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Original scientific paper

# Effects of 1-MCP and dynamic controlled atmosphere on apple fruit rot caused by *Fusarium avenaceum*

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**SUMMARY**

*Fusarium* species are increasingly detected as the causal agents of decay of stored apple fruits. *Fusarium avenaceum* is particularly significant due to its predominant occurrence among *Fusarium* species in stored apple fruits and its ability to produce mycotoxins. Treatments with 1-methylcyclopropene (1-MCP) and different storage conditions affect the aggressiveness of *F. avenaceum* and development of fungal-caused decay in stored apple fruits. In this study, apple fruits (cv. 'Granny Smith') were treated with 1-MCP, and artificially inoculated with *F. avenaceum*. The isolate used for inoculation, originating from apple fruit, was identified based on morphological characteristics and by polymerase chain reaction (PCR) using a species-specific primer pair (FA-ITSF and FA-ITSR) for *F. avenaceum*. After inoculation, treated and untreated fruits were stored at room temperature and cold-stored under dynamic controlled atmosphere (DCA). Diameters of necrotic lesions were measured after 7, 14 and 21 days of incubation on fruits stored at room temperature, while necrosis diameters on DCA-stored fruits were measured immediately at the end of storage period (143 days), and after 7, 14 and 21 days of additional incubation at room temperature. The results show that treatment with 1-MCP inhibits the development of *F. avenaceum* on apple fruits during storage under DCA. However, after storage, i.e. during incubation at room temperature, no significant difference between 1-MCP-treated and untreated fruits was observed. On fruits stored at room temperature only, no difference between 1-MCP-treated and untreated fruits was observed. However, 1-MCP-treated fruits stored at room temperature only developed significantly smaller necrosis lesions compared to 1-MCP-treated and DCA stored fruits. It infers that both 1-MCP treatment and DCA storage inhibit fungal decay caused by *F. avenaceum* on apple fruits. However, the effects do not persist after storage.

**Keywords**: 1-methylcyclopropene: Ethylene inhibitor; Dynamic controlled atmosphere; Storage; Apple fruit rot

## INTRODUCTION

Apple is a highly nutritious fruit, widely used in human diet. Due to its aptness to stay in storage for a long time, apple fruits are available for consumption all year round. However, maintaining high fruit quality is not the only issue during storage; it is also important to ensure microbiological safety of fruit during and after storage. Pathogenic microorganisms that develop on stored apple fruits can cause significant losses, while mycotoxin-producing fungi can also adversely affect human health (Juhneviča et al., 2011).

Apple fruits are susceptible to decay in storage, and storing can result in significant post-harvest losses. Apart from physiological disorders, losses are also caused by plant pathogenic fungi, ranging from 5 to 25% in developed countries and even up to 50% in developing countries (Marković et al., 2011; Ewekeye et al., 2013).

*Fusarium* species are frequently detected as the causal agents of decay of stored apple. Symptoms occur as soft, circular, brown necrosis of different extent, either with or without visible sporulation on fruit surface. The most common causal agents of decay of stored apple and pear fruits in the genus *Fusarium* are: *F. avenaceum*, *F. culmorum*, *F. lateritium*, *F. solani*, as well as *F. pseudograminearum*, *F. semitectum*, *F. crookwellense*, *F. proliferatum* and *F. compactum* (Konstantinou et al., 2011; Sever et al., 2012; Kou et al., 2014; Wenneker et al., 2016). According to Sørensen et al. (2009), *F. avenaceum* produce high amounts of mycotoxins in naturally and artificially inoculated apple fruits, and therefore, monitoring of its occurrence on stored apples is of high importance. As with other postharvest pathogens of apple, control of *F. avenaceum* is difficult because postharvest use of synthetic fungicides is prohibited in many countries, including Serbia. Therefore, alternative control strategies are desired in order to retain the achieved levels of yield. Dynamic controlled atmosphere (DCA) keeps apple fruits in good condition during storage, making them less susceptible to pathogens. Moreover, Juhnevica-Radenkova et al. (2016) reported that treatment of climacteric fruits with the ethylene inhibitor 1-methylcyclopropene (1-MCP) delayed their ripening process, which is associated with biochemical and physiological changes, inferring that it was important to ascertain the impact of 1-MCP on microbial growth because clear relevant research data were missing.

It is well-known that control by gaseous atmosphere in storage improves postharvest quality of fruits and vegetables, and that such effect is much more pronounced than control with temperature and humidity only (Thompson, 2016). The DCA is a storage system where $O_2$ concentration is gradually decreased and dynamically optimized during storage, and kept above lower oxygen limit (LOL) (Maxin, 2012; Prange et al., 2013). The LOL is the concentration of oxygen at which cell metabolism changes from aerobic to fermentative (Wright et al., 2012). Dynamic controlling is possible through monitoring of ethanol production or chlorophyll fluorescence (DCA-CF) based on detection of a sudden change in fluorescence at the LOL (Zanella et al., 2008; Wright et al., 2012; Prange et al., 2013).

Ethylene is a plant hormone that accelerates ripening in apples. Binding to ethylene receptors in plant tissue 1-MCP inhibits its action. Thus, 1-MCP suppresses ethylene mediated ripening of apple fruits and decreases ethylene production in treated apple fruits (Mao et al., 2007). 1-MCP treatment combined with optimal harvest time prevents the occurrence of superficial scald and helps in retention of fruit quality during shelf life (Akbudak et al., 2009; Magazin et al., 2010), and, also, it is able to extend the storage period in normal atmosphere (NA) storage for at least 3 months, and even two weeks after removal from cold storage (Tomic et al., 2016). According to McCormick et al. (2012), 1-MCP treated apple fruits could be successfully stored under higher storage temperatures, resulting in energy savings and lower costs, as well as environmental benefits (reduced carbon footprint).

The aim of this paper was to investigate the effects of storage conditions and 1-MCP treatment on the aggressiveness of the phytopathogenic fungi *F. avenaceum*.

## MATERIALS AND METHODS

The experiment was carried out during autumn 2015 and winter 2015/2016.

### *Fungal pathogen*

The effects of storage conditions and 1-MCP treatment on disease aggressiveness were tested on an isolate of *F. avenaceum* (KA12) obtained from the apple cultivar 'Granny Smith'. Apple fruits were harvested during autumn 2015. The pathogen was isolated using standard phytopathogenic techniques, and was cultured on sterile potato dextrose agar medium (PDA) at 25°C for seven days.

The pathogen was identified based on pathogenic and morphological characteristics, and identification was confirmed by PCR using the species-specific primer pair

FA-ITSF (5'-CCA GAG GAC CCA AAC TCT AA-3') and FA-ITSR (5'-ACC GCA GAA GCA GAG CCA AT-3') (Schilling et al., 1996; Turner et al., 1998). DNA was extracted from fungal mycelia using DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer's instructions. Sterile water was used as negative control. The reaction mixture for PCR contained 1 µL of DNA extract, 12.5 µL Paq5000 Hotstart PCR Master Mix (Agilent Technologies, Inc., USA), 1 µL of each primer (25 pmol $\mu L^{-1}$), and 9.5 µL of water. PCR reaction was performed in Eppendorf Master Cycler, and the reaction conditions were: initial denaturation for 2 min at 94°C, 30 cycles of 1 min at 94°C, 1 min at 59°C, and 2 min at 72°C, followed by 5 min final extension at 72°C. PCR products (7 µL) were separated by horizontal gel electrophoresis in 1.5% agarose gel, stained in ethidium bromide, and visualized under UV light.

### *Aggressiveness assay*

Apple fruits, cv. 'Granny Smith' (firmness 8.99 kg $cm^{-2}$, starch index 6.2 [scale 1–10], total soluble solids 12.9%, titratable acidity 0.97%), were used in the trial.

Thirty fruits were treated with 1-MCP (commercially available as SmartFresh™) and another thirty were left untreated. The fruits were surface-sterilized, injured with a sterile cork borer (Ø 4 mm and 3 mm deep) and artificially inoculated with seven-days old mycelium plugs (Ø 3 mm) of *F. avenaceum* (isolate KA12) cultured on PDA. Treated and untreated fruits inoculated with sterile PDA plugs were used as a negative control. The fruits were placed in disinfected, plastic chambers, non-hermetically closed and stored either at room temperature (21 ± 2°C) or in a cold-storage under DCA conditions (atmosphere composition 0.5% $O_2$ and 0.8% $CO_2$, air temperature 1 ± 0.5°C, relative humidity 92-94%). On fruits stored at room temperature, the diameters of necrotic lesions caused by *F. avenaceum* were measured after 7, 14 and 21 days of incubation. On DCA stored fruits, necrosis diameters were recorded immediately after storage period (143 days), and after 7, 14 and 21 days of additional incubation at room temperature (21 ± 2°C).

Data obtained by measuring necrosis diameters developed on untreated fruits and fruits treated with 1-MCP immediately after cold storage and after additional incubation at room temperature were compared with the data obtained by measuring necrosis diameters on treated and untreated fruits incubated at room temperature. Necrosis diameters developed during cold storage were subtracted from the necrosis diameters developed after incubation at room temperature for each assessment (7, 14 and 21 days), and the data were compared with necrosis diameters developed on fruits incubated at room temperature only.

Necrosis development rate (NDR) (mm $day^{-1}$) was calculated as the ratio between necrosis diameter (mm) and duration of observation period.

### *Statistical analysis*

The obtained data were processed by factorial *ANOVA* using *STATISTICA 13* software (StatSoft Inc., USA). Fisher's LSD test was used to test the significance of differences ($p \leq 0.05$) in the diameters of developed necrotic lesions.

### *Fruit storage details*

Apple fruits were chamber stored from October 19th until October 29th, 2015. Storage temperature was 4°C. Treatment with 1-MCP was conducted on October 30th. After treatment, the fruits were shortly removed from the storage (<12 h) for inoculation purposes and then returned. Storage temperature was gradually decreased, 0.2°C per day, until it reached 1.5°C. After 4 weeks in storage at this temperature, a decrease in oxygen level was initiated on December 3rd. Ultra Low Oxygen (ULO) parameters were achieved on December 18th ($O_2$ = 0.8%, $CO_2$ = 1%). DCA parameters were achieved for the first time on January 5th ($O_2$ = 0.5%, $CO_2$ = 0.8%) when monitoring of ethanol production in samples was initiated. On March 21th the fruits were removed from storage.

## RESULTS

### *Fungal pathogen*

Morphological and cultural characteristics of the isolate KA12 were tested on PDA and carnation leaf agar (CLA) media. Its growth was more pronounced on PDA, while sporulation was more pronounced on CLA medium. The isolate KA12 formed sporodochia on CLA medium. Sporodochia were orange to brown and formed at the central part of the colony. Aerial mycelia were abundant, white to gray-pink. The type of conidiophores that formed on mycelia was monophialides and polyphialides with 2-3 conidiogenous sites. Macroconidia were curved, needle-like, multicellular, with 3-7 septa. Based on these findings, the isolate was identified as *F. avenaceum*. The identification was confirmed by PCR and the amplified fragments were of the expected size of 272 bp.

*Aggressiveness assay*

During cold storage, necrosis developed on artificially inoculated apple fruits regardless of whether they were treated with 1-MCP or not. However, on the day of storage termination, there were statistically significant differences between treated and untreated fruits ($p < 0.01$). On treated fruits, necrosis diameters were significantly smaller compared to untreated apples (Figures 1 and 2).


**Figure 1.** Necrosis development on 1-MCP-treated (left) and untreated fruits (right)

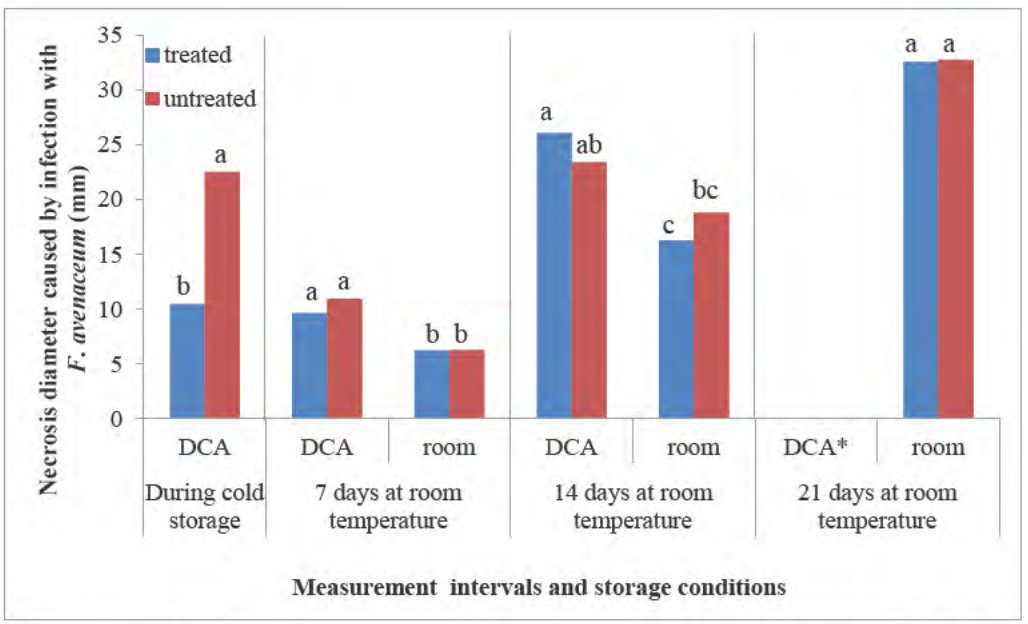


**Figure 2.** Mean values of necrosis diameter caused by *Fusarium avenaceum* during DCA storage, and after 7, 14 and 21 days of incubation at room temperature on 1-MCP-treated and untreated fruits

*Note.* Necrosis diameters that developed during cold storage were subtracted from diameters developed after the incubation period at room temperature; bars marked with different letters point to significantly different values according to Fisher's LSD test at $p \leq 0.05$ within a given period

*Fruits decayed completely

After seven days of incubation at room temperature, necrosis developing on fruits stored under DCA conditions and at room temperature was compared. The fruits stored at room temperature only developed significantly smaller necrosis diameter compared to those kept in DCA storage ($p \leq 0.01$), regardless of the treatment applied. There were no significant differences between the diameters that developed on 1-MCP-treated and untreated fruits stored at room temperature only ($p = 0.98$). Although fruits stored under DCA conditions and treated with 1-MCP developed smaller necrosis diameter compared to untreated fruits stored under DCA, data analyses showed there were no statistically significant differences between the treated and untreated fruits ($p = 0.4$).

Significant differences between necrosis diameters measured on fruits stored at room temperature only and fruits from DCA storage were observed after 14 days of incubation. The smallest necrosis diameter was recorded on 1-MCP-treated fruits stored at room temperature only, and the largest diameter was observed on 1-MCP-treated fruits stored under DCA conditions ($p < 0.01$). No significant differences in necrotic lesion diameter were observed between untreated fruits stored at room temperature only and under DCA conditions ($p = 0.12$).

After 21 days of incubation at room temperature, there were no significant differences in necrotic lesion diameters between 1-MCP-treated and untreated fruits ($p = 0.96$). The fruits stored under DCA conditions decayed completely and necrosis diameter could not be measured (Figure 2).

No symptoms were observed on uninoculated control apple fruits, regardless of treatment or storage condition.

The results regarding NDR (mm day$^{-1}$) are presented in Figure 3. The lowest NDRs were found during the 143 days of storage under DCA conditions. The NDR of fruits treated with 1-MCP was significantly lower compared with the NDR of untreated fruits ($p < 0.01$). After 7 days of incubation at room temperature, the NDR of fruits stored only at room temperature was significantly lower compared to fruits from DCA storage ($p \leq 0.03$). The NDR of both treated and untreated fruits stored at room temperature were at the same level of significance ($p = 0.98$). Also, there were no significant differences between the NDRs of treated and untreated fruits from DCA storage ($p = 0.4$). After 14 days of incubation at room temperature, significant differences in NDR data were observed. Fruits stored at room temperature and treated with 1-MCP had the lowest NDR, while the NDRs of treated fruits stored under DCA conditions were significantly higher ($p < 0.01$). After 21 days of incubation, there were no statistically significant differences between treated and untreated fruits stored at room temperature ($p = 0.96$).

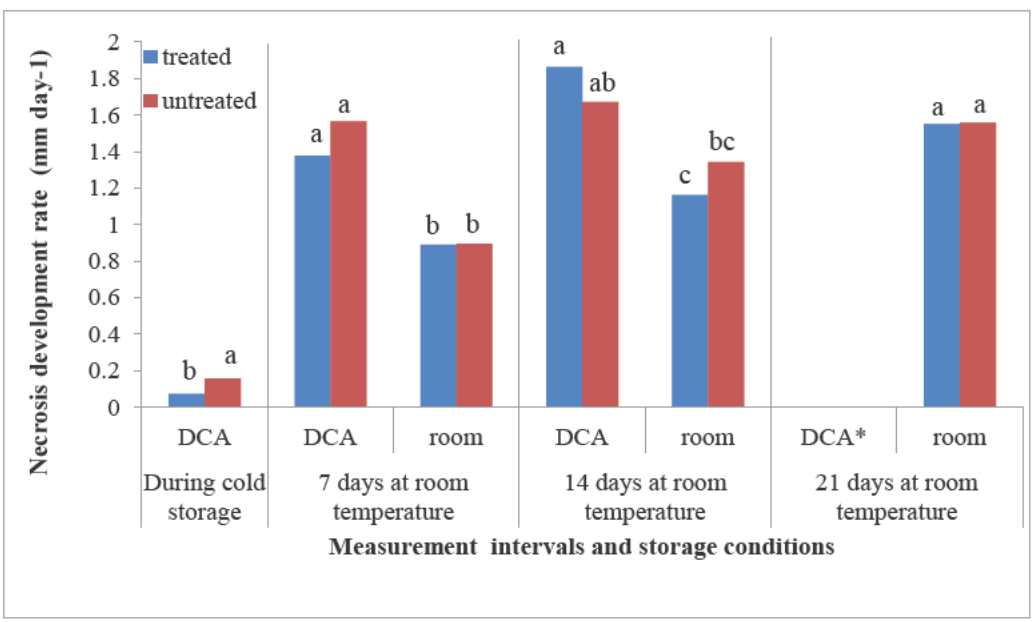


**Figure 3.** Necrosis development rate (NDR) caused by *Fusarium avenaceum* during dynamically controlled atmosphere (DCA) storage, and after 7, 14 and 21 days of incubation at room temperature

*Note.* For abbreviations, see Figure 2

## DISCUSSION

In this study, necrosis development on apple fruits was significantly affected by 1-MCP treatment. Necrosis on apple fruits treated with 1-MCP during cold storage was significantly smaller compared to untreated fruits. The results are consistent with findings reported by Jeziorek et al. (2010) showing that treatment with 1-MCP usually reduces losses caused by fungi, mainly *Botrytis cinerea*, *Neofabrea perennans* and *Penicillium expansum*. On the other hand, according to Kittemann et al. (2015), the incidence of fungal rots was slightly higher under ULO conditions on 1-MCP-treated cv. 'Jonagold' fruits at 5°C, but it was strongly reduced on cv. 'Pinova'. The ability of 1-MCP to reduce rotting of apple fruits varies depending on the stage of fruit maturity, and there were little or no effects on fruits that were harvested late (Lafer, 2006). It has been observed that 1-MCP can reduce postharvest decay of tomato fruits as well, caused by *Alternaria alternata*, *B. cinerea* and *Fusarium* spp. over a certain storage period (Su & Gubler, 2012). Moreover, McArtney et al. (2011) reported a significant effect of 1-MCP treatments in terms of reduction of rots caused by phytopathogenic fungi on apple fruits. Also, apple fruit quality was improved by 1-MCP because treated fruits contained more vitamin C, the loss of titrable acids was delayed, soluble solid content (SSC) decreased, and fruit rot consequently decreased 30% (Moor et al. 2007). Li et al. (2017) found that 1-MCP treatment enhances the oxidative damage of spores and mycelia of *P. expansum* and destroys the integrity of plasma membrane of spores, and thus inhibits blue mold on apple fruits.

According to Gago et al. (2015), shelf life fruit rot after storage period plus 7 days was lower in 1-MCP-treated fruits than in untreated fruits. Köpcke (2015) found that 1-MCP and DCA delayed ripening, and prolonged storage life, while 1-MCP was more effective in prolonging shelf life than DCA. These claims are partially in agreement with the results obtained in this study. After fruits were removed from storage, the 1-MCP-treated fruits stored in DCA developed slightly smaller necrosis diameter after 7 days compared to untreated fruits from DCA, but after 14 days 1-MCP-treated fruits from DCA storage developed larger necrosis than both untreated fruits from DCA and fruits stored at room temperature. It can be assumed that 1-MCP inhibits the development of necrosis during storage, but after storage period is over, the inhibitory effect of 1-MCP ceases. This is in line with the findings of Fallik et al. (2001), who reported that rapid necrosis development began when fruits were moved out of cold storage. However, after six-months of cold storage, Juhnevica-Radenkova et al. (2016) reported no significant difference in the number of colonies forming units (CFU) of microorganisms present on 1-MCP-treated cold-stored apple fruits compared to untreated ones. Grahovac et al. (2016) reported an inhibitory effect of long-term cold storage on the aggressiveness of *Colletotrichum gleosporoides* and *Colletotrichum acutatum*. However, the same authors noted that unless aggressiveness is completely inactivated, symptoms would occur after four days of incubation at room temperature.

The NDR varied depending on storage conditions and treatment with 1-MCP. The lowest NDR was noted on treated fruits during the 143 days of storage under DCA conditions. These results are in line with the findings of Tarlanović et al. (2017), noting that NDRs were very low during cold storage. Moreover, the NDR was found to increase rapidly during incubation at room temperature. The highest NDR was recorded after 14 days of incubation at room temperature on treated fruits stored under DCA conditions, and after 21 days on both treated and untreated fruits stored only at room temperature. The findings indicate that both 1-MCP and storage conditions are able to inhibit fungal decay on apple fruit, but the strength of inhibition highly depends on pathogen characteristics

## CONCLUSION

Treatment with 1-MCP can slow down the development of *F. avenaceum* on apple fruits of the 'Granny Smith' cultivar during storage under DCA conditions. However, after incubation at room temperature the effects of 1-MCP cease. Conversely, 1-MCP-treated fruits stored at room temperature only developed smaller necrosis during incubation compared to DCA stored fruits. Also, the NDR caused by *F. avenaceum* on apple fruits was inhibited by 1-MCP treatment during storage under DCA conditions, but it rapidly increased during the incubation period. The results indicate that both 1-MCP and DCA storage can inhibit fungal decay caused by *F. avenaceum* on apple fruits, but the effects do not persist after storage, i.e. at room temperature.

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# Delovanje 1-MCP i dinamički kontrolisane atmosfere na trulež jabuke prouzrokovanu gljivom *Fusarium avenaceum*

**REZIME**

Vrste roda *Fusarium* sve češće se javljaju kao prouzrokovači truleži plodova jabuke u skladištima. Vrsta *Fusarium avenaceum* je od posebnog značaja zbog dominacije u odnosu na ostale *Fusarium* vrste na uskladištenim plodovima, kao i sposobnosti da proizvodi mikotoksine. Upotreba 1-metilciklopropena (1-MCP) i različiti uslovi skladištenja utiču na agresivnost *F. avenaceum* i pojavu truleži plodova prouzrokovane gljivama. U ovom radu, plodovi jabuke sorte 'Granny Smith' tretirani su sa 1-MCP i veštački inokulisani izolatom vrste *F. avenaceum*. Izolat *F. avenaceum* korišćen za inokulaciju je poreklom iz ploda jabuke i identifikovan je na osnovu morfoloških karakteristika i lančanom reakcijom polimeraze (PCR) upotrebom para prajmera specifičnog za vrstu *F. avenaceum* (FA-ITSF i FA-ITSR). Nakon inokulacije, tretirani i netretirani plodovi su čuvani na sobnoj temperaturi i u hladnjači sa dinamički kontrolisanom

atmosferom (DCA). Ocena razvoja nekroze na plodovima čuvanim na sobnoj temperaturi vršena je nakon 7, 14 i 21 dan inkubacije, a na plodovima čuvanim u DCA hladnjači odmah nakon skladištenja (143 dana), te nakon 7, 14 i 21 dan dodatne inkubacije na sobnoj temperaturi. Ostvareni rezultati pokazuju da tretman sa 1-MCP inhibira razvoj *F. avenaceum* na plodovima jabuke tokom čuvanja u DCA hladnjači. Međutim, po iznošenju jabuka iz skladišta i inkubaciji na sobnoj temperaturi, efekat 1-MCP prestaje i razlike u razvoju nekroze na tretiranim i netretiranim plodovima nisu značajne. Na plodovima koji su čuvani samo u uslovima sobne temperature nisu registrovane razlike u razvoju nekroze između netretiranih i plodova koji su tretirani sa 1-MCP. Tokom inkubacije na sobnoj temperaturi, na tretiranim plodovima koji su čuvani samo u uslovima sobne temperature, razvoj nekroze je značajno manji u odnosu na tretirane plodove prethodno skladištene u DCA hladnjači. Može se zaključiti da tretiranje sa 1-MCP i DCA uslovi skladištenja inhibiraju pojavu truleži plodova jabuke prouzrokovanu vrstom *F. avenaceum* tokom skladištenja, ali se efekti ne zadržavaju nakon iskladištenja.

**Ključne reči**: 1-metilciklopropen; Inhibitor etilena; Dinamički kontrolisana atmosfera; Skladištenje; Trulež plodova jabuke