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Biochemical Characteristics of Apple Rot Caused by *Macrophoma* sp. II. Phenolic Compound Content in Infected Fruits

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Macrophoma sp.에 依한 사과 腐敗의 生化學的特性 Ⅱ. 感染과일의 페놀含量

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ABSTRACT

Changes in levels of phenolic compounds such as total phenols, flavonols and anthocyanins in *Macrophoma*-infected apples were studied at various developmental stages of apple fruits.

The amounts of total phenols in apple fruit flesh and peel drastically decreased as apples became mature. Apple rot resulted in concentration of total phenols somewhat lower than those of healthy apple flesh. The decline in amount of total phenols was distinct in infected fruit flesh of the cultivar Fuji, which was more susceptible to *Macrophoma* sp. than the cultivar Miller. Higher amounts of total phenols were found in infected than in heathy fruit peel. In the case of the cultivar Miller, increased accumulation of total phenols was pronounced in infected peel.

Apple rot resulted in concentrations of flavonols much higher than those of healthy apples. In particular, the drastically increased accumulation of flavonols was detected in infected peel at the first collection on 10 July, when the cultivars tested were completely resistant to *Macrophoma* sp. Production of anthocyanins was increased considerably by apple rot: anthocyanins in infected fruits of the cultivar Miller increased markedly as compared with their concentration from healthy fruits.

These results suggest that the altered phenolic metabolism in apple fruits may be associated with the development of apple rot.

INTRODUCTION

An earlier paper has reported the levels of total soluble carbohydrates and amino acids in healthy and *Macrophoma*-infected apples at various stages of maturity.[3] With the maturing of apples, the concentration of total soluble carbohydrates was gradually increased, whereas the amount of total soluble amino acids declined in the fruit flesh. Sitterly and Shay[7] reported that the infusion of apple fruit with fructose and sucrose caused rotting by *Botryosphaeria ribis* earlier in the same season. Wallace et al.[9][10] suggested that immature fruit resistance to *B. ribis*

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was due to the presence of protein-pectin-polyvalent cation complexes in the cell walls of apple fruits. Cole and Wood[1] reported the inhibition of pectionolytic enzymes of *Sclerotinia fructigena* by phenol oxidation products in apples.

Preformed inhibitory substances present in plants and/or antimicrobial compounds, phytoalexins, may play an important role in host-parasite relations. Numerous reports has been published on the appearance an accumulation of phenolic compounds on plant tissue in response to infection[4]. Huang and Agrious[2] indicated that changes in phenolic metabolism were associated with the events observed in development of scar skin and dapple apple diseases.

The objective of our work was to determine the levels of total phenols, flavonols and anthocyanins in healthy and *Macrophoma*-infected apples at various stages of maturity and to note possible correlations between the change in level of phenolic compounds and the expression of immature fruit resistance to apple rot.

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MATERIALS AND METOHDS

Macrophoma sp. isolate and apple cultivars.

The isolate of *Macrophoma* sp. used in this study was obtained from the Department of Plant Pathology, ORD, Suweon, Korea. The fungus was grown on oatmeal agar at 25±1°C, unless indicated otherwise.

The experiments were carried out with apple fruits of the cultivars Fuji and Miller growing in the University's orchard at Duckso. The cultivar Fuji (red) is harvested at the end of October and for Miller (dark red) at the beginning of October.

Inoculation and examination of apple rot.

Apple fruits at different developmental stages were inoculated every 30 days from 30 June through 31 August, 1982. Agar disks 7mm in diameter cut from 7-day-old cultures were inoculated by inserting into holes (7mm ϕ) on the apple fruits cut with a cork borer. The apples inoculated were covered with a vinly wrapper. On the 10th day after inoculation, healthy and infected apple fruits were collected 10 July, 10 August and 10 September for analysis of phenolic compounds.

Extraction and determination of phenolic compounds-Total phenols.

Immediately after the apples were peeled with a razor, 2g fresh weight of peel or 10g of flesh was boiled in 60% (v/v) ethanol for 10 minutes (three changes). The peel extract then was filtered through Whatman No. 1 filter paper. The final volume of the extract was made to 50m*l* of 60% ethanol. The flesh extract was decanted and 10m*l* of 60% ethanol was added to the residues, which were macerated in a mortor. The macerated preparation and decanted supernatants were pooled and centrifuged at 14,000g for 15min. The final volume of the resulting supernatant was made to 100m*l* of 60% ethanol. Aliquots of the ethanol water extracts were used in determining the amounts of total phenols, according to the method of Swain aud Hillis.[8] The 0.4m*l* of the extract was diluted with 7m*l* distilled water. The contents were well mixed, 0.5m*l* of the Folin-Denis reagent was added, and the tubes were thoroughly shaken again. Exactly 3min. later, 1.0m*l* of saturated sodium carbonate solution was added. After 1hr, the absorptivity was determined at 725nm in a Beckman spectrophotometer.

Total anthocyanins and flavonols.

Ten grams of apple peel were macerated with 30 m*l* of extracting solvent [95% ethanol:1.5N HCl (85 : 15)] in a mortor. The macerate was transfered to a 200m*l* beaker, covered with parafiim and stored overnight at 4°C. The sample was filtered through Whatman No. 1 filter paper. The final volume of the extract was made to 100m*l* with extracting solvent. Aliquots of the extract were used for the quantitative determination of the anthocyanins and flavonols by the method of Lees and Francis.[6] The extract was stored in the dark for 2 hrs at room temperature. The optical density was measured at 535 nm for the total anthocyanin content and 374 nm for the total flavonol content in a Beckman spectrophotometer. The quantities of pigments were calculated as follows: Total anthocyanins (mg/10g) =

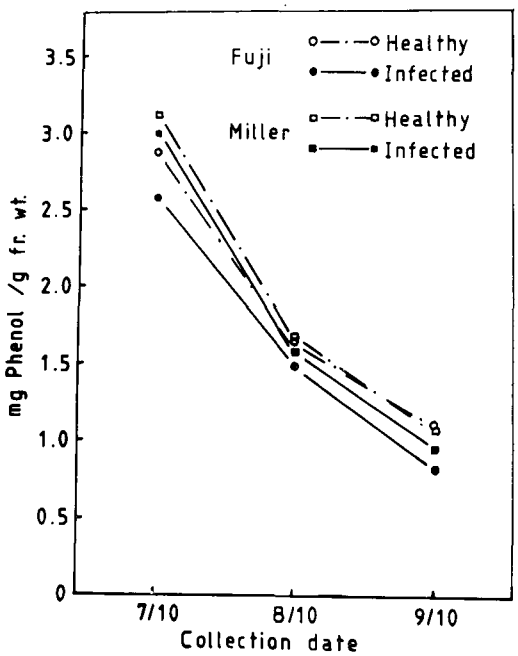

Fig. 1. Levels of total phenols in flesh of healthy and *Macrophoma* sp.-infected apples (cultivars Fuji and Miller) at different developmental stages.

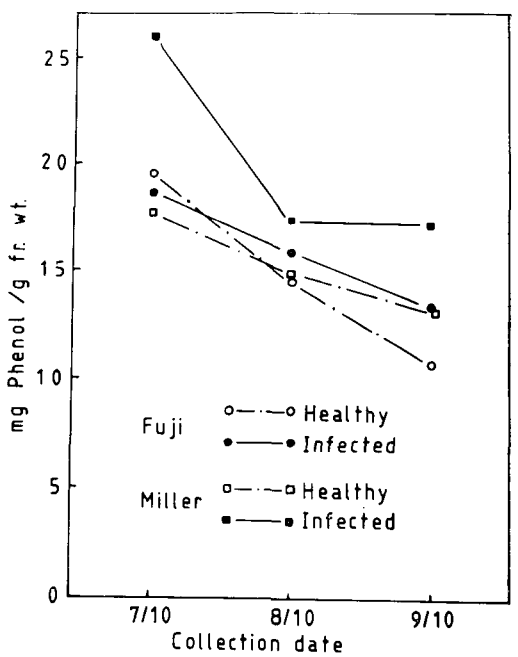

Fig. 2. Levels of total phenols in peel of healthy and *Macrophoma* sp.-infected apples (cultivars Fuji and Miller) at different developmental stages.

(Sample O.D.× Dilution factor)/9.82 and Total flavonols (mg/10g)=(Sample O.D.×Dilution factor)/7.65.

RESULTS AND DISCUSSION

Immature fruits of the cultivars Fuji and Miller tested were completely resistant until 10 July[3]. When inoculated on 31 July, apples became susceptible to *Macrophoma* sp. This fungus grew better on Fuji than Miller. The healthy and infected apple fruits which were collected at various stages of maturity were used for analysis of phenolic compounds.

Effects of apple rot on total phenols.

In both cultivars Fuji and Miller, the amounts of total phenols in apple fruit flesh drastically decreased as apples became mature (Fig. 1). Apple fruit flesh at the first collection on 10 July had ca. 3 times more total phenols than that at the last collection on 10 September. A slight difference in the amount of total phenols was observed between healthy and infected apples. Apple rot resulted in concentration of total phenols somewhat lower than those of healthy apples. The decline in amount of total phenols was distinct in infected tissue of the cultivar Fuji, which was more susceptible to *Macrophoma* sp. than the cultivar Miller.

Figure 2 illustrates the pattern of changes in concentration of total phenols in apple fruit peel during the maturity of apple fruits. The decrease in amounts of total phenols for fruit peel in matured apples was like that for fruit flesh in Figure 1. On the other hand, higher amounts of total phenols were found in infected than in healthy fruit peel. In the case of the cultivar Miller, increased accumulation of total phenols was pronounced in infected peel. The data indicates that the higher amounts of total phenols in flesh and peel of immature apple fruits may exert directly or indirecty an inhilbitory effect on the growth of *Macrophoma* sp. Also, the inhibitory effect of the phenols already present in the apple flesh may be stimulated in part by an incerase in content of amino acids, as shown in our earier study[3] Some amino acids such as phenylalanine and tyrosine has been known to be the precursors

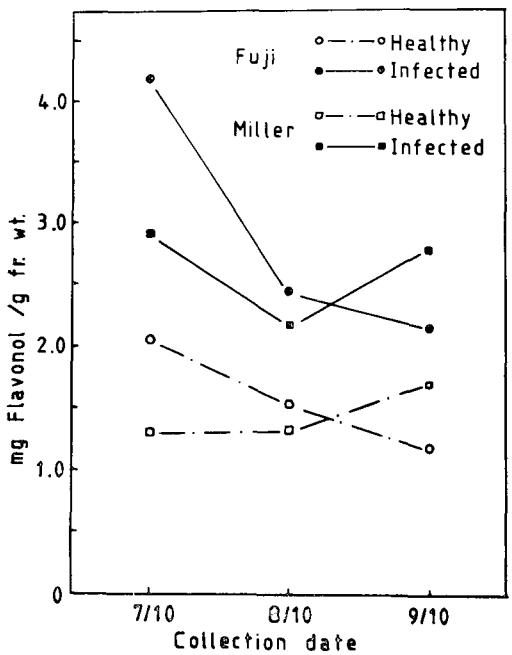

Fig. 3. Levels of flavonols in peel of healthy and *Macrophoma* sp.-infected apples (cultivars Fuji and Miller) at different developmental stages.

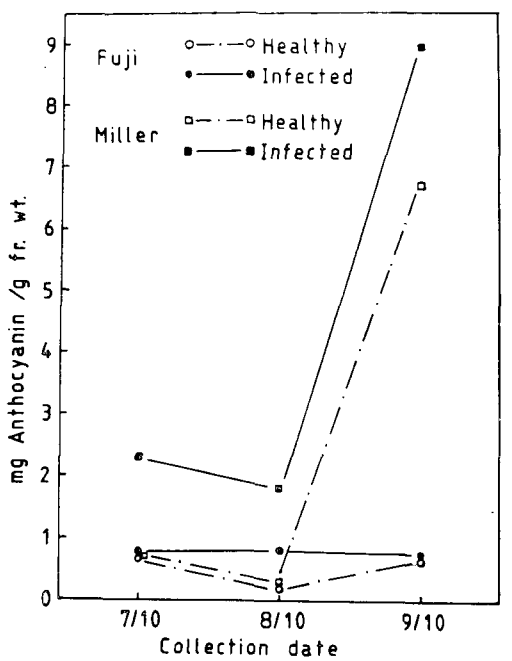

Fig. 4. Levels of anthocyanins in peel of healthy and *Macrophoma* sp.-infected apples (cultivars Fuji and Miller) at different developmental stages.

for synthesis of phenolic compounds. Kuć et al.[5] reported that no fungitoxic compounds were detected in the resistant apples and oxidation products of phenolic substances did not inhibit growth of *B. ribis*. They further observed that oxidation products of phenolic substances were inhibitory to pectinolytic enzymes in culture filtrates of *B. ribis*.

The interesting fact from our results is that as compared with the healthy tissues of apples, the infected peel had a higher amount of total phenols, whereas their concentration was lower in infected apple flesh. This suggests that the phenolic compounds which accumulated in peel of apple fruits inoculated with the fungus may be expressed as a disease reaction during the symptom development.

Effect of apple rot on flavonols and anthocyanins.

The amounts of flavonols in apple peel of the cultivars Fuji and Miller did not change significantly during the development of apples (Fig. 3). Apple rot resulted in concentrations of flavonols much higher than those of healthy apples. In particular, the drastically increased accumulation of flavonols was detected in infected peel at the first collection, when the cultivars tested were completely resistant to *Macrophoma* sp.

For healthy fruit peel, the amount of anthocyanins in the cultivar Fuji did not change greatly until the last collection on 10 September, but a rapid synthesis of anthocyanins occurred in the cultivar Miller at the last collection (Fig. 4). Production of anthocyanins was increased considerably by apple rot: anthocyanins in infected fruits of the cultivar Miller increased markedly as compared with their concentration from healthy fruits.

The apples growing in Korea owe its attractive coloration mainly to the red anthocyanin pigments and to a less extent to the yellow flavonol pigments. The red-colored apples, i.e., Miller, Starkrimson and Hongok, have been observed to be more resistant to *Macrophoma* sp. than the yellow-colored apples, i.e., Golden Delicious, Mutsu and Orei (Kim, Seong Bong, 1982, personal communication). When inoculated with the fungus on immature fruits, the cultivar Miller became red-colored around the lesion

than the cultivar Fuji, indicating more accumulation of anthocyanins in apple peel.

Accumulation of flavonols in *Macrophoma*-infected apples is in accordance with the findings of Huang and Agrios from scar skin-affected Red Delicious apples[2]. In contrast to our results, they also reported that scar skin and dapple apple caused significant reduction in fruit anthocyanins. The accumulation of flavonols and anthocyanins in *Macrophoma*-infected apples in this study could account for the altered phenolic metabolism accompanied by development of apple rot.

摘 要

사과 腐敗病菌(*Macrophoma* sp.)에 感染된 과일에서의 總페놀, flavonol, anthocyanin 含量의 變化를 사과의 여러 發育時期에서 調査하였다.

사과가 成熟됨에 따라 果肉, 果皮의 總페놀含量은 급격히 減少하였다. 腐敗病菌에 感染된 果肉에서 健全한 果肉보다 다소 總페놀含量이 낮았으며 品種밀러보다 더 感受性인 후지의 感染된 果肉에서 이의 減少가 뚜렷하였다. 健全한 果皮에서 보다 感染된 果皮에서 높은 페놀含量을 보였으며 밀러에서 이의 增加가 顯著하였다.

腐敗된 사과에서 flavonol 含量이 健全사과보다 높았으며 7月 10日에 *Macrophoma*에 완전 抵抗性이었던 사과의 感染된 果皮에 flavonol이 크게 蓄積되었다. anthocyanin 生成도 感染된 果皮에 상당히 增加되었으며 品種 밀러에서 뚜렷했다.

이들 結果에서 미루어 사과의 페놀代謝變動은 사과腐敗病進展과 關係가 있을지 모른다.

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