USE OF THERMOFOGGING FOR DPA AND FUNGICIDES APPLICATIONS IN CHILE

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INTRODUCTION

Superficial scald, one of the most common postharvest physiological disorders of apples, is characterized by irregularly shaped blotches in the skin of the fruit; they vary from light to dark brown, depending on cultivar and severity of the disorder. The symptoms usually appear after long-term storage (more than 4 months), and the damage is associated to the oxidation of “alpha-farnesene” into a class of compounds known as conjugated trienes hydroperoxides.

On the other hand, postharvest fungicides are frequently applied to fruits in order to avoid rotting during storage. Main apple postharvest diseases in Chile are grey mold and calyx end rot (*Botrytis cinerea*); blue mold (*Penicillium expansum*); moldy core (*Alternaria* spp.) and recently Gloeosporium or bitter rot (*Colletotrichum gloeosporioides*). The usual practice of spraying the orchard with fungicides would probably not substantially affect the susceptibility to decay or reduce the amount of inoculum, which may help reduce postharvest losses. Therefore, natural and synthetic chemicals have to be used to control postharvest decay. However, there are relatively few effective chemicals available for postharvest treatments, besides in apples there is only one fungicide (thiabendazole) registered.

Both diphenylamine (DPA) and fungicides are commonly applied in water solution by drenching the fruit for 30 to 40 seconds. Efficacy of the treatments is based on adequate concentration at the tank solution, and uniform distribution of the chemicals on the fruit surface. But, drenching has some disadvantages: fungal diseases can be aggravated by dipping or drenching the fruit; dirt and dust in the chemical solution can reduce the effectiveness of the treatments; disposal of DPA solutions is a major environmental problem.

Using thermofogging, a process that converts a liquid chemical into a fine mist, DPA and fungicides could be directly applied to the fruit into the cold storage facility. This would allow a uniform distribution of the product, as well as the use of very small amounts of chemicals, and the possibility of several applications during the storage period.

During the last two seasons, trials have been conducted in order to determine: the effect of a second DPA application, after 2, 4 and 6 months of storage on the incidence of superficial scald; and to evaluate thermofogging for DPA and fungicides application.

DPA APPLICATION

2001/2002 season

Granny Smith apples coming from two geographical regions were harvested at two dates: March 1st and 25th in San Fernando (34° 40´ Southern Lat; 71° Western Lat.) and March 13th and 27th in Curicó (35° 20´Southern Lat.; 71° Western Lat.).

Fruit was subjected to different DPA treatments (both at harvest and during storage) and set under regular air (RA: 0 °C) or controlled atmosphere (CA: 1.8 to 2%O_2 and 1.3 to 1.5% CO_2), (Table 1).

Initial DPA treatment was applied (2000 ppm) by drenching at a commercial packinghouse. Reapplications (after 2, 4 and 6 months of storage) were made either by dipping the fruit in a DPA solution (2000 ppm) or by fogging (in San Fernando, first harvest). For the latter treatment, a cold truck was used, where 1 ton of fruit was placed and left to stabilize at 0 °C, with no condensation on its surface. Then a "canister" containing the DPA powder formulation was placed in the truck and ignited. The truck was immediately sealed and opened after 24 hours, transferring the fruit into storage.

Table 1. DPA treatments applied during 2001/2002 season to Granny Smith apples under refrigerated air (RA) or controlled atmosphere (CA) storage.

Storage	***Number of DPA applications***	***Treatment***
RA 0ºC	0	RA
	1 (harvest)	RA+DPA*h*
CA 1.3-1.5% CO_2 1.8-2.0% O_2	0	CA
	1 (harvest)	CA+DPA*h*
	2 (harvest & 2 months)	CA+DPA*h+2m* *(drenching or fogging)*
	2 (harvest & 4 months)	CA+DPA*h+4m* *(drenching or fogging)*
	2 (harvest & 6 months)	CA+DPA*h+6m* *(drenching or fogging)*

2002/2003 season

During this season thermofogging was tested either as an initial treatment or as a reapplication (Table 2), in Royal Gala, Scarlet, Granny Smith, Fuji and Braeburn apples, as well as in Packham´s Triumph pears.

These trials involved a higher amount of fruits; thermofogging was applied using a Xeda fogger (Figure 1) placed inside cold trucks and also in commercial CA chambers. Before applying the thermofogging, top bins were covered with a sheet of plastic to avoid condensation on the roof of the cold store that might drip onto the fruit.

Table 2. Moment of application of DPA treatments during 2002/2003 season, to apples and pears under refrigerated air (RA) or controlled atmosphere (CA) storage.

Treatment	**At harvest**	**2m storage**
0	0	---
Di	Drenching	---
Di+F2	Drenching	Fogging
Fi	Fogging	---
Fi+F2	Fogging	Fogging

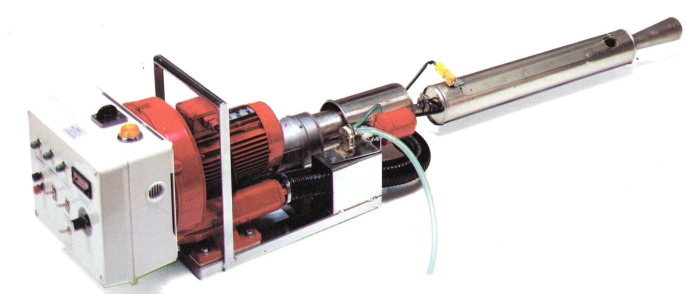

Figure 1. Xeda Fogger utilized for the thermofogging treatments.

EVALUATIONS AND RESULTS

Scalded fruit was evaluated after 6 and 8 months of storage, plus 10 days at room temperature to enhance symptoms. Incidence was determined as percentage (%) of affected fruits (3 reps of 100 fruits each): severity was rated on a three-category scale based on the percentage of the surface area affected, where slight = 1 to 25%, moderate = 26 to 50%, and severe = 51 to 100 %.

Samples of 20 fruits per replicate were drawn for DPA residue analysis, during the second season, in some treatments: after initial drenching, after initial fogging, before reapplication, after reapplication, and after 3 to 6 months of storage.

Scald incidence and severity were analysed using analysis of variance, previous data transformation to correct heterogeneity of variances, when needed. Means separations were made by HSD test ($p \leq 0.05$).

First Season

a) San Fernando orchard

Results showed that the highest scald incidence occurred in fruit with no DPA, both under RA or CA storage, with almost 100% of damage (Table 3). Additionally in CA scald was less severe as compared to RA fruit (Figure 2).

Table 3. Scald incidence (%) in Granny Smith apples, after 8 months of storage. San Fernando orchard. 2002/2003 season.

		Scald (%)	
Harvest	**Treatment**	**8m + 1 d.**	**8m + 10 d.**
01 March	RA	100.0 c	100.0 c
	RA+DPAc	11.3 b	67.5 b
	CA	98.8 c	100.0 c
	CA+DPAc	0.0 a	5.0 a
	CA+DPAc+DPA 2 m	0.0 a	0.0 a
	CA+DPAc+DPA 4 m	0.0 a	2.5 a
	CA+DPAc+DPA 6 m	1.3 a	3.8 a
	CA+DPAc+Fogg.2 m	0.0 a	1.3 a
	CA+DPAc+Fogg.4 m	0.0 a	1.3 a
	CA+DPAc+Fogg.6 m	0.0 a	0.0 a
	Significance	**	**
25 March	FC	100.0 d	100.0 e
	FC+DPAc	48.8 b	75.0 bc
	AC	98.8 d	98.8 e
	AC+DPAc	83.8 c	95.0 de
	AC+DPAc+DPA 2 m	12.5 a	21.3 a
	AC+DPAc+DPA 4 m	42.5 b	52.5 b
	AC+DPAc+DPA 6 m	77.5 c	87.5 cd
	Significance	**	**

**= significant $p \leq 0.01$. Within a column, values followed by the same letter do not differ significantly according to HSD test ($p \leq 0.05$).

After 8 months of storage, all treatments from the first harvest, where CA and DPA were involved, were almost free of scald, and no statistical differences were detected between one or two antioxidant applications.

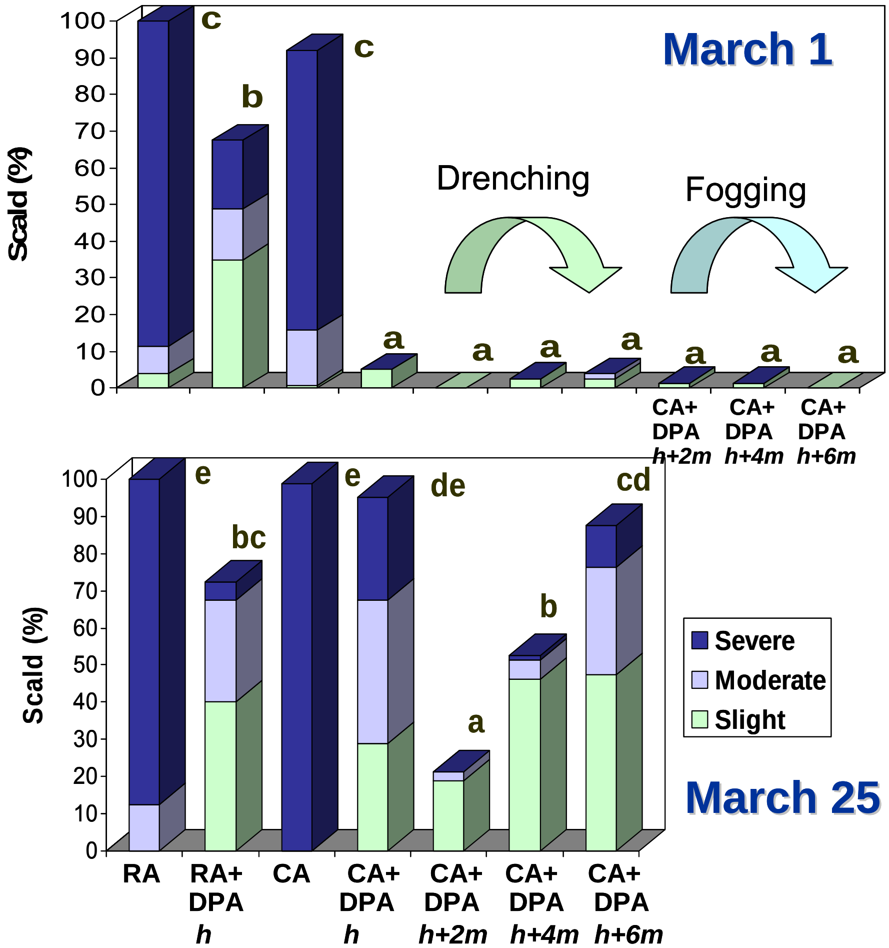

Figure 2. Severity of Scald in Granny Smith apples, after 8 months storage + 10 days at 20 °C. Fruit coming from the orchard of San Fernando.

Fruit from the second harvest, developed more scald than the first one, and here, effect of the reapplication was evident. Apples coming from CA+DPAh harvest had 84 and 95% scald after 1 and 10 days at room temperature respectively. The smallest amount of scald was found in fruit stored under CA+DPAh+DPA 2 m (12.5 and 21% for 1 and 10 days at room temperature). Reapplications made after 4 and 6 months were more effective than a single one, but less than the one after 2 months.

b) Curicó orchard

The highest scald incidence for this orchard occurred in treatments: RA, RA+DPAh and CA (Table 4, Figure 3).

Although no statistical differences were found among treatments coming from CA, that received DPA, (fruit from March 13th harvest); apples from CA+DPAh+DPA2m had 0% scald on day 1 at room temperature. This tendency was confirmed by means of statistical differences after 10 days at room temperature (1.3% incidence for treatment CA+DPAh+DPA2m vs. 38% scald for treatment CA+DPAh). A similar situation was found in fruit of the second harvest. It has to be noticed that treatment with reapplication after 2 months was 100% scald free.

Table 4. Scald incidence (%) in Granny Smith apples, after 8 months of storage. Curicó Orchard 2002/2003 season.

		Scald (%)	
Harvest	**Treatment**	**8m + 1 d.**	**8m + 10 d.**
13 March	RA	100.0 c	100.0 c
	RA+DPAc	82.5 b	92.5 c
	CA	98.8 bc	100.0 c
	CA+DPAc	12.5 a	35.0 b
	CA+DPAc+DPA 2 m	0.0 a	1.3 a
	CA+DPAc+DPA 4 m	7.5 a	8.8 a
	CA+DPAc+DPA 6 m	12.5 a	36.3 b
	Significance	**	**
27 March	FC	88.8 c	88.8 c
	FC+DPAc	20.0 b	29.1 b
	AC	97.5 c	100.0 d
	AC+DPAc	3.8 ab	10.0 b
	AC+DPAc+DPA 2 m	0.0 a	0.0 a
	AC+DPAc+DPA 4 m	10.9 ab	10.9 ab
	AC+DPAc+DPA 6 m	15.0 b	22.5 b
	Significance	**	**

**= significant $p \leq 0.01$. Within a column, values followed by the same letter do not differ significantly according to HSD test ($p \leq 0.05$).

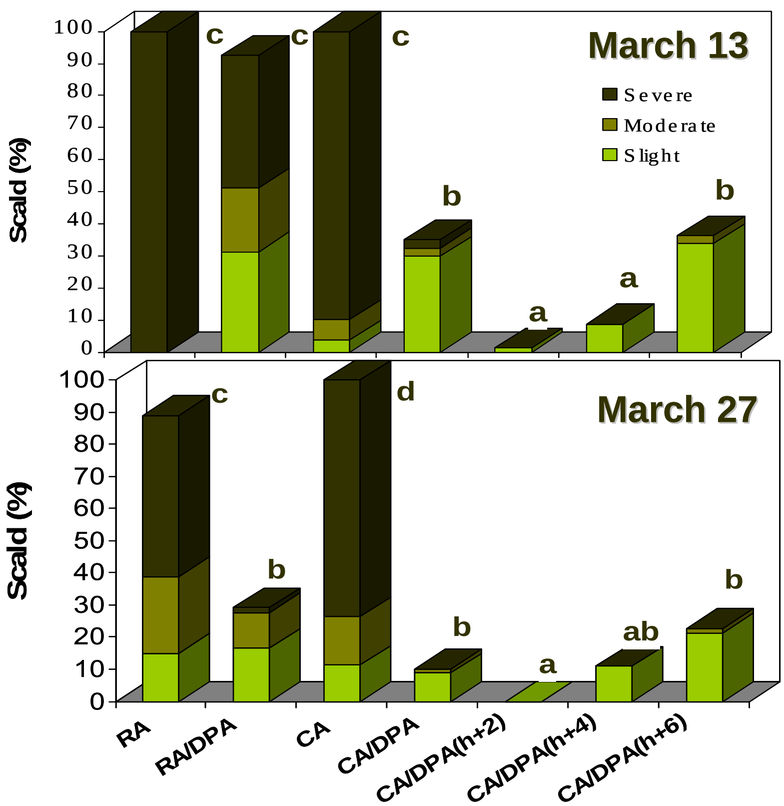

Figure 3. Severity of Scald in Granny Smith apples, after 8 months storage + 10 days at 20°C. Fruit coming from the orchard of Curicó.

Second season

a) Pears

Results from this season are preliminary, since some cultivars are still under evaluation (Table 5). In general, scald incidence was mostly controlled on Packham's Triumph pears by two DPA applications, being the most effective Fi+F2 treatment.

Table 5. Scald incidence (%) for Packham´s Triumph pears and Fuji and Braeburn apples, after 6 months of storage plus 10 days at 20 °C.

		Scald (%)	
Treatment	**P.Triumph**	**Fuji**	**Braeburn**
Control	34.3 b	13.1 b	4.22
Di	11.3 a	6.6 ab	0.44
Di+F2	11.3 a	13.0 b	3.16
Fi	13.0 a	3.5 a	2.58
Fi+	5.3 a	4.7 a	3.35
Significance	*	*	n.s

*Significant $p \leq 0.05$; ns: not significant. Within a column, values followed by the same letter do not differ significantly according to HSD test ($p \leq 0.05$).

Initial drenching of pears with DPA resulted in 6 ppm residues, whereas for fogging this value was around 2 ppm. The reapplication after 2 months of storage allowed residues to rise to 4 to 5 ppm, which stayed almost the same up to 6 months of storage (Figure 4).

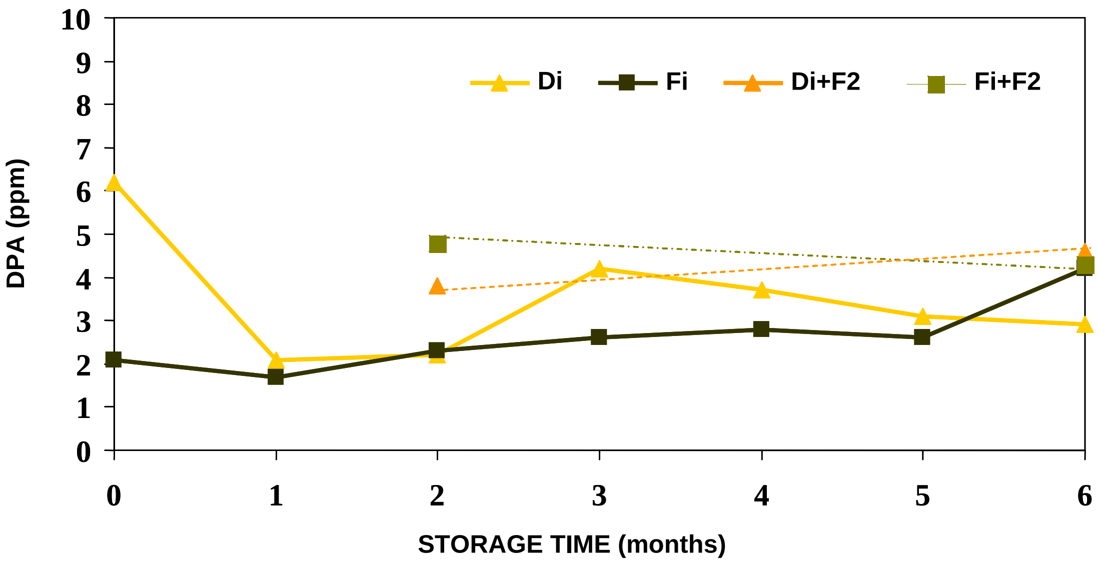

Figure 4. DPA residues for Packham´s Triumph pears, treated by drenching and thermofogging.

b) Apples

In Braeburn apples, control fruit had very low scald incidence (4.2%), and there were no statistical differences among treatments (data not shown). On the other hand, Fujis developed 11.1% of scald in the control treatment, and the disorder decreased to 3.5 and 4.7% with Di+F2 and Fi+F2 treatments.

Royal Gala apples, which are not treated with DPA in Chile, did not show any scald symptoms. Nevertheless, fruit treated with Fi significantly diminished the amount of rot as compared to drenching treatments (Table 6).

Table 6. Rot incidence (%) of Royal Gala apples treated with DPA, after 6 months of storage plus 10 days at 20°C.

Treatment	Rot Incidence (%)
Control	4.7 ab
Di	11.0 c
Di+F2	0.1 a
Fi	9.7 bc
Fi+F2	4.3 a
Significance	*

* Significant $p \leq 0.05$. Within a column, values followed by the same letter do not differ significantly according to HSD test ($p \leq 0.05$).

Very little scald was developed on Granny Smith apples this season (less than 2% on control treatments that were stored along with treated fruit; data not shown). Residue analysis showed that initial concentration was over 6 ppm for RA and CA storage, both for fogging and drenching treatments. The reapplications showed an increase up to 7.1 ppm for the Fi+F2 treatment and up to 9.7 ppm for Di+F2, in fruit under CA. Apples that were stored in RA showed a higher increase in residues: 22.6 ppm for Di+F2 and 35.7 for Fi+F2 (data not shown). These high concentrations resulted in toxicity of fruits of some specific replicates.

Three fruit industries in Chile applied DPA by thermofogging this season in full commercial storage chambers and no symptoms of toxicity were detected. Therefore, it is most likely that injured fruit (in the trials) was not properly placed in the chamber during fogging, or it might not have been completely covered with plastic before the application.

FUNGICIDE APPLICATION

2002/2003 Season

The effect of a thermofogging application of pyrimethanil on suppression of grey rot was investigated on Royal Gala and Fuji apples and Packham's Triumph pears. Fruits were wounded and inoculated with conidia of B. *cinerea* (106 conidia mL^{-1}), which were collected in sterile water containing 0.05% Tween 20, quantified microscopically and adjusted to the required concentration. The thermofogging treatment of pyrimethanil (Xedathane A) and imazalil (Xedazil A) was performed by using a Xeda fogger inside a refrigerated truck, in the same way as described previously for DPA treatments. Controls with both inoculated fruit without fungicide and DPA alone were also included. Furthermore, the effect of the fungicide application on non-inoculated fruit was also tested. Finally for Fuji apples, fungicide application through drenching was also compared with the thermofogging technique. After treatments, fruits were stored at 0 °C for three months. Disease incidence and severity were both analysed using analysis of variance, previous data transformation to correct heterogeneity of variances when needed. HSD test and orthogonal contrasts were used to determine the effect of the fungicide treatments on grey rot.

EVALUATIONS AND RESULTS

For Gala apples and Packham's Triumph pears, a highly significant fungicide effect was detected (Tables 7 and 8). HSD test indicated that pyrimethanil applied by thermofogging was more effective in reducing the incidence of grey rot in inoculated fruit than the control and imazalil or DPA alone. The same tendency was found in rot severity, where the weight and volume of the rotted lesion in Gala apple and the fungal rot colonisation in Packham's Triumph pears were significantly less in pyrimethanil treated fruits than in those of the control and imazalil or DPA alone.

Table 7. Rot incidence (%) and severity on Gala apples inoculated with *B. cinerea* after thermofogging application of imazalil and pyrimethanil. Fruits were examined after 90 days at 0°C.

	Incidence (%)	Rot severity	
		Weight (g)	Volume (cm^3)
Control	90.8 b	160.4 b	27.5 b
Fogging			
DPA	93.3 b	160.0 b	21.4 b
DPA + imazalil	85.3 b	78.7 ab	20.2 b
DPA + pyrimethanil	37.8 a	33.9 a	0.9 a
Significance	**	*	**

** Highly significant $p \leq 0.01$; * Significant $p \leq 0.05$. Within a column, values followed by the same letter do not differ significantly according to HSD test ($p \leq 0.05$). n=100.

Table 8. Rot incidence (%) and severity on Packham's Triumph pears after three months at 0°C, inoculated with *B. cinerea* and treated by thermofogging with imazalil and pyrimethanil.

	Incidence (%)	Severity
		Rot colonisation (%)
Control	96.2 b	87.6 b
Fogging		
DPA	93.3 b	81.4 b
DPA + imazalil	91.4 b	82.1 b
DPA + pyrimethanil	14.8 a	11.3 a
Significance	**	**

** Highly significant $p \leq 0.01$; * Significant $p \leq 0.05$. Within a column, values followed by the same letter do not differ significantly according to HSD test ($p \leq 0.05$). n=100.

For Fuji apples, the orthogonal contrast analysis showed that it was better to apply pyrimethanil through drenching than thermofogging, in order to significantly reduce the grey rot incidence on inoculated fruit. However, this difference was not seen when the fruit was not artificially inoculated with conidia of B. *cinerea* (Table 9). In the same way, pyrimethanil applied through drenching showed a significant greater effect than through the commercial treatment thiabendazole (TBZ). Again, this better performance was not kept when the fruit had not been inoculated. Finally, imazalil was not able to significantly reduce grey rot incidence, in both inoculated and non-inoculated Fuji apples.

Table 9. Rot incidence (%) on Fuji apples after three months at 0°C, inoculated with *B. cinerea* and treated with fungicides by drenching and thermofogging.

Treatments	Rot incidence (%)	
	Inoculated	**No inoculated**
T1:Control	86,3	12,4
T2:Tf [x] DPA 15%	73	11,5
T3: Tf DPA 15% + TF pyrimethanil 16%	56,7	4,6
T4: Dr [y] DPA + Imazalil 75 SG	60	6,7
T5: Dr DPA + Imazalil 75 SG + pyrimethanil 40 WV	51	2,3
T6: Dr DPA + TBZ (Tecto 50%)	36,3	4,8
T7: Dr DPA	35,3	12,7
T8: Dr DPA + pyrimethanil 40 WV	13,3	1
Significance	**	**
Orthogonal contrasts		
T1 vs T2-T8	**	**
T3 vs T8	**	*NS*
T2-T3 vs T7-T8	**	*NS*
T4 vs T5	*NS*	*NS*
T6 vs T8	**	*NS*
T6 vs T4	**	*NS*
T5 vs T8	**	*NS*

** Highly significant $p \leq 0.01$; * Significant $p \leq 0.05$. *NS* No Significant
[x]: thermofogging
[y]: drenching

FINAL COMMENTS

Reapplication of DPA can be highly effective in reducing scald development in susceptible fruit, being the best moment for this treatment, between 2 and 3 months. Furthermore, it decreases severity of any injured fruit.

Thermofogging is capable of raising DPA residues in the fruit when applied after two or three months of storage, but a threshold for DPA should be established in order to determine which fruit needs a second application, based on its degradation in storage.

Further studies are needed to determine factors which can cause toxicity in treated fruit.

The fungicide pyrimethanil is effective in reducing postharvest losses due to Botrytis cinerea in apples and pears. Moreover, the thermofogging technique showed to be efficient in transporting the fungicide to the fruit surface, therefore, it should be feasible to use in postharvest management of fruits and vegetables.

DPA and fungicides can be applied in commercial storages by thermofogging, since this eliminates the necessity of disposing drench solutions. Additionally, it allows a second application of the products during storage.

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