J. Mycopathol. Res. 38(2) : 59–64 (2000) Printed in India

Physiology of post-harvest spoilage of sweet potato (*Ipomoea batatas* L.)

SWAYAM PRAVA PATI AND R. C. RAY
Regional Centre of Central Tuber Crops Research Institute, Bhubaneswar 751019, Orissa

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

Key words : Java black, soft rot, sweet potato, *Botryodiplodia theobromae, Rhizopus oryzae*

INTRODUCTION

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

Like any other vegetable crop, sweet potato is subjected to several forms of post-harvest wastage such as physical damage, weight loss, pathological decay, sprouting and weevil (*Cylas formicarius*) infestations (Wagner *et al.*, 1983 ; Ray *et al.*, 1991 ; Ray and Balagopalan, 1997). Spoilage or rot is the most significant form of wastage in sweet potato caused by either fungi or bacteria which accounts for 20-25 percent of post-harvest loss (Ray and Balagopalan, 1997). Although there are several studies on the physiological and bio-chemical changes associated with post harvest microbial infection in horticultural crops (Prasad, 1977 ; Thomson, 1979 ; Otazu and Secor, 1980 ; Thornton and Workman, 1987), very little is known on the physiology of sweet potato spoilage (Uritani *et al.*,1984). From our previous studies, it was reported that the fungi *Botryodiplodia theobromae* Pat. and *Rhizopus oryzae* Went & Prins. Geerl. are two important fungi associated with sweet potato spoilage or rot. The present paper deals with the post-harvest physiology of sweet potato infected with the above two fungi, particularly in relation to starch-sugar, proline and ascorbic acid metabolism.

MATERIALS AND METHODS

Sweet potato tubers

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

Fungal isolates

The isolates of *B. theobromae* (IMI 361230) and *R. oryzae* (IMI 361235) used in these studies were previously isolated from the post harvest decay of tuberous roots of sweet potato (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.

Physiology of spoilage

Freshly harvested and healthy tubers were taken, washed in tap water, air dried and surface sterilized with 70 per cent alcohol. These roots were inoculated with mycelial discs of the fungi (*B. theobromae* or *R. oryzae*) following 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± 2°C) for 15 days. Similar number of roots were stuffed with agar discs (in place of fungus) and kept as control. Both inoculated and non-inoculated (control) roots were analyzed for starch, total sugar, proline and ascobic acid contents at five days intervals.

Extraction of total sugar and starch

Since tuberous roots of sweet potato 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 borosillicate test tubes (18 × 150 mm). The tubes were kept on a water bath at 80-85°C for 10 min, cooled and then centrifuged at 4,000 rpm for 15 min. The supernatants were preserved and the extraction process was repeated for 3 times. All the supernatants were combined and kept under fan at room temperature (30± 2°C) to evaporate off ethanol. Then the concentrate(s) were diluted with 5 ml of distilled water and were transferred to centrifuge tubes. These test tubes were then centrifuged at 4,000 rpm for 10 min and supernatants were collected for analysis of total sugar.

The residues remaining after alcoholic extraction were dried under fan. Then 2 ml of distilled water was added to the residue followed by 2 ml of 2N HCl. The tubes were kept on a boiling water bath for about 20 min with occasional stirring. The completion of hydrolysis was checked by absence of blue colour 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. These extracts were used for estimation of starch, as glucose equivalent.

Estimation of total sugar

The total sugar was estimated using the anthrone reagent. The anthrone reagent was prepared as follows. Two gm of anthrone reagent was dissolved in 1 liter of concentrated H_2SO_4. Two ml of freshly prepared anthrone reagent was taken in a wide-mouth test tube and chilled in ice bath. Then 1 ml of sugar extract solution was layered carefully over the reagent and was allowed to chill. Tubes were then shaken vigorously while still emerged in the ice-bath. Then the tubes were brought to room temperature and boiled in water bath for about 10 min. A reagent blank was prepared similarly. After cooling, the absorbancy was taken at 625 nm. Total sugar content was calculated following a standard curve drawn with 0-50 µg of D-glucose per assay.

Estimation of starch

The starch content was calculated in terms of glucose equivalent by the anthrone reagent method using a conversion factor of 0.9 to convert the values of glucose to starch (Mahadevan and Sridhar, 1996).

Proline extraction and assay

About 1 g tissues (cortex and pith) were homogenized with 5 ml of 3% of aqueous sulphosalicylic acid and the homogenate was centrifuged at 4,000 rpm for 10 min. Proline content of the supernatant was estimated following the method described by Bates *et al.* (1973). Following reagent was prepared for the purpose of estimation. Acid ninhydrin reagent-1.25 g of 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 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 tubes were vertexed 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 upper toluene phase was carefully taken out by a dropper and the absorbancy was taken at 520 nm. The proline content was

calculated from a standard curve prepared with 0-20 µg of proline per assay.

Ascorbic acid extraction and assay

Ascrobic acid (AA) was extracted by the method described by Mahadevan and Sridar (1986). About 2 g of tissue (from cortex and pith) was homogenized with 10 ml of 0.4% oxalic acid and centrifuged at 5°C for 15 min in a refrigerated centrifuge. The clear supernatant was taken for ascorbic acid estimation.

Five ml extract(s) taken in a 100 ml beaker were titrated against the 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 calculated by using the formula :

$I \times S \times D/A \times 100/W$ = mg AA/100g tissue (fwb)

where, I = ml of Indophenol reagent used in the titration ; S = mg of standard AA 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 by INDOSTAT softwere using completely randomized block design. Mean comparison within treatments was performed by least significant differences (LSD) test at p=0.05 level (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

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

Changes in starch and total sugar contents

Tables 1 and 2 show the changes in starch and total sugar contents in four sweet potato varieties (Gouri, 606, 701 and 704) following infection by *B. theobromae* and *R. oryzae*. In general, there was a gradual decline in starch content in sweet potato during storage for a period of 15 days. The decline in starch content was further accentuated in tuberous root infected with the 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. Preliminary studies with these fungi revealed that they produced α-amylase in substantial quantity (unpublished results) which might have converted sweet potato starch to sugars. In contrast to the gradual declining trend 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 also follow a definite trend (Tables 1 and 2).

Table 1 : Changes in starch content of sweet potato tubers infected with *Botryodiplodia theobromae*

Variety	Parameters	Starch (g/100 g fwb)* 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)	16.7(4.8)	14.4(10.3)	10.0(10.6)

**LSD at 0.05 level between treatments is 1.57 (starch) and 0.69 (sugar)

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

Table 2 : Changes in starch content of sweet potato tubers infected with *Rhizopus oryzae*

Variety	Parameters	Starch (g/100 g fwb)* Days after infection			
		0	5	10	15
Gouri	Uninfected	22.7(6.8)**	19.5(4.8)	12.6(7.5)	9.9(4.3)
	Infected	22.7(6.8)	14.7(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(6.2)	11.2(5.3)
	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/100g fresh weight basis (fwb))

There are some studies on starch-sugar metabolism of horticultural crops infected by plant pathogens. Raman and Sankaran (1989) reported in

grapes infected with *Curvularia* that there was a decline in total sugar during storage and such decline was more pronounced in infected berries than healthy ones. Likewise, Otazu and Secor (1980) 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. Our studies showed that infected tuberous roots of sweet potato suffered a faster degradation of starch as compared with the non-infected roots, in conformity with the results obtained form other horticultural crops.

Changes in proline content

Proline accumulation is considered to be a parameter of stress (Stewart, 1973 ; Singh *et al.*, 1972) imposed upon the plants/plant parts due to adverse environment (Mohanty and Sridhar, 1982) such as drought (Singh *et al.*, 1972 ; Blum and Ebercon, 1976 ; Boggess *et al.*, 1976), salt (Stewart and Lee, 1974 ; Chu *et al.*, 1976 ; Cavalieri and Huang, 1979), temperature (Chu *et al.*, 1974 ; 1978) and pathogen infection (Sinha *et al.*, 1984). Since fungal infection of tubers alters the host metabolism in several ways comparable to abiotic stresses like water or salt stress, it was presumed that infection by fungi would induce proline accumulation in tubers. Tables 3 and 4 show results on proline accumulation in fungi-infected and uninfected tuberous roots. Contrary to the hypothesis that accumulation of proline is an indicator of stress due to microbial infection, there

Table 3 : Changes in proline content of sweet potato tubers infected with *Botryodiplodia theobromae*

Variety	Parameters	Proline (mg/100 g fwb)* Days after infection			
		0	5	10	15
Gouri	Uninfected	1.07	2.26	3.07	2.65
	Infected	1.07	1.7	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 content of sweet potato tubers infected with *Rhizopus oryzae*

Variety	Parameters	Proline (mg/100 g fwb)* 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	2.32	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

was a reverse trend observed in proline accumulation. In most of the samples, accumulation of proline was 1.5-2 folds higher in uninfected tubers as compared with infected tuberous roots. These results, therefore, do not corroborate with the earlier findings by several researchers. For example, accumulation of free proline was reported in sugarcane leaves infected with *Colleotrichum falcatum* (Bhansali *et al.*, 1983 ; Sinha *et al.*, 1984).

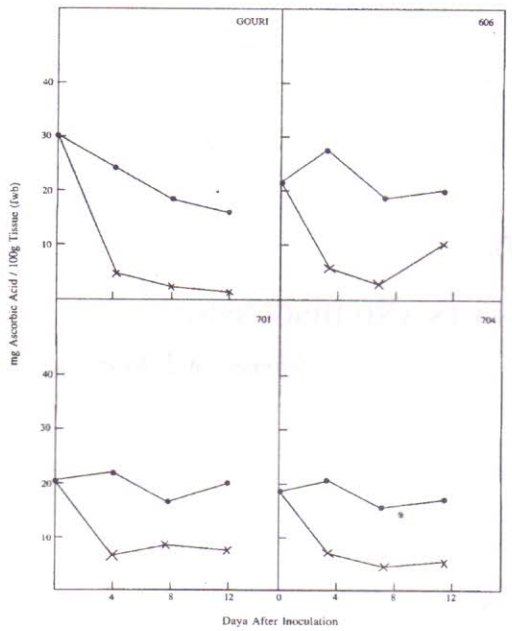

Fig. 1 : Changes in Ascorbic Acid content in sweet potato following infection by *Botryodiplodia theobromae.*

Similarly, Mohanty and Sridhar (1982) reported increase in proline content in rice leaves infected with tungro virus. Although these reports are concerned with plant leaves only, there is no reason

why the same principle will not hold good for roots. Our studies, therefore, indicate that accumulation of proline may not always stand as a 'reliable' indicator of imposed stress on plants, particularly in connection with host-pathogen interactions.

Changes in ascorbic acid content

Figs. 1 and 2 show changes in ascorbic acid content in tuberous roots of sweet potato infected with *B. theobromae* and *R. oryzae*, respectively. The hydroascorbic acid content in the four varieties studied was almost non-significant (3-5 mg/100g fresh weight basis (fwb). The α-ascorbic acid content of the four varieties under study varied from 17 mg to 30 mg /100 g fwb. But, there was a gradual decrease in ascorbic acid content in tuberous roots following harvest. For example, in 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 content respectively after 12 days of harvest.

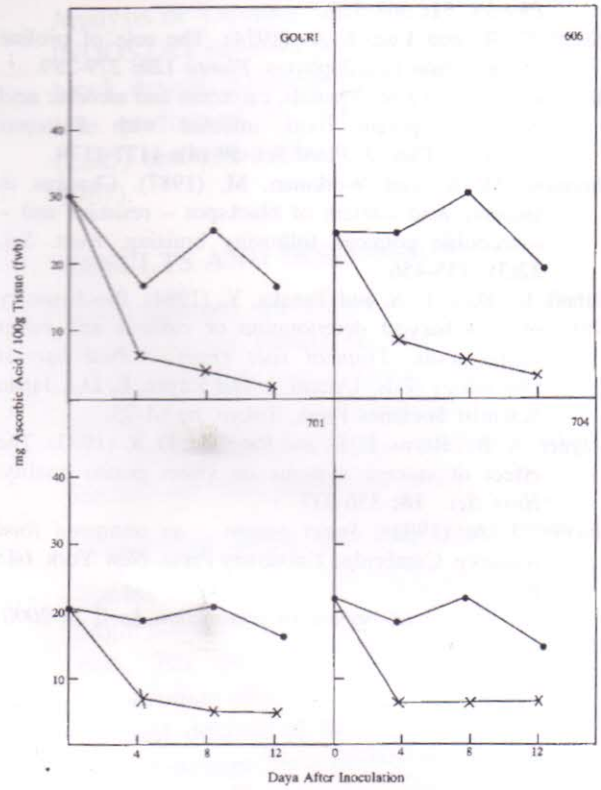

Fig. 2 : Changes in Ascorbic Acid content in sweet potato following infection by *Rhizopus oryzae.*

Moreover, infection by either *B. theobromae* or *R. oryzae* drastically decreased the ascorbic acid content to very low levels. In variety 'Gouri'. ascorbic acid content decreased from 30 mg/100 g fwb (0 day) to 3 mg/100 g fwb after 12 days of harvest in *B. thebromae* or *R. oryzae* infected roots (Figs.1 and 2). The trend was similar also for other varieties.

There are several reports in literature to show that ascorbic acid content in plant decreased following pathogen infection (Agarwal and Ghose, 1979 ; Thornton and Workman, 1987 ; Chile and Vyas, 1987). Prasad (1977) reported that banana fruits inoculated with *Helminthosporium spiciferum* (*Cochliobolus spicifer*) showed a decline in ascorbic acid content compared with uninoculated. Agarwal and Bisan (1976) reported that apple fruits inoculated with *Aspergillus niger* and *Alternaria tenuis* showed drastic decline in ascorbic acid content compared to uninoculated fruits. Thompson (1979) reported the ascorbic acid level in sweet potato infected with *Rhizopus stolonifer* to be only 20 mg/100 g compared to 32 mg/100 g (fwb) in healthy tissues. Results from our studies confirm the trend that fungal infection decreases ascorbic acid content in plants, fruits or vegetables.

Thus, there was a gradual decline in starch and ascorbic acid content in sweet potato roots following harvest. This decline was further increased as a consequence of infection by either *B. theobromae* or *R. oryzae.* The proline content did not increase in fungal-infected tissues which disagree with the 'hypothesis' that accumulation of proline is an indicator of stress either by salt, water or pathogen infection.

ACKNOWLEDGEMENTS

We thank our director for facilities. The work is funded by an adhoc grant from Indian Council of Agricultural Research, New Delhi (Code No. 10(3)/ 94-Hort II).

REFERENCES

Agarwal, G. P. and Bisan, P. S. (1976). Changes in the ascorbic acid contents of some cultivars of stored apples infected by *Aspergillus niger* and *Alternaria tennis. Phytopath Medit.* **15:** 126-127.

Agarwal, G. P. and Ghose, K. (1979). Post-infection changes in ascorbic acid content in lemon, musambi and

orange fruits induced by *Collectorichum gloeosporioides. Indian Phytopath* **32:** 108-109.

Bates, L. S. ; Waldren, R. P. and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* **39:** 205-207.

Bhansali, R. R. ; Sinha, O. K. and Singh, K. (1983). Accumulation of free proline in sugarcane leaves infected with *Colleotrichum falcatum. Indian Phytopath.* **36:** 367-368.

Blum, A. and Eberson, A. (1976). Genetic responses in sorgum to drought stress. III. Free proline accumulation and drought resistance *Crop Sci.* **16:** 428-431.

Boggess, S. F. ; Aspinall, D. and Paleg, L. G. (1976). Stress metabolism. IX. The significance of end-product inhibition of proline biosynthesis and of compartmentation in relation to stress-induced proline accumulation. *Aust. J. Plant Physiol.* **3:** 513-525.

Cavalieri, A. J. and Huang, A. H. C. (1979). Evaluation of proline accumulation in the adaptation of diverse species of marsh haloyphytes to the saline environment. *Am. J. Bot.* **66:** 307-312.

Chile, S. K. and Vyas, K. M. (1989). Metabolism of ascorbic acid and *Piper betel* leaves under Phytophthora-pathogenesis. *Perspective in Mycological Research* (Vol 1) – (Eds. Harija, S. K. ; Rajak, R. C. ; Singh, S.M.), Today and Tomorrow Printers and Publishers, New Delhi. pp. 143-150.

Chu, T. M. ; Aspinall, D. and Paleg, L. G. (1974). Stress metabolism. VI. Temperature stress and accumulation of proline in barley leaves. *Aust. J Plant Physiol.* **1:** 87-97.

Chu, T. M. ; Aspinall, D. and Paleg, L. G. (1976). Stress metabolism. VII. Salinity and proline accumulation in barley. *Aust. J. Plant Physiol.* **3:** 319-328.

Chu, T. M. ; Jusaitis, M. ; Aspinall, D. and Paleg, L. G. (1978). Accumulation of free proline at low temperature. *Physiol. Plant.* **43:** 254-260.

Mahadevan, A. and Sridhar, R. (1996). *Methods in Physiological Plant Pathology* (4th edn.), Sivakami Publications, Madras.

Mohanty, S. K. and Sridhar, R. (1982). Physiology of rice tungro virus disease : proline accumulation due to infection. *Physiol. Plant.* **56:** 89-93.

Otazu, V. and Secor, G. A. (1981). Soft rot susceptibility of potatoes with high reducing sugar contents. *Phytopathology,* **71:** 290-295.

Panse, V. G. and Sukhatma, P. V. (1967). *Statistical methods for agriculture workers.* Indian Council of Agricultural Research, New Delhi, P. 347.

Prasad, M. M. (1977) Post-infection changes in vitamin-C contents of banana fruits. *Current Science.* **46:** 197-198.

Raman, T. and Sankaran S. (1989). Biochemical changes during fruit rot of grapes caused by *Curvularia. Indian. J. mycol. and Pl. Pathol.* 19(2) **:** 211-212.

Ray, R. C. ; Bhat, P. S. ; Kumar, P. P. and Nedunzhiyan, M. (1991). Sweet potato in Orissa, *Sweet potato in South Asia : Post harvest handling, storage, process and use.* (Eds. Dayal, T. R., Scott, G. J. Kurup, G. T. & Balagopalan, C.), CIP-C.T.C.R.I., Trivandtum, pp. 83-89.

Ray, R. C. and Balagopalan, C. (1997). Post harvest spoilage of sweet potato. *Technical bulletin series* 23, Central Tuber Crops Research Institute. Trivandrum. 36 pp.

Ray, R. C. and Misra, R. S. (1995). Spoilage of sweet potato tubers in tropics. I. Microorganisms associated. *Adv. Hort. Sci.* **8:** 159-163.

Ray, R. C. and Punithalingman, E. (1996). Spoilage of sweet potato tubers in tropics. II. Java black rot by *Botryodiplodia theobromae* Pat. : Growth studies and mode of infection. *Adv. Hort. Sci.* **10:** 151-157.

Singh, T. N. ; Aspinall, D. and Paleg, L. G. (1972). Proline accumulation and varietal adaptability to drought in barley : a potential metabolic measure of drought resistance. *Nature, New Biol.* **236:** 188-190.

Sinha, O. K. ; Bhansali, R. R. and Singh, K. (1984). Free proline accumulation in response to infection by *Colleotrichum falcatum* in sugarcane. *Curr. Sci.* **53:** 493-494.

Stewart, C. R. (1973). The effect of wilting on proline metabolism in excised bean leaves in the dark, *Plant Physiol.* **51:** 508-511.

Stwart, C. R. and Lee, J. A. (1974). The role of proline accumulation in halophytes. *Planta* **120:** 279-289.

Thompson, D. P. (1979). Phenols, carotenes and ascorbic acid in sweet potato roots infected with *Rhizopus stolonifer. Can. J. Plant Sci.* **59 (4):** 1177-1179.

Thornton, M. K. and Workman, M. (1987). Changes in ascorbic acid content of blackspot – resistant and – susceptible potatoes following bruising, *Hort. Sci.* **22(3):** 455-456.

Uritani, I. ; Data, E. S. and Tanaka, Y. (1984). Biochemistry of post harvest deterioration of cassava and sweet potato roots. *Tropical root crops : Post harvest Physiology* (Eds. Uritani, I. and Reyes, E. D.), Japan Scientist Societies Press, Tokyo, pp 61-75.

Wagner, A. B. ; Burns, E. E. and Paterson, D. R. (1983). The effect of storage systems on sweet potato quality. *Hort. Sci.* **18:** 336-337.

Woolfe, J. A. (1992). *Sweet potato : an untapped food resource.* Cambridge University Press, New York. 643 pp.

(Accepted for publication April 28 2000)