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Biochemical Characteristics of Apple Rot Caused by *Macrophoma* sp. (I)

Disease Development, Carbohydrate and Amino Acid Contents in Infected Fruits

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Macrophoma sp.에 依한 사과 腐敗의 生化學的 特性 (I)

病進展과 感染과일의 炭水化物, 아미노酸 含量

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Abstract: Immature apple fruits of cultivars Fuji and Miller were completely resistant to *Macrophoma* sp. until 10 July. When inoculated on 31 July, apples became susceptible to this fungus, irrespective of cultivar. This fungus grew better on Fuji than Miller. Lesion enlargement on apple at 25°C was remarkably pronounced relative to 20°C. In both cultivars, the amount of total soluble carbohydrates or reducing sugars in apple fruit flesh was gradually increased as apples became mature. The healthy apples of cultivar Fuji had a higher amount of carbohydrates than those of Miller. The infected apples contained more soluble carbohydrates in comparison to the healthy ones. In healthy apples, there were no consistent differences between Fuji and Miller in amount of total soluble amino acids. With increasing age of apples, the amount of soluble amino acids declined in the fruit flesh of both cultivars. Levels of carbohydrates and amino acids in apple fruits were discussed in association with the immature fruit resistance to *Macrophoma* sp.

Introduction

A rot of apple fruits caused by *Macrophoma* sp. is one of the most important limiting factors in apple production in Korea. It has not been reported about the time when this disease occurred in Korea. Late in the 1970', apple rot began to cause a damage in apple orchard. Recently, the rotting of apple fruits by *Macrophoma* sp. increased rapidly in apple growing areas (Lee, 1981). In apple fruit diseases, there are three rots, *i.e.*, black rot, white rot and bitter rot that cannot be easily diagnosed on the basis of symptoms (Starkey and Hendrix, 1980). There are so far much contraversy about the perfect stage of the causal fungus, *Macrophoma* sp. in Korea. Kim et al. (1982) reported that the causal fungus of this apple rot may be *Botryosphaeria ribis*, in view of the symptom strikingly resemble to white rot and the identification of this fungus by Commonwealth Mycological Institute in England.

Considerable literature has been published regarding the etiology, and cultural characteristics of *Botryosphaeria ribis* and the rotting of apple fruits by this fungus (Fenner, 1925; Luttrell, 1948; Sitterly and Shay, 1960; Fulkerson, 1960; Wallace *et al.*,

1962; Wallace *et al.*, 1962).

The objectives of the research reported herein were: (1) to examine whether the susceptibility of developing apple fruits to infection by *Macrophoma* sp. varies with the stage of development or not; and (2) to determine the levels of total soluble carbohydrates and amino acids on healthy and infected apples at various stages of maturity.

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Materials and Methods

***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. The temperature effect on apple rot was examined on the cultivar Fuji harvested at November, 1981.

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 apple fruits inoculated were covered with a vinyl wrapper. The lesion enlargement on apples was measured at intervals after inoculation. On the 10th day after inoculation, healthy and infected apple fruits were collected 10 July, 10 August and 10 September for analysis of carbohydrates and amino acids.

Extraction and determination of total ethanol-soluble carbohydrates and amino acids: Immediately after the apples were peeled with a razor, 10g fresh weight of apple flesh was boiled in 60%(v/v) ethanol for 10 min. The flesh extract was decanted and 10 ml 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 14,000 g for 15 min. The final volume of the resulting supernatant was made to 100 ml of 60% ethanol. Aliquots of the ethanol-water extracts were used in determining the amount of total carbohdrates and amino acids. Total carbohydrate analysis was made according to the phenol-sulphuric acid method of Dubois et al. (1951). Concentrations of reducing sugars were assayed by the arsenomolybdate method (Nelson, 1944). The contents of total amino acids were determined by ninhydrin method of Yemm and Cocking (1955).

Results and Discussion

Disease development: To determine whether the susceptibility of developing apple fruits to infection by *Macrophoma* sp. varies with the stage of development or not, apple fruits of two cultivars Fuji and Miller were inoculated by an isolate of *Macrophoma* sp. at various maturity stages of apples in the orchard. The changes in lesion size during the maturing of apples is presented in Fig. 1. Immature fruits of both cultivars Fuji and Miller were completely resistant until 10 July. In this period, the isolate of *Macrophoma* sp. did not grow on apple fruits. When inoculated on 31 July, apples became susceptible to *Macrophoma* sp., irrespective of cultivar. This fungus grew better on Fuji than Miller. The rotting of apples by this fungus in September was slower than that in August. This phenomenon may be explained by reduced fungal growth in the apples due to decreased temperature (ca. 20°C) in September as compared with August. (ca. 30°C).

The enlarging areas of apple rot (Cultivar Fuji) at 20°C and 25°C under controlled conditions were examined 3 and 7 days after inoculation with *Macrophoma* sp. (Fig. 2). Lesion enlargement on apple at 25°C was remarkably pronounced relative to 20°C.

The data obtained in this investigation indicate that the susceptibility of apple fruits to *Macrophoma* sp. varied with the stage of development. This fact is in agreement with the findings of Sitterly and

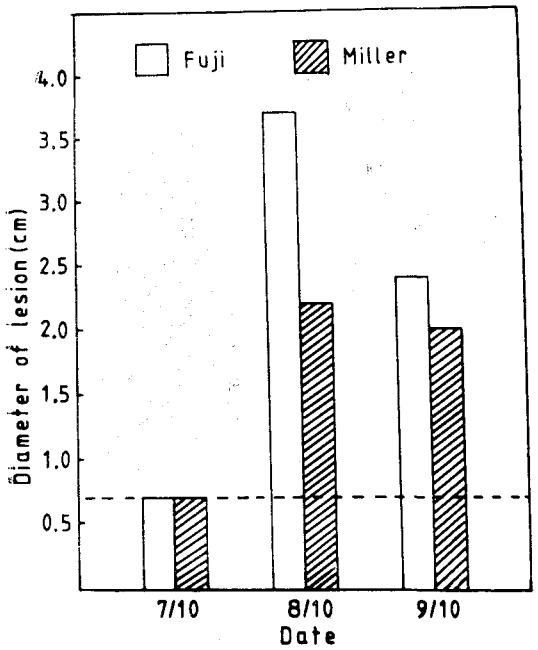

Fig. 1. Changes of lesion size in differently maturing apple fruits of cultivars Fuji and Miller inoculated by *Macrophoma* sp. in the orchard. Lesion sizes were measured on 10th day after inoculation with the mycelial agar disks (7mm ϕ).

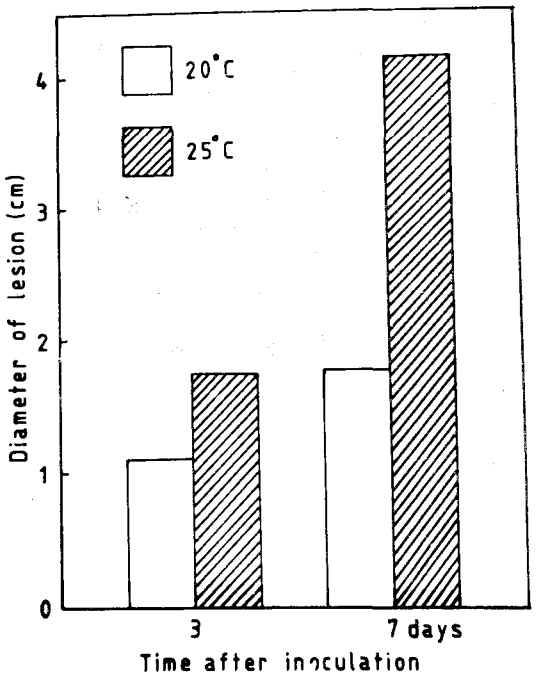

Fig. 2. Lesion enlargement in apple fruits of cultivar Fuji infected by *Macrophoma* sp. at 20°C and 25°C.

Shay (1960). This characteristic of delay of the natural occurrence of fruit rot until the fruit becomes mature may be associated with several physiological and structural changes in the apple fruit.

Carbohydrate and amino acid content in healthy and infected apples: Substantial changes in carbohydrate and amino acid contents of apple fruits infected by *Macrophoma* sp. may reflect the alterations in various chemical constitution favorable or unfavorable for fungal development. Figure 3 shows the levels of total ethanol-soluble carbohydrates in healthy and infected apples of the cultivars Fuji and Miller at various stages of maturity. In both cultivars, the amount of total soluble carbohydrates in apple fruit flesh was gradually increased as apples became mature. The healthy apples of cultivar Fuji had a higher amount of carbohydrates than those of Miller. The infected apples contained more soluble carbohydrates than the healthy ones. In particular, the high amounts of soluble carbohydrates were

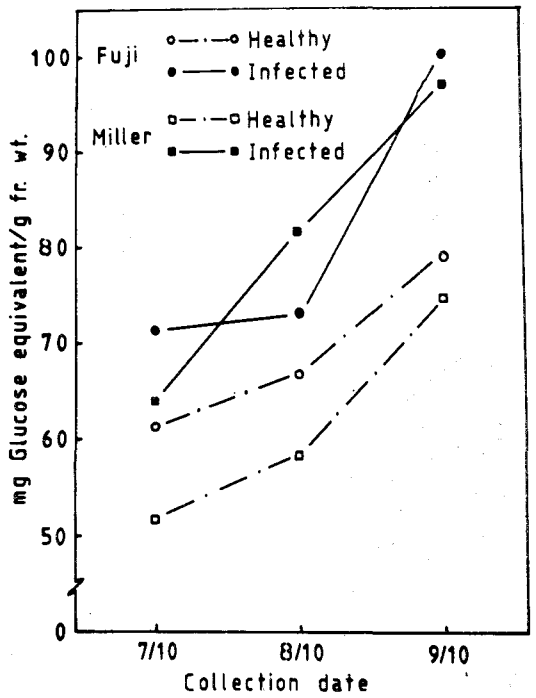

Fig. 3. Levels of total soluble carbohydates in flesh of healthy and *Macrophoma* sp. -infected apples (Cultivars Fuji and Miller) at different developmental stages.

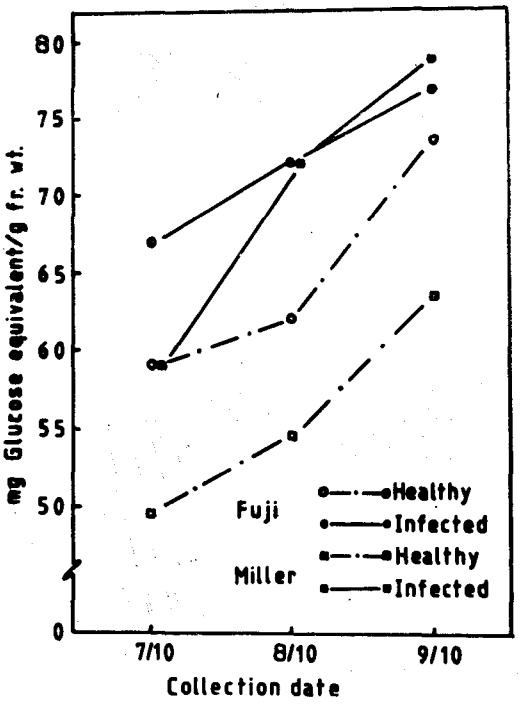

Fig. 4. Levels of reducing sugars in flesh of healthy and *Macrophoma* sp.-infected apples (Cultivars Fuji and Miller) at different developmental stages.

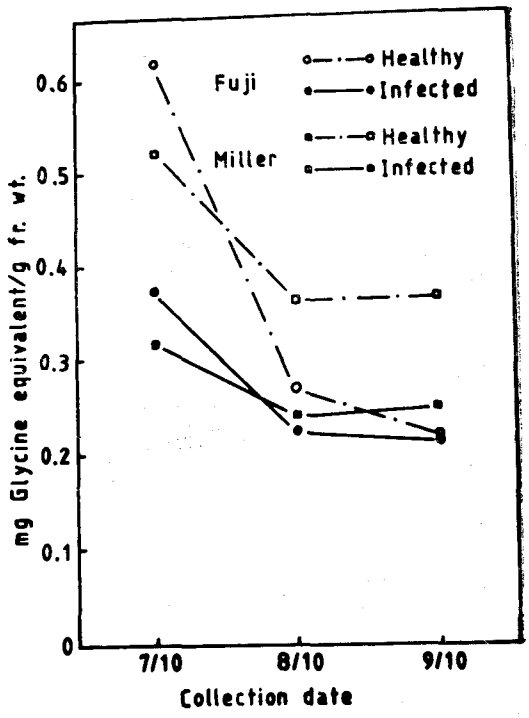

Fig. 5. Levels of total soluble amino acids in flesh of healthy and *Macrophoma* sp.-infected apples (Cultivars Fuji and Miller) at different developmental stages.

present in the apple flesh infected on 10 September. The concentrations of ethanol-soluble reducing sugars altered during the maturity of apples (Fig. 4). In healthy apples, Fuji showed a higher amount of reducing sugars as compared with Miller. The increase in these sugars was remarkably great in the late developmental stages of apples. The amount of reducing sugars increased markedly in infected apples of both cultivars.

There are mainly fructose, glucose and sucrose, as soluble carbohydrates, in apples. Sugar contents in American apple cultivars are fructose 3.8~6.8%, glucose 0.9~2.7%, sucrose 1.4~4%. Starch in apples is present in a very small amount. The average cellulose content in apple fruit flesh amounts to 0.44% (Drews, 1968).

The lack of critical concentration of total soluble carbohydrate and reducing sugars in immature fruits suggests that some soluble sugars such as fructose, glucose or sucrose may be partially involved in immature fruit resistance to *Macrophoma* sp. In infected apple fruits, a rise in soluble carbohydrate or reducing sugar concentrations may be due to the increased conversion of polysaccharides such as cellulose or pectin to the soluble sugars for the nutrient utilization by this fungus.

The levels of total ethanol-soluble amino acids in healthy and infected apples of the cultivars Fuji and Miller shown in Fig. 5. In health apples, there were no consistent differences between Fuji and Miller in amount of total soluble amino acids. With increasing age of apples, the amount of soluble amino acids declined in the fruit flesh of both cultivars. As compared with the healthy apples, the lower amounts of soluble amino acids were found in immature apples inoculated with *Macrophoma* sp. on 30 June.

The amino acids may has direct effect on the fungus, as a fungicide, or may be involved with the metabolism of the host in association with increased resistance of the plant (Van Andel, 1966). Aspara-

gine, aspartic acid and glutamic acid, as free amino acids, are present in apples (Burroughs, 1957). Kuc et al. (1957) reported that leaves of apple cultivars susceptible to *Venturia inaequalis* showed a resistant reaction after injection of phenylalanine into the petiole. From our study, it seems likely that the increase in content of amino acids in immature apple fruits may contribute to the inhibitory effect of some substances already present in the leaves on *Macrophoma* growth or may has a direcct effet on this fungus. The decrease in amino acid content in infected apple fruits may be due to uptake of amino acids from host into the fungal cells. However, it is difficult to decide whether or not changes in amino acid content is associated with the immature fruit resistance to *Macrophoma* sp. Further studies on biochemical and structural components of apple fruits will be necessary to establish more reliable criteria for the characterization of resistance of apple to *Macrophoma* sp.

要 約

사과品種 후지와 밀러의 未熟果는 7月 10日까지 사과 腐敗病菌(*Macrophoma* sp.)에 對해 완전히 抵抗性이었다. 7月 31日에 接種하였을 때 品種에 關係없이 感受性이 되었고 이곰팡이는 밀러보다 후지에서 더 잘자라났다. 25°C에서 사과腐敗가 20°C에 比하여 뚜렷하였다.

兩品種의 사과 果肉에서 總炭水化物, 還元糖은 사과가 成熟됨에 따라 점차 增加했다. 健全한 과일에서는 후지가 밀러보다 더 높은 炭水化物을 含有했으며 感染된 사과가 健全果에 比해서 水溶性 炭水化物을 더 많이 含有했다.

健全果에서 總水溶性 아미노酸含量에 후지와 밀러사이에 差異가 없었고 사과가 成熟함에 따라 總아미노酸含量은 果肉에서 減少되었다.

果肉에서 炭水化物, 아미노酸 含量을 사과腐敗病에 對한 未熟果의 抵抗性과 聯關시켜 論하였다.

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