Mating Groups in Fusarium moniliforme

W. H. Hsieh, S. N. Smith, and W. C. Snyder

Former Graduate Student, Plant Pathologist, and Professor Emeritus, respectively, Department of Plant Pathology, University of California, Berkeley, CA 94720. Present address of senior author: Taiwan Sugar Experiment Station, 54 Sheng Chaan Road, Tainan, Taiwan, Republic of China.

Accepted for publication 4 March 1977.

ABSTRACT

HSIEH, W. H., S. N. SMITH, and W. C. SNYDER. 1977. Mating groups in Fusarium moniliforme. Phytopathology 67: 1041-1043.

Crossing attempts with 60 *Fusarium moniliforme* isolates on a variety of media, yielded 19 isolates that formed the *Gibberella* stage. These cultures fell into three mating groups that were not interfertile, in that perithecia were not formed by mating any two cultures of different mating groups regardless of their sex and compatibility characteristics. For perithecium production for two mating groups, V-8 juice agar was an excellent medium, whereas perithecia in the other mating group were produced more abundantly on rice straw or other natural media in PDA. Perithecia formed most abundantly at 20 C under 12-hr alternating light and dark than at higher or lower temperatures, constant light, or constant darkness.

Additional key words: corn, rice, sugar cane.

Fusarium moniliforme (Sheld.) emend. Snyd. & Hans. (8) has a wide host range and is widespread throughout the world; only heterothallic types are known (1). The well-known diseases caused by this fungus are: bakanae and foot rot or stunting of rice, pokkah boeng of sugarcane, corn ear rot, stalk rot and leaf blight of corn, stalk rot of sorghum, endosepsis of fig (1), and crown rot of asparagus (3). The fungus not only causes considerable damage on many plants, but also is parasitic on plants without production of visible symptoms.

The *Gibberella* stage of the fungus was found first by Wineland (11) when she put two compatible mating types together in culture. The occurrence of *Gibberella moniliformis* Wineland in nature has been reported in Japan (5), Taiwan (7), the United States (10), Australia (2), and according to Snyder (W. C. Snyder, *unpublished*) the perfect stage is produced on corn in Mexico, central Europe, and Italy. The purpose of this work was to test interfertility of isolates from various sources.

MATERIALS AND METHODS

Isolates of *Fusarium moniliforme* were collected from many plants and locations in California and Taiwan during 1972-1973. In addition, isolations were made from plant material sent from other states and countries to the laboratory in Berkeley. Isolates from 12 countries and eight states in the USA were used in the study. Potato-dextrose agar (PDA) was used for maintaining all of the isolates. Single conidia were isolated directly from sporodochia produced on diseased plants or transferred from cultures in the collection.

Fusarium moniliforme often was obtained from healthy-looking corn roots and stalks. The fungus seemed to be parasitic mostly on epidermal tissue, seldom invading more than a few cell layers deep until the tissues became mature.

Potato-dextrose agar, water agar, V-8 juice agar (pH 6.8), lima bean agar, Sachs' agar (calcium nitrate, 1.0 g; dipotassium hydrogen phosphate, 0.25 g; magnesium sulfate, 0.25 g; ferric chloride, trace; calcium carbonate, 4.0 g; agar, 20 g; and water, 1 liter) plus rice straw, and 33 different kinds of straw and other natural plant materials (dried stems, leaves, seeds, wood, etc.) were tested for their effect on formation of the *Gibberella* stage. The natural materials were sterilized with propylene oxide (4) and were added to PDA or water agar in deep slants. To determine compatibility and sex, isolates were intercrossed as soon as the fungus growth covered the slant of the various test agars. Sterilized water was used to wash spores of one isolate (acting as male) onto the surface of another isolate (acting as female). Each cross was repeated two or three times. After crossing, the cultures were incubated at room temperature 36 cm beneath two cool-white fluorescent tubes (approximately 1,900 lux) under natural light. All crosses were inspected for formation of perithecia and ascospores during an incubation period of up to 2 mo.

To test the effect of light and temperature on the formation of the *Gibberella* stage, two mating cultures, M-1a (♂-) and M-1e (♀+), grown on Sachs' agar plus rice straw were crossed. Some crosses were incubated out-of-doors (in Berkeley) during August to September (1972), others were incubated at 15, 20, 25, and 30 C under constant light (36 cm beneath two cool-white fluorescent tubes, approximately 1,900 lux) or with a 12-hr photoperiod, and still others were incubated at 15, 20, 25, and 30 C in the dark. Three wk later, cultures were inspected for perithecia and ascospore formation.

RESULTS

Mating types and sex.—Nineteen of the 60 isolates crossed successfully with one or more of the isolates. They segregated into three mating groups designated A, B, and C (Table 1). Five of the 19 clones proved to be hermaphroditic and 14 were unisexual; of the latter, eight were male and six were female. The compatibility ratio was 11 (+) to 8 (−). In mating group B, the sex of M-15a, 15b, 15d, and 15f remains unknown; when crossed with known mating types these isolates formed perithecia without asci.

None of the isolates in this study produced the perfect stage alone, nor did isolates from one mating group cross with isolates of either of the other two mating groups, under conditions of our tests. Mating group A included isolates from corn, wheat, rye, and pine, and ants, aphids, and lady bugs associated with corn plants in the field, whereas mating groups B and C included only isolates from sugarcane and rice, respectively. The ascospores of groups A and C were two-celled, and those of group B were two- to six-celled. All mature asci contained eight ascospores.

Light and temperature requirements for the production of perithecia.—Perithecia were produced most abundantly in vitro at 20 C when exposed to diffuse light for 12-hr intervals. Fewer perithecia were produced out-of-doors, under constant light, or at 25 C. No perithecia formed at the other temperatures or in the dark.

Effect of media on formation of perithecia.—The V-8 juice agar was the best medium for formation of the *Gibberella* stage in mating groups A and B. For mating group C, Sachs' agar plus rice straw, or dried banana leaves, carrot leaves, or rice straw added to PDA were best. The V-8 juice was unfavorable to perithecium production in group C. Likewise, media suitable for perithecial production in mating group C were unsuitable for fruiting of mating groups A and B. Perithecia of isolates in mating group A and B formed on V-8 juice agar 7-10 days after crossing, whereas in mating group C they formed on natural media 3-6 wk after crossing.

DISCUSSION

The phenomenon of mating groups is not unique to the species *F. moniliforme*. For example, *F. solani* f. sp. *cucurbitae* race 1 cannot fertilize *F. solani* f. sp. *cucurbitae* race 2 nor *F. solani* f. sp. *pisi* (6). Recently Ueyama and Tsuda (9) also found that there are two mating groups in *Cochliobolus miyabeanus* which behave similar to *Gibberella moniformis* in our findings.

Since the mating groups thus far encountered are restricted to separate hosts, the question arises as to whether they represent genetically distinct forms incapable of intergroup crossing or whether they vary simply because they developed in ecologically different niches and intergroup crossing may be dependent on the proper nutrition.

Fusarium moniliforme does not exhibit great cultural and morphological variation, but in the present work a beginning has been made to define natural variation of this fungus.

TABLE 1. Characteristics and origin of mating groups of *Fusarium moniliforme*

Mating group and clone	Origin	Crop	Sex	Compatibility
Mating Group A:				
M-2a	Minnesota	corn kernels	♀ ♂	+
M-2d	California, Modesto	corn cobs	♂	−
M-4	Georgia	rye kernels	♀ ♂	+
M-9	Missouri	corn roots	♂	−
M-9b	Taiwan	corn stalks	♀ ♂	−
M-9c	California, Watsonville	corn roots	♂	−
M-20	California, Sacramento	wheat roots	♂	+
M-21	California, Sacramento	ants in corn	♀	+
M-22	California, Sacramento	lady bugs		+
M-23	California, Modesto	aphids in corn	♀ ♂	+
M-28	Taiwan	pine	♂	+
Mating Group B				
M-15a	Taiwan	sugarcane	?[a]	+
M-15b	Taiwan	sugarcane	?	+
M-15c	Taiwan	sugarcane	♀ ♂	−
M-15d	Taiwan	sugarcane	?	−
M-15e	Taiwan	sugarcane	♀ ♂	+
M-15f	Taiwan	sugarcane	?	−
M-15g	Taiwan	sugarcane	♀	−
Mating Group C				
M-1a	Taiwan	rice	♂	−
M-1b	Taiwan	rice	♀ ♂	+
M-1c	Taiwan	rice		−
M-1d	Taiwan	rice	♀	+
M-1e	Taiwan	rice	♀	+

[a]Assignment to mating group B was tentative because perithecia did not mature.

All of our isolates produced macroconidia, and in almost all of the isolations made from nature, microconidia were formed in chains. In some, microconidia formed both in chains and in false heads in the same culture. Sometimes, however, chains were produced only during the first day or two after isolation from nature; thereafter, only false heads were seen in the culture and in subsequent transfers. This observation raises a question about some isolates described as *F. moniliforme* var. *subglutinans*. The possibility exists that by daily observation of most cultures from the time of the original isolation, chains may be noted.

LITERATURE CITED

1. BOOTH, C. 1971. The genus Fusarium. Commonw. Mycol. Inst., Kew, Surrey, England. 237 p.
2. EDWARDS, E. T. 1935. Studies on Gibberella fumikuroi var. subglutinans the hitherto undescribed ascigerous stage of Fusarium moniliforme var. subglutinans and on its pathogenicity on maize in New South Wales. N. S. W. Dep. Agric. Sci. Bull. 49:1-68.
3. ENDO, R. M., and E. C. BURKHOLDER. 1971. The association of Fusarium moniliforme with the crown rot complex of asparagus. Phytopathology 61:891 (Abstr.).
4. HANSEN, H. N., and W. C. SNYDER. 1947. Gaseous sterilization of biological materials for use as culture media. Phytopathology 37:369-371.
5. ITO, S., and J. KIMURA. 1931. Studies on the 'bakanae' disease of rice plant. Hokkaido Agric. Exp. Stn. Rep. 27:1-99.
6. REICHLE, R. E., W. C. SNYDER, and J. MATUO. 1964. Hypomyces stage of Fusarium solani f. pisi. Nature (Lond.) 203:664-665.
7. SAWADA, K. 1927. Beitrage uber Formosa-Pilze No. 14, Trans. Nat. Hist. Soc. Formosa 31:31-133.
8. SNYDER, W. C., and H. N. HANSEN. 1945. The species concept in Fusarium with reference to discolor and other sections. Am. J. Bot. 32(10):657-666.
9. UEYAMA, A., and M. TSUDA. 1976. Mating type and sexuality of Cochliobolus miyabeanus, the perfect stage of Helminthosporium oryzae. Ann. Phytopathol. Soc. Jap. 42:1-6.
10. VOORHEES, R. K. 1933. Gibberella moniliformis on corn. Phytopathology 23:368-378.
11. WINELAND, G. O. 1924. An ascigerous stage and synonymy for Fusarium moniliforme. J. Agric. Res. 28:909-922.