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THE EFFECT OF PROTECTION FILM ON THE ADAPTATION OF 'HANFU' APPLE TREES UNDER LOW TEMPERATURE

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ABSTRACT

In this study, 'Hanfu' apple trees were used as a test material to determine the effect of a functional protective film on the ability of the trees to withstand temperatures below zero. The functional protective film is a quaternary copolymer prepared by emulsion polymerization of vinyl acetate (Vac), butyl acrylate (NbA), methyl methacrylate (MMA) and acrylic acid (AA). The resulting copolymer was modified by adding a thermal insulation material (ceramic powder) to enhance the insulating properties of the film. Compared with a control, application of various quantities of the protective film resulted in as much as 2.5℃ decrease in the semi lethal temperature for 'Hanfu' trees. The insulating protective coating effectively reduced the MDA (malondialdehyde) content and significantly increased the activities of antioxidant-enzyme in the tree branches, which improved the low-temperature adaptability of the plants helping them to maintain normal physiological functioning. In the four treatments, protective films with the addition of 4% and 6% thermal insulation offered better protective effects.

Key words: Protection film, Thermal insulation, 'Hanfu' Apple, Low-temperature stress, Semi lethal temperature, Antioxidant enzyme activity.

INTRODUCTION

In nature, plants often experience harsh conditions such as temperatures below zero which result in plant injuries. These types of injuries result in inhibition of plant growth and development, and can result in the death of the plant. In China, the cold weather damage to fruit trees in the temperate and frigid zones, as well as periodic low temperatures in the tropics and subtropics occasionally occur (Ma *et al*., 2010; Peng *et al*., 2011; Schwindt, 2010; Li *et al*., 2011). In response to this situation, development of protective films for fruit trees has progressed to help deal with cold stress in agriculture (Mart, 2010; Joseph, 2002; Sun, 1996; Satake and Mikio, 1994; Wongshereea *et al*., 2009; Wan and Li, 2000; Shinji, 1996; Zhang and Guo, 2011). A fruit tree protective film is a type of polymer latex that is synthesized using emulsion copolymerization. The protective film forms a thin layer after being sprayed on the tree and provides a multi-aspect protection against adverse environmental conditions, providing a beneficial value from a practical application.

In this study, one-year branches of 'Hanfu' apple trees (*Dongguang*×*Fuji*) were used as the subject materials to study the effects of protective film modified with a thermal insulator.

MATERIALS AND METHODS

Experiment 1 Materials: This experiment was carried out from 2011 to 2012. Based on the method of Tongyu (2007), raw materials including vinyl acetate (Vac), butyl acrylate (NbA), methyl methacrylate (MMA) and acrylic acid (AA) were used as main components for copolymerization of the protective film. Moreover, $(NH_4)_2S_2O_8$, $NaHSO_3$, $NaHCO_3$ and membrane additives were included in preparation of the films.

Experiment 1 Methods: The film was synthesized using a basic emulsion process that consisted of: 1. A protective colloid (Polyvinyl alcohol), Sodium dodecyl sulfate and OP-10 were mixed in a flask which was maintained in a water bath at 30±2℃. All monomers were then added and a quantity of initiator was added after the monomers were completely emulsified. The mixture was maintained at 30 ℃ for 2h and then cooled down to room temperature.

The protective film was prepared as follow: dispersant (Sodium Hexametaphosphate, Sodium Polyacrylate), wetting agent (APM-95), film former (CH_3CH_2), antifreeze (Ethylene Glycol) and fungicides (Thiophanate-Methyl) were added to reaction kettle, evenly mixed with water and then a pigment, and thickener were added and the mixture was homogenized. This homogenate was then added to the polymer basic emulsion after filtering, low-speed stirred mixture until evenly dispersed. This constituted the final protective film.

Experiment 2 Materials: In 2012, one-year dormant branches were collected in 'Hanfu' apple orchard of Shenyang agricultural university. The branches were treated as follows: Treatment 1 (T1): branches were coated with the basic compolymer protective film; Treatment 2 (T2): branches were coated with protective film + 2% ceramic powder; Treatment 3 (T3): branches were coated with protective film + 4% ceramic powder; Treatment 4 (T4): branches were coated with protective film + 6% ceramic powder; The branches with no protective film were used as a control (CK). 2%, 4% or 6% means the ceramic powder was 2%, 4% or 6% of total film quality.

The insulating ceramic powder was a white powder synthesized from inorganic mineral, nonmetal oxide and metal oxides made by calcining, pH 6~8, which has good heat insulating properties. All of the branch samples used for low-temperature experiments were collected from the orchard when the lowest outdoor temperature was -20℃.

Experiment 2 Methods: Based on the method of Li (2000), the branch samples covering the experimental protective film and were placed into zip-lock bags and then into a thermal-humidity incubator (the change of temperature and humidity can be programmed). The low-temperature treatment began at -20℃and was decreased to -45℃ with the gradient of 2℃. The samples were tested for their physiological indices, including, relative electric conductivity, MDA (malondialdehyde) content, and activities of antioxidant-enzyme such as SOD, POD and CAT.

Data Analysis: The each result came from three experimental replications. The ANOVA and linear-regression calculation of experimental data were done by using SPSS 19.0 software (*SPSSInc.*, USA). The Excel 2010 was used for data processing and mapping.

RESULTS AND DISCUSSION

Property Analysis of Protection Film Emulsion: The protection film emulsion, as described in experiment 1, was painted on one-year branches of 'Hanfu' apple in the autumn when temperature was close to 0 ℃. This protection film emulsion formed a water-white membrane after the water in the film vaporized. Hence, the protection film emulsion was able to form a white layer of white and heat insulating membrane if the white pigment and thermal insulation were added to emulsion. From November 2011 to April 2012, the protection film was intact and without cracking. It exhibited strong resistance to rain-water flushing and external force. Data for some emulsion's properties are exhibited in Table1.

Table1. Polymer emulsion properties.

Test Item	Test Result	Test Item	Test Result
Gel rate (%)	1.4	Polymerization Stability	Result is Stable, without gel
Emulsion Appearance	Milk white with blue light	Standing Stability	Less Layered
Minimum Forming Temperature (℃)	0	Freeze-thaw Stability	Passed
Test Item	Test Result	Test Item	Test Result
pH	6~7	Dilution Stability	Passed
Solid Content (%)	44.5	Ca^{2+} Stability	Passed
Water Absorption (%)	19.66	Film Forming Ability	Good result

Influence of Protection Film on the Water Content and Water Loss Ratio: Water is very important to the plant protoplasm structure, cell turgor pressure and the fruit tree temperature. Water is vital solvent and life medium for biochemical cell reactions. In the shoots of the fruit tree, the water content can be as high as 50% of the mass. The change of 'Hanfu' apple branches' water content is shown on graph A (Fig 1). Graph B (Fig 1) shows the water loss rate of the 'Hanfu' apple branches. The water loss rate of the control branches was higher the loss from the treated branches which were 91.7% for T1, 89.6% for T2, 90.5% for T3 and 92.6% for T4. These results demonstrate that the protective film is air permeable and reduces water loss helping to maintain the water content of the branch. Consequently, the protective film, plays a role in stabilizing cell structure and maintaining physiological activity. Among the four treatments, T2 and T3 exhibited results.

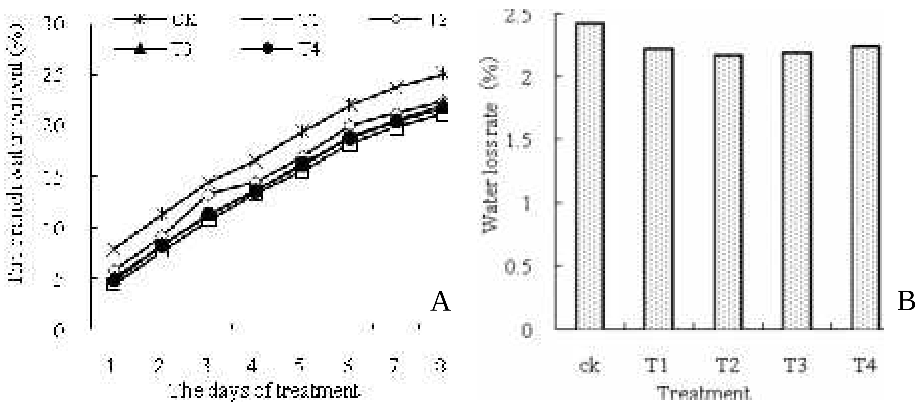


Fig 1. The changes of water content with different treatment in annual branches of 'Hanfu' apple

Influence of the Protection Film on Relative Electric Conductivity: The change of the relative electric conductivity in branch exhibited an 'S'- type curve (as showed in Fig2) at low-temperature. When the temperature decreased, relative electric conductivity in branches increased and then decreased.

These data indicate that the relative electrical conductivities were different for these four coating treatments at low-temperature. When the ambient temperature reached -33℃, relative electric conductivities of coating treatments were markedly lower than that of control, especially between the control and T3 and T4. When the temperature dropped to -43℃, the relative electrical conductivity of control increased 82.76%. By contrast, the relative electrical conductivities of T1, T2, T3 and T4 were 16.8%, 9.1%, 8.7% and 9.1% lower than that in control. As the temperature decreases, an increase in electrical conductivity is an indication of inadequate temperature adaptability of the plant.

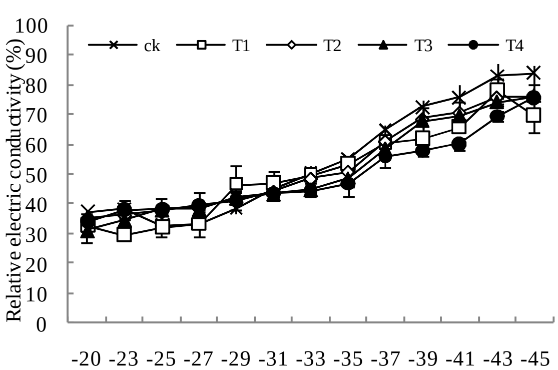


Fig 2. The changes of 'Hanfu' apple annual branches relative electric conductivity rate with different treatment and temperature

Therefore, since coating film with the added thermal insulation decreased the relative electric conductivity, this would indicate that the coating protective film played an important role in protecting branches from membrane lipid peroxidation at low-temperature. T3 and T4 retard the low temperature stress on membrane damage of 'Hanfu' apple one-year branches, and maintain heat in the branches.

The Logistic Equation fitting by Relative Electric Conductivity: The LT_{50} is a critical temperature for a plant. This is a measurement of the temperature at which 50% of a population of plants will die. When the temperature reaches LT_{50}, the plant is in a semi lethal state. As the temperature decreases, the damage that plant suffers can be unrecoverable, leading to the death of the plant. The curve of the relative electric conductivity of 'Hanfu' apple one-year branches is similar to a Logistic curve. The Logistic equation is a typical 'S' curve, based on the method of Zhu *et al*. (1986).

According to the relative electric conductivity resulting from different low-temperature treatments, semi lethal temperature and Logistic equation of 'Hanfu' apple one-year branches are shown in Table 2.

The method of using relative electric conductivity to fit a Logistic equation has been used for a variety of fruit trees, such as European pear (Li *et al.*, 2007), apricot (Yang *et al.*, 2011), table grape (Wang *et al*., 2007) and *Prunusavium* (Zhang *et al.*, 2012)

Logistic curve equation $y=k/(1+ae^{-bx})$ accurately fits the data, fitting ry'x was between 0.914 and 0.968. In this study, semi lethal temperature was significantly different for each of the coating treatments. The LT_{50} of control was -30.7℃, and LT_{50} of T1, T2, T3 and T4 were -31.51℃, -31.5℃, -32.29℃ and -33.22℃, respectively. These results suggest that the coating film with adding thermal insulation forms a protective polymer layer which effectively alleviates the damage to the branch from freezing damage and maintains a steady-state environment in the cells, leading to improved low-temperature adaptability.

Table 2. 'Hanfu' apple LT_{50} of coating treatments

Treatment	LT_{50}	Fitted equation	Equation parameter		Degree of fitting
			A	b	
CK	-30.73	y=3.166-0.103x	23.57	0.103	0.944 **
T1	-31.51	y=2.615-0.083x	13.60	0.083	0.914 *
T2	-31.52	y=2.585-0.082x	13.20	0.082	0.966 **
T3	-32.29	y=2.680-0.083x	14.51	0.083	0.968 **
T4	-33.22	y=2.193-0.066x	8.92	0.066	0.946 **

Note: * and ** indicate the significance of rate level of 0.05 or 0.01 respectively.

The Influence of Protective Film on MDA Content: In most organisms, free radicals effect lipid peroxidation and lead to production of malondialdehyde (MDA). The MDA causes cross-linked polymerization of macromolecules such as proteins and nucleic acids and is cytotoxic. Low-temperature stress exacerbates membrane lipid peroxidation, which damages the membrane structure and function, leading to potential cell death.

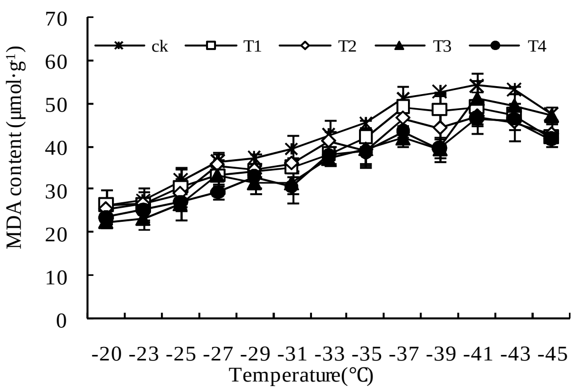
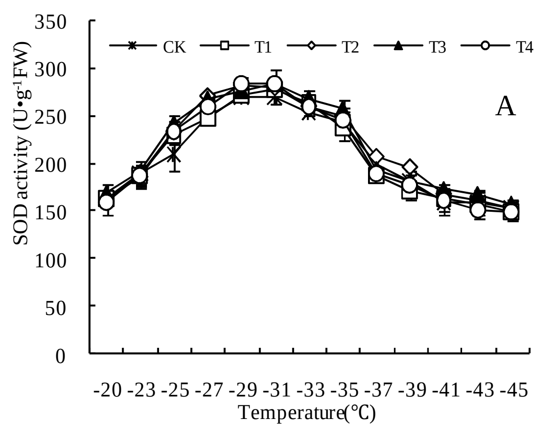


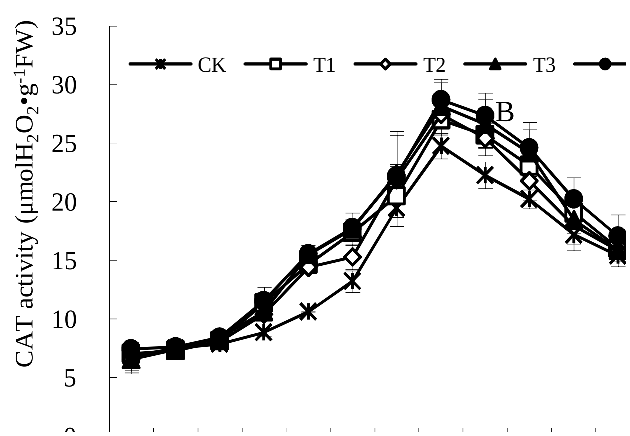
Fig 3. The changes of MDA content with different treatment and temperature in annual branch of 'Hanfu' apple

At low-temperature, MDA content in the control and coated branches increased as the temperature decreased in Fig 3. The MDA content of the control reached a maximum (54.12 μmol·g-1) at -41℃ , and was 1.1 times higher than at -20℃.The MDA contents in T1, T2, T3 and T4 were 9.5%, 13.2%, 5.4% and 14.0% lower than in control and the variations between the control and coated samples were significant.

Under low-temperature stress, the MDA content in the flower bud of the 'Hanfu' apple tree increased as the temperature decreased. The protective film with additional insulation effectively protected the cell membrane from lipid peroxidation in 'Hanfu' apple branches. Moreover, the MDA content began to slowly decrease after the branch temperature reached -41℃, suggesting that branch tissues had already died under extreme low temperature.

The Influence of Protective Film on Antioxidant-Enzyme Activity: Low temperatures can destroy the cell membrane antioxidant system, which affects fatty oxidase activity. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are collectively referred to as the cell protective enzyme system. The changing trend of SOD, POD and CAT activities were similar as they increased and then decreased with temperature drop (Fig 4).





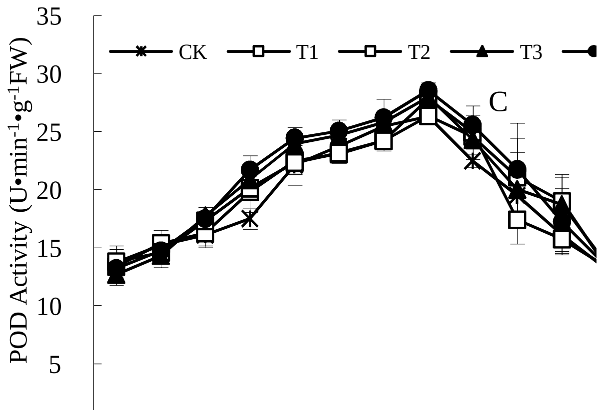


Fig 4A,B,C. The changes of SOD, CAT, POD activity with different treatment and temperature in annual branches of 'Hanfu' apple

From -20℃ to -27℃, there was no significant difference in the antioxidant-enzyme activities between the control and treated branches. However, in the range from -31 ℃ to -35 ℃, the enzyme activity changed significantly. The peak of the SOD activity was observed at -31℃. SOD activity of T1, T2, T3 and T4 were 3.6%, 4.3%, 5.8%, and 5.4% more than the control, respectively (Fig 4A). The peak of CAT and POD activity were observed at -35℃. CAT activity of T1, T2, T3 and T4 was 9.0%, 11.1%, 13.8%, and 16.2% more than the control, respectively (Fig 4B). POD activity of T1, T2, T3 and T4 were 0.2%, 5.8%, 6.3%, and 8.4% more than the control, the difference were significantly (Fig 4C).

SOD activity is considered to be an important indicator of how a plant copes with adversity (Sun *et al*., 2011). The main function of the protective enzyme systems, such as POD and CAT, is H_2O_2 enzymatic degradation. This reaction decompose H_2O_2 into water and oxygen and avoids excessive accumulation of H_2O_2 which can generate hydroxyl free radicals ($\cdot OH^-$) which damage the cell membrane structure (Breusegem *et al*., 2001; Fu and Huang, 2001; Poirier *et al*., 2006). The coating treatment and added insulation materials, can preserve heat and form an internal environment, which will induce increased SOD activity. Peroxisome continues to produce a large amount of H_2O_2 which induces CAT and POD enzyme activity increase, thereby eliminating H_2O_2. Antioxidant enzyme activities of T3 and T4 were relatively higher under low-temperature stress. Compared with the control, the protective film effectively reduced the degree of damage of low-temperature stress.

Conclusions: The protective film prepared in this work, together with added thermal insulation material (ceramic powder) was intact without cracking, exhibited strong resistance to rain-water and external force during long-term observation. The protective film reduced water loss and maintained water content of apple tree branches.

The LT_{50} of the branches following addition of2%, 4% and 6% insulation material were lower than the control of 0.79℃, 1.56℃ and 2.49℃. The protection insulating film reduced the content of MDA in the branches and increased the content of soluble substances. The protective film increased the activities of SOD, CAT and POD thereby improving the adaptation of the plant at temperatures below zero centigrade. The protective film is containing 4% thermal insulation material exhibited the most effective result.

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