Effect of Ozone Exposure on Phytopathogenic Microorganisms on Stored Apples

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SUMMARY

The aim of our study was to clarify the effect of ozone exposure on several phytopathogenic fungi on stored apple fruits under different storage conditions. The study was conducted at Bistrita, Romania, in the storehouse of an experimental apple orchard in 2002 and 2003. Two widely grown apple cultivars ('Jonathan' and 'Golden Delicious') were used. General microbial examination of the fruits was made during storage in order to identify the most important storage pathogens. Efficacy of six ozone treatments was evaluted on fruit decay caused by phytopathogenic fungi. Monthly observations (January, February, March and April) were made of the degree of decay and three measurements were assessed (disease frequency, disease intensity and degree of attack). Our results showed that the most important phytopathogenic fungi during storage was blue mold, caused by species of Penicillium*. Disease frequency of apple fruits was very high on cv. 'Jonathan', much higher than on cv. 'Golden delicious'. Ozone treatments (25 ppm ozone for 0.5 and 1.5 hours in November) caused significantly lower disease incidence on stored apple than all other ozone treatments. For longer storage, it seems that additional ozone treatments in February increased treatment efficacy. Cv. 'Golden delicious' seemed to be more resistant to storage diseases than cv. 'Jonathan' both on the untreated and treated fruits. The effect of the ozone treatments was also the most effective when 25 ppm ozone was applied for 0.5 and 1.5 hours in November.*

Keywords: *stored fruit, sanitation,* Malus domestica*, blue mold, ozone, apple fruits, Jonathan, Golden delicious*

INTRODUCTION

During fruit storage, a depreciation of fruit quality occurs due to the metabolic processes inside the fruit and the negative activity of pathogen microorganisms (Mari et al., 2003). Qualitative depreciation is a normal process during storage, but the level of degradation depends on storage conditions and the storage technologies used (Hulea et al., 1982; Mari et al., 2003). Pathogen microorganisms are one of the most important agents causing fruit quality degradation during storage. Fungi pathogens cause considerable losses during storage, which results in lower fruit quality and lower market prices (Crişan, 1973; Spotts and Cervantes, 1992; Smilanick et al., 2002). Several fungal pathogens such as *Monilia* spp., *Botrytis* spp., *Fusarium* spp., *Rhizopus stolonifer*, *Trichotecium roseum*, *Mucor* spp., *Penicillium* spp., *Gloeosporium* spp., *Sphaeropsis* spp. and *Phomopsis* spp. can cause serious storage damage to fruits if storage temperature or relative humidity is higher than required (Spotts and Cervantes, 1992; Perez et al., 1999; Skog and Chu, 2001; Palou et al., 2001; van Leeuwen et al., 2002; Smilanick et al., 2002; Holb, 2003, 2004; Mari et al., 2003). Pathogen microorganisms in storage can originate from the stored fruit from the field at harvest or from itself the storage place due to imprecise storage sterilization. Several methods are used to reduce pathogen microorganisms during storage, including physical, mechanical, biological and chemical methods (Mari et al., 2003). Several studies deal with the effect of ozone on fungal pathogens in storage; however these disagree on the effectiveness of ozone on phytopathogenic microorganisms. Some studies recommend that ozone as a strong oxidizer and an efficient material for reducing decay of stored products during the storage period (Spotts and Cervantes, 1992; Skog and Chu, 2001). However, other studies (Perez et al., 1999; Smilanick et al., 2002; Palou et al., 2002) reported that ozone had little or no effect on e.g. green and blue molds on stored citrus fruit.

The aim of our study is to clarify the effect of ozone exposure on several phytopathogenic fungi on stored apple fruits under different storage conditions.

MATERIAL AND METHODS

Conditions and plant materials

The study was conducted at Bistrita, Romania, in the storehouse of an experimental apple orchard in 2002 and 2003. Two widely grown apple cultivars ('Jonathan' and 'Golden Delicious') were used for this study. The apples were harvested in October, and healthy fruits that exhibited no macroscopical symptoms were stored. Every sample contained at least 3 kg apples for each cultivar.

General microbial examination

For each cultivar, a general microbial examination was was made in three replications, as follows:

a) macroscopic identification of the pathogens according to the external symptoms and sporulation of the fungus;
b) incubation of different sections of a fruit (epidermis, pulp and seminal loculus) on wet blotting paper at 25^{o}C in climate chambers, then studying the developed mycelia and sporulation with a stereo and light microscope;
c) finally, identification of the fungi were also studies on malt and Czapek mediums.

Ozone treatments

Every ozone treatment was conducted in three replications, so 3 samples were used for each cultivar in each treatment. Ozone treatments were applied as follows:

a) V1 – treatment with 10 ppm ozone/1 hour in November 2002;
b) V2 – treatment with 25 ppm ozone/0.5 hour in November 2002;
c) V3 – treatment with 25 ppm ozone/1 hour in November 2002;
d) V4 – treatment with 25 ppm ozone/1.5 hour in November 2002;
e) V5 – treatment with 50 ppm ozone/1 hour in November 2002;
f) V6 – untreated control, without ozone exposure;
g) V1r-V5r – treatment as V1-V5 and an unique ozone dose of 25 ppm/1 hour in February 2003.

Disease measures during storage

Apples were monitored from the beginning of storage, November 2002 (zero point – 26 November) until the end of storage, April 2003. Monthly observations (January, February, March and April) were made about the degree of decay and three measurements were assessed (disease frequency, disease intensity and degree of attack).

a) Disease frequency (F%) was determined by dividing the number of diseased apples (n) by the total number of apples (N) from each sample: F%= n / N x 100.
b) Disease intensity (I%) was calculated as I%= (i x f) / n, where (i) is the percentage of diseased area, (f) is the number of cases with symptoms and (n) is the number of diseased apples.
c) Degree of attack (DA%) is calculated by combining the disease level of the above two formulas 'a' and 'b', calculated as: DA%= (F% x I%) / 100.

Significant differences between ozone treatments were obtained from ANOVA using LSD t test at P= 0.05, 0.01 and 0.001 significance levels.

RESULTS AND DISCUSSIONS

General microbial examination

Results of the macroscopic and microscopic studies revealed several fruit rot pathogens on the surface and inside the fruit originated from the field or from the storage site (*Table 1*).

Pathogens which originated from the field are the following: *Spilocaea pomi* (*Venturia inaequalis*), *Monilia fructigena* (*Monilinia fructigena*), *Gloeosporium fructigenum* (*Glomerella cingulata*) and *Sphaeropsis malorum* (*Physalospora cydoniae*). Most of fungi were found on the surface and inside the fruits, and only few in the seminal loculus such as *Monilinia fructigens*. A larger number of pathogens were isolated from cv. 'Jonathan' than from cv. 'Golden'. Only *Monilinia* and *Fusicladium* could be isolated from cv. 'Golden delicious'.

Pathogens which infect during the seasons and produce symptoms during storage were more abundant in cv. 'Jonathan', than cv. 'Golden delicious' (*Table 1*). The most common pathogens were *Phomopsis mali* (*Diaporthe perniciosa*) on cv. Jonathan; *Gloeosporium album* (*Pezicula malicorticis*) and *Cytospora pomicola* on both cvs. 'Jonathan' and 'Golden'; *Cylindrocarpon mali* (*Nectria galligena*) sporadically on cv. 'Jonathan' and *Botrytis cinerea* on cv. 'Golden delicious'.

Pathogens which infect and produce rot during storage were developed on both cultivars. The disease frequency of blue mold (*Penicillium* spp.) was the highest for both cultivars, both outside and inside the apples. Our results corresponded to those obtained in several earlier studies (Palou et al., 2001), which reported that the most important postharvest decay agent in stored fruits is the blue mold fungus. *Fusarium* spp., *Rhizopus stolonifer* developed sporadically on cv. 'Jonathan' and *Trichotecium roseum* on cv. 'Golden delicious'.

Table 1

Phytopathogen fungi isolated from stored apples (cv. Jonathan, Bistrița, 2002/2003)

Conidian stage (anamorph)	Perfect stage (teleomorph)	Localization			Mold symptom			Frequency	
		S	I	L	P	T	M	J	G
Spilocaea pomi	*Venturia inaequalis*	+						+	+
Gloeosporium fructigenum	*Glomerella cingulata*	+	+		+	+	+	++	
Monilia fructigena	*Monilinia fructigena*	+	+	+	+	+	+	++	+
Sphaeropsis malorum	*Physalospora cydoniae*	+	+		+			++	
Cylindrocarpon mali	*Nectria galligena*	+	+	+	+	+	+	+	
Gloeosporium spp.	*Pezicula* spp.	+	+		+			+	+
Cytospora pomicola		+	+		+			+	+
Phomopsis mali	*Diaporthe perniciosa*	+	+	+	+	+	+	+	
Rhizopus stolonifer		+	+		+	+		+	
Fusarium spp.		+		+	+	+		+	
Botrytis cinerea		+	+		+	+	+		+
Penicillium spp.		+	+		+	+		+++	++
Trichotecium roseum		+		+	+	+	+	+	+

S= surface of fruit, I= inside, L= seminal loculus, P= partial, T= total, M= mummy, + (F%= 1-5%), ++ (F%= 5-25%), +++ (F%= 25-50%)

The effect of ozone treatment on disease measurements

The effect of ozone treatment on disease frequency of fungal pathogens is presented in *Table 2* for cv. 'Jonathan' and *Table 3* for cv. 'Golden delicious'. On cv. 'Jonathan', a progressive increase in disease frequency was observed on stored apples. In January, the disease frequency was lower in all treatments compared to the untreated control; however, it was statistically different only in treatments V4 and V5. After the second treatment with ozone, treatments V1, V2r, V4r and V5 showed very low disease frequency (between 13.8 and 19.05%) in March, compared to the untreated control. At the end of the storage period in April, disease frequency was very high in all treatments of cv. 'Jonathan' fruits (between 22.4% at V2r and 58.8% at V5r). The lowest disease frequency (less than 25%) was observed in the treatments of V2r (25 ppm/hour), of V4 (25 ppm/1.5 hour), and of V4r retreated in February.

Table 2

Frequency (F%) of fungal attack on stored Jonathan apples treated with ozone (F%= 0, in November 2002)

Treatments	January			February			March			April		
	F%	Dif.	S.	F%	Dif.	S	F%	Dif.	S	F%	Dif.	S
V1	14.5	-4.2	-	18.2	-6.4	-	19.1	-13.3	0	33.3	-17.2	0
V1r							27.8	-4.6	-	28.7	-21.8	0
V2	12.7	-6	-	13.3	-11.3	0	24.2	-8.2	0	32.1	-18.4	0
V2r							13.8	-18.6	0	22.4	-28.1	0
V3	17.2	-1.5	-	17.8	-6.8	-	26.2	-6.2	0	31.2	-19.4	0
V3r							34	1.6	-	36	-14.5	0
V4	8.3	-10.4	0	12	-12.6	0	17.5	-14.9	0	24.7	-25.8	0
V4r							14	-18.4	0	24	-26.5	0
V5	10.4	-8.3	0	14.8	-9.8	0	17.3	-15.1	0	40.4	-10.1	-
V5r							33.3	0.9	-	58.8	8.3	-
V6	18.7	-	Ct.	24.6	-	Ct.	32.4	-	Ct.	50.5	-	Ct.
$LSD_{0.05}$	5.73			6.89			6.21			13.26		
$LSD_{0.01}$	7.35			9.35			9.87			18.13		
$LSD_{0.001}$	8.78			11.12			13.02			23.12		

On cv. 'Golden delicious' (*Table 3*), an increase of disease frequency could be observed on the stored apple fruits. The values of disease frequency were much lower than for cv. 'Jonathan' apples. Until February, disease frequency was almost zero in all treatments, except for treatments V3 (25 ppm/hour, F%= 1%). In March, the highest disease frequency was observed in treatments V1 and V5. Minimal increase of disease frequency was found in treatments V3r and V4. At the end of the experiment in April, the lowest values of disease frequency were observed in tretaments V2, V4 and V3r (F% around 3%). Maximum disease frequencies were above 10% in treatments V1, V2r, V5, V5r, and in the untreated control.

Table 3

Frequency (F%) of mycotic attack on stored Golden apples treated with ozone (F%= 0, in November 2002)

Treatments	January			February			March			April		
	F%	Dif.	S.	F%	Dif.	S	F%	Dif.	S	F%	Dif.	S
V1	2.5	0	-	4.6	-	-	7.2	-0.3	-	10	-9.2	-
V1r							5.3	-2.2	0	5.5	-12.7	0
V2	1	-1.5	0	2	-2.5	0	2.4	-5.1	0	2.9	-16.3	0
V2r							3.1	-4.4	0	10.9	-8.3	-
V3	0.5	-2	0	1	-3.5	0	3	-4.5	0	9.1	-10.1	0
V3r							1.6	-5.9	0	2.9	-16.3	0
V4	1	-1.5	0	2.1	-2.4	0	2.1	-5.4	0	3.1	-16.1	0
V4r							3.2	-4.3	0	6.4	-12.8	0
V5	2	-0.5	-	3.5	-1	-	6.5	-1	-	11.4	-7.8	-
V5r							6.2	-1.3	0	14.1	-5.1	-
V6	2.5	-	Ct.	4.5	-	Ct.	7.5	-	Ct.	19.2	-	Ct.
$LSD_{0.05}$	0.86			2.01			1.12			9.47		
$LSD_{0.01}$	1.75			3.15			3.57			11.35		
$LSD_{0.001}$	2.39			4.36			5.26			14.96		

The effect of ozone treatment on the disease intensity of fungal pathogens is presented in *Figure 1* for cv. 'Jonathan'. Disease intensity considerably increased when storage time was longer. On cv. 'Jonathan', the intensity of disease reached high values by the final assessment date (70-87%). On cv. 'Golden delicious', the lowest intensity could be observed for V3 treatments, and it was higher in all other treatments (data not shown). The disease intensity was very high in the untreated control treatments for cv. 'Golden delicious' (80%).

The effect of ozone treatment on the degree of attack of fungal pathogens is presented in *Figure 2* for cv. 'Jonathan' and in *Figure 3* for cv. 'Golden delicious'. The degree of attack was similar to those of disease intensity. The lowest values of degree of attack were observed in the same treatments, V2, V4, V4r and V1r. On cv. 'Golden delicious', degree of attack was very low during storage, less than 3%, in most treatments. The degree of attack increased only by April 2003, therefore, only assessment data observed in April is shown in *Figure 3*. We observed a very low degree of attack, less than 5% for six treatments. The maximum degree of attack was observed in treatments V5 and V5r, and in the untreated control.

Comparing our research with other studies, e.g. Mari et al. (2003) stated that sanitizing products such as ozone have considerable fungicidal effect against *P. expansum* and *M. piriformis*, depending on the concentration of chemical product and the duration of exposure to the treatment. Palou et al. (2001) stated that exposure to ozone (1.0 +/- 0.05 ppm ozone at 10°C in an export container for 2 weeks) did not reduce final incidence of green or blue mold (*Penicillium* spp.), although incidence of both diseases was delayed by about 1 week and infections developed more slowly under ozone. In agreement with our study, Palou et al. (2001) also showed that sporulation was prevented or reduced by gaseous ozone without noticeable ozone phytotoxicity to the fruit. Spotts and Cervantes (1992) demonstrated that spore inhibition of *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* directly correlated with ozone concentration in 1 to 5 minute exposure times. However, in the same study, it was also noted that in pears wound-inoculated with *P. expansum* and then treated with water containing up to 5.5-mμ-g of ozone per milliliter for 5 minutes, levels of decay were similar to those of a control treated with water alone. Similarly, Smilanick et al. (2002) showed that green mold and sour rot on citrus fruit, caused by *Penicillium digitatum* and *Geotrichum citri-aurantii*, respectively, were not reduced by 20 minutes immersion in 10 ppm ozone. However, they also indicated that the average natural incidence of brown rot on peach cultivars, caused by *Monilinia fructicola*, was reduced from 10.9 to 5.4% by 1 minute immersion in 1.5 ppm ozone. A treatment of 15 minutes with 5 ppm ozone further reduced decay to 1.7%, but consistent control of brown rot was associated only with this severe treatment and it caused shallow pits on the fruit. Palou et al. (2002) demostrated that continuous ozone exposure at 0.3 ppm (v/v) inhibited aerial mycelial growth and sporulation on cv. 'Elegant Lady' peaches wound inoculated with *Mondinia fructicola*, *Botrytis cinerea*, *Mucor piriformis*, or *Penicillium expansum* and stored for 4 weeks at 5°C and 90% relative humidity (RH). However, the same authors also showed that ozone exposure did not significantly reduce the incidence and severity of decay caused by these fungi with the exception of brown rot. Similarly, Perez et al. (1999) indicated that ozone treatment was ineffective in preventing fungal decay in strawberries after 4 days at 20°C.

Figure 1: **Disease intensity (%) of stored apple fruits in 6 ozone treatments (cv. Jonathan, Bistrița, 2002/2003)**

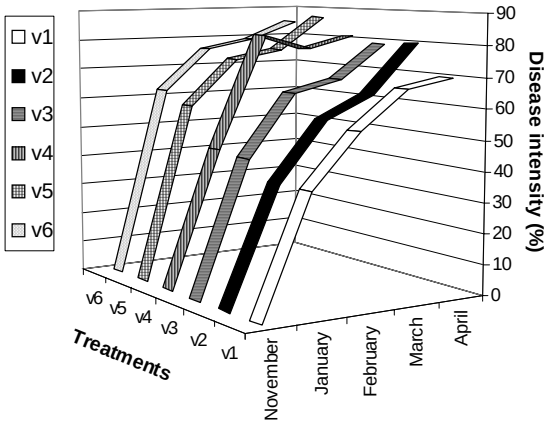


Figure 2: **Degree of attack (%) of stored apple fruits in 6 ozone treatments (cv. Jonathan, Bistrița, 2002/2003)**

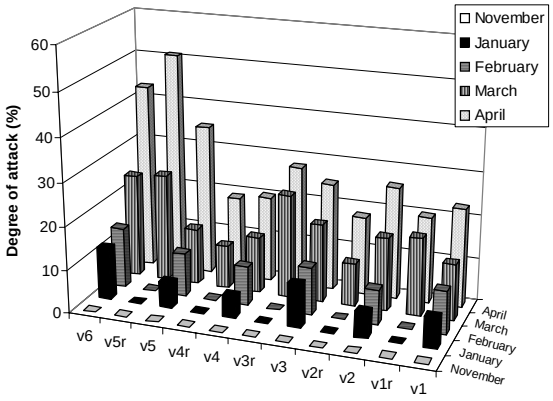
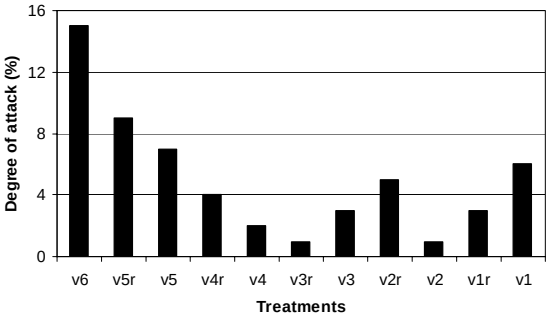


Figure 3: **Degree of attack (%) of stored apple fruits in 6 ozone treatments in April 2003 (cv. Golden delicious, Bistrița)**



CONCLUSIONS

Disease frequency of stored apples was much higher on cv. 'Jonathan' compared to cv. 'Golden delicious' during the storage period. The most important phytopathogenic fungi during the storage was blue mold caused by species of *Penicillium*. Disease frequency of apple fruits was very high on cv. 'Jonathan' much higher than on cv. 'Golden delicious'. Ozone treatments (25 ppm ozone in time of 0.5 and 1.5 hours in November) caused significantly lower disease incidence on stored apple than all other ozone treatments. For a longer storage, it seems that additional ozone treatments in February increased treatment efficacy. Cv. 'Golden delicious' seemed to be more resistant to storage diseases than cv. 'Jonathan' both on untreated and treated fruits. The effect of the ozone treatments was also the most effective when 25 ppm ozone was applied for 0.5 and 1.5 hours in November.

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