A Black, Readily Sporulating Mutant of Stemphylium solani

Homer D. Wells, Ian Forbes, and Charles R. Markham

Research Plant Pathologist, Research Agronomist, and Agricultural Research Technician, respectively, Crops Research Division, ARS, USDA, Tifton, Georgia 31794.

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ABSTRACT

A mutation for black pigment and abundant spore production occurred in one of our cultures (61-20-E) of *Stemphylium solani* from blue lupine, *Lupinus angustifolius*. The mutant, isolates 61-20-E-1 and 61-20-E-2, sporulates readily in complete darkness and is equal to the parent strain in pathogenicity to susceptible biotypes of blue lupine. Phytopathology 61:575-577.

Additional key words: gray leaf spot.

Stemphylium solani Weber was first reported as a causal organism of gray leaf spot on blue lupine, *Lupinus angustifolius* L., in 1956 (10). Hackbarth (7) states that gray leaf spot is potentially the most damaging disease in northern and northwestern Germany, where the seed harvest was reduced to zero in 1962 and 1965. We have successfully used a number of *S. solani* isolates in surveying blue lupine accessions for sources of resistance to the pathogen, in genetic studies of host resistance, and in incorporating genes for resistance into two disease-resistant forage cultivars (Rancher and Frost) (3, 4, 5, 6). In all of these studies, we artificially inoculated seedlings in the greenhouse with spores produced in vitro. We encountered the same problem of poor sporulation on artificial media that other workers have reported with this fungus (1, 2, 8, 9). Our period of active work with the cultures was from November to February each year. The stock cultures were maintained during the remainder of the year under mineral oil in culture tubes on 20% V-8 juice agar (V-8A).

Materials and Methods.—One of our *S. solani* isolates, designated 61-20-E, originated from a single conidium taken from a leaf spot on blue lupine from Newberry, Fla., in April 1961. The 61-20-E isolate is light gray, and sporulates only sparsely unless given special treatment. In the fall of 1969, when we made transfers from the stock cultures maintained under mineral oil, four of the transfers of 61-20-E appeared typical, but two contained black sectors (Fig. 1). At first we thought the black sectors were contaminants. A microscopic examination, however, revealed the black sectors to be a sporulating biotype of *S. solani.* The two black sectors designated 61-20-E-1 and 61-20-E-2 appeared identical, and perhaps resulted from a mutation while the culture was under mineral oil between February and November 1969. We had older stock cultures of 61-20-E under mineral oil from 1966, 1967, and 1968. These were viable, but produced only the typical 61-20-E colonies when transfers were made from them. Our spore production technique for 61-20-E consisted of growing the *S. solani* for 7 days in culture dishes on V-8A at ambient temperatures (20-25 C) in the laboratory, aseptically scraping the aerial mycelium from the cultures with a scalpel, and placing the scraped cultures approx 36 cm below 40-w daylight-type fluorescent bulbs for 10 hr/day for a period of 7 days. This technique resulted in the production of an abundance of virulent conidia. Conidia of 61-20-E-1 and 61-20-E-2 were produced without scraping or supplemental light on 7-day-old transfers on V-8A. Aqueous suspensions of the conidia were sprayed on 14-day-old blue lupine seedlings growing in 10-cm clay pots in the greenhouse. These plants were placed in a moisture chamber for 48 hr and rated for disease reaction 5 days after inoculation. Seedlings of the blue lupine genetic stocks, with genotypes in parentheses, tested for reaction to the three sources of conidia included: *S. solani* susceptible Blanco ($Gl_1\ Gl_1$, $Gl_2\ Gl_2$); resistant Rancher ($gl_1\ gl_1$, $Gl_2\ Gl_2$); resistant Frost ($Gl_1\ Gl_1$, $gl_2\ gl_2$); and several hybrid populations segregating for susceptibility versus resistance. The two recessive gene pairs, $gl_1\ gl_1$ and $gl_2\ gl_2$, are independent, and either pair conditions near-immunity to *S. solani* injury (5, 6).

Evaluations of the light requirements for sporulation of the mutant isolates consisted of growing transfers of single conidia of the parent culture, 61-20-E, and mutant isolates, 61-20-E-1 and 61-20-E-2, on V-8A and potato-dextrose agar (PDA) in 100-mm culture dishes under various light regimes at ambient temperatures (20-25 C) in the laboratory. Light regimes consisted of (i) total darkness (in a photographic changing bag); (ii) normal laboratory light; and (iii) 10 hr/day at a distance of 36 cm below daylight-type fluorescent bulbs. Each treatment was replicated 4 times.

Results.—In all instances, the disease reactions from inoculations with the two mutant isolates, 61-20-E-1 and 61-20-E-2, were identical to those obtained from inoculations with 61-20-E. All resistant phenotypes tested were resistant to all three isolates; all susceptible phenotypes were susceptible to all three isolates.

There were no noticeable differences in colony diam among isolates in different light regimes on the different media. In all three light regimes and on both V-8A and PDA, the mutant isolates, 61-20-E-1 and 61-20-E-2, were black and produced an abundance of conidia. There was no difference in colony color or spore density of the two mutant isolates that could be related to light. The parent isolate, 61-20-E, however, was almost completely white when grown in the photographic

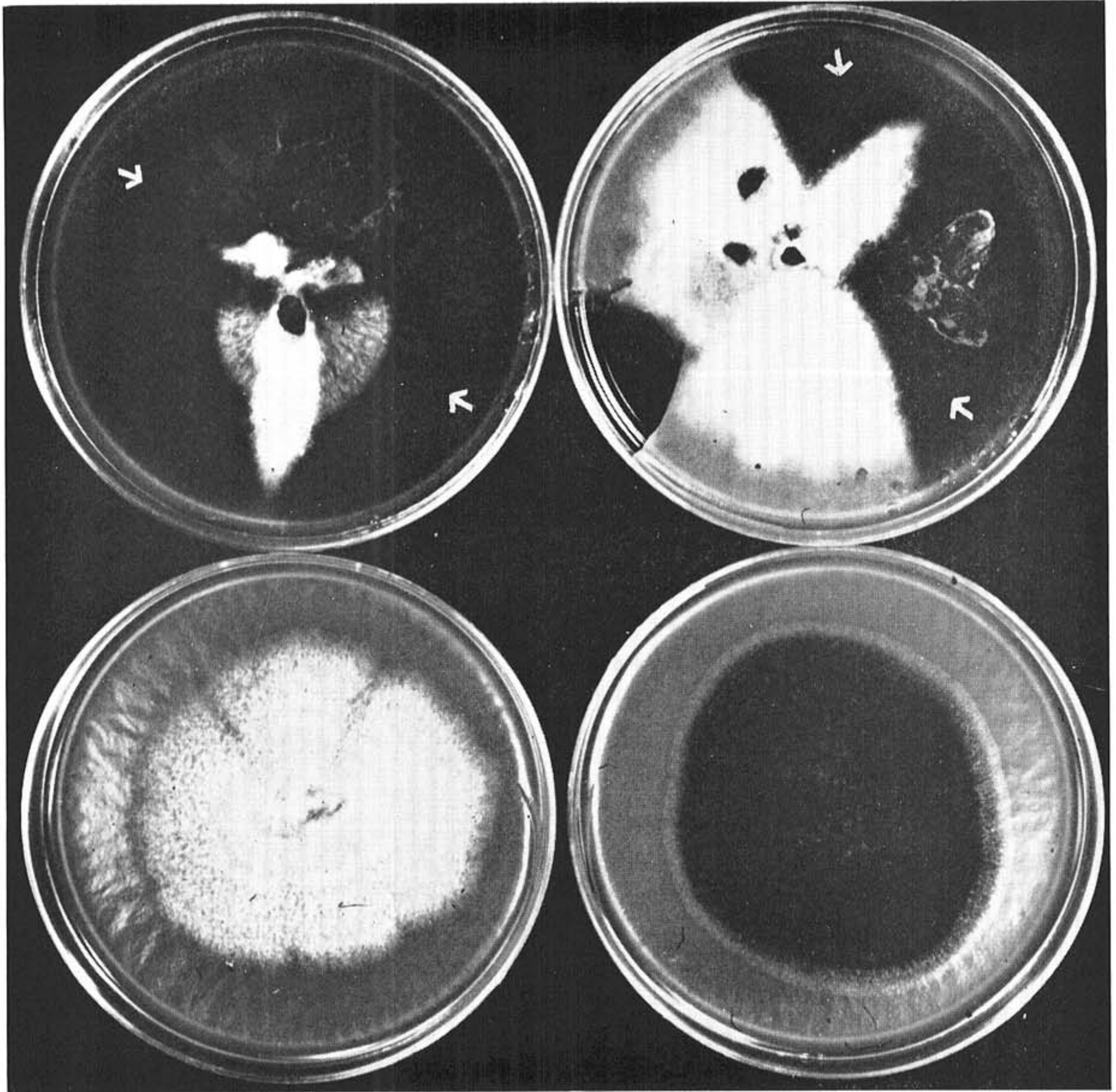

Fig. 1. (Upper left) Transfer from culture 61-20-E of *Stemphylium solani* showing dark pigment sector designated 61-20-E-2; (upper right) transfer from 61-20-E showing dark pigment sector designated 61-20-E-1; (lower left) 61-20-E after 10 days on V-8A in a photographic changing bag; (lower right) 61-20-E-1 after 10 days on V-8A in a photographic changing bag.

changing bag and no spores were produced. A comparison of color difference between 61-20-E and 61-20-E-1, grown on V-8A in the photographic changing bag, is shown in Fig. 1. Under normal laboratory lighting conditions, the parent culture (61-20-E) was light gray, and a few spores were present after 10 days on V-8A but not on PDA. At 10 hr/day under the daylight-type fluorescent light, the parent culture (61-20-E) was dark gray and produced a few more spores than were produced under normal laboratory lighting conditions on V-8A. No spores were produced on PDA, and the numbers of spores produced on the V-8A were infinitesimal as compared to the spores produced by the black mutants on either media.

Discussion.—The black mutant isolates, 61-20-E-1 and 61-20-E-2, have not changed in virulence from that of the parent, 61-20-E. The relative simplicity of producing abundant, virulent conidia from the mutant makes it a valuable biotype for use in genetic studies. It is also practical for use in breeding programs at other locations, where *S. solani* is a serious pathogen of blue lupine. The mutation appears to affect only colony pigment and spore production. The parent culture is characteristic of most *S. solani* isolates, and apparently re-

quires a light reaction to produce conidia. The mutation permits the fungus to produce conidia in total darkness. The dark pigment may be associated with the light requirement change; i.e., both characters may be pleiotropic effects of the same mutant gene.

We have the normal parent culture, 61-20-E, and the two mutant isolates, 61-20-E-1 and 61-20-E-2, and will make them available to researchers for use in studying light requirements, biochemistry of spore production of fungi, and other related phenomena.

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