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Effect of Preharvest Bagging on Fruit Epidermis Epiphyte Community Structure of 'Red Fuji' Apple

[1]S.J. Qin, [1]F.D. Li, [1]D.G. Lv and [1,2]W.S. Gao

[1]College of Horticulture/Key Laboratory for Northern Fruit Trees Cultivation and Physiology Ecology of Shenyang City, Shenyang Agriculture University, Liaoning, China
[1,2]Fruit and Tea Technique Supervising Station of Shandong Province, Shandong, China

Abstract: 'Red Fuji' apple was used as material to study the effect of preharvest bagging on epiphyte community structure of fruit epidermis through fruit development stages and on different epidermis positions. The results showed that Alternaria is the main sort of epiphytes in three sorts of bags ('Kobayashi', 'Tongle' and 'Qianwei') at different fruit development phases, as well as in different epidermis positions. Penicillium, Trichoderma and Aspergillus could be separated till July. Comparing the epiphyte community structure of bagged and non-bagged fruits, epiphyte community structure of non-bagged fruits did not have Aspergillus, which showed no overt pathogenicity in this experiment. Meanwhile, the occurrence rate of black-dot disease and red-dot disease of bagging fruits were little higher than that of non-bagging fruits. The results indicate that the epiphytes in the micro-environment of fruit bag do not directly influence the occurrence rate of black-dot diease and red-dot diease.

Key words: *Alternaria* % Black-dot diease % Fruit developing phases % Lenticel % *Penicillium* % Red-dot diease

INTRODUCTION

The practice of preharvest bagging has been extensively used in several fruit crops, such as apple [1, 2], pear [3, 4], peach [5, 6], mango [7, 8], longan [9], to improve the commercial value of the fruit, namely, improving fruit coloration [10], reducing splitting [11], mechanical damage [12] and sunburn [13] of the skin. Preharvest bagging also reduces pesticide residues in the fruit [12] and improves insect [14], disease [15] and bird damage control [12]. Therefore, preharvest bagging had been an important technical measure in improving the commercial value and promoting the export of the fruit.

Apple is the second most important fruit produced in temperate parts of the world after grape. China is a world leader in apple production and accounts for 47.8% of total yield [16]. Preharvest bagging has been applied wildly in apple production, especially for 'Red Fuji' apple, which is a major apple cultivar with a bagging rate of over 75% in main production areas of both Shandong and Shaanxi in China [17]. So, lots of studies on preharvest bagging of 'Red Fuji' apple have done by mang researchers and focused on the effects on fruit internal and appearance quality, plant diseases and insect pests emergence law, bagging technique, fruit physiological property [1, 18-20] and pesticide residues in the fruit [21]. However, microorganism of bagging fruit epidermis and inner-bag had little been involved. The micro-environment of fruit epidermis would change obviously after bagging, such as temperature, humidity and ventilation [22]. Moreover, some spot disease appeared or even worsen and fruit intrinsic traits debased. All these made people be aware that more detailed studies of micro-environment characteristic in fruit bag were necessary to elucidate the effect of preharvest bagging on fruit intrinsic quality. Based on this situation, we investigated the epiphyte community emerging of pericarp, lenticel epidermis and non-lenticel epidermis of fruit and inner-layer bags in the micro-environment of bagged fruit, with different paper bags, at different fruit development phases. Furthermore,

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Equal contributions were made by the first two authors.
Corresponding Author: D.G. Lv, College of Horticulture, Shenyang Agriculture University, Liaoning, China.
Tel: +86 02488487144.

the relationship between preharvest bagging and the occurrence rate of black-dot diease and red-dot diease was discussed. The purpose of this study was to references for both safety production and solving the problem of black-dot diease and red-dot diease of bagging fruit.

MATERIALS AND METHODS

The experiment was carried out at Tiechang orchard of Lijia village, Suizong county, Huludao city, Liaoning province in China. The soil in this orchard was umber and well ventilated. 'Red Fuji' apple trees used in this experiment were 26 years old, which grown in medium vigorous. The orchard is managed in relatively high level.

Three types of bags were used for treatment, 'Kobayashi' bag with double layer produced by QingDao Kobayashi bag Manufacture Limited Company (brown outer-layer outboard, black outer-layer inboard, size of 150 mm × 182 mm; red inner-layer with cere, size of 147 mm × 164 mm), 'Tongle' bag with double layer produced by TongLe Fruit Bag Manufacture Factory in Wafangdian city, Liaoning province (brown outer-layer outboard, black outer-layer inboard, size of 150 mm × 182 mm; red inner-layer with cere, size of 147 mm × 164 mm) and 'Qianwei' bag with double layer produced by Huludao QianWei Fruit Bag Factory (gray outer-layer outboard, black outer-layer inboard, size of 152 mm × 182 mm; black inner-layer without cere, size of 147 mm × 164 mm), non-bagged fruit as control.

Five apple trees were selected for bagging treatment, which grew in well state and had similar yield and all fruits were bagged in the same day. Bagging of fruit at June. 7 (sunny day), 40 days after full bloom (DAF), drop until Sep. 22, before 30 days of harvest, Oct. 21. Sampling was collected from the 40 DAF to harvest every 14-16 day. Three fruits per tree were randomly picked each time and used for microorganism incubation, observation and identification.

Epiphytes were separated by MS medium and purified in PDA medium [23] under the following process, 1) removing the bag gently from the fruits at clean bench, 2) cut some clean epidermis with lenticel and some without lenticel with sterilized blade and then put the epidermis on medium with exterior side downwards, 3) some inner layer paper was sheared and put on medium with interior side downwards. All experiments were repeated five times. All implements were sterilized in autoclave sterilizer (120 Pa, 125**9**) for 20 min.

The colony morphology of epiphyte was described in Rose Bengal medium and PDA medium, the mycelium morphology of epiphyte, through tabletting slice method, was observed with microscope and all kinds of epiphytes were classified and identified according to the 'Manual of fungi taxonomy' [24] and 'The morphological and classification of fungi' [25].

RESULTS

Changing of community structure in fruit epidermis were analysed mainly because of it was similar to epiphyte in inner-bag from Table 1. Four kinds of epiphytes were isolated in this study, *Alternaria*, *Penicillium*, *Trichoderma* and *Aspergillus*. Non-bagged fruit epiphyte community structure mainly included *Alternaria* and *Penicillium* and *Trichoderma* was separated only at Oct. 8, 16 days after removing bags. However epiphyte community structure and the emerging time of epiphytes changed after fruit bagged. The emerging time of *Penicillium* and *Trichoderma* in bagged fruits was obviously earlier than in non-bagged fruits. In bagged fruit, Penicillium appeared at Jul. 7 and Trichoderma at Aug. 7, in non-bagging fruits, they existed till Aug. 22 and Oct. 8 respectively. Furthermore, different types of bags also affected epiphyte community structure. Four kinds of epiphyte were separated from Pericarp of fruit with 'Kobayashi' bag, which was separated the most kinds of epiphyte in this experiment. Had there are three kinds of epiphytes (*Alternaria*, *Penicillium* and *Trichoderma*) in 'Tongle' bag and only two (*Alternaria* and *Penicillium*) in 'Qianwei' bag. Whereas, the ebb and flow trends of epiphyte community in three kinds of bags was basically consistent.

Epiphyte species of lenticel and non-lenticel epidermis were separated and identified (Table 2). The results showed that bagged fruit had marked effect on epiphyte community structure of the two positions and there is difference among three types of bags. 'Kobayashi' and 'Tongle' bag were generally consistent in epiphyte s pecies and change trend of lenticel and non-lenticel epidermis before removing bag, including *Alternaria*, *Penicillium* and *Trichoderma*, but *Aspergillus* was only found in non-lenticel epidermis at Oct. 8. At the same separating stage, epiphyte species of lenticel were incompletely consistent with that of non-lenticel epidermis, *Trichoderma* was not separated in non-lenticel epidermis while it emerged in lenticel. The change of epiphyte species in 'Qianwei' bag had a distinct dissimilarity in epiphyte species change of other two bags ('Kobayashi' and 'Tongle' bag). And epiphyte community structure both of lenticel and of non-lenticel epidermis was basically consistent, including *Alternaria*

Table 1: The effect of bagging on epiphyte community structure in fruit bag micro-environment

	Kobayashi bag		Tongle bag		Qianwei bag		Non bagging
Date	Pericarp	Inner layer	Pericarp	Inner layer	Pericarp	Inner layer	Pericarp
Jun 7	-	-	-	-	-	-	1
Jun 22	1	1	1	1	1	1	1
Jul 7	1,2	1,2	1,2	1	1,2	1,2	1
Jul 22	1,2	1,2	1,2	1,2	1,2	1	1
Aug 7	1,2,3	1,2,3	1,2,3	1,2,3	1,2	1	1
Aug 22	1,2,3	1,2	1,2	1,2	1,2	1	1,2
Sep 7	1,2,3	1.2	1.2.3	1.2	1.2	1	1,2
Sep 22	1,2,3	-	1,2,3	-	1,2	-	1,2
Oct 8	1,2,4	-	1,2,3	-	1,2	-	1,2,3
Oct 21	1,2	-	1,2	-	1,2	-	1,2

Note: 1. *Alternaria*; 2. *Penicillium*; 3. *Trichoderma*; 4. *Aspergillus*

Table 2: Change of epiphyte community structure on lenticel and non-lenticel pericarp during fruit development

	Kobayashi bag		Tongle bag		Qianwei bag		Non bagging	
Date	Lenticel	Non-lenticel	Lenticel	Non-lenticel	Lenticel	Non-lenticel	Lenticel	Non-lenticel
Jun 7	-	-	-	-	-	-	-	1
Jun 22	-	1	-	1	-	1	-	1
Jul 7	-	1,2	-	1,2	-	1,2	-	1
Jul 22	-	1,2	-	1,2	-	1,2	-	1
Aug 7	1,2	1,2,3	1,2	1,2,3	1	1,2	1	1
Aug 22	1,2,3	1,2	1,2	1,2	1	1,2	1	1,2
Sep 7	1,2,3	1,2	1,2,3	1,2	1,2	1,2	1,2	1,2
Sep 22	1,2	1,2,3	1,2,3	1,2,3	1,2	1,2	1,2	1,2
Oct 8	1,2	1,2,4	1,2	1,2,3	1,2	1,2	1,2,3	1,2
Oct 21	1,2	1,2	1,2	1,2	1,2	1,2	1,2	1,2

Note: 1. *Alternaria*; 2. *Penicillium*; 3. *Trichoderma*; 4. *Aspergillus*

Table 3: Effect of fruit bags on occurrence rate of epiphyte diseases in Red Fuji apple fruits

		Black-dot disease		Red-dot disease	
Bags	No. of checked fruits	No. of infected fruits	Occurrence rate (%)	No. of infected fruits	Occurrence rate (%)
Kobayashi bag	100	5	5	2	2
Tongle bag	100	8	8	3	3
Qianwei bag	100	6	6	3	3
Control	100	3	3	0	0

and *Penicillium*. From the results, we speculated that the difference of epiphyte community of three fruit bags would have correlation with character of fruit bag, especially that of inner layer of fruit bag. Compared with bagging fruit, there were mainly *Alternaria* and *Penicillium* separated from lenticed and non-lenticel epidermis and *Trichoderma* was only found in lenticel at Oct. 8.

Four kinds of epiphytes had been identified from bagging fruits, which were *Alternaria*, *Penicillium*, *Trichoderma* and *Aspergillus* (Table 1 and Table 2). The fact, *Alternaria* existing in lenticel, non-lenticel of fruit of all treatment and inner bag of three bags, at every stage, indicate that *Alternaria* was one of the main epiphytes at fruit surface. *Penicillium* ranks the second, which was first found in non-lenticel epidermis on Jul. 7 and in 'Kobayashi' and 'Tongle' bag at the time of lenticel formation in epidermis, althouth it was first found in 'Qianwei' bag from lenticel on Sep. 7. Regardless of bag type and positions, *Penicillium* is observed till harvest. In addition, *Trichoderma* was found from 'Kobayashi' and 'Tongle' bag and the law of ebb and flow of *Trichoderma* was consistent in two types of bags. *Trichoderma* of two bags was separated from non-lenticel epidermis and inner layer of fruit bag firstly and then from lenticel. *Aspergillus* was only found in non-lenticel epidermis of 'Kobayashi' bag at Oct. 8 and its ebb and flow law would need further research.

Fruit spot diseases were investigated before harvest. Two kinds of spot diseases were identified, which were black-dot disease and red-dot disease. Black-dot disease existed in both bagging fruits and non-bagging fruits and occurrence rate of black-dot disease in bagging fruits was higher than that of in non-bagging fruits. Red-dot disease was separated only in bagging fruits and its occurrence rate has less difference among three kinds of bags. Based on the comparison of those two diseases, occurrence rate of black-dot disease was little higher than that of red-dot disease in three kinds of bags.

DISCUSSION AND CONCLUSSION

Bagging apple fruits was one of necessary techniques for producing high quality fruits, which had been universally adopted in apple production [17]. Furthermore, some countries importing apple from China, such as Mexico, Chile, Argentina, require that apple fruits must be bagged. Fruit preharvest bagging could effectively improve fruit coloration [26, 27], markedly lower pesticide residual [28] and avoid eating by birds and insects [1]. Due to bad ventilation of fruit bag, air exchange between inside and outside of fruit bag is blocked after bagging, consequently inducing high temperature and high humidity in fruit bag [22]. Fruits might not acclimatize to the micro-environment of fruit bags, or after removing the bag at unseemly time [22]. In these cases, some diseases would be induced or worsen at such kind of particular condition. The humidity of inside fruit bags was 20.3%-50.7% higher than that of outside [22], such high humidity and high temperature might worsen physiological diseases induced by pathogenic epiphytes. According to former statistic data, *Alternaria*, one of main epiphytes separated from micro-environment of inside fruit bag in this experiment, could induce black-dot disease of Ya pear, which was one of the important reason for sharply cutting down Ya pear's export in China [29, 30]. And some other studied had proved that *Alternaria* is also one of the main pathogenies of apple black-dot diseases [31-34]. In this study, we did not find *Trichothecium roseum*, another pathogen resulting of apple black-dot disease. Further study is needed to elucidate why *Trichothecium roseum* was not observed in our study.

Only *Alternaria* was separated at early stage of fruit bagging and the other three kinds of epiphyte was not separated until Jul. It might be due to that there was no raining all along Jun. after fruit bagging in the area where our experiment was done. Another reason might be the modest temperature. When it came into Jul., the area was in high temperature and rainy, which kept high temperature and humidity of inside fruit bag. At this stage, high temperature and humidity provided suitable living environment for epiphytes, with the addition of contact between pericarp and bactericide were decreased, so that more epiphytes emerged accordingly. Different micro-environment restricted different kinds of epiphyte, the ebb and flow law of different epiphytes and the main reason of the changing in epiphyte community structure is changing of micro-environment in inner-bag or cut off function of bags to bactericides need more studies to elucidate.

Epiphyte community structure of 'Qianwei' bag was basically consistent with that of non-bagging fruits, which was less than that of the other two bags ('Kobayashi' and 'Tongle' bag) during the period of fruit development. Those results indicated that micro-environment of inside 'Qianwei' bag was similar to outside. However, 'Kobayashi' bag, considered to be one of the best quality bags in market, had the most epiphytes. 'Tongle' bag had the similar results to 'Kobayashi bag'. More experiments need to be done to explain whether epiphytes separated from fruit bags was pathogenic and whether epiphyte community structure of inside fruit bags had relationship with the texture of bag (inner-layer of different fruit bags had different technics).

Although it had much more rain when it came into Jul., there was little black-dot disease in apple fruits with bagging and non-bagging and all treatment had *Alternaria* from preharvest bagging until harvest. It indicated that *Alternaria*, as one of primary pathonies of black-dot disease, could be inhibited in a certain extent through improving cultural measure. Comparing with non-bagging fruits, *Aspergillus* was the only one that non-bagging fruits did not have, which was separated merely from 'Kobayashi' bag after removing bags and had no overt pathogenicity. From our results, we considered that epiphyte community of micro-environment induced by bagging had no direct effect on black-dot diease and red-dot diease. Consequently, happening of scab maybe caused by unbalance of physiological metabolism which result from changes of micro-environment after bagged.

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