The impact of the degree of ripeness of an apple to the infectivity and pathogenicity of the *Colletotrichum* microfungi during storage

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MOLECULAR PLANT BIOLOGY

INTRODUCTION

Colletotrichum is a genus, which contains plantpathogenic microfungi. The genus is estimated to be the eighth largest cause of plant diseases in the world, leading to large agricultural losses annually. The subspecies belonging to *C. gloeosporioides* and *C. acutatum* species complexes cause storage disease called bitter rot in apple fruits.

The *Colletotrichum* infection is not necessarily noticeable in apples growing in apple orchards because *Colletotrichum* is still in the biotrophic stage where it does not kill the host apple cells it feeds on. The symptoms of the disease become apparent after harvest when the apple ripens during the storage and the disease moves to the necrotic stage. The symptoms of the apple bitter rot often include circular growing brown lesion, which sinks into the apple. The black spots on the surface of the lesion are sporangium structures containing spores.

Many plant pathogenic fungi form a resistance for fungicides easily, which might lead to fungicides becoming useless quickly and therefore finding new, more effective but safe antifungal agents is crucial. The formation of the resistance can be slowed down by taking care of sanitation at the apple orchards and by applying the fungicide the proper way and at the proper times. Because fungicides might lose their effectiveness, it is important to study and breed more resistant varities for different microfungi causing diseases in apples.

The research was conducted to find out which factors associated with ripening of the apple fruit contributed to the infectivity and pathogenicity of the *Colletotrichum* fungi in stored apples. The tolerance to the apple bitter rot disease in different apple varieties cultivated in Finland was also studied.

MATERIALS & METHODS

The apple varieties used in the study were Amorosa, Rubinola, Lobo and Rajka. The apples were harvested at three different levels of ripeness. 20 apples from each harvested apple patch were spore-inoculated. In addition, 30 apples were used for destructive measurement methods of apple firmness, starch content and sugar content. All the apples were stored at +2°C in high relative humidity. The diameter of the lesion caused by *Colletotrichum* was measured every two weeks for 12 weeks and starch, brix, DA and firmness of the apple were measured from six of the stored apples at the same day.

In addition, a set of 10 apple-pathogenic isolates of Colletotrichum spp. were used to wound-inoculate fruits. Per each isolate, ten evenly ripe fruits of cultivar Amorosa were wounded and wounds inoculated with a small disc of the *Colletotrichum* culture. Before the inoculation, the apples were disinfected with 70% ethanol and the wound was sealed with gelatin. The apples were put to the storage and the growth of the lesion was measured every two weeks for 12 weeks.

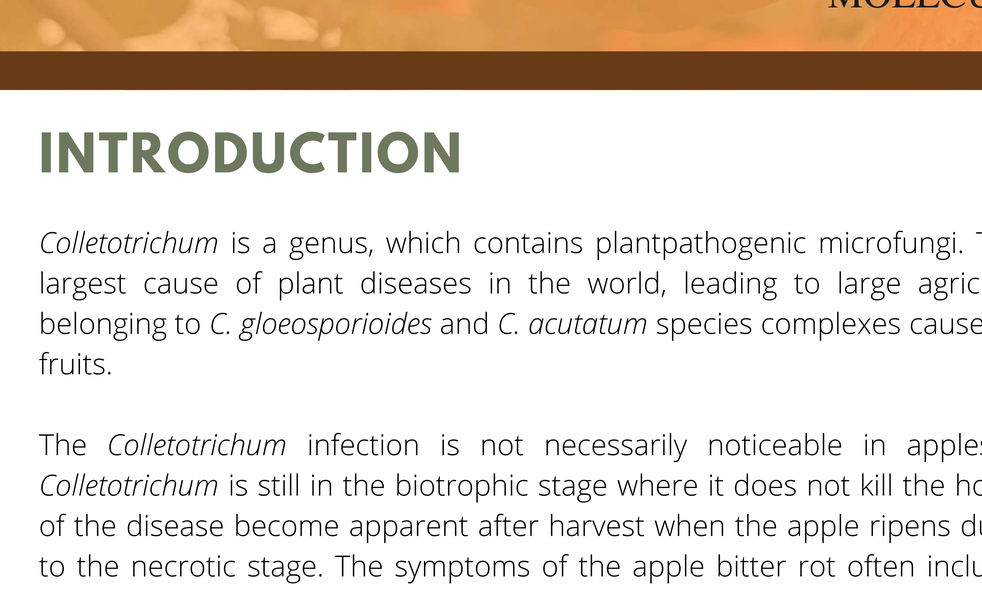

Figure 2. The bitter rot appeared around sixth week after the wound inoculation in the Amorosa apples. The diameter grew logistically during the storage period.

a.

b.

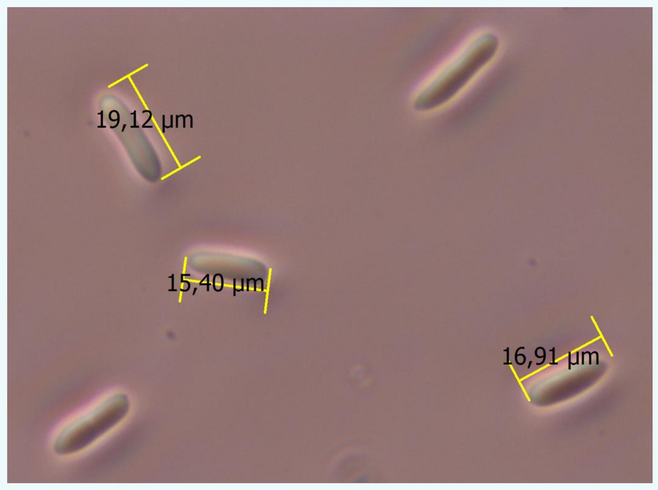

Figure 1. a. Bitter rot disease in an apple caused by *Colletotrichum spp*. b. The spores isolated from the inoculated and stored apples at the end of the study were shaped like cylindrical with rounded ends.

RESULTS & CONCLUSIONS

The first bitter rot symptoms appeared around the sixth week after the inoculation at the stored apples and the lesions started to grow logistically from there on (Figure 2.). The lesion was ensured to be bitter rot by comparing the symptoms with known symptoms and by checking if the spores match the original spores the inoculation was performed with (Figure 1.).

Preliminary results show that of the variables measuring apple ripeness, sugar and starch content (>0,05) influence disease progression in the wound infected apples. The chlorophyll content and the firmness of the fruit on the other hand were found not to be significant (p<0,05) for the growth of the lesion caused by *Colletotrichum*.

None of the variables measuring apple ripeness or the chlorophyll content were found significant (p<0,05) for the bitter rot in apples infected with *Colletotrichum* spores in the preliminary results. Further, no difference for the tolerance to the bitter rot disease was found between the apple varieties or the harvest ripeness.

The spore inoculation did not seem to work for *Colletotrichum* spores as most of the apples did not show any symptoms of bitter rot disease. It is possible that *Colletorichum* will infect developing fruits more efficiently compared to ripe apples ready to harvested. Further research using different study design is therefore needed for detecting possible varietal differences.