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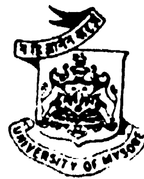

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Health Status of Seeds of ICRISAT Mandate Crops in Relation to Plant Quarantine

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Summary

This paper describes in brief ICRISAT's treatment methodology for quarantine inspection, which includes fumigation, dry seed examination, seed washing test, blotter test, ELISA and chemical seed treatment. Between June 1982 and June 1989, microflora associated with 431492 seed samples of sorghum, pearl millet, chickpea, pigeonpea and groundnut crops were monitored through the procedures mentioned above. Fungi of no quarantine significance dominated the seed microflora. However, microflora of quarantine importance such as *Ascochyta rabiei*, *Claviceps fusiformis*, *Colletotrichum cajani*, *Fusarium oxysporum* f. sp. *ciceri*, *Perenosclerospora sorghi*, *Sclerospora graminicola*, *Sphacelotheca cruenta*, *S. reiliana*, *S. sorghii*, *Tolyposporium ehrenbergii*, *T. penicillariae*, *Xanthomonas campestris* and peaunt mottle virus were also recorded. A few hitherto undescribed microflora such as *Glococercospora sorghi*, *Glomerella cingulata*, *Panagrolaimus* sp., *Xanthomonas campestris*, *Colletotrichum gloeosporioides* and *C. dematium* have been intercepted. By inspecting and treating the seeds, ICRISAT's Plant Quarantine Unit (PQU) filters out the diseased seeds and then gives the importing country a first line of defense which is 'exclusion', thus fulfilling the objective of plant quarantine.

Introduction

Testing of ICRISAT seed for quarantine is connected with the movement of its mandate crops (sorghum, pearl millet, chickpea, pigeonpea and groundnut) for use in breeding, conservation or other research projects. Quarantine methods are used to prevent movement of a pathogen to an area where it is not known to occur or is of limited distribution. The spectacular growth of international institutes, where plant genetic resources are conserved and evaluated has accelerated the tempo of plant material exchange. ICRISAT, keeping in view its commitment for a large scale and a speedy exchange of its germplasm signed a Memorandum of Agreement with the Government of India (GOI) in 1972, as a result of which an Export Certification Laboratory with post-entry quarantine isolation area and a greenhouse were created at the

Institute. The work of this laboratory is closely monitored jointly by the scientists of the National Bureau of Plant Genetic Resources (NBPGR) and ICRISAT.

During recent years, the ICRISAT's plant Quarantine Unit (PQU) has adopted improved detection and treatment methodology to provide a sound biological basis to exchange its germplasm and to minimize the risk of introducing a pathogen into new geographical areas. This includes improved methods of virus detection, seed health testing, thermotherapy, chemotherapy etc. This paper describes ICRISAT's treatment methodology and examines the results of various detection methods in use since 1982, so as to ascertain the effectiveness of the methods and to determine the health status of its seeds.

Material and methods

A total of 431492 seed samples exported to 145 countries included 222570 sorghum, 52389 pearl millet, 77842 chickpea, 29492 pigeonpea and 49209 groundnut seeds. While inspecting seeds for export certification by various seed health testing methods, microflora intercepted were recorded as percentage infection for each organism separately. The total number of samples tested during June 1982 to June 1989 were used to calculate the percentage of seed microflora for each crop separately. The seed material received for export was subjected to the following seed health testing procedures, in this order.

Fumigation: Sorghum, chickpea and pigeonpea seeds received at the PQU are first subjected to fumigation under vacuum at a pressure of 125 mm mercury, with a methyl bromide dosage of 32 g m^{-3} for 4 h; pearl millet and groundnut are fumigated at normal atmospheric pressure with aluminium phosphide dosage of 3 g m^{-3} for 5 days (Varma and Ravi, 1984; Joshi, 1988).

Dry seed examination: After fumigation, each seed sample is carefully examined under illuminated floating desk magnifier of 2X magnification to remove the admixtures of plant debris, sclerotia, galls, insects, smut sori discolored and moldy seeds. Apparently healthy looking and clean seeds are selected and those of poor quality are rejected.

Seed-washing test: This is used to test fungi that do not grow on the seed during incubation but may be carried externally through seeds as spores. This test was carried out to detect the presence of oospores of downy mildew and teliospores of smut fungi on the surface of sorghum and pearl millet. Fifty seeds from each accession were randomly drawn into the test tubes separately. Distilled water (10 mL) and a few drops (10-20) of 95% ethyl alcohol or a detergent were added to the test tubes. The tubes were shaken in a mechanical shaker for 10 min and the suspension was centrifuged at 3000 rpm for 10 min. Discarding the supernatant, the pellet was resuspended in 2 ml of sterile water. The suspension from each test tube was placed on the microscope slides for examination under the bright field, and the data was recorded (Table 1).

Blotter test : Blotters were soaked in sterile distilled water and placed in petri plates after draining off the excess water. For each seed accession, ten seeds of cereal crops and five seeds of legume crops were plated in each petri plate. Seeds were arranged in the petri plate equidistant from one another and kept for incubation at 22° ± 2°C under Near Ultra Violet (NUV) light with 12 hours alternate cycles of light and dark period for seven days (ISTA., 1976). After incubation, the seeds in each petri plate representing an individual seed accession were examined under stereobinocular microscope at 50X magnification and the microflora observed were recorded. Fungi were identified up to the genus level based on mycelial growth, length, colour, and the ornamentation of conidia on conidiophores. Identification of species was confirmed by sending single spore cultures of the fungus to the Commonwealth Mycological Institute (CMI) and the data on microflora was recorded as described elsewhere.

Enzyme-linked immunosorbent assay (ELISA): Groundnut seed accessions and cuttings submitted for export were tested by ELISA for the detection of Peanut Mottle Virus (PMV). Double antibody sandwich (DAS) or direct antigen coating (DAC) methods can be used to detect the viral antigens. At ICRISAT, the DAC system, standardized by Hobbs *et al.* (1987), was followed. The seeds which were found to be free from PMV were further tested through the blotter method for detecting the seed microflora.

Seed treatment : After completing the detection tests of microflora mentioned above, seed accessions free from quarantine pathogen or pathogens important to the planting value were selected for fungicidal treatment. Dry seed dressing of the fungicide was applied to each seed accession at the recommended dosage as follows. Seed accessions of all the five mandate crops were treated with benomyl (50 WP) and thiram (75 SD) in a ratio of 3:2 @4.5 g kg^{-1} seed (Haware *et al.*, 1978 ; Vidhyasekaran, 1983). In addition, sorghum and pearl millet were also treated with metalaxyl (35 SD) and mancozeb (45 WP) @ 4 g kg^{-1} and 2 g kg^{-1} (Anahosur, 1980 ; Williams and Singh, 1981) seed respectively, while chickpea seeds were treated with thiabendazole (TBZ) 60 WP@3 g kg^{-1} seed (Kaiser *et al.*, 1973). However, seeds intended for specific studies such as disease resistance or response to different strains of *Rhizobium* or chemical analysis were exported without treatment.

Results

Sorghum [*Sorghum bicolor* (L.) Moench]

The frequency and infection percentages of microflora showed that sorghum seeds were infected by 129 different species of fungi and bacteria belonging to 62 genera (Table 1). Among the various fungi recorded, 34 of them have been reported as seed-borne in this crop causing the disease under field conditions (Richardson, 1979). One of the interesting observations made during this study was the occurrence of sclerotia of *Gloeocercospora sorghi* on

seeds during the dry seed examination. Sclerotia of this fungus closely resemble the pycnidia, but they can be differentiated by their characteristic flat, spindle or ovoid to ellipsoid shape; they were immersed or erumpant, and scattered all over the seed surface, whereas the pycnidia appear as black globose pin head eruptions in concentric rings around the stylar region.

In the seed-washing test, oospores of *Peronosclerospora sorghi*, teliospores of *Sphacelotheca sorghi*, *S. cruenta*, *S. reiliana*, *Tolyposporium ehrenbergi*, conida of *Sphacelia sorghi* and uredospores of *Puccinia purpurea* were recorded (Table 1)

We recorded the occurrence of *Glomerella cingulata* on the seed in blotters. We have not been able to differentiate the perithecia of this fungs from the pycnidia under stereoscopic microscope. However, the CMI has confirmed our identification. This shows that *Colletotrichum graminicola*, the fungus which causes anthracnose and red rot disease in the field could also be carried on seed as a teleomorph.

Pearl millet [*Pennisetum glaucum* (L.) R. Br]

Pearl millet seeds were found to be naturally infested by 89 species of microflora belonging to 46 genera. During the course of dry seed inspection, pearl millet seeds were found to be infested by *Panagrolaimus* sp, a free living nematode (Panchbhai *et al*., 1986). Nematode-infested seeds were elongated, with longitudinal fissure, approximately two third the length of one side. There was a small slit on the micropyle of the hilum region. Infested seeds were shrivelled dark grey or greyish-black, and weighed less than the healthy ones. During seed-washing tests, oospores of *Sclerospora graminicola*, teliospores of *Tolyposporium penicillariae*, conidia of *Claviceps fusiformis*, and uredospores of *Puccinia penniseti* were detected. Seed microflora encountered are given in Table 1.

Chickpea (*Cicer arietinum* L.)

A total of 67 microflora belonging to 49 different genera were recorded on chickpea seeds in blotters. Seed microflora of quarantine importance as well as pathogens that reduce the planting value of the seed have been recorded in varying percentages (Table 1). One of the important findings is the detection of a seed-borne bacterial pathogen, *Xanthomonas campestris* (Reddy *et al*., 1988). The bacterium was found to be pathogenic, and seed transmissible.

Pigeonpea [*Cajanus cajan* (L.) Millsp]

Pigeonpea seed accessions were found to be naturally infected by 52 species belonging to 35 different genera of microflora. Pathogens of quarantine importance as well as planting value of seed were recorded in varying percentage of infection (Table 1).

Groundnut (*Arachis hypogaea* L.)

Groundnut seed accessions numbering 4815 were tested for PMV through ELISA, out of which 58 (1.2%) samples showed positive results. In addition

to the seeds, groundnut cuttings of 25 different accessions were tested and two accessions showing positive reaction to PMV were discarded.

Microflora encountered in the blotters are given in Table 1. Among the various seed-borne fungi recorded, the most frequently occurring fungi were *Aspergillus flavus* and *A. niger*. One of the important findings was the interception of *Colletotrichum gloeosporioides* and *C. dematium* on the seeds of *Arachis villosa*, and *A. chiquitana*, but these fungi have not been recorded on any of the cultivated *Arachis* sp. tested.

Discussion

A review of our records of various quarantine methods revealed that there are 157 microflora belonging to 74 different genera occurring on the seeds of sorghum, pearl millet, chickpea, pigeonpea and groundnut crops, and there are at least 54 host/pathogen interactions in which the pathogen is reported to be seed-borne in one or more than one of these crops. Out of these, only 13 are known to be of quarantine importance (Harinath Naidu and Nirula, 1979). Our plant quarantine precautions or the quarantine export control is related to the epidemiological potential of the pathogens. Pathogens that have a high or considerable epidemic potential are counteracted by complete prohibition against introduction of seed material. The microflora included in this group are downy mildews, rusts of sorghum and pearl millet, *Xanthomonas campestris* of chickpea and peanut mottle virus of groundnut. For these pathogens, the principle of exclusion is adapted, and as such no tolerances are acceptable in consignment submitted for export.

A second category of pathogens are those that have a moderate to low epidemic potential. Microflora included in this group are *Colletotrichum* spp., *Exserohilum turcicum*, ergots, smuts, *Gloeocercospora sorghi* (sorghum and pearl millet), *Ascochyta rabiei*, *Fusarium oxysporum*, *F. solani* (chickpea), and *F. udum* (pigeonpea). Seed accessions found to be completely free of these pathogens are selected for export.

The third category consists of 50 instances of microflora that affect the quality of stored seeds and are not considered, under most regulatory definitions, as pathogens (e.g. *Penicillium* spp., *Aspergillus* spp., etc).

Tolerances of such frequently occurring pathogens are accepted. Out of the 50 instances, some of them have rather low inoculum potential, and as such, may not be harmful to the planting value of the seeds at lower percentages of infection or contamination in the seed. Decisions on the export of such consignments is based on the importance of material for scientific purposes. However, at higher percentages of infection, seeds are discarded from export.

Concrete selection of quarantine objects required by a country may be difficult, although more than 100 countries have published regulations for quarantine (Neergaard, 1979). Due to lack of trained personnel and adequate facilities in many countries, the imported material is released after visual

inspection, thus giving every chance for hidden infections or infestations to be introduced (Neergaard, 1989). In the absence of specific quarantine regulations for the seeds of mandate crops, ICRISAT's PQU has adapted additional precautions and safeguards by subjecting its export seed material through various tests. The PQU also updates its methodology from the practical research programs as well as by accumulating data for quarantine objects drawn from a wide spectrum of scientists. The fact that so far, there has not been a single instance where plant material cleared by us has been found infected with quarantine objects by the importing country proves that our inspection methodology aimed at 'exclusion' fulfils the basic objective of plant quarantine.

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TABLE 1. **Microflora recorded on seeds of sorghum, pearl millet, chickpea, pigeonpea and groundnut exported from ICRISAT between June 1982 and June 1989**

Organism	Percentage Infection				
	Sorghum	Pearl millet	Chickpea	Pigeonpea	Groundnut
1	2	3	4	5	6
Acremonium spp.	α0.01[c]	α0.01[c]	0.01	α0.01[c]	—
A. strictum	α0.01[ac]	—	—	—	—
Alternaria spp.	33.80[ac]	6.21[c]	10.2[c]	1.02[c]	—
A. alternata	9.08	0.26	26.84	58.04	α0.01
A. dauci	α0.01	—	—	—	—
A. longissima	0.01	α0.01	—	0.11	—
Arthobotrys spp.	0.12	α0.01	α0.01	—	—
Aspergillus spp.	0.18	5.32	4.62	4.02	0.94
A. flavus	10.21[c]	10.87	28.7	46.25	46 84[c]
A. glaucus	—	—	—	—	α0.01
A. niger	16.64[ac]	8.64	35.22	44.06	48.0[c]
A. parasiticus	—	—	—	—	0.04
Ascochyta sorghina	α0.01[a]	—	—	—	—
A. rabiei	—	—	α0 01[abc]	—	—
A. subalphina	α0.01	—	—	—	—
Aurcobasidium spp.	0.01	—	0.01	0.05	—
Bacillus spp.	2.24[c]	0 08[c]	10.02[c]	11.09[c]	6.81[c]
Beauveria alba	0.04[c]	0.08[c]	—	—	—
Bipolaris cynodontis	α0.01	—	—	—	—

(Continued)

1	2	3	4	5	7
B. hawaiiensis	0.65[a]	0.35	0.12	0.01	—
B. papendorfii	α0.01	—	—	—	—
B. setariae	α0.01	α0.01[a]	—	—	—
B. sacchari	α0.01	0.01[a]	α0.01	—	—
B. sorghicola	0.08[a]	0.02	—	—	—
B. sorokiniana	0.07	0.04	—	—	—
B. specifera	α0.01[a]	0.02[a]	—	—	—
B. tetramera	0.72[a]	0.64	0.36	0.28	0.01
Bispora sp.	—	—	α0.01	—	—
Botryosphaeria ribis	α0.01	—	—	—	—
Botrytis spp.	α0.01	α0.01	—	α0.01	—
B. cinerae	—	—	α0.01[a]	—	—
Ceratocystis spp.	—	α0.01	α0.01	—	—
Cercospora spp.	0.05	α0.01	—	1.09	—
C. sorghi	0.04[a]	—	—	—	—
Cephalosporium spp.	0.11[c]	0.57[c]	0.27[c]	1.08	2.16[c]
Chaetomium spp.	0.13	0.01	0.04	0.06	0.02
C. globosum	α0.01	α0.01[a]	—	—	—
Cladosporium spp.	26.24[c]	20.20[c]	23.83[c]	58.08[c]	3.41[c]
Claviceps sp.	α0.01[a]	—	—	—	—
Claviceps fusiformis	-	α0.01[b]	—	—	—
Coleophoma empetri	—	—	α0.01	—	—
Colletotrichum cajani	—	—	—	α0.01[b]	—
C. dematium	—	—	α0.01[a]	—	α0.01[a]
C. gloeosporioides	—	—	—	—	α0.01[a]
C. graminicala	0.01[a]	—	—	—	—
Curvularia spp.	1.8[c]	1.18[c]	0.65[c]	0.41[c]	0.08[c]
C. andropogoni	α0.01	—	—	—	—
C. borreriae	α0.01	—	—	—	—
C. clavata	0.02	0.08	—	—	—
C. cymbopogonis	α0.01	—	—	—	—
C. eragrostidis	0.26[c]	0.09[c]	0.08[c]	0.01[c]	—
C. geniculata	α0.01[a]	—	—	—	—
C. inaequalis	0.04	0.07	—	0.06	—
C. intermedia	α0.01	α0.01	—	—	—
C. lunata	14.28[ac]	5.82[ac]	2.48	4.21[a]	0.06
C. oryzae	α0.01	—	α0.01	—	—
C. pallescens	0.24[c]	0.61[c]	0.07[c]	0.04[c]	—
C. penniseti	α0.01[a]	0.09[a]	—	—	—
C. prasadii	0.01	—	—	—	—
C. protruberata	0.25	0.45	—	—	—
C. robusta	0.03	α0.01	—	—	—

(Continued)

1	2	3	4	5	6
C. senegalensis	α0.01	—	—	—	—
C. siddiquii	α0.01	—	—	—	—
C. trifolii	0.14	0.03	0.06	—	—
C. tuberculata	α0.02[a]	α0.01	—	—	—
Dendrophoma spp.	α0.01	—	—	—	—
Diplodia sp.	α0.01	α0.01	—	—	α0.01[a]
Drechslera spp.	0.12	0.87	0.09	—	—
D. bicolor	0.03	0.01[a]	—	α0.01	—
D. ellisii	α0.01	α0.01	—	—	—
D. frumentacei	α0.01	—	—	—	—
D. holmii	α0.01	—	—	—	—
D. maydis	α0.01[a]	α0.01	—	—	—
D. micropa	α0.01	—	—	—	—
D. oryzae	0.10	0.01	—	—	—
D. stenospila	α0.01	—	—	—	—
D. urochloae	α0.01	—	—	—	—
D. victoriae	0.06[a]	α0.01[a]	—	—	—
Epicoccum spp.	0.64	0.07	0.09	—	0.91
Exserohilum curvatum	α0.01	—	—	—	—
E. gedarefensis	α0.01	—	—	—	—
E. holmii	α0.01	—	—	—	—
E. longirostratum	0.18[ac]	0.18[c]	—	—	—
E. rostratum	3.00[ac]	4.05[c]	0.31	—	—
E. turcicum	0.09[a]	0.01	—	—	—
Fusarium spp.	14.87[c]	7.09[c]	1.31[c]	1.08	0.04[c]
F. acuminatum	0.01[c]	0.26[c]	—	—	—
F. chlamydosporum	0.04[c]	0.44[c]	—	—	—
F. dimerum	0.01[c]	0.01	—	—	—
F. equiseti	0.56[c]	1.27[c]	0.07[c]	0.02[c]	0.03[c]
F. graminearum	0.27[a]	0.07[c]	—	—	—
F. lateritium	0.01[c]	0.01[c]	—	—	—
F. moniliforme	18.61[ac]	12.28[a]	2.02[c]	4.09[c]	0.62[c]
F. oxysporum	0.05[c]	0.29[c]	1.42[abc]	α0.01[c]	0.31[c]
F. pallidoroseum	2.02[c]	3.94[c]	0.68[c]	0.61[c]	0.02[c]
F. solani	0.18[c]	0.34[c]	1.64[ac]	0.28[ac]	6.84[ac]
F. udum	—	α0.01	α0.01	α0.01[ac]	—
Geotrichum spp.	0.06	0.05	0.01	0.04	—
Gloeocercospora sorghi	0.74[ac]	—	—	—	—
Glomerella cingulata	α0.01[a]	—	—	—	—
Gonatobotrys spp.	0.01	—	—	0.02	0.05
Hyalodendron spp.	—	—	—	0.01	—
Lasiodiplodia theobromae	α0.01[c]	—	0.01[c]	0.02[c]	0.03[ac]

(Continued)

1	2	3	4	5	6
Macrophomina phaseolina	0.02[ac]	0.04[ac]	0.24[c]	0.09[c]	6.41[ac]
Melanospora spp.	0.04	α0.01	—	—	—
Mamnoniella spp.	0.09	—	0.07	0.06	—
M. echinata	—	0.05	—	—	—
Metarhizium spp.	—	0.01	α0.01	—	—
Mucor spp.	0.31	1.01	3.67	5.61	3.85
Myrothecium spp.	0.04	—	α0.01	0.02	α0.01
M. roridum	α0.01	—	—	—	—
Nigrospora spp.	0.0[ac]	0.25[c]	α0.01	—	0.04
N. oryzae	0.02[c]	0.01[c]	—	—	0.01
Oedocephalum spp.	0.62[a]	9.26[c]	0.26[c]	0.49[c]	0.04[c]
Oidiodendron spp.	0.01	—	—	—	—
Panagrolaimus sp.	—	α0.01[c]	—	—	—
Penicillium spp.	2.45[c]	5.04[c]	21.04[c]	7.46[c]	14.91[c]
Peronosclerospora sorghi	α0.01[ab]	—	—	—	—
Periconia spp.	α0.01	α0.01	α0.01	α0.01	—
P. byssoides	0.80	—	—	α0.01	—
P. hispendula	—	—	α0.01	—	—
Pestalotia spp.	α0.01[c]	α0.01	α0.01	—	—
Phoma spp.	11.72[c]	4.81[c]	7.94[c]	4.48[c]	1.26
Phomopsis spp.	—	—	α0.01[a]	0.5[a]	—
Pithomyces spp.	0.23	0.16	0.07	0.42	—
P. sacchuri	α0.01	—	—	—	—
Puccinia penniseti	—	α0.01	—	—	—
P. purpurea	α0.01	—	—	—	—
Pyricularia sp.	—	α0.01	—	—	—
P. gresia	α0.01	α0.01	—	—	—
Pyrenochaeta sp.	α0.01[c]	α0.01[c]	α0.01[c]	—	—
Rhizoctonia sp.	0.08[c]	0.01[c]	0.16[c]	α0.01[c]	1.42[c]
R. bataticola	0.07[ac]	—	—	—	1.86[ac]
R. solani	—	—	α0.01[ac]	—	0.04[ac]
Rhizopus spp.	1.06[c]	1.02[c]	5.64[c]	7.80[c]	24.61[c]
Sclerospora graminicola	—	α0.01[ab]	—	—	—
Sclerotium rolfsii	—	—	0.01[ac]	—	α0.01[ac]
Scolecobasidium spp.	—	α0.01	—	—	—
Sphacelia sorghi	α0.01[a]	—	—	—	—
Sphacelotheca cruenta	α0.01[ab]	—	—	—	—
S. reiliana	α0.01[ab]	—	—	—	—
S. sorghi	α0.01[ab]	—	—	—	—
Spegazzinia spp.	α0.01	—	—	—	—
Stachybotrys spp.	0.42	3.08	1.06	0.21	0.02
Stemphylium spp.	0.08[c]	—	0.01[c]	—	—

(Continued)

1	2	3	4	5	6
Streptomyces spp.	0.17	0.67[c]	α0.91	—	—
Tolyposporium ehrenbergii	α0.0[ab]	—	—	—	—
T. penicillariae	—	α0.0[ab]	—	—	—
Torula spp.	0.21	—	0.51	0.02	—
Trichoconis spp.	α0.01	—	—	—	—
Trichoconis padwiki	α0.01	—	—	—	—
Trichoderma spp.	0.06	0.08	0.21	—	—
Trichothecium spp.	0.01[c]	1.34[c]	0.62	2.12[c]	0.02
Trichurus spp.	—	—	α0.01	—	—
Ulocladium spp.	α0 01	α0.01	—	—	—
Verticillium spp.	0.01[c]	0.01[c]	α0.01	α9.99[c]	α9.01[ac]
Xanthomonas spp.	—	—	—	0.27[c]	—
X. campestris	—	—	α0.01[abc]	—	—
Peanut mottle virus	—	—	—	—	1.20[ab]

a = Seedborne microflora

b = Seedborne microflora having quarantine importance

c = Microflora important to the planting value of the seed but not quarantine

— = Organism not recorded

α = Less than

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