SWEET POTATO TUBER ROT DISEASE IN THE PHILIPPINES

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ABSTRACT

Tuber rot or Java black rot, caused by *Diplodia theobromae* (Pat.) Nowell is the most prevalent storage disease of sweet potato. Affected tubers become dry, hard and coal-black in color. Isolations made from stored sweet potato revealed a high incidence (69%) of *D. theobromae* alone and in combination with other fungi. The occurrence of *Aspergillus, Rhizopus* and *Fusarium* in tubers was limited. *D. theobromae* was artificially inoculated to healthy tubers from field-planted sweet potato accessions. BNAS-51 variety was used as check throughout the experiment. Preliminary results indicate that 78 cultivars were resistant, 13 were moderately resistant and 78 were susceptible to tuber rot.

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INTRODUCTION

Losses due to infection of micro-organisms is probably the most serious problem in tropical root crops. The pathogen is often predisposed by some physical and physiological damage like bruises and cuts during harvesting and handling, and thawing and water loss during storage of tubers.

The role of fungi in causing tuber rot of stored sweet potato tubers has not been extensively studied. Although no reliable estimates of losses due to storage rots are available locally, their presence and probable increase in incidence could become a threat to the development of the sweet potato industry. Consequently, knowledge of the identity and pathogenicity of the pathogens is helpful in formulating control measures that are economical and practical.

This study presents the incidence

and identity of the most prevalent fungi found in stored sweet potato tubers, a method of inoculating tubers with the pathogen causing tuber rot and the reaction of sweet potato cultivars to tuber rot.

MATERIALS AND METHODS

Survey of Fungi Causing Tuber Rot.

Visual reading on stored sweet potato. — Fresh sweet potato tubers were stored in a wooden box lined with newspapers in the screen-house. Observation and visual readings were made periodically thereafter.

In another experiment, sweet potato tubers were taken from storage sheds in ViSCA, Baybay, Leyte. The infected tubers which showed signs and symptoms of the disease were brought to the laboratory for examination. The percentage incidence of the rot due to a particular fungal pathogen(s) was determined using the formula:

$$\% \text{ Incidence} = \frac{\text{No. of infected tubers}}{\text{Total no. of infected tubers sampled}} \times 100$$

Laboratory test. — Fresh healthy tubers were taken to the laboratory and thoroughly washed. Ten slices of about 2 mm^2 portion of each of the cortex and skin were sliced and disinfected with 10% calcium hypochlorite, while another 10 slices were not disinfected. The tissues were planted on the center of solidified potato dextrose agar (PDA) in Petri plates containing the newly-planted tissues and incubated at room temperature (28°C). Observations were initially made 24 hr after incubation and continued for at least six days or until the plate was covered with the mycelia.

Isolation of Fungal Pathogens Associated with Tuber Rot of Sweet Potato. — Mycelia, spores and other fruiting bodies and structures on infected tubers were directly picked using a previously heated dissecting needle. They were placed on plated PDA wherein three drops of 10% lactic acid were previously added before solidification to prevent possible bacterial contamination. Pure cultures of the most common pathogens isolated from rots were mass produced for the pathogenicity tests.

Artificial Inoculation of Sweet Potato Tubers with Diplodia, Aspergillus and Rhizopus. — Initial tests using uninjured tubers were unsuccessful. Therefore, in succeeding experiments to test the pathogenic potential of the three fungal pathogens, 240 healthy tubers of BNAS-51 sweet potato were inoculated, 80 tubers for each fungal isolate. Inoculation was made by cutting two one-cm holes on tubers, filling them with bits of PDA containing spores and mycelia of the respective fungus, and covering the inoculum with cellotape. The same procedure was done with 60 tubers that served as control except that clean solidified agar cubes were used. In both

cases, the uninjured tubers were sprayed individually with 10% calcium hypochlorite prior to inoculation.

The inoculated tubers were placed inside plastic bags lined with wet tissue paper and incubated at room temperature. Observation of tubers for possible development of the fungi was continued until the sixth week after inoculation. During this time the inoculum was expected to have reached its maximum potential in infecting the tubers.

Screening of Sweet Potato Accessions for Resistance to Tuber Rot. — Sweet potato accessions obtained from the germplasm collection of the Philippine Root Crop Research and Training Center, Baybay, Leyte were evaluated for their reaction to tuber rot. Five healthy tubers from each accession were taken from the field. Samples were taken only from 10 accessions per day to make sure that the tubers were fresh when inoculated. The tubers were brought to the laboratory, thoroughly washed and allowed to dry. Inoculations were done following the procedure described earlier.

RESULTS AND DISCUSSION

Survey of Fungi Associated with Rots of Stored Sweet Potato Tubers.

In the laboratory test using sliced skin and cortex of freshly harvested sweet potato tubers, *Fusarium* was found to be the most common fungus, followed by *Rhizopus* (Table 1). The latter was observed after 24 hr with mycelia covering the Petri plates after six days. Relatively more fungi were recorded on the skin than on the cortex and on non-disinfected compared to disinfected samples. In storage, however, *Diplodia* was the prevalent genus found associated with tuber rot of sweet potato (Table 2). Out of 600 tubers sampled, 39.2% were infected with *Diplodia* alone, 22.2% with *Aspergillus* and 24.0% with *Rhizopus*. The results also showed that the incidence of *Diplodia* alone and in combination with other fungi was very high in stored sweet potato.

Aspergillus and *Rhizopus* were only found in some isolated parts of the tubers colonized by *Diplodia*. They were either found on the tips of tubers or around restricted areas on the surface of the infected tubers. Their occurrence did not reach an alarming stage as compared to *Diplodia*.

The tubers colonized by *Diplodia* were characterized by the presence of many pimple-like or wart-like growths on the surface of the tubers which were coal-black in color and almost always in aggregates (Fig. 1). These wart-like growths were the pycnidia of the fungus. On the surface of these pycnidia were the spores of the fungus which were oval in shape, brown in color and with clear cross-walls at the center or midpoint (Fig. 2). These spores, upon landing on a pile of newly-stored tubers of sweet potato, may start another infection on these tubers.

It was also found that fully

Table 1. Frequency of fungi on disinfected and nondisinfected skin and cortex of freshly harvested sweet potato.

	NUMBER OF TUBERS WITH FUNGI											
	REPLICATION										AVE	
	I		II		III		IV		V			
	ND	D	ND	D	ND	D	ND	D	ND	D	ND	D
Cortex												
Fusarium	6	1	7	7	10	5	1	5	0	0	4.8	3.6
Rhizopus	4	0	10	4	3	0	10	5	0	0	5.4	1.8
Curvularia	0	0	2	2	1	0	0	4	0	0	0.6	1.2
Aspergillus	0	0	2	0	0	0	4	0	4	1	2.0	0.2
Penicillium	1	0	0	0	0	0	1	0	0	0	0.4	0
Unidentified	0	0	0	0	1	5	2	2	3	0	1.2	1.4
Total	11	1	21	13	15	10	18	16	7	1	14.4	8.2
Skin												
Fusarium	8	7	10	9	10	7	3	6	0	0	6.2	5.8
Rhizopus	6	2	7	5	2	1	8	8	0	0	4.6	3.0
Curvularia	0	0	4	3	1	0	0	4	0	0	1.0	3.0
Aspergillus	0	0	3	2	1	0	4	0	4	2	2.5	0.8
Penicillium	0	0	2	0	0	0	0	0	0	0	0.4	0
Unidentified	0	1	0	0	2	6	0	2	2	2	0.8	2.2
Total	14	10	26	19	16	14	15	20	6	4	15.5	13.2

ND = not disinfected
D = disinfected with calcium hypochlorite

Table 2. Incidence of tuber rot fungi associated with sweet potato in storage sheds in ViSCA, Baybay, Leyte.

Genus of Tuber Rot Fungi	No. of Infected Tubers	% Incidence
Diplodia	235	39.2
Aspergillus	13	2.2
Rhizopus	23	3.8
Diplodia - Aspergillus	135	22.2
Diplodia - Rhizopus	144	24.0
Aspergillus - Rhizopus	17	2.8
Diplodia - Aspergillus - Rhizopus	33	5.5
Total % incidence		99.7
Total no. of tubers sampled	600	

colonized or even half-infected tubers were likewise attacked by a grayish unidentified bettle about 0.46 mm long belonging to Family Bruchidae. They were found dwelling beneath or inside the infected tubers. Once these small beetles were disturbed, they immediately surfaced out and crawled over the surface of the pycnidia of the fungus. They became covered with dust of spores and, when they flew off to adjoining tubers, carried the spores to new uninfected tubers.

The aforementioned situation was observed in the storage sheds where old and new stocks of sweet potato tubers were kept. The insects alighting on newly-stored tubers may carry the spores of the fungus. It is not surprising, therefore, that tuber rot due to *Diplodia* was abundant in storage.

Aspergillus infection was usually observed on the tips of infected tubers. Their presence on stored

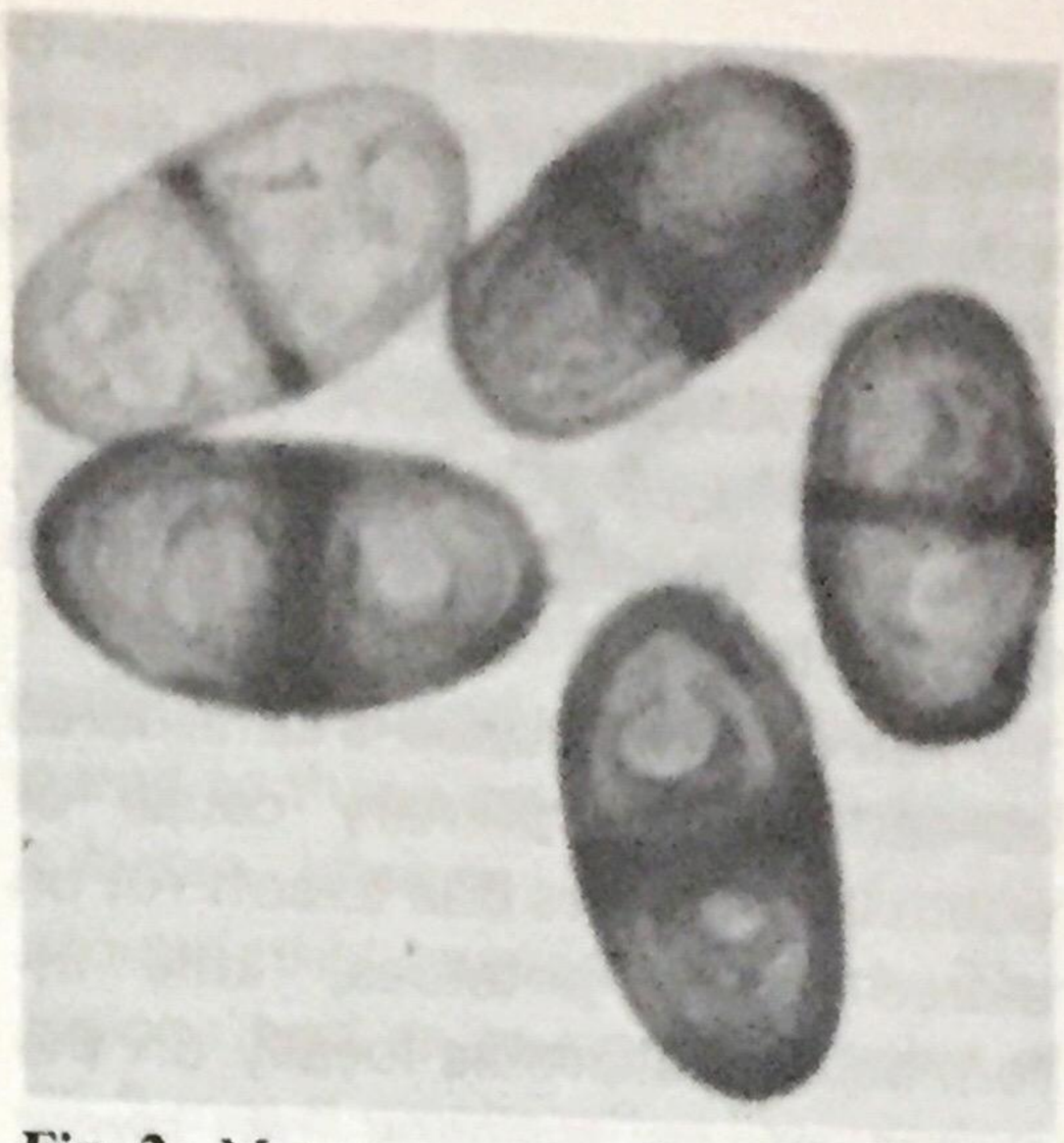

Fig. 2. Mature spores produced by *Diplodia theobromae* from infected tubers. Spores are oval in shape with cross-walls at the midpoint (X-1000).

sweet potato tubers has not been extensively studied, being mentioned in passing or together with other fungi causing tuber rots but never cited as the main causal organism. The fungus has often been observed associated with stored grains, although there were

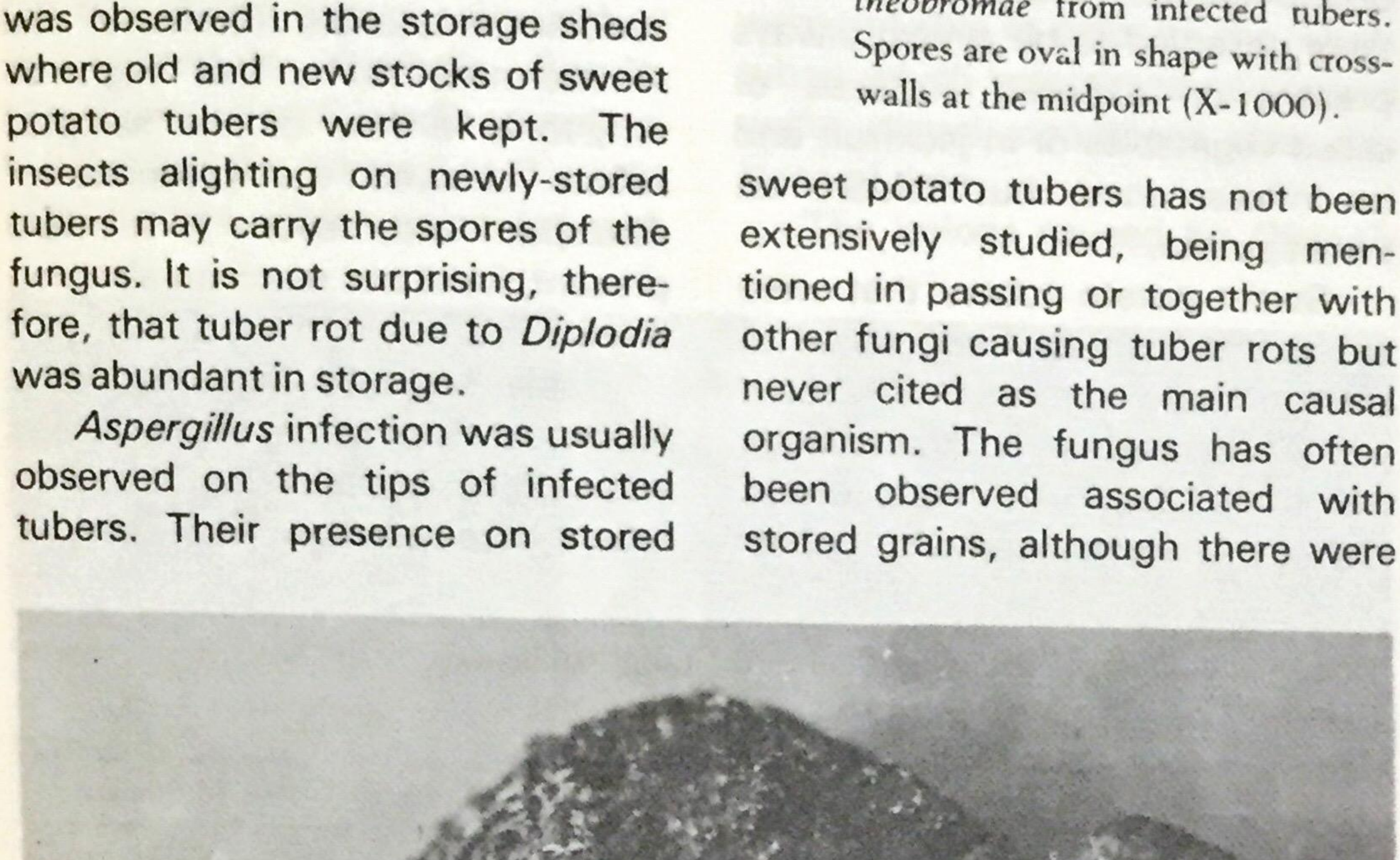

Fig. 1. Sweet potato tuber fully colonized by *Diplodia theobromae*. Note the coal-black wart-like growths on the surface of the tuber which are the pycnidia of the fungus.

reports that around five species of *Aspergillus* contaminate tubers of cassava about to be processed into flour, namely *Aspergillus flavus, A. flavipes, A. japonicus, A. niger* and *A. ochraceous* (Clerk and Caurie, 1975). No species has been reported on sweet potato.

Although *Rhizopus* is considered abroad as the primary cause of postharvest losses due to soft rot on stored sweet potatoes, little information is available locally on the extent of its damage on sweet potato tubers. *Rhizopus* structures were observed to be almost always present on exposed surfaces of sliced vegetables or in jackfruit and its inflorescence, but seldom on sweet potato.

Sweet potato tubers that were cut during harvest and placed in storage were not easily infected with *Rhizopus*. Instead, the wounds had some sap on the surface and edges, and seemed corky in appearance. This healing of the wounds may be due to the rapid formation of a corky layer that helped prevent the entrance of the soft rot organisms. During the process, suberine is formed. Suberine is a compound which possesses the remarkable property of retarding the escape of water from and preventing the entrance of the soft rot fungus into the flesh of the tubers underneath the wounds. A periderm develops when the corky layer is formed, which is composed of suberine, tannin and other water and fungus-resistant materials (Edmond and Ammerman, 1971). This might be why only a few of the sampled tubers showed the presence of *Rhizopus* and, even if it were present, it did not reach an alarming level.

Table 3 shows the result of a follow-up study of the survey which was conducted earlier. Two-hundred tubers were harvested from the field

Table 3. Percentage incidence of tuber rot fungi found associated with sweet potato stored in the screenhouse.

Genus of Tuber Rot Fungi	No. of Tubers	% Incidence
Diplodia	**94**	**24.7**
Aspergillus	**26**	**6.8**
Rhizopus	**34**	**8.9**
Diplodia - Aspergillus	**20**	**5.2**
Diplodia - Rhizopus	**41**	**10.7**
Aspergillus - Rhizopus	**19**	**5.0**
Diplodia - Aspergillus - Rhizopus	**24**	**6.3**
Total % incidence		**67.6**
Total no. of infected tubers	**258**	
Total no. of tubers without infection	**122**	
Total no. of tubers stored	**200**	

and were brought into the screen-house for storage. Symptoms of *Diplodia* infection become visible one month after storage as brown and water-soaked lesions starting at the tips of the tubers and progressing slowly. The infected tubers were further observed until the pycnidia of the fungus became visible on the surface of the infected tubers, 6 weeks after storage. Fruiting structures of *Aspergillus* and *Rhizopus* were observed earlier than those of *Diplodia.* One week after storage, they were already visible mostly at the tips of the tubers colonized or uncolonized by *Diplodia.* Results also showed that *Diplodia* completely colonized the tubers 6 to 8 weeks after storage, which coincided with the report of Chiu (1976). Forty-three percent of the stored tubers did not show symptoms of the disease nor signs of the pathogen.

Sweet potato tubers inoculated with *Diplodia theobromae* (Pat.) Nowell showed typical symptoms of the disease (Fig. 3), except that the lesions produced were smaller compared to those on naturally infected tubers. Lesions developed only around the inoculated areas. The lesions had an average diameter of 26.3 mm 6 weeks after inoculation. The lesions did not advance and were not able to colonize the whole tuber, which was already colonized under natural conditions after this lapse of time.

The lesions caused by *Diplodia*

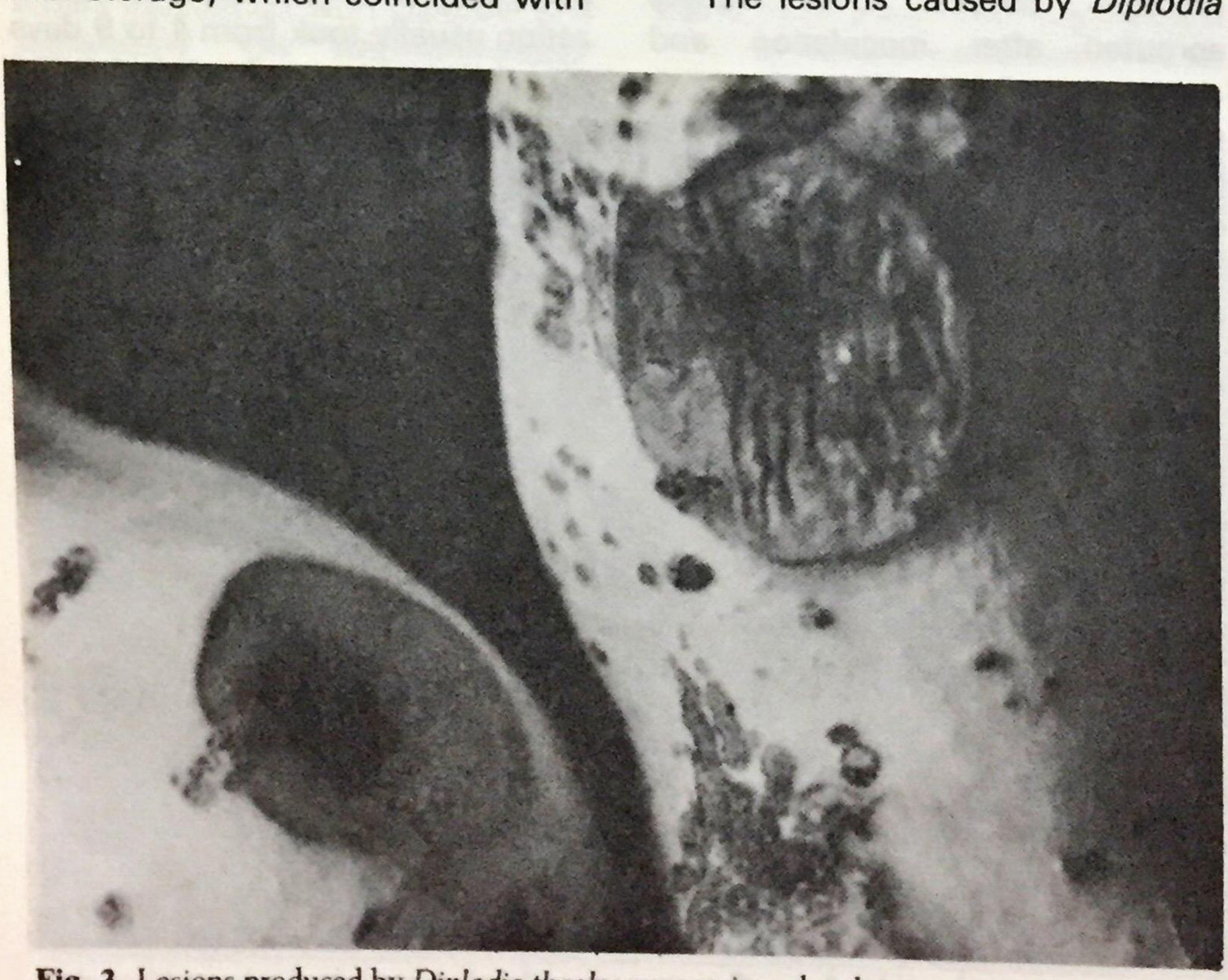

Fig. 3. Lesions produced by *Diplodia theobromae* on inoculated sweet potato tubers. Note dried up, shriveled and sunken lesions.

became either sunken or dried or both. Most of them stopped progressing while a few developed very slowly until the observation was terminated 6 weeks after inoculation. Three reasons could be advanced for this reaction: (1) The environment in the laboratory was different from the natural conditions outside; (2) In the laboratory, the source of inoculum was only those inoculated into the tubers. However, when the tubers were exposed to natural inoculation, the supply of inoculum was continuous. The first inoculum that landed on the tubers was reinforced by incoming inoculum, and; (3) *Diplodia* usually does not infect actively developing and growing tissues. The tubers sprouted after inoculation and became active organs, so that the cells were actively differentiating during the development of the sprouts.

Artificial inoculation of sweet potato tubers with *Aspergillus* resulted in infection which usually started a week after inoculation (Fig. 4). It readily developed around the inoculated areas, infecting 42 of the 80 treated tubers 4 weeks after inoculation. Infection did not progress further; thus, the fungus was not able to colonize the tubers.

When inoculated on sweet potato tubers *Rhizopus* readily colonized 37 of the tested tubers 9 days after inoculation. Other tubers were either contaminated or did not produce any infection at all. Colonization usually took from 4 to 9 days

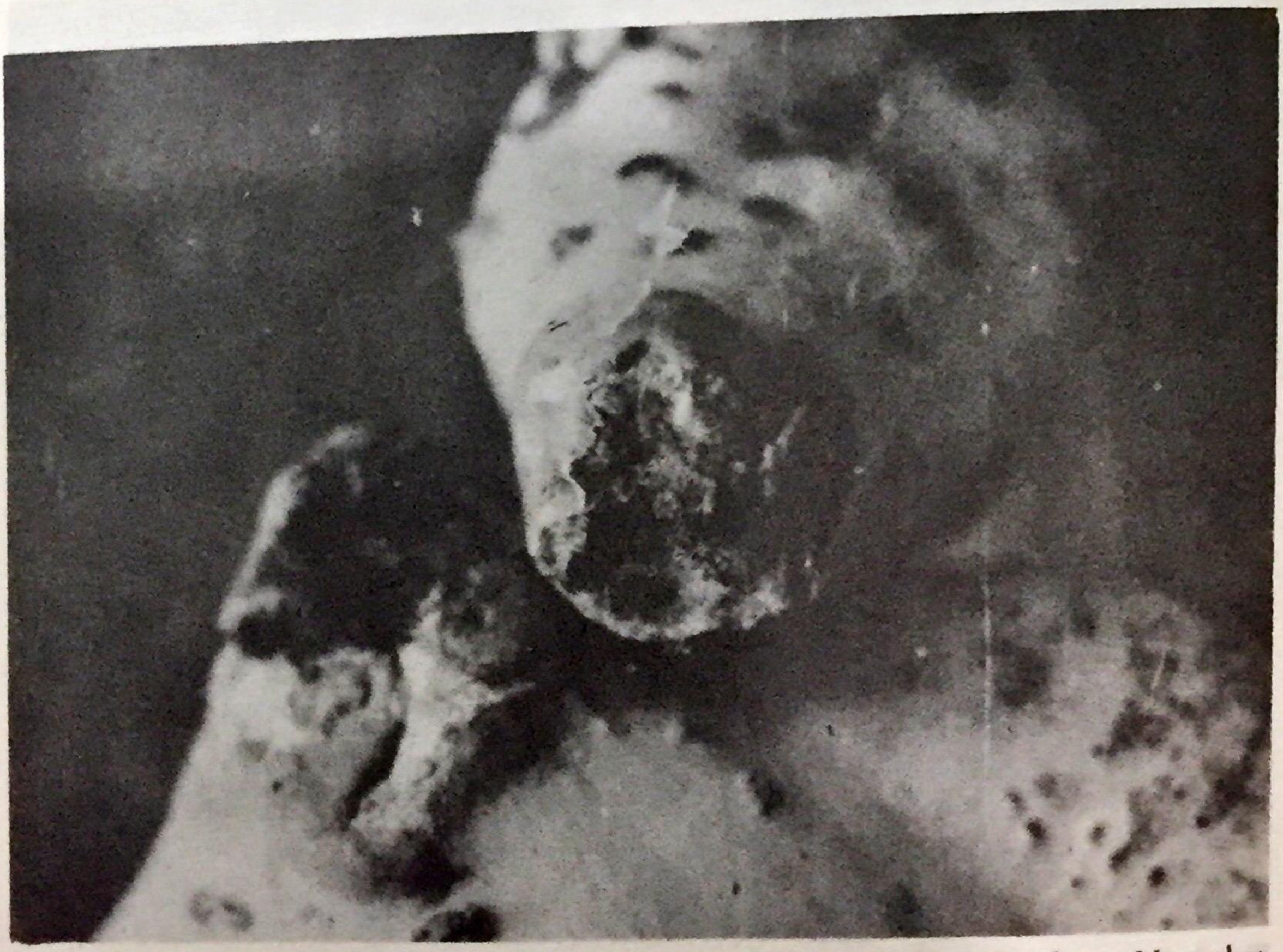

Fig. 4. Lesions produced by *Aspergillus* sp. on inoculated sweet potato tubers. Note that lesions are confined around the inoculated portion of the tubers only.

after inoculation. Infected tubers were characterized by the presence of coarse, aerial mycelia with black sporangia covering the inoculated portion and progressing very rapidly toward the other end of the tuber making the tuber very soft (Fig. 5).

Inoculated tubers were also attacked by a fungal contaminant which overcame the inoculated fungus. The contaminated tubers were covered with white mycelia (Fig. 6) which grew profusely around the infected tubers. The decaying process was accompanied by a very disagreeable odor. The fungus did not produce any fruiting structure so it was not identified. This contaminant infected the tubers inoculated with *Diplodia* and *Aspergillus*. On *Rhizopus*-inoculated tubers, *Aspergillus* was the usual contaminant.

Fig. 6. Sweet potato tuber fully colonized by the funfal contaminant. Note the white, thick mycelia covering the colonized tuber.

Fig. 5. Mycelia and sporangia produced by *Rhizopus* sp. on inoculated and fully colonized tuber of sweet potato.

Table 4. Reaction of different sweet potato accessions after artificial inoculation with *Diplodia theobromae*.

Acc. No.	Common Name	Acc. No.	Common Name	Acc. No.	Common Name	Acc. No.	Common Name
RESISTANT							
2	(Julian)	80	(Kinarusa)	150	—	251	(Kenangkong)
4	(Samar Big Yellow)	82	—	152	—	254	—
5	(Señorita)	83	—	157	—	256	—
11	(Jervel)	93	—	158	—	259	—
13	(Tapol)	98	—	159	—	261	—
14	(Kapening)	103	—	171	—	272	—
16	(Baka-bakahan)	104	(Kabacan Native)	173	(Patatas)	273	—
18	(Tiririming)	106	(Kalugti	177	(HDK - I)	279	(Surao)
20	(Georgia Yellow Yam)	108	(MIT - I)	195	(UPLB 8 (34)	280	(Ubi Inengkanto)
22	(Callieburon)	109	(Kabacan I)	209	(UPLB - 78)	281	(Inapod)
54	(Kaitong)	110	(Big Samar Yellow)	210	(UPLB - 79)	283	(Kamalig camote)
56	(Makabuhi)	113	(MIT - 2)	211	(Taiwan 467)	289	(Karedian puti)
63	—	121	—	221	(Taiwan 477)	293	(Maligayan pula)
64	(Winaray)	122	—	225	(Taiwan 481)	297	—
69	(Norminac)	123	—	227	(Taiwan 483)	299	—
70	(Kasincio)	126	—	230	(Taiwan 483)	304	(Inengkanto)
71	(Inanahaw)	140	—	240	(Centennial)	311	(Bacon)
74	(Kaagbon)	145	—	241	(Karja)	313	(Kamalig gaping)
75	(Karaso)	146	—	245	—		
76	(Chinese potato)	147	—	248	(San Agustin - 1)		
MODERATELY RESISTANT							
1	Salvacion (Kabutho)	55	(Kinabaw)	107	(BPA - 4)	141	—
19	(Ligan Yellow)	65	—	127	(Local - A)		
32	—	67	(Norminac)	133	(Karja)		
40	—	72	(Karingkit)	134	(Local - B)		
SUSCEPTIBLE							
3	(Manobo)	42	—	87	—	143	—
6	(Kaimay)	43	(Elorde)	88	—	148	—
7	(Kaindoy)	44	—	89	—	151	—
8	(Decolores)	45	—	90	—	153	(Karigis)
9	(Kagbon)	46	—	91	—	154	(Kinampay)
10	(BNAS-51)	47	—	95	—	155	(Parihado)
12	(R Bohol)	48	—	96	—	160	—
15	(Viscaya)	49	(Katalo)	97	—	161	—
23	(Cabgan)	50	(Senador)	99	—	162	(Kapasa)
24	(Banaue)	51	(Tinugabang)	100	—	186	(L. C. Taiwan)
25	(Americano)	53	(Katuka)	102	(BPA - 2)	192	(UPCA - 1)
26	(Karingkit)	57	(Tainung)	105	(UPR)	204	(UPLB - 73)
27	(Kapoy)	60	—	111	(Garcia Yellow)	205	(UPLB - 74)
28	(Kaputyong)	62	—	114	(Davao - 1)	306	(BNAS - 51)
33	—	66	—	116	(Davao - 3)		(Georgia - 1)
34	—	68	—	117	—		(Hsinchu - 1)
36	—	73	(BNAS - 51)	118	—		(Tinangkong)
37	—	77	(Kabutho)	119	—		(BPA - 1)
39	—	84	—	124	—		
41	—	86	—	142	—		

Preliminary Screening for Resistant Cultivars.

Since tuber rot may occur before harvest, the field performance of sweet potato tubers was initially assessed. However, no fungal infection was observed; thus, screening was done by artificial inoculation of tubers in the laboratory.

Out of 268 cultivars planted in the field, 74 did not produce tubers while 25 succumbed to an unidentified bacterial soft rot disease. From the 169 cultivars that were tested, 78 were evaluated to be resistant to tuber rot (Table 4). Cultivars classified as resistant did not develop

symptoms of the disease, while those that were moderately resistant produced rotting only in the inoculated portion. The 78 cultivars that were susceptible produced well-developed lesions typical of the disease. BNAS-51, which has been recently called the "supercamote", is susceptible to the disease and was used as control throughout the experiment.

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