Isolation of *Fusarium lateritium* from Sweetpotato Seed

C. A. CLARK and M. W. HOY, Department of Plant Pathology & Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge 70803-1720

ABSTRACT

Clark, C. A., and Hoy, M. W. 1994. Isolation of *Fusarium lateritium* from sweetpotato seed. Plant Dis. 78:585-587.

Fusarium lateritium, incitant of sweetpotato chlorotic leaf distortion, was isolated from acid-scarified, surface-disinfested seed of sweetpotato collected from different locations in North America, South America, Africa, Australia, and Asia. Frequency of isolation ranged from nondetectable to 58% of seed. Relatively high levels of contamination were found in seed as old as 10 yr. The data presented suggest that *F. lateritium* may be widespread in association with sweetpotato.

Chlorotic leaf distortion (CLD) is a recently described disorder of sweetpotato (*Ipomoea batatas* (L.) Lam.) caused by *Fusarium lateritium* Nees:Fr. (4). The causal fungus has a unique association with the plant—it colonizes a mucilagelike material produced by the plant on the apical meristem and young, folded leaves but does not penetrate the plant (1). In determining the etiology of CLD, it was found that *F. lateritium* could be isolated from NaOCl-treated apical meristems, axillary buds, young folded leaves, unopened flower buds, various flower parts, and true seed but not from roots (4).

In commerce, sweetpotato is propagated vegetatively. However, true seed is produced naturally in small numbers at some latitudes. The efficiency of seed production is poor, and considerable effort is required to produce seed for breeding purposes (5). Because of potential problems with viruses, vegetative parts are generally subject to quarantine restrictions. Sweetpotato seed has not previously been shown to be an important source of pathogens. Therefore, it has been recommended, where possible, that true seed be used for international exchange of sweetpotato germ plasm (12). Little has been published about the geographic distribution of CLD or the causal fungus since its first report in Louisiana (4). CLD also has been reported in North Carolina, South Carolina, and Kenya (9,11,14). This study was undertaken as part of a program to assess the potential and importance for dissemination of CLD by *F. lateritium*-infested sweetpotato seed and to learn more about the geographic distribution of CLD. A preliminary account has been presented (3).

Approved for publication by the director of the Louisiana Agricultural Experiment Station as manuscript 93-38-7342.

Accepted for publication 1 March 1994.

MATERIALS AND METHODS

In a preliminary experiment, a seed lot produced at Baton Rouge, Louisiana, on sweetpotato clone L85-237 that had a high level of infestation with *F. lateritium* was divided into two equal lots. The seed that floated in water (presumed nonviable) were separated from those that sank (presumed viable). One-half of the seed from each group was then scarified with concentrated sulfuric acid for 20 min with continual stirring; the other subsample was not scarified. Scarification is routinely used to improve germination of sweetpotato seed (7). The seed were allowed to air-dry, then were dipped briefly in 95% ethanol and surface-disinfested in 0.525% NaOCl for 5 min. The seed were rinsed in sterile distilled water (SDW) and allowed to stand in SDW overnight. The seed were then transferred to acidified potato-dextrose agar (APDA) and incubated at room temperature for 4 days.

Sweetpotato seed were solicited from numerous sweetpotato breeders around the world, and information was obtained on maternal parent, location and year of seed production, and any postharvest treatment of seed. After receipt, seed were stored at 4 C until they could be assayed, usually within 1–2 mo, for fungal contamination, following the standard procedure. Depending on the number of seed available, 48 seed were randomly selected from each seed lot for processing; when isolation frequency was low in the first subsample and sufficient additional seed were available, a second subsample of 48 seed was processed. Seed were not separated by floating. Seed were acid-scarified in concentrated H_2SO_4 for 20 min. They were then rinsed in running tap water to remove the acid and surface-disinfested in 0.525% NaOCl for 10 min. The seed were rinsed in SDW, and each seed was placed in a separate well in sterile 96-well, tissue culture low-evaporation-lid clusters (No. 3595, Costar, Cambridge, MA) containing 150 μl of SDW in each 6.4-mm-diameter well. The seed were allowed to imbibe at room temperature overnight and were transferred to APDA (up to 10 seed per 9-cm-diameter petri dish). The plates were incubated at room temperature for 7 days, and the percent seed germination, percent seed producing any fungal colonies, and percent seed producing colonies similar in gross appearance to *F. lateritium* were recorded.

Depending on the incidence of isolation, as many as five putative *F. lateritium* isolations per seed lot were mass-transferred to PDA. Single-conidium transfers were then made, and each isolate was grown on carnation leaf agar in petri dishes placed 25 cm under a combination of fluorescent and black lights at room temperature (approximately 23–26 C). The cultures were examined with a microscope, and those that produced three- to five-septate macroconidia, 3–4 × 25–60 μm in dimension, with a beak, bend, or nipple in the apical cell were identified as *F. lateritium* (13).

RESULTS

Isolation percentages in the preliminary experiment from nonscarified floaters, nonscarified sinkers, scarified floaters, and scarified sinkers were 70, 34, 83, and 16%, respectively, for *F. lateritium* and 93, 45, 97, and 32%, respectively, for all fungi. The fungus was isolated both from germinated seed and from seed (mostly floaters) that did not germinate. In fact, many seedlings continued to grow despite development of colonies of *F. lateritium* and could be transplanted to soil in a greenhouse and retain their viability (C. A. Clark, *unpublished*). Some of the sinkers did not swell and their seed coats did not crack even after 7 days of incubation on APDA. Generally, *F. lateritium* was not recovered from these seed and often did not grow out of other sinkers until after the seed coat cracked.

Because seed lots provided by cooperating scientists generally had a small proportion of floaters or did not include floaters, data were not kept separately for sinkers and floaters. Seed germination percentages and the isolation frequency of *F. lateritium* from seed received from around the world were highly variable (Table 1). The fungus was not detected from many seed lots, and the greatest frequency of isolation was 58%. Although the isolation frequency was generally higher from seed lots from the United States, high levels of contamination also were found in certain seed

lots from Brazil, Indonesia, and several African countries. Seed lots provided from Charleston, South Carolina, had been produced at the same location in different years by A. Jones and P. Dukes. The percent isolation of *F. lateritium* by year of seed production was: 1976, 0%; 1978, 2%; 1979, 2%; 1980, 3%; 1981, 22%; 1982, 16%; 1983, 2%; 1984, 0%; 1985, 29%; 1986, 48%; 1987, 4%; 1988, 8%; and 1990, 4% (isolations conducted in 1991).

DISCUSSION

F. lateritium was isolated from sweetpotato seed produced in many countries from which CLD has not yet been reported. It appears that the fungus is widely distributed in association with sweetpotato because it was found in seed from Africa, Asia, and South America as well as the United States. This fungus also has been isolated from seed of *I. hederacea* (L.) Jacq. and *I. setosa* Kerr from the field in Louisiana (C. A. Clark, *unpublished*). Another *Fusarium* species, *F. moniliforme* J. Sheld., has been isolated from sweetpotato flower parts and was described as the cause of a disease of pollen mother cells (6,10). It also may have reduced seed production (8). *F. moniliforme* was not isolated from any of the seed in this study and was isolated only once (from a floral bud) in an earlier study (4). *F. equiseti* (Corda) Sacc., *F. oxysporum* Schlechtend.:Fr., *F. proliferatum* (T. Matsushima) Nirenberg, *F. semitectum* Berk. & Ravenel, and *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun, & Marasas each was isolated from a small percentage of seed from one to three locations. However, *F. lateritium* was the predominant *Fusarium* species isolated from sweetpotato seed, and none of the other species induced symptoms on sweetpotato vines in pathogenicity tests (*unpublished*).

It is not known what effect weather conditions have on seed contamination by *F. lateritium*, but the year-to-year variation in isolation frequency from seed produced at one location suggests that weather may influence contamination. Likewise, the effect of maternal genotype on seed infection is unknown. In this study, virtually all seed lots were produced on different sweetpotato genotypes. Thus, although absence of the fungus is one possible reason for failure to isolate *F. lateritium* from seed from some locations, unfavorable weather and a resistant parent might also be factors. It is likely that the isolation methods used in this study give a conservative estimate of the occurrence of *F. lateritium* in sweetpotato seed, since only scarified seed was used and since the number of seed lots available was limited in most cases to seed produced in 1 yr. However, combined with reports of CLD from Kenya (11) and anecdotal observations of CLD symptoms on sweetpotato from Peru (T. Icochea, *personal communication*) and Brazil (V. Duarte, *personal communication*), it is likely that CLD will be found to be a widespread disease even though there are few formal reports of its occurrence in the literature.

The fact that *F. lateritium* was recovered from scarified seed that was subsequently disinfested with NaOCl and that it seemed to grow out of viable seed more frequently after the seed coat cracked suggests that the fungus is present internal to the seed coat. This is in contrast to histological observations, which indicate that the fungus does not enter the vegetative shoot where symptoms develop (1). Although properly stored sweetpotato seed can maintain viability for many years (7), it is clear that the fungus can persist in the seed for many years, as indicated by the fact that 22% of a 10-yr-old seed lot was contaminated.

The isolates of *F. lateritium* obtained in this study have provided a useful culture collection to study diversity of the fungus. Preliminary results indicate that isolates vary in vegetative compatibility, pathogenicity, and double-stranded RNA content and that there are minor variations in morphological characteristics (2). Further research is in progress to determine the similarity and relatedness of isolates obtained from different locations to provide evidence on how the fungus might have been disseminated and to further evaluate the impact of CLD on international exchange of sweetpotato seed.

Table 1. Germination and isolation of *Fusarium lateritium* and other fungi from sweetpotato seed

Origin	Sites (no.)	Seed lots (no.)	Total seed (no.)	Percent seed germination (min.-mean-max.)	Percent isolation of other fungi (min.-mean-max.)	Percent isolation of *F. lateritium* (min.-mean-max.)	Seed lots with *F. lateritium* (no. positive/no. tested)
North America							
United States							
Louisiana, South Carolina	2	18	1,115	31–62–80	0–2.5–14	0–17–58	15/18
Puerto Rico	1	2	97	25–48–71	0–1.0–2	15–18.5–22	2/2
South America							
Argentina	1	4	190	4–33–63	0–11–32[a]	0–1–2	2/4
Brazil	2	16	479	8–41–92	0–7.9–27[b]	0–5.4–54	4/16
Peru	1	12	594	51–88–100	0–1.2–8	0–0.3–2	2/12
Africa							
Burundi	1	4	140	38–59–70	7–25–45	9–17–24	4/4
Cameroon	1	10	215	0–8–35	0	0	0/10
Ghana	1	3	113	72–83–95	5–19.7–32	7–13.7–21	3/3
Kenya	3?	7	223	0–52–100	0–3–15	0–6.3–42	2/7
Nigeria	1	11	541	0–44–94	0–7–46	0–3.7–20	4/11
Rwanda	1	10	297	52–82–100	0–1.6–7[c]	0–1.8–17	5/20
South Africa	1	6	326	31–41–56	0	0	0/6
Zambia	1	5	239	10–54–87	0	0–2.0–6	3/5
Australia	1	1	25	88	0	0	0/1
Asia							
China (PR)	1	3	148	24–73–100	0–1–2	0	0/3
Indonesia	3	9	428	0–34–81	0–26–81[d]	0–7.8–19	8/9
Japan	1	6	354	27–72–100	0	0	0/6
Papua New Guinea	1	1	98	55	0	1	1/1
Philippines	6	16	768	6–37–71	0–2.7–10[e]	0–1.9–10	5/16
Taiwan (ROC)	2	3	281	37–66–96	0	0–1–3	1/3

[a] Includes three isolations of *Fusarium equiseti* and one probable isolation of *F. semitectum*.
[b] Includes one isolation of *F. equiseti* and two isolations of *F. oxysporum*.
[c] Includes one isolation of *F. equiseti*.
[d] Includes one isolation of *F. proliferatum* and two isolations of *F. oxysporum*.
[e] Includes four isolations of *F. subglutinans*.

ACKNOWLEDGMENTS

We thank P. E. Nelson, Fusarium Research Center, The Pennsylvania State University, for confirming identifications of *Fusarium lateritium* isolates. We are grateful to the International Potato Center (CIP) and the Louisiana Sweet Potato Advertising and Development Commission for financial support. We also thank the following providers of sweetpotato seed: A. Jones and P. Dukes, USDA-ARS, Charleston, South Carolina; L. Skoglund and N. Smit, CIP, Nairobi, Kenya; T. Ichochea, CIP, Lima, Peru; C. Bester, VOPRI, Pretoria, South Africa; A. Boy, INTA, San Pedro, Argentina; Z. Chongwen, Xuzhou Sweet Potato Research Center, People's Republic of China; E. Chujoy, CIP, Manila, Philippines; V. Duarte, UFRGS, Porto Alegre, Brazil; S. K. Hahn, IITA, Ibadan, Nigeria; G. Hartman, AVRDC, Shanhua, Taiwan; H. Kukimura, KBI, Kagoshima, Japan; D. La Bonte and L. Rolston, LAES, Baton Rouge, Louisiana; C. Lopes, EMBRAPA, Brasilia, Brazil; I. Mok, CIP, Bogor, Indonesia; J. Ngeve, IRA, Yaounde-Messa, Cameroon; R. Soenarjo, RTIP, Mansa, Zambia; M. Soto, PRAPAC, Ruhengeri, Rwanda; A. Villordon, VISAYAS, Baybay, Leyte, Philippines; and H. Vinck, ISABU, Moso, Burundi.

LITERATURE CITED

1. Clark, C. A. 1992. Histological evidence that *Fusarium lateritium* is an exopathogen on sweetpotato with chlorotic leaf distortion. Phytopathology 82:656-663.
2. Clark, C. A., Hoy, M. W., Nelson, P. E., and McCormack, S. 1993. Diversity among isolates of *Fusarium lateritium* from sweetpotato. Page 132 in: Abstr. Int. Congr. Plant Pathol. 6th.
3. Clark, C. A., Hoy, M. W., and Stine, B. S. 1992. Isolation of *Fusarium lateritium* from sweetpotato true seed from different countries. (Abstr.) Phytopathology 82:1073.
4. Clark, C. A., Valverde, R. A., Wilder-Ayers, J. A., and Nelson, P. E. 1990. *Fusarium lateritium*, causal agent of sweetpotato chlorotic leaf distortion. Phytopathology 80:741-744.
5. Edmond, J. B., and Ammerman, G. R. 1971. Sweet Potatoes: Production, Processing, Marketing. AVI Publishing, Westport, CT.
6. Jones, A. 1964. A disease of pollen mother cells of sweetpotato associated with *Fusarium moniliforme*. Phytopathology 54:1494-1495.
7. Jones, A., and Dukes, P. D. 1982. Longevity of stored seed of sweet potato. HortScience 17:756-757.
8. Jones, A., Dukes, P. D., and Cuthbert, F., Jr. 1977. Pesticides increase true seed production of sweet potato. HortScience 12:165-167.
9. Jones, A., Dukes, P. D., Schalk, J. M., Hamilton, M. G., and Baumgardner, R. A. 1987. 'Southern Delite' sweet potato. HortScience 22:329-330.
10. Jones, A., and Jackson, C. R. 1968. Fungi from floral parts of sweet potato (*Ipomoea batatas* (L.) Lam.). HortScience 3:76-77.
11. Kihurani, A. W., Skoglund, L. G., and Gatumbi, R. W. 1993. Occurrence of sweetpotato chlorotic leaf distortion caused by *Fusarium lateritium* in Kenya. Plant Dis. 77:1260-1261.
12. Moyer, J. W., Jackson, G. V. H., and Frison, E. A., eds. 1989. FAO/IBPGR Technical Guidelines for the Safe Movement of Sweet Potato Germplasm. FAO, United Nations, Rome.
13. Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press, University Park.
14. Ristaino, J. B., Averre, C. W., and Abad, G. 1991. Occurrence of sweetpotato chlorotic leaf distortion (*Fusarium lateritium*) in North Carolina. Plant Dis. 75:750.