

Biochemistry of postharvest spoilage of sweet potato (*Ipomoea batatas* L.): Changes in starch, total sugar, proline and ascorbic acid content

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ABSTRACT

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Java black rot and soft rot are two important postharvest diseases of sweetpotato caused by *Botryodiplodia theobromae* Pat. and *Rhizopus oryzae* Went & Prins. Geerl., respectively. There was a gradual decline in starch and ascorbic acid contents in sweet potato roots following harvest and this decline was further aggravated by infection by these putative fungi *B. theobromae* and *R. oryzae*. However, the total sugar and proline contents did not exhibit much variation between fungi-infected and uninfected tubers.

Keywords: *Botryodiplodia theobromae*, *Ipomoea batatas* L., java black rot, *Rhizopus oryzae*, soft rot, spoilage, sweetpotato

INTRODUCTION

Sweetpotato (*Ipomoea batatas* L.) is one of the important root crops grown in India and many other parts of the world, being cultivated in more than 100 countries (Woolfe, 1992). Besides being a rich source of starch, proteins, minerals and vitamins, the crop produces high amount of food per unit area per unit time and is capable of growing in marginal conditions.

Like any other vegetable crop, sweetpotato is subjected to several forms of post harvest wastages such as physical damage, weight loss, pathological decay, sprouting and weevil (*Cylas formicarius*) infestations (Wagner *et al.*, 1983; Ray and Balagopalan, 1997; Ray *et al.*, 1991). Spoilage or rottage is the most significant form of wastage in sweetpotato caused by either fungi or bacteria, which accounts for 20-25 percent of post harvest losses (Ray and Balagopalan, 1997). Although there have been several studies on physiological and biochemical changes associated with postharvest microbial infection in horticultural crops (Prasad, 1977; Thornton and Workman, 1987), very little is known about the biochemistry of sweetpotato spoilage (Uritani *et al.*, 1984). From our previous studies, we reported that the fungi *Botryodiplodia theobromae* Pa. and *Rhizopus oryzae* Went & prins. Geerl. are two important fungi associated with sweetpotato spoilage. The present paper deals with the postharvest physiology of sweetpotato infected with the above two fungi, particularly in relation to starch-sugar, proline and ascorbic acid metabolism.

MATERIALS AND METHODS

Sweetpotato tubers

Freshly harvested sweetpotato (var. Gouri, 606, 701 and 704) tubers, were collected from the experimental farm of the Regional Center of CTCRI, Bhubaneswar during the year 1990-2000 (January-March). The roots were of medium size (100-180g) and were used within 24 h after harvest.

Fungi isolates

The isolates of *B. theobromae* (IMI 361230) and *R. oryzae* (IMI 361235) used in these studies were previously isolated from the postharvest decay of sweetpotato tubers (Ray and Misra, 1995). Spore suspensions of *B. theobromae* and *R. oryzae* were prepared from 7 day old cultures grown at 30°C on potato-dextrose agar (PDA). Spores were harvested in sterile distilled water and diluted to a concentration of 5.5×10^6 spores/mL. The same concentration was used throughout the experiments.

Biochemistry of spoilage

Freshly harvested and healthy roots were taken, washed in tap water, air dried and surface sterilized with 70 % alcohol. These roots were inoculated with mycelial discs of fungi (*B. theobromae* or *R. oryzae*) following the method described by Ray and Misra (1995) and Ray and Punithalingam (1996). Ten roots were inoculated for each replication and three such replications were maintained at room temperature ($30 \pm 2^\circ\text{C}$) for 15 days. Similar number of roots were stuffed with agar (in place of fungus) and kept as control. Both inoculated and non-inoculated (control) roots were analyzed for starch, total sugar, proline and ascorbic acid contents (Mahadevan and Sridhar, 1998) at five-day interval.

Estimation of total sugar and starch

Since sweetpotato roots are rich in starch and sugar to the extent of 20-25% and 5-10% of raw dry matter, respectively (Woolfe, 1992), only 10 mg of the tissues (from cortex and pith) were homogenized with 5 ml of 80% ethanol and transferred to borosilicate test tubes (18x150 mm). The tubes were kept on a water bath at $80-85^\circ\text{C}$ for 10 min., cooled and then centrifuged at 4000 rpm for 15 min. The supernatants were preserved and then extraction process was repeated 3 times. All the supernatants were combined and kept overnight at room temperature ($30 \pm 2^\circ\text{C}$) to evaporate off the ethanol content. Then the concentrates were diluted with 5 ml of distilled water and were transferred to centrifuge tubes. These test tubes were then centrifuged at 4000 rpm for 10 min and supernatants were collected for analysis of total sugar.

The residues (starch) remaining after alcoholic extraction were dried overnight and then added with 2 ml of distilled water followed by 2 ml of 2N HCl. The tubes were kept in boiling water bath for about 20 min with occasional stirring. The completion of hydrolysis was checked by the absence of blue color with N/10 iodine solution. The tubes were cooled and centrifuged at 4,000 rpm for 10 min. Finally the supernatants were made up to the volume of 10 ml by addition of distilled water. The extract was used for estimation of starch as glucose equivalent.

Estimation of total sugar

Total sugar was estimated using the anthrone reagent. The reagent was prepared by dissolving 2 grams of anthrone in a liter of concentrated H_2SO_4 . Two ml each of anthrone reagent placed in test tubes (25 x 100 mm) were chilled in ice bath. Then 1 ml of the extract was layered carefully over the reagent and was allowed to chill. The tubes were then shaken vigorously while still emerged in ice bath. They were then brought to ambient temperature and boiled in water bath for about 10 minutes. A reagent blank was prepared similarly. After cooling, the absorbance was read at 625 nm. Total sugar content was calculated using a standard curve drawn with 0-50 $\mu g/ml$ of D-glucose.

Estimation of starch

Starch content was estimated as glucose equivalent by the anthrone reagent method as described above. The starch content was calculated in terms of glucose equivalent and a conversion factor of 0.9 was used to convert the values of glucose to starch (Mahadevan and Sridhar, 1998).

Proline extraction and assay

Tissues (from cortex and pith) weighing about 1g were homogenized with 5 ml of 3% aqueous sulphosalicylic acid and the homogenate was centrifuged at 4,000 rpm for 10 min. The following reagents were prepared for the purpose of proline estimation.

Acid ninhydrin was dissolved in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 M ortho-phosphoric acid with agitation and slight warming. The reagent was stored in a refrigerator and used within 24 h. The assay mixture for proline estimation consisted of 2 ml of supernatant of standard proline solution, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid.

The contents of the tubes were mixed thoroughly and heated in a boiling water bath for 1 h. The reaction was terminated in an ice bath followed by addition of 4.0 ml of toluene. The contents were mixed vigorously and then allowed to separate into phases.

The chromatophore containing proline (upper toluene phase) was carefully taken out by a dropper and the absorbance was read at 520 nm. The proline content was calculated from a standard curve prepared with 0-20 $\mu\text{g/ml}$ of proline.

Ascorbic acid extraction and assay

Tissues (from cortex and pith) weighing about 2 g were homogenized with 10 ml of 0.4% oxalic acid and centrifuged at 50°C for 15 min in a refrigerated centrifuge. The clear supernatant was taken for ascorbic acid estimation.

Five ml extract in a 100 ml beaker was titrated with a standardized sodium 2,6-dichlorophenol indophenol reagent until the solution became pink which persisted for at least 30 sec. The ascorbic acid contents of the extract were computed using the formula:

$$I \times S \times D/A \times 100/W = \text{mg ascorbic acid/ 100g tissue fresh weight basis where,}$$

I= ml of indophenol reagent used in the titration

S= mg of standard ascorbic acid reacting with 1 ml of indophenol reagent

D= volume of the extract in ml

A= the aliquot titrated in ml

W= the weight of the samples in g

fwb= fresh weight basis

Analysis of variance (ANOVA) was performed using the INDOSTAT software following completely randomized block design. Mean comparison within treatments was performed by least significant differences (LSD) test at $p=0.05$ (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Botryodiplodia theobromae and *Rhizopus oryzae* were reported earlier as two predominant fungi associated with sweetpotato spoilage (Ray and Balagopalan, 1997; Ray and Misra, 1995; Ray and Punithalingam, 1996). Further studies were carried out on these two fungi.

Table 1. Changes in starch * (g/100g fwb) content of sweetpotato roots infected with fungus *Botrydiplodia theobromae*

Variety	Parameter	Days after infection			
		0	5	10	15
Gouri	Uninfected	22.7(6.8)**	21.0(7.4)	18.6(5.3)	16.9(2.5)
	Infected	22.7(6.8)	18.7(6.0)	11.7(6.7)	9.5(11.0)
606	Uninfected	18.2(10.3)	17.6(10.3)	14.0(4.5)	12.4(10.8)
	Infected	18.2(10.3)	9.5(4.8)	6.8(10.0)	4.7(18.6)
701	Uninfected	31.5(9.3)	30.3(11.0)	22.5(14.8)	19.4(14.8)
	Infected	31.5(9.3)	22.1(4.9)	21.0(4.7)	20.9(11.3)
704	Uninfected	16.7(4.3)	16.7(7.0)	15.8(8.2)	16.0(6.4)
	Infected	16.7(4.3)	16.4(4.8)	14.4(4.8)	10.0(10.9)

* LSD at 0.05 level between treatments is 1.77 (starch) and 0.69 (sugar)

** Corresponding total sugar content (g/100 g fresh weight basis (fwb)) in parenthesis.

Changes in starch and total sugar content

Tables 1 and 2 show the changes in starch and total sugar contents of four sweetpotato varieties (Gouri, 606, 701 and 704) following infection by *B. theobromae* and *R. oryzae*, respectively. In general, there was a gradual decline in starch content during the storage for a period of 15 days. This was due to the natural process of aging of the roots due to storage (Uritani, 1999). The decline in starch content was further accentuated in roots infected with fungi. The enhanced decline in starch content was expected as the fungi could have degraded some amount of starch to sugar for its normal metabolism. Unpublished results of preliminary studies revealed that these fungi produced α -amylase in vitro which possibly converted sweetpotato starch to sugar (Collins and Walter, 1985). In contrast to gradual decline in starch content, there was no particular pattern of variation in total sugar content. Further, the variation in total sugar content between infected and non-infected samples did not follow a definite trend (Tables 1 and 2). This might be due to the utilization of some sugars in normal metabolism of the roots and pathogens as well.

Table 2. Changes in starch* (g/100 g fwb) content of sweetpotato tubers infected with fungus *Rhizopus oryzae*

Variety	Parameter	Days after infection			
		0	5	10	15
Gouri	Uninfected	22.7(6.8)**	19.4(4.8)	12.6(7.5)	9.9(4.3)
	Infected	22.7(6.8)	14.4(4.5)	11.9(7.9)	10.4(8.9)
606	Uninfected	18.2(10.3)	10.1(2.4)	13.7(7.1)	17.1(4.3)
	Infected	18.2(10.3)	9.4(4.3)	12.6(5.5)	10.5(8.7)
701	Uninfected	31.5(9.3)	27.0(6.8)	22.7(7.8)	22.7(7.8)
	Infected	31.5(9.3)	24.5(6.8)	21.8(8.5)	14.1(10.0)
704	Uninfected	16.7(4.3)	12.7(8.4)	18.5(8.2)	11.2(6.4)
	Infected	16.7(4.3)	13.4(5.5)	10.0(8.6)	8.9(10.5)

* LSD at 0.05 level between treatments is 1.77 (starch) and 0.69 (sugar)

** Corresponding total sugar content (g/100 g fresh weight basis (fwb)) in parenthesis.

Previous studies on starch-sugar metabolism of horticultural crops infected by plant pathogens showed related results. Raman and Sankaran (1989) reported that in grapes infected with *Curvularia* there was a decline in total sugar during storage and such decline was more pronounced in infected berries than in healthy ones, perhaps due to utilization of some of the sugars by the microorganism. Our results corroborated these studies. Likewise, Otazu and Secor (1981) showed that there was a highly positive significant correlation between reducing sugar content and soft-rot severity in potato tubers over a wide range of conditions like temperature, cultivars, tuber portion and tuber age. Total sugar and non-reducing sugars tended to decrease and reducing sugar increased in radish with prolongation of storage period as well as infection by *Aspergillus flavus* (Prasad *et al.*, 1990). Our studies showed that sweetpotato infected roots suffered a rapid degradation of starch as compared with the non-infected ones, in conformity with the results of Prozedziecki and Murawa, 1991).

Changes in proline contents

It has been reported that plants synthesize and accumulate amino acid proline (Bogges *et al.*, 1974) under stress environment such as drought, salt and pathogen infection (Chu *et al.*, 1974, and 1978). Hence, proline accumulation is considered to be a parameter of stress (Singh *et al.*, 1972; Stewart, 1973) imposed upon the plant/plant parts due to adverse environments (Mohanty and Sridhar, 1982) such as drought (Blum and Ebersson, 1976; Singh *et al.*, 1972) salt (Cavalieri and Huang, 1979) Chu *et al.*, 1976; Stewart and Lee, 1974), temperature (Chu *et al.*, 1974) and pathogen infection (Sinha *et al.*, 1984) leading to aging and senescence (Uritani, 1999). Since fungal infection of tubers alters the host metabolism in several ways comparable to abiotic stresses like water or salt stress (Mohanty and Sridhar, 1982), it was presumed that infection by fungi would induce proline accumulation in sweetpotato roots. Tables 3 and 4 show that there was no definite trend in proline accumulation in fungi-infected and uninfected roots. In most of the samples, accumulation of proline was 1.5-2 folds higher in uninfected roots compared with the infected ones. These results, therefore, do not corroborate with the findings by several researchers. For example, accumulation of free proline was reported in sugarcane leaves infected with *Colletotrichum falcatum* (Bhansali *et al.*, 1983). Similarly, Mohanty and Sridhar (1982) reported an increase in proline content in rice leaves infected with tungro virus. The earlier belief (Singh *et al.*, 1972; Thomas and Stoddart, 1980) that proline accumulation by stressed plants is a metabolic adaptation to help them withstand adverse environmental conditions, a view based on the contention that proline acts as a nitrogen reserve and is a transportable nitrogen-containing material during stress, has often been questioned (Hanson *et al.*, 1977, 1979; Tully *et al.*, 1979). Although proline is considered to function as an organic solute in the stressed tissues (Greenway and Munns, 1980), the physiological significance of its accumulation is not clear, either during abiotic (Tully *et al.*, 1979) or biotic stress (pathogen infection) as shown by our study

Table 3. Changes in proline* (g/100g fwb) content of sweetpotato roots infected with fungus *Botrydiploia theobromae*

Variety	Parameter	Days after infection			
		0	5	10	15
Gouri	Uninfected	1.07	2.26	3.07	2.65
	Infected	1.07	1.70	2.23	2.08
606	Uninfected	2.00	2.18	2.15	3.75
	Infected	2.00	1.91	1.45	2.49
701	Uninfected	2.32	3.26	3.35	3.35
	Infected	2.32	1.55	1.35	1.71
704	Uninfected	3.04	2.86	3.30	4.23
	Infected	3.04	3.49	2.06	3.16

*LSD at 0.05 level between treatments is 0.23

Table 4. Changes in proline* (g/100g fwb) content of sweetpotato roots infected with fungus *Rhizopus oryzae*

Variety	Parameter	Days after infection			
		0	5	10	15
Gouri	Uninfected	1.07	4.43	2.06	3.08
	Infected	1.07	2.28	1.08	1.60
606	Uninfected	2.00	2.65	2.73	3.89
	Infected	2.00	1.36	1.18	0.90
701	Uninfected	2.32	3.05	4.28	4.04
	Infected	2.33	3.28	3.68	0.70
704	Uninfected	3.04	3.99	2.88	4.15
	Infected	3.04	3.87	2.29	4.59

*LSD at 0.05 level between treatments is 0.12

Changes in ascorbic acid content

Vitamin C is a vital component of sweetpotato roots having therapeutic values (Woolfe, 1992). It occurs in both the reduced (ascorbic acid) and oxidized (dehydroascorbic acid) forms. The dehydroascorbic acid content in the four varieties studied was almost non-significant (3-5 mg/100g fwb). However, their ascorbic acid content varied from 17 mg to 30 mg/100 g fwb (Figs. 1 and 2). But there was a gradual decrease in ascorbic acid content in roots following harvest. For example, in the variety "Gouri", the ascorbic acid content decreased from 30 mg/100 g fwb (at harvest) to 15 mg/100 g fwb after 12 days of harvest. Likewise, in varieties 606, 701, and 704, the ascorbic acid content decreased to 77, 70 and 67 percent of the initial contents, respectively, after 12 days of harvest. This is normal since the decline in ascorbic acid content is considered as a parameter of aging roots following storage (Woolfe, 1992). Moreover, the infection by either *B. theobromae* or *R. oryzae* drastically decreased the ascorbic acid content to very low levels. For example, in the variety "Gouri", ascorbic acid content decreased from 30 mg/100 g fwb (0day) to 3 mg/100 g fwb after 12 days of harvest either in *B. theobromae* or *R. oryzae* infected roots (Figs. 1 and 2). The trend was likewise similar with the other varieties.

Several earlier reports showed that ascorbic acid content in plants decreased following pathogen infection (Agrawal and Ghose, 1979; Chile and Hyang, 1979; Thornton and Workman, 1987). Prasad (1977) reported that banana fruits inoculated with *Helminthosporium spiciferum* (*Cochliobolus spicifer*) showed a decline in ascorbic acid content as compared with uninoculated fruits. This was perhaps due to the utilization of ascorbic acid, in part, by the pathogen as well as acceleration of aging following pathogen infection (Uritani, 1999). Agrwal and Bisan (1976) reported that apple fruits inoculated with *Aspergillus niger* and *Alternaria tenuis* showed drastic decline in ascorbic acid content compared with uninoculated fruits. Results from our studies confirm the trend that fungal infection decreases ascorbic acid content in plants, fruits and vegetables more than the healthy tissues.

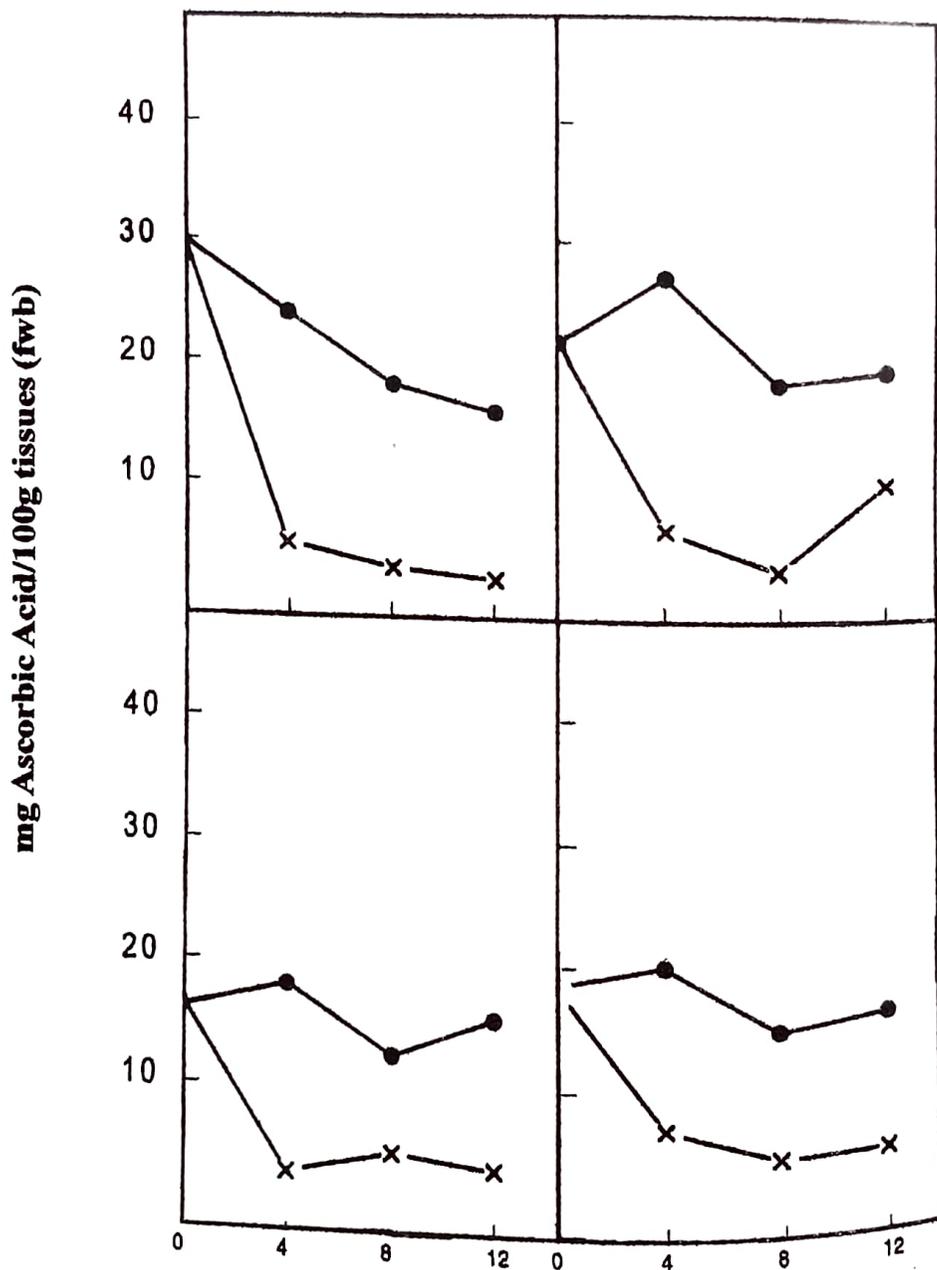


Fig.1. Changes in ascorbic acid content in sweet potato roots (var. Gouri, 606, 701 and 704) following infection by *Botrydiplodia theobromae* (●—●—●, uninfected; x---x---x, infected with the fungus)

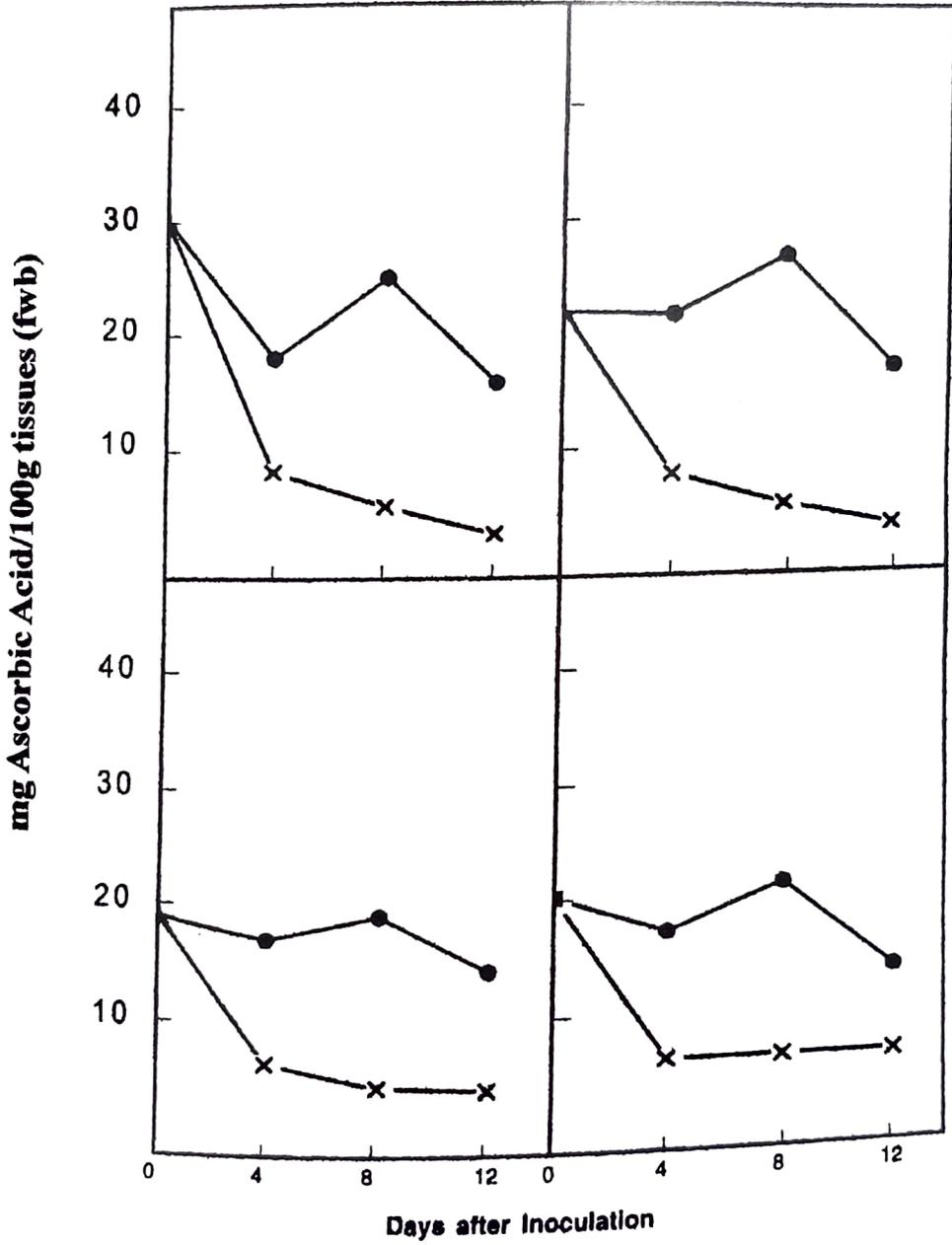


Figure 2. Changes in ascorbic acid content in sweetpotato roots (var. Gouri, 606, 701 and 704) following infection by *Rhizopus oryzae* (●---●---●, uninfected; x---x---x, infected with fungus).

To summarize, there was a gradual decline in starch and ascorbic acid content in sweetpotato following harvest. This decline was further enhanced as a consequence of infection by either *B. theobromae* or *R. oryzae*. But neither proline nor total sugar content increased in fungus-infected tissues as compared with healthy tissues.

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