by either 6-min hot water treatments or by 3-min DCNA treatments at 54.5°C.

Decay of naturally infected fruit treated with hot water and DCNA.—Naturally-occurring decay of peaches and nectarines was reduced from a mean of 65% in the untreated lots to 7 to 14% in lots treated with hot water plus DCNA (Table 2). A 1.5-min treatment with 225 ppm DCNA at 51.5° reduced decay to a mean of 7.8% ($s_{\bar{x}}/\pm$ 2.9%) and caused no injury to the fruit. There was no significant statistical difference in decay between 0.5- and 1.5-min treatments at 51.5°C, nor between 225 or 450 ppm DCNA treatments at any temperature. Combination heat and DCNA treatments, however, were generally more effective than heat treatments alone.

The late-maturing cultivars of fruit used in these tests were apparently more sensitive to treatment injury than the early cultivars. Treatments with 225 ppm DCNA for 3 min at 49°C or higher, or with 450 ppm DCNA for 1.5 min at 51.5°C and 54.5°C caused a noticeable darkening of skin color and a loss of surface glossiness.

Infections due to *Monilinia* accounted for most of the decay observed, but infections due to *Rhizopus* were occasionally as numerous in lots treated with hot water alone (Table 3). Combination heat-DCNA treatments, in addition to reducing the number of *Monilinia*-infected fruit, completely controlled the incidence of *Rhizopus* decay, and did not result in a significant increase in miscellaneous infections.

Plums required heat treatments with longer exposure times or higher DCNA concentrations than peaches or

TABLE 2. Percentage decay in five cultivars of nectarines and peaches treated with hot water and 2,6-dichloro-4-nitroaniline (DCNA), stored for 17-21 days at 2°C, and ripened for 5-7 days at 21°C

			% Decay[a]					
			Nectarines			Peaches		
Water temp	DCNA concn.	Exposure time	Gold King	Regal Grand	Sept. Grand	Fiesta	Halloween	Mean decay[b]
C	*ppm*	*min*	%	%	%	%	%	%
24	0	1.5	86	56	53	38	97	66.0 e
24	450	1.5	94	33	45	31	65	53.6 d
49	0	3.0	11	11	15	11	30	15.6 ab
49	225	3.0[c]	9	3	4	2	17	7.0 a
51.5	0	0.5	48	7	25	11	37	25.6 c
51.5	0	1.5	33	15	10	6	22	17.2 bc
51.5	225	0.5	26	8	17	10	10	14.2 ab
51.5	225	1.5	8	4	9	6	12	7.8 ab
51.5	450	0.5	10	5	30	13	7	13.0 ab
51.5	450	1.5[c]	13	7	7	2	11	8.0 ab
Dry Check Lot			91	35	61	45	93	65.0 e

[a] Percentage of 100 fruit infected by *Rhizopus stolonifer, Monilinia fructicola, M. laxa, Penicillium* sp., or *Aspergillus* sp.
[b] Means not followed by the same letter are significantly different at the 5% level.
[c] These treatments caused slight skin darkening and loss of surface luster of the fruit.

TABLE 3. Incidence of various types of decay on nectarines treated with hot water and 2,6-dichloro-4-nitroaniline (DCNA), stored for 17-21 days at 2 C, and ripened for 5-7 days at 21 C

Treatment			Mean percent decay[a]			
Water temp	DCNA concn.	Exposure time	*Monilinia*	*Rhizopus*	*Penicillium-Aspergillus*	Mean decay[b]
C	*ppm*	*min*	%	%	%	%
24	0	1.5	47	18	1	66 e
24	450	1.5	53	4	0	57 d
49	0	3.0	9	2	1	12 ab
49	225	3.0	3	0	2	5 a
51.5	0	1.5	14	4	2	20 c
51.5	225	1.5	5	0	2	7 a
54.5	0	0.5	14	22	1	35 c
54.5	225	0.5	8	0	1	9 ab
Dry Check Lot			59	3	0	62 de

[a] Means of three experiments with Gold King, Regal Grand, and September Grand nectarines using 100 fruit/treatment.
[b] Means not followed by the same letter are significantly different at the 5% level.

nectarines to reduce naturally-occurring decay significantly. A 1.5-min treatment at 51.5°C with 450 ppm DCNA reduced decay to a mean of 8.4 ± 3.2%, and with 900 ppm DCNA to 3.8 ± 1.4% compared to a mean of 14.8 ± 4.1% for the untreated lots. Treatments did not affect the appearance of the plums.

Residues of DCNA on treated fruit.—DCNA residues on nectarines and plums increased linearly with increasing exposure times (Fig. 4-A), and with increasing concentrations of DCNA in the treatment solution (Fig. 4-B). However, residues were greater on fruit treated with the hot water plus DCNA than with unheated water plus DCNA. Residues from a 225-ppm heated DCNA treatment (3.1 ppm) were approximately the same as those (2.7 ppm) from a 900-ppm unheated DCNA treatment. A tolerance of 20 ppm DCNA has been established by the Food and Drug Administration (FDA) for preharvest applications on nectarines (1). DCNA tolerances for postharvest application on plums and nectarines are pending.

Residues on peaches were similarly influenced by exposure times, DCNA concentrations, and temperature, but were generally higher than on the other fruits. The residue from a 1.5-min treatment at 51.5°C with 225 ppm DCNA was 12.3 ppm, and was equivalent to that from an unheated 900-ppm DCNA treatment (12.8

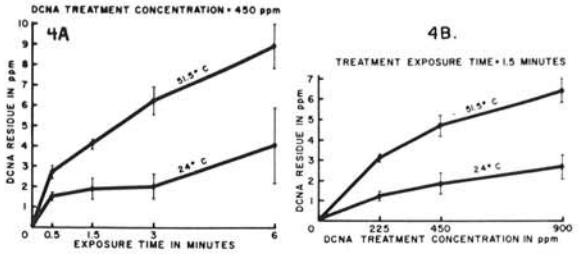

Fig. 4. Residues on nectarines and plums treated in hot water (51.5°C) and unheated water (24°C) plus 2,6-dichloro-4-nitroaniline (DCNA), as a function of **A)** exposure time, with the concentration of DCNA constant at 450 ppm, and of **B)** DCNA concentration, with exposure time constant at 1.5 min. Each point represents a mean of residue determinations from the nectarine cultivars Gold King and September Grand, and the plum cultivar Casselman. Brackets define the standard error of the means.

ppm). The FDA tolerance is 20 ppm DCNA for postharvest applications on peaches (1).

DISCUSSION.—Heat, as a fungicidal agent, is able to penetrate host tissues and act against established infections. The length of exposure to heat, however, is restricted to times that do not injure the host. Six-minute heat treatments, which are at, or close to, the injury thresholds of the fruit, retard but do not completely inactivate *Rhizopus* or *Monilinia* infections. Surviving spores or mycelia begin to grow again after several days, and decay then progresses normally (7). The use of DCNA in combination with heat enhances the fungicidal effect of the treatment, and provides the additional protection of a chemical residue on the fruit.

DCNA residues which are great enough to provide adequate protection against postharvest decay of smooth-skinned or glabrous fruit, such as nectarines and plums, are difficult to attain with conventional spray or dip treatments (5). The application of DCNA in hot water increased the residues on the treated fruit. Unusually low concentrations of the fungicide in the treating tank could thus be applied with a high degree of effectiveness. Similar results have been obtained with hot water and captan (*unpublished data*).

Treatments that were effective with naturally-infected fruit were only partly effective with inoculated fruit. This result suggests that a large proportion of naturally-occurring decay is due to superficial infections that are easily inactivated by heat treatments. Also, decay resulting from inoculations is probably equivalent to an advanced stage of decay resulting from natural infection. Experiments with inoculated fruit, therefore, constituted a severe test of the effectiveness of heat treatments as a control for brown rot and Rhizopus rot.

The holding conditions for tests with noninoculated fruit (3 to 4 weeks of storage and ripening at high humidities) are probably more severe than those usually encountered in commercial practice. The fact that treatments were effective under these severe conditions suggests that results would be satisfactory under most commercial situations.

LITERATURE CITED

1. ANONYMOUS. 1968. Official FDA tolerances. Nat. Agr. Chem. News Pesticide Rev., Vol. 26.
2. KILGORE, W. W., K. W. CHENG, & J. M. OGAWA. 1962. Fungicide residues. Extraction and determination of 2,6-dichloro-4-nitroaniline in processed fruits. J. Agr. Food Chem. 10:339-401.
3. LUEPSCHEN, N. S. 1964. Effectiveness of 2,6-dichloro-4-nitroaniline-impregnated wraps in reducing Rhizopus decay losses. Phytopathology 54:1219-1222.
4. OGAWA, J. M. 1965. Control of pre- and postharvest fruit decays in relation to residues of 2,6-dichloro-4-nitroaniline (DCNA). Botran Symposium, Tuco Products Company, Division of the Upjohn Company, Nov. 3-5, 1965. Kalamazoo, Michigan. Loose leaf. n.p.
5. OGAWA, J. M., J. H. MATHRE, D. J. WEBER, & S. D. LYDA. 1963. Effects of 2,6-dichloro-4-nitroaniline on Rhizopus species and its comparison with other fungicides on control of Rhizopus rot of peaches. Phytopathology 53:950-955.
6. ROSE, D. H., D. F. FISHER, C. BROOKS, & C. O. BRATLEY. 1950. Market diseases of fruits and vegetables: Peaches, plums, cherries, and other stone fruits. USDA Misc. Pub. 228, 22 p. Illus.
7. SMITH, W. L., JR., C. S. PARSONS, R .E. ANDERSON, & R. D. BASSETT. 1964. Reduction of postharvest decay of peaches and nectarines with heat treatments. USDA Mktg. Res. Rep. 643. 22 p.
8. SMITH, W. L., & W. H. REDIT. 1968. Postharvest decay of peaches as affected by hot-water treatments, cooling methods, and sanitation. USDA Mktg. Res. Rep. 807. 9 p.
9. USDA, AGRICULTURAL RESEARCH SERVICE. 1965. Losses in agriculture. USDA Handbook 291. 120 p.
10. USDA, AGRICULTURAL RESEARCH SERVICE. 1968. Progress Rep. Market Quality Res. Div. 134 p.