Mutations at the *SMO* Genetic Locus Affect the Shape of Diverse Cell Types in the Rice Blast Fungus

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ABSTRACT

Teflon film surfaces are highly conducive to the formation of infection structures (appressoria) in the plant pathogenic fungus, *Magnaporthe grisea*. We have utilized Teflon films to screen and select for mutants of *M. grisea* that are defective in appressorium formation. This approach and several others yielded a group of 14 mutants with a similar phenotype. All the mutant strains make abnormally shaped conidia and appressoria. When two mutant strains are crossed, abnormally shaped asci are formed. Ascus shape is normal when a mutant strain is crossed with a wild-type strain. Despite dramatic alterations in cell shape these strains otherwise grow, form conidia, undergo meiosis, and infect plants normally. This mutant phenotype, which we have termed Smo$^-$, for abnormal *s*pore *mo*rphology, segregates in simple Mendelian fashion in crosses with wild-type strains. Some ascospore lethality is associated with *smo* mutations. In genetic crosses between mutants, *smo* mutations fail to recombine and do not demonstrate complementation of the abnormal ascus shape phenotype. We conclude that the *smo* mutations are alleles of a single genetic locus and are recessive with regard to the the ascus shape defect. Mutations at the *SMO* locus also permit germinating *M. grisea* conidia to differentiate appressoria on surfaces that are not normally conducive to infection structure formation. A number of spontaneous *smo* mutations have been recovered. The frequent occurrence of this mutation suggests that the *SMO* locus may be highly mutable.

THE ascomycetous fungus *Magnaporthe grisea* Barr [*Pyricularia* spp.] (BARR 1977; VALENT *et al.* 1986) is a pathogen of grasses that causes a devastating disease of rice called rice blast disease (for review see OU 1985). *M. grisea* is well suited as an experimental system for genetic and biochemical investigation of fungal pathogenicity towards plants (see MATERIALS AND METHODS). This paper reports an effort to initiate a genetic analysis of one of the early events in fungal phytopathogenesis, formation of the specialized infection structure known as an appressorium.

Asexual spores, or conidia, of the fungus infect plants under conditions of high relative humidity. The apical cell of a hydrated conidium expels a mucilage that attaches the spore to the leaf surface (HAMER *et al.* 1988). The conidium rapidly germinates by the growth of a hyphal element called a germ tube from one or more of the three cells comprising the conidium. The tip of the elongating germ tube enlarges and forms a dome-shaped, melanized infection structure, the appressorium (UCHIYAMA *et al.* 1979; T. BOURETT and R. J. HOWARD, manuscript in preparation). The appressorium elaborates a thin infectious hypha that penetrates directly through the plant cuticle and enters the underlying plant cell. Hyphae grow through and between leaf cells, and a conidiating lesion is observed five days after inoculation with a virulent strain of *M. grisea* (for review see LATTERELL 1975).

M. grisea conidia can be induced to form appressoria *in vitro*. We have shown that germinating conidia in contact with a hydrophobic surface, such as Teflon film, form appressoria efficiently. However, appressoria are never formed in liquid media and they are rarely formed when germinating conidia are in contact with a smooth, cleaned glass surface (HAMER *et al.* 1988). Many other plant pathogenic fungi can form infection structures *in vitro*, and considerable research has been devoted to the biochemistry and ultrastructure of appressorium formation (for review see EMMETT and PARBERRY 1975). Staples and colleagues have documented changes in protein and RNA synthesis during infection structure formation in the rust, *Uromyces* spp. (for reviews see STAPLES 1985; HOCH and STAPLES 1987; STAPLES and HOCH 1987), and they have described the substratum surface parameters necessary to trigger infection structure formation (HOCH *et al.* 1987). The availability of techniques for mutant isolation and genetic analysis in *M. grisea* should allow the identification of gene products critical for infection structure formation in plant pathogenic fungi.

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TABLE 1

Magnaporthe grisea strains

Strain	Description	Source
4091-5-8	*Mat1-2*; laboratory wild-type strain, pathogen of weeping lovegrass and goosegrass	VALENT *et al.* (1986)
4136-4-3	*Mat1-1*; laboratory wild-type strain, pathogen of weeping lovegrass and goosegrass	VALENT and CHUMLEY (1987)
4136-1-2	*Mat1-1*; laboratory wild-type strain, pathogen of goosegrass and weak pathogen of weeping lovegrass	VALENT and CHUMLEY (1987)

The three-part number for the strains shown above and in the text denotes strains that are progeny from crosses (VALENT *et al.* 1986).

TABLE 2

Summary of Smo$^-$ strains

Strain	*smo*$^-$ Allele	Mating type	Parental origin	Method of induction; other mutations
CP750	*smo1-1*	2	4091-5-8	UV
CP751	*smo1-2*	2	4091-5-8	UV
CP665	*smo1-3*	1	4136-1-2	Spontaneous (*ben1-24*)
CP777	*smo1-4*	2	4091-5-8	UV, nonstick enrichment
CP778	*smo1-5*	2	4091-5-8	UV, nonstick enrichment
CP785	*smo1-7*	2	4091-5-8	Spontaneous
CP786	*smo1-8*	2	4091-5-8	UV, nonstick enrichment
CP787	*smo1-9*	2	4091-5-8	UV, nonstick enrichment
CP788	*smo1-10*	2	4091-5-8	UV, nonstick enrichment
CP789	*smo1-11*	2	4091-5-8	UV, nonstick enrichment
CP790	*smo1-12*	2	4091-5-8	UV, nonstick enrichment
CP824	*smo1-13*	2	4091-5-8	Spontaneous
CP892	*smo1-14*	2	4379-R-16	Spontaneous (*alb-25*)

Smo$^-$ strains used in this study. Another Smo$^-$ strain (CP784, *smo-6*) was obtained in the nonstick enrichment but was not characterized further. Strains designated "CP" are original mutant isolates.

Employing Teflon films, we pursued two approaches to identify genetic loci that play a role in infection structure formation. First, we screened a collection of prototrophic survivors of mutagenesis for individuals that might fail to form appressoria on Teflon film. Second, we attempted to isolate mutants that failed to attach to the surface of dishes made from Teflon films. In a third and separate approach, we attempted to identify mutants with cytoskeleton defects that might alter appressorium formation. Although we did not exhaust the potential of any of these approaches, we isolated a single class of mutants from all three procedures. We report the identification of a genetic locus in *M. grisea* that plays a role in appressorium formation and the determination of cell shape. Strains carrying mutations at this locus produce conidia, appressoria and asci with altered shapes. In addition, these mutations alter the ability of germinating conidia to discriminate appropriate surfaces for appressorium formation. Our results suggest that common pathways or processes are involved in the formation of these distinct cell types in *M. grisea*.

MATERIALS AND METHODS

Strains, media and genetic analysis: *Magnaporthe grisea* is a haploid, filamentous, heterothallic Ascomycete. *M. grisea* can be cultured on standard laboratory media (CRAWFORD *et al.* 1986). Vegetative growth results in the formation of numerous conidiophores bearing conidia with three isogenic nuclei (YAMASAKI and NIIZEKI 1965). Genetic analysis is accomplished by growing strains of opposite mating type in mixed culture on solid media. Perithecia are produced after 12 days' incubation, and segregation analysis can be conducted using tetrads or random ascospores (YAEGASHI and HEBERT 1976; YAEGASHI 1977; TAGA *et al.* 1979; NAGAKUBO *et al.* 1983). Numerous auxotrophic, drug resistant and color mutants have been isolated, and mutations have been shown to segregate appropriately in crosses (CRAWFORD *et al.* 1986). An integrative transformation system has been developed that facilitates the complementation of mutants with cloned, exogenous DNA (PARSONS, CHUMLEY and VALENT 1987).

M. grisea wild-type strains are listed in Table 1. Mutant strains are listed in Table 2. All mutant strains were purified by clonal propagation from a single conidium. Strain numbering, growth, storage, mutagenesis, genetic crosses, and media for *M. grisea* are described by CRAWFORD *et al.* (1986). Plates used to select and identify benomyl resistant mutants contained benomyl (supplied by Du Pont Agricultural Products Dept.) added to a final concentration of 2.5 μg/ml in 2YEG medium (2 g/liter yeast extract, 10 g/liter glucose). Teflon dishes were made by thermal molding 10 mil Teflon-PFA film (Du Pont Co.).

Infection assays: Infection assays for weeping lovegrass (*Eragrostis curvula*) were performed in a manner similar to that described by VALENT *et al.* (1986). Conidia were harvested from oatmeal agar cultures in 0.2% gelatin and filtered through two layers of cheese cloth. The concentration of the conidial suspension was adjusted to 5×10^5/ml with 0.2% gelatin solution, and 4 ml of the conidial suspension was used to inoculate 11–14-day-old plants using an artist's air brush. Plants were covered with plastic bags during inoculation and for 24 hr after inoculation in order to maintain humidity. After 24 hr the plants were removed from the bags and placed in a growth chamber. After 5 to 7 days plants were inspected for disease symptoms.

Spore germination and appressorium formation: Spore germination and appressorium formation were monitored on 10 mil Teflon film cut into 2 cm × 2 cm squares as well as on plastic or glass microscope coverslips. Conidia were harvested from oatmeal agar cultures in 0.2% gelatin, centrifuged at 1000 × *g*, resuspended in 10 mM phosphate buffer, pH 6.5, centrifuged again, and finally resuspended in phosphate buffer at a concentration of $1–2 \times 10^5$ conidia/ml. Conidia were deposited on glass or Teflon surfaces in 100 μl aliquots. Germination and appressorium formation were monitored microscopically as previously described (HAMER *et al.* 1988).

To screen large numbers of mutagenized colonies for their ability to form appressoria, prototrophic survivors of mutagenesis were first picked to oatmeal agar medium and allowed to grow for 3–4 days to produce conidiating colonies. A 100-μl droplet of water was added to the middle of each colony and the conidia were released by gentle agita-

tion with a toothpick. The conidial suspension was transferred to a strip of 10 mil Teflon film that was placed on a wet, dense sponge cut to fit a large stainless steel pan. The pans were covered to prevent drying and incubated at 23° for 14–24 hr. The Teflon strips were then inverted onto glass microscope slides and appressoria were observed using a Leitz phase contrast microscope.

Nonstick enrichment: *M. grisea* strain 4091-5-8 (Table 1) was mutagenized using UV light as described by CRAWFORD *et al.* (1986). Irradiated conidia were divided into eight pools of 10^7 conidia and plated on minimal medium. All pools were treated independently in subsequent manipulations. Conidia were harvested from minimal plates as described above, washed once in sterile water, resuspended at a concentration of 10^5 spores/ml in 50 ml of sterile water and poured into dishes formed from Teflon-PFA film. The dishes were covered and incubated for 18–24 hr at 23°. Unattached cells were removed by pouring off the liquid from the dishes and concentrating the cells by centrifugation. Cells were plated on oatmeal agar medium to produce more conidia. This cycle of enrichment was repeated from 2 to 6 times and the cells from the final wash were diluted and plated on plates with complete medium containing 3% sorbose. Conidia from individual colonies were tested for their ability to form appressoria on Teflon strips as outlined above.

Microscopy: Differential interference contrast microscopy was performed using a Zeiss Axiomat microscope. Phase contrast microscopy was performed using a Leitz Laborlux microscope. All photographs were taken using Kodak Technical Pan film and processed according to the manufacturer's suggestions.

RESULTS

Selection of wild-type strains: We have used laboratory strains of *M. grisea* to begin a genetic analysis of infection structure (appressorium) formation. These strains are the result of a breeding program that involved genetic crosses among field isolates and the selection of progeny with high fertility, stable culture phenotypes and suitable pathogenicity characteristics (VALENT *et al.* 1986). The strains used in this study are aggressive pathogens of weeping lovegrass (*E. curvula*). They form appressoria on rice leaves, just as on weeping lovegrass, and they penetrate the rice leaf epidermis, but they do not cause disease symptoms on rice (R. HOWARD, unpublished results; M. HEATH, unpublished results). These observations suggest the genetic loci that govern infection structure formation in these laboratory strains will have homologues in strains that infect rice.

Isolation of Smo$^-$ mutants: To isolate mutants defective in appressorium formation, we screened for prototrophic survivors of UV mutagenesis that failed to form appressoria on Teflon-PFA films. We previously demonstrated that Teflon films are a preferred substratum for the induction of infection structures in *M. grisea* (HAMER *et al.* 1988). In screening approximately 3000 mutagenized individuals, we observed two strains (CP750 and CP751) that formed aberrantly shaped spores. Wild-type spores of *M. grisea* are pyriform in shape and are bilaterally symmetrical about a long axis (Figure 1a). Strains CP750 and CP751 produced conidia of various shapes with no visible axis of symmetry (Figure 1, b and c). Appressoria formed by strains CP750 and CP751 also exhibited aberrant morphology, as discussed in RESULTS, below. Despite drastic changes in spore morphology (Smo$^-$ phenotype), CP750 and CP751 exhibited no change in spore viability, rate of germination or radial growth rate on Petri plates when compared to wild type (data not shown). We scored the segregation of the Smo$^-$ phenotype in crosses with wild-type laboratory strains.

Table 3 shows the results of crosses between CP750 or CP751 (Smo$^-$) and the laboratory wild-type strain 4136-4-3 (Smo$^+$). In the cross CP750 × 4136-4-3, fifteen asci where four or more ascospores had germinated were scored for the Smo phenotype. Four of these asci contained eight viable ascospores that segregated 4 Smo$^+$:4 Smo$^-$. Although the remaining asci showed a deficiency of Smo$^-$ progeny, we did not recover any tetrads with more than four Smo$^+$ spores. A similar result was observed for cross CP751 × 4136-4-3, where six asci with eight viable ascospores were obtained, and they all segregated 4 Smo$^+$:4 Smo$^-$. No asci were obtained with more than four Smo$^+$ spores. These results show that the Smo$^-$ phenotype in strains CP750 and CP751 is due to single gene mutations designated *smo-1* and *smo-2*, respectively. In both crosses we also observed a deficiency of Smo$^-$ progeny when random ascospores were scored for the segregation of the Smo phenotype (Table 3). Taken together these results suggest that the *smo* mutations reduce ascospore viability or that they are linked to a second mutation that reduces ascospore viability.

Strains containing *smo* mutations are selected in an enrichment for nonsticking mutants: Enriching for individuals that failed to attach to Teflon film after appressorium formation might select strains that fail to make normal appressoria. The MATERIALS AND METHODS outlines the enrichment procedure used to select for nonsticking mutants. After three rounds of enrichment, 19 of 76 colonies screened from one population and 25 of 64 colonies screened from a second population had the Smo$^-$ phenotype. Conidia from the remaining colonies that were screened in these populations appeared to make normal appressoria. After five rounds of enrichment, all the colonies tested in the remaining populations had the Smo$^-$ phenotype. A single monoconidial Smo$^-$ isolate was obtained from each population and crossed with a wild-type laboratory strain. In all cases the Smo$^-$ phenotype segregated as a single gene mutation (data not shown), yielding results similar to those presented in Table 3. All Smo$^-$ strains obtained from the nonstick enrichment appeared to have spore morphology sim-

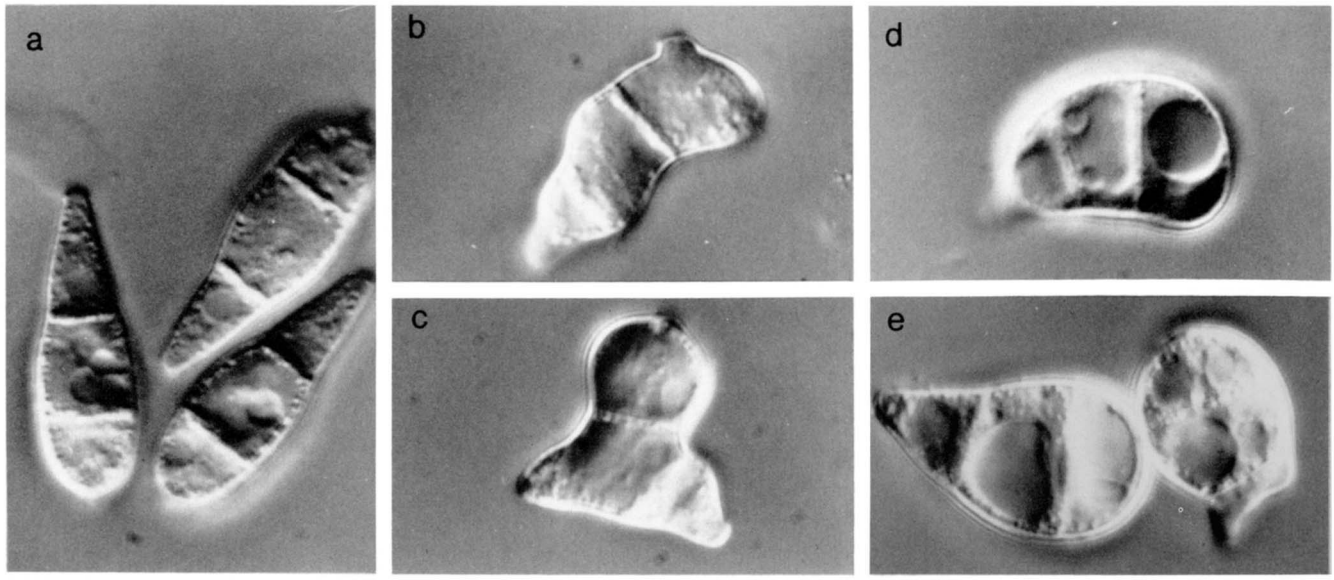

FIGURE 1.—Differential interference light micrographs of conidia from wild-type and Smo⁻ strains. Conidia were harvested and washed as described in the MATERIALS AND METHODS. Conidia were not fixed and were mounted and photographed immediately. (a) Wild-type *M. grisea* conidia. (b and c) Conidia from a strain that contains the *smo1-1* mutation. (d and e) Conidia from a strain that contains the *smo1-3* mutation (×1600).

TABLE 3

Segregation of two independently isolated *smo* mutations in crosses with *M. grisea* wild-type strain 4136-4-3

	Strains crossed and ratio of progeny	
Tetrad	4136-4-3 × CP750 Smo⁺:Smo⁻	4136-4-3 × CP751 Smo⁺:Smo⁻
1	3:2	4:4
2	4:1	4:2
3	3:2	4:3
4	4:1	4:4
5	4:0	3:4
6	4:0	4:4
7	4:2	4:3
8	4:4	3:4
9	4:3	4:4
10	4:2	4:4
11	4:2	4:4
12	4:1	2:3
13	4:4	4:3
14	4:4	4:3
15	4:4	4:3
Random ascospores	65:41	72:23

Strains are described in Tables 1 and 2. Strain CP750 carries *smo1-1* and strain CP751 carries *smo1-2*. Crosses were performed as described by VALENT *et al.* (1986). The Smo⁻ phenotype was scored as described in the MATERIALS AND METHODS.

ilar to the strains containing the *smo-1* and *smo-2* mutations (Figure 1).

Identification and isolation of spontaneous Smo⁻ mutants: The cytoskeleton is believed to play a role in the recognition of surfaces and the formation of infection structures in some plant pathogenic fungi (HOCH and STAPLES 1983). We examined this possibility in *M. grisea* by isolating strains that carried non-lethal mutations in a locus involved in cytoskeletal structure. In Ascomycetes, mutations that confer resistance to the fungicide, benomyl, occur in tubulin genes (MORRIS 1986). In *M. grisea*, an analysis of 25 independently isolated spontaneous benomyl-resistant (Ben^R) mutants demonstrated that resistance segregated as a single gene mutation (J. BODEAU and F. CHUMLEY, unpublished results). Sensitive recombinants could not be obtained from crosses of various Ben^R strains, suggesting that a single genetic locus conferred resistance to benomyl in *M. grisea*.

All the Ben^R strains we tested produced appressoria on Teflon films, although one strain (CP665) carrying the *ben1-24* mutation, had a phenotype reminiscent of the previously isolated Smo⁻ mutants (Figure 1, d and e). Table 4 shows the results of a cross between strain CP665 and the wild-type laboratory strain, 4091-5-8 (Table 1). Tetrad and random spore analysis were used to follow the segregation of benomyl resistance and the Smo phenotype. Tetrad analysis (Table 4) showed a segregation ratio of 3 PD:4 NPD:22 T, demonstrating that the Smo⁻ phenotype in strain CP665 was due to a second spontaneous mutation, *smo-3*, not linked to *ben1-24*. Analysis of 43 randomly isolated ascospores from the same cross demonstrated that although Ben^R segregated 1:1 (21 resistant to 22 sensitive), the Smo phenotype segregated with a deficiency of Smo⁻ progeny (28 Smo⁺:15 Smo⁻). Because the *smo-3* mutation was obtained spontaneously, it seemed unlikely the deficiency of Smo⁻ progeny was due to a second, linked, ascospore lethal mutation. This observation suggested the low viability of Smo⁻ ascospores may be a consequence of the *smo* mutation.

TABLE 4

Genetic crosses with spontaneous Smo$^-$ isolates

Cross	Segregation ratios	
1. CP665 (Smo$^-$ Ben^R) × 4091-5-8 (Smo$^+$ Ben^S)		
Tetrads	3 PD:4 NPD:22 T	
Random ascospores	Smo$^+$ Ben^R	11
	Smo$^-$ Ben^R	10
	Smo$^+$ Ben^S	17
	Smo$^-$ Ben^S	5
2. CP892 (Alb$^-$ Smo$^-$) × 4091-5-8 (Alb$^+$ Smo$^+$)		
Tetrads	2 PD:2 NPD:10 T	
Random ascospores	Alb$^+$ Smo$^+$	23
	Alb$^+$ Smo$^-$	10
	Alb$^-$ Smo$^+$	12
	Alb$^-$ Smo$^-$	13

The results presented are pooled from several crosses between the strains indicated. Crosses were performed as described in the text. Benomyl resistance was scored by observing growth after 1 week of incubation on plates containing benomyl (2.5 μg/ml). The pigmentation phenotype was observed after colony formation on oatmeal agar plates, and the Smo phenotype was scored as described in the MATERIALS AND METHODS.

TABLE 5

Allelism and complementation of *smo* mutations

smo$^-$ allele[a]	Smo$^-$ progeny	Smo$^+$ progeny	Zygotic complementation[b]
smo-2	124	0	−
smo-3	145	0	−
smo-4	163	0	−
smo-5	162	0	−
smo-7	151	0	−
smo-8	126	0	−
smo-9	77	0	−
smo-10[c]	232	1	−
smo-11	105	0	−
smo-12	68	0	−
smo-13	90	0	−
smo-14	63	0	−

[a] Smo$^-$ strains carrying the mutations indicated (as listed in Table 2) were crossed either with strain 4316-R-43 (*smo1-1 mat1-1*; a progeny from a cross between CP750 and 4136-4-3) or strain CP750 (*smo1-1 mat1-2*). All crosses were performed as described by VALENT *et al.* (1986). As expected, mating type segregated 1:1 among the progeny of several crosses that were checked. Benomyl resistance was scored in progeny of the cross between the *smo1-3* strain (CP665) and CP750; the ratio of Ben^R:Ben^S progeny was 1:1.

[b] Zygotic complementation (Figure 2) was determined by observing the morphology of asci in dissected perithecia. *SMO*$^+$ × *smo*$^-$ crosses yield normal long, slender asci, indicating zygotic complementation of the *smo*$^-$ defect. All *smo*$^-$ × *smo*$^-$ crosses yielded abnormal short, stubby asci.

[c] The results presented for *smo1-10* are pooled from two crosses. A single Smo$^+$ recombinant was observed after scoring 96 progeny in a first cross. A second cross, in which 136 progeny were scored, failed to produce a Smo$^+$ recombinant. Therefore recombination between *smo1-1* and *smo1-10* is approximately 0.4%.

The *smo-3* mutation appeared to have an effect on the shape of conidia that was less severe than that of *smo-1* or *smo-2* (Figure 1, d and e). Strains containing the *smo-3* mutation produced conidia that were more rounded and stubby in appearance than the wild type. This strain did not produce conidia with the highly irregular shapes seen in strains carrying the *smo-1* or *smo-2* mutations. We tested the possibility that the *smo-3* mutation arose in strain CP665 as a suppressor of some sublethal phenotype associated with the *ben1-24* allele. Strains carrying either the *smo-3* or *ben1-24* or both mutations were tested for growth at 16°, 21°, 25° or 30°. All mutant strains had growth rates that were indistinguishable from wild-type strains grown at these temperatures. We failed to isolate additional *smo* mutations by screening another collection of 107 benomyl-resistant strains. The original *smo-1* (CP750) and *smo-2* (CP751) containing strains were not benomyl resistant. From these experiments we conclude that there was no obvious selection for *smo*$^-$ mutations in strain CP665.

Wild-type *M. grisea* colonies are light to dark gray in color, and spontaneous color mutations have been identified as either buff (Buf$^-$) or albino (Alb$^-$) sectors or papillae. Genetic crosses have demonstrated that these color variations segregate as single gene mutations (CRAWFORD *et al.* 1986; B. VALENT and F. G. CHUMLEY, unpublished results). A spontaneous albino papilla occurred in laboratory strain 4379-R-16, and the spores of this albino mutant had a phenotype similar to the previous Smo$^-$ isolates. A sampling of the conidiophores surrounding the albino papilla demonstrated that the wild-type gray conidiophores had normally shaped spores, suggesting that the mutations to alter conidial shape (*smo*) and mycelial pigmentation (*alb*) may have occurred simultaneously. A monoconidial isolate was obtained from this papilla (CP892) and was crossed to a wild-type laboratory strain. Tetrad analysis demonstrated a ratio of tetrad types 2 PD:2 NPD:10 T, indicating that these mutations are unlinked and segregate as single gene defects (Table 4). Random ascospore analysis confirmed this result, and we again observed a deficiency of Smo$^-$ segregants among the progeny. Other Alb$^-$ strains do not carry *smo* mutations (B. VALENT, unpublished results). The *smo* mutation in strain CP892 was designated *smo-14* and was found to be allelic with *smo-1* (Table 5). This strain was not used further in characterizing the Smo$^-$ phenotype, because the parent strain, 4379-R-16, had a significantly different genetic background than strain 4091-5-8.

Several irregular-shaped spores were observed by coworkers during hemocytometer counting of otherwise normal wild-type spore preparations. The spore preparations that contained abnormally shaped spores were spread onto water agar plates (CRAWFORD *et al.* 1986) and putative Smo$^-$ spores were cut out and subcultured. Colonies derived from these spores had the Smo$^-$ phenotype. Two of these strains, CP785 (*smo-7*) and CP824 (*smo-13*), were used in this study. Both

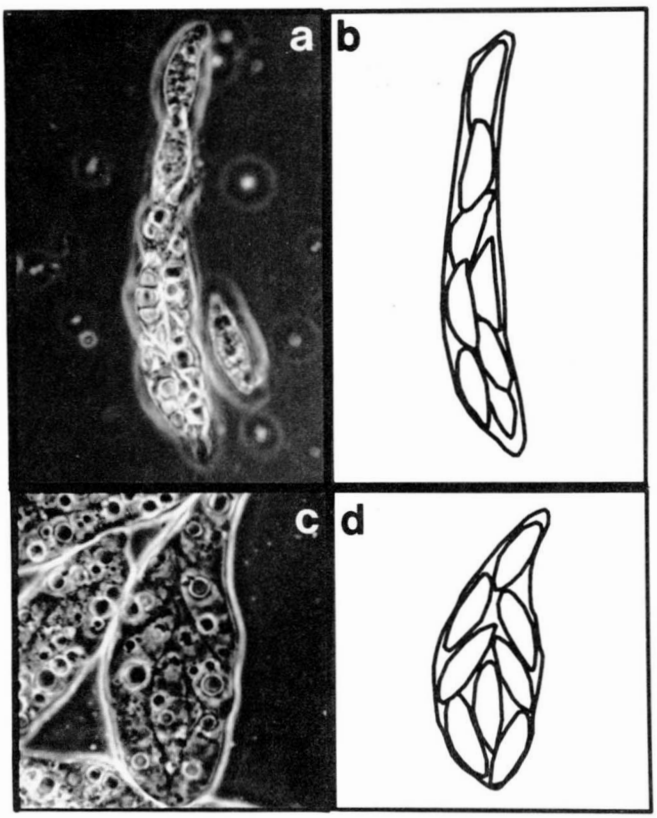

FIGURE 2.—Phase contrast light micrographs of wild-type and mutant asci. Asci were dissected from 14-day-old perithecia, mounted in sterile water and photographed. (a) Wild-type ascus from a Smo$^-$ × Smo$^+$ cross. (b) Line tracing of the ascus shape and ascospore position in part A. (c) Abnormally shaped ascus from a Smo$^-$ × Smo$^-$ cross. (d) Line tracing of ascus shape and ascospores in part c. Note that ascospore shape is not affected by *smo* mutations (×360).

mutations segregated as single gene mutations and had spore morphologies similar to strains containing the *smo-1* mutation (Figure 1, b and c).

Allelism and zygotic complementation of *smo* mutations: We conducted tests of allelism between *smo* mutations by crossing a strain containing the *smo-1* mutation with the other Smo$^-$ mutants (Table 2). The results of these crosses are presented in Table 5. With one exception, crosses between *smo-1* strains and the other *smo*$^-$ strains failed to produce wild-type recombinant progeny. The one exception was a cross between a strain containing the *smo-10* mutation and a strain containing the *smo-1* mutation, where a single Smo$^+$ recombinant was obtained among 232 progeny. These results show that *smo* mutations are allelic, or may reside in a group of tightly linked genes.

In all *smo*$^-$ × *smo*$^-$ crosses, we observed a striking zygotic phenotype shown in Figure 2. Wild-type *M. grisea* asci are slender, tapered and contain eight crescent shaped ascospores (Figure 2, a and b; HEBERT 1971; YAEGASHI and HEBERT 1976; KATO and YAMAGUCHI 1982). In crosses where a *smo* mutation was carried by only one of the parents (*SMO*$^+$ × *smo*$^-$), the asci were wild-type in shape. When *smo* mutations were carried by both parents in a cross (*smo*$^-$ × *smo*$^-$), the asci appeared stubby and rounded (Figure 2C). Despite this alteration in ascus shape, Figure 2, c and d, shows that the ascospores were normally shaped in these asci. In dissecting many asci from *smo*$^-$ × *smo*$^-$ crosses, we always observed eight ascospores per ascus. We conclude that the *smo* mutations are recessive with regard to the ascus phenotype, and that product(s) encoded by the *SMO* locus play a role in ascus formation. Failure to observe zygotic complementation in any *smo*$^-$ × *smo*$^-$ cross (Table 5) suggests that all *smo* mutations affect the same gene.

TABLE 6

Segregation of *smo* mutations in crosses with wild-type *M. grisea* strains

Allele	Random ascospore analysis	
	1st Generation[a] Smo$^+$:Smo$^-$	2nd Generation[b] Smo$^+$:Smo$^-$
smo1-1	65:41	57:32
smo1-2	T	72:23
smo1-3	28:15	52:20
smo1-5	T	57:9
smo1-7	59:28	49:16
smo1-8	T	45:27
smo1-9	57:17	ND[c]
smo1-12	58:34	ND

All crosses were performed and scored as described in the MATERIALS AND METHODS.

[a] First generation segregation ratios for *smo* alleles *1*, *2*, *5*, *7*, *8*, *9* and *12* were generated by crossing the Smo$^-$ mutants listed in Table 2 with wild-type strain 4136-4-3 (Table 1). Strain CP665, carrying *smo1-3*, was crossed with wild-type strain 4091-5-8 (Table 1). A "T" indicates that only tetrad analysis was used to score segregation in the first generation. All asci with eight viable progeny contained 4 Smo$^+$ and 4 Smo$^-$ ascospores. Smo$^-$ progeny from these tetrads were used in subsequent crosses.

[b] Second generation segregation ratios were obtained by crossing first generation Smo$^-$ progeny with wild-type strain 4091-5-8.

[c] Not determined.

Ascospore inviability in Smo$^-$ strains: We investigated whether or not the reduced viability of *smo*$^-$ ascospores in crosses between *SMO*$^+$ and *smo*$^-$ strains was a property of the *smo* mutations. Table 6 shows the segregation of the Smo phenotype in crosses involving eight *smo* alleles. In all of the crosses tested there was a marked deficiency of Smo$^-$ progeny. In the six cases tested, this deficiency was still seen in second generation crosses with a different wild-type strain. We conclude that the failure of some Smo$^-$ ascospores to germinate is a property of the *smo* mutations.

Random ascospore analysis of *smo*$^-$ × *SMO*$^+$ crosses demonstrated that in most cases the ratio of Smo$^+$ to Smo$^-$ progeny was approximately 2:1 (Table 6). This observation suggested that *smo* mutations might be due to a chromosome rearrangement, such as a terminal translocation including the *SMO* locus. Such a rearrangement would yield one chromosome deleted

for part of its normal genetic complement and another chromosome carrying a translocated segment. In a genetic cross with a wild-type strain, alternate centromere disjunction at meiosis would result in deletion and duplication progeny. Presumably, progeny containing the deletion chromosome (without the translocation chromosome) would be inviable. Progeny with the translocation chromosome would contain a *smo*$^-$ allele and a *SMO*$^+$ allele and would exhibit the Smo$^+$ phenotype. Random ascospore progeny from a cross of this sort would segregate 2 Smo$^+$:1 Smo$^-$. Because of the absence of genetic markers linked to the *SMO* locus, we attempted to detect the presence of Smo$^+$ duplication progeny as follows.

We dissected asci from a cross between a *smo1-1* strain and the wild-type laboratory strain, 4091-5-8. Asci were obtained that segregated 4 Smo$^+$:0 Smo$^-$. Smo$^+$ spores from these asci would be expected to carry the hypothetical duplication. If such Smo$^+$ progeny were back-crossed to the original *smo1-1* parent, the putative translocation would be homozygous in the cross, and the viability of Smo$^-$ ascospores should be normal. Consequently, the Smo phenotype should segregate 1 Smo$^+$:1 Smo$^-$ among random ascospores. From four asci that segregated 4 Smo$^+$:0 Smo$^-$, we tested Smo$^+$ progeny for the presence of a duplication by crossing them to the original *smo1-1* strain. In all six crosses that we examined, Smo continued to segregate with a deficiency of Smo$^-$ progeny (data not shown). We conclude that it is unlikely that the Smo$^+$ progeny used in these crosses carried duplications of the *SMO* locus. This experiment does not rule out the possibility that some of the *smo* mutations may be due to chromosome rearrangements. We are currently analyzing other *smo* alleles for the presence of chromosome rearrangements using the same genetic approach, together with electrophoretic chromosome separation techniques, because the absence of genetic markers and cytogenetics makes conventional methods of detecting chromosome rearrangements difficult in *M. grisea* at this time.

Appressorium formation in Smo$^-$ mutants: We observed that appressorium formation is altered in Smo$^-$ mutants (Figure 3). Conidia from Smo$^+$ strains produce short germ tubes before forming appressoria on a Teflon film surface (Figure 3, a and b, HAMER *et al.* 1988). In contrast, conidia from Smo$^-$ strains produce germ tubes that grow extensively before forming appressoria that are often abnormally shaped (Figure 3, c and d). *M. grisea* appressoria become melanized by the deposition of a phenolic pigment in the appressorium wall (WOLOSHUK *et al.* 1980; CHIDA and SISLER 1987). The presence of this material in the appressorial wall can be observed using bright field microscopy (Figure 3b; R. J. HOWARD, unpublished results). Figure 3d shows that although appressoria from a Smo$^-$ strain are abnormally shaped, they still appear melanized under bright field microscopy. This result suggests that processes necessary to localize material to the appressorial wall are functional in Smo$^-$ mutants.

Because conidia from Smo$^-$ strains produce long germ tubes prior to appressorium formation, we thought that Smo$^-$ strains might not discriminate as effectively as Smo$^+$ strains between conducive and nonconducive surfaces for appressorium formation. To test this idea we measured the extent of appressorium formation on glass and Teflon in strains carrying different *smo*$^-$ mutations. Conidia from a Smo$^+$ strain (4091-5-8) and conidia from strains harboring various *smo* alleles were deposited on glass coverslips and Teflon film strips as described in the MATERIALS AND METHODS. After 18–20 hours' incubation, the coverslips and Teflon strips were inverted onto microscope slides, and the average number of appressoria in five microscopic fields at ×200 was determined. The results are shown in Figure 4. Similar results were obtained when this experiment was repeated using different progeny strains containing the same *smo* alleles. Strains carrying the *smo1-3* and *smo1-8* mutations were tested a third time with the same results. In the experiment shown in Figure 4 no appressoria were observed in the five microscopic fields that were counted for a strain carrying the *smo1-9* mutation. However, examination of the entire surface showed a few appressoria had formed.

The results show that conidia from the wild-type laboratory strain 4091-5-8 germinate but form very few appressoria on glass, although numerous appressoria are formed when conidia germinate on a Teflon surface (Figures 3a and 4; HAMER *et al.* 1988). Conidia from different Smo$^-$ strains vary in their ability to form appressoria on Teflon. Figure 4 shows that germinating conidia from all Smo$^-$ strains produce more appressoria on glass than germinating conidia from 4091-5-8. In particular, conidia from strains carrying the *smo1-8* mutation produce more appressoria on glass than on Teflon (Figure 4). The results suggest that the extensive germ tube growth prior to appressorium formation in Smo$^-$ strains is due to the inability of these strains to discriminate appropriate surfaces for appressorium formation.

The function of infection structures in plant pathogenic fungi is to breach the outer plant surface. To test the function of appressoria produced by Smo$^-$ strains we performed infection assays. Infection assays were conducted using weeping lovegrass as previously described (VALENT and CHUMLEY 1987) with conidia at a concentration of 5.0×10^5/ml. At this inoculum concentration we did not detect any differences between plants infected with either Smo$^+$ or Smo$^-$ strains (data not shown). To see whether or not we could

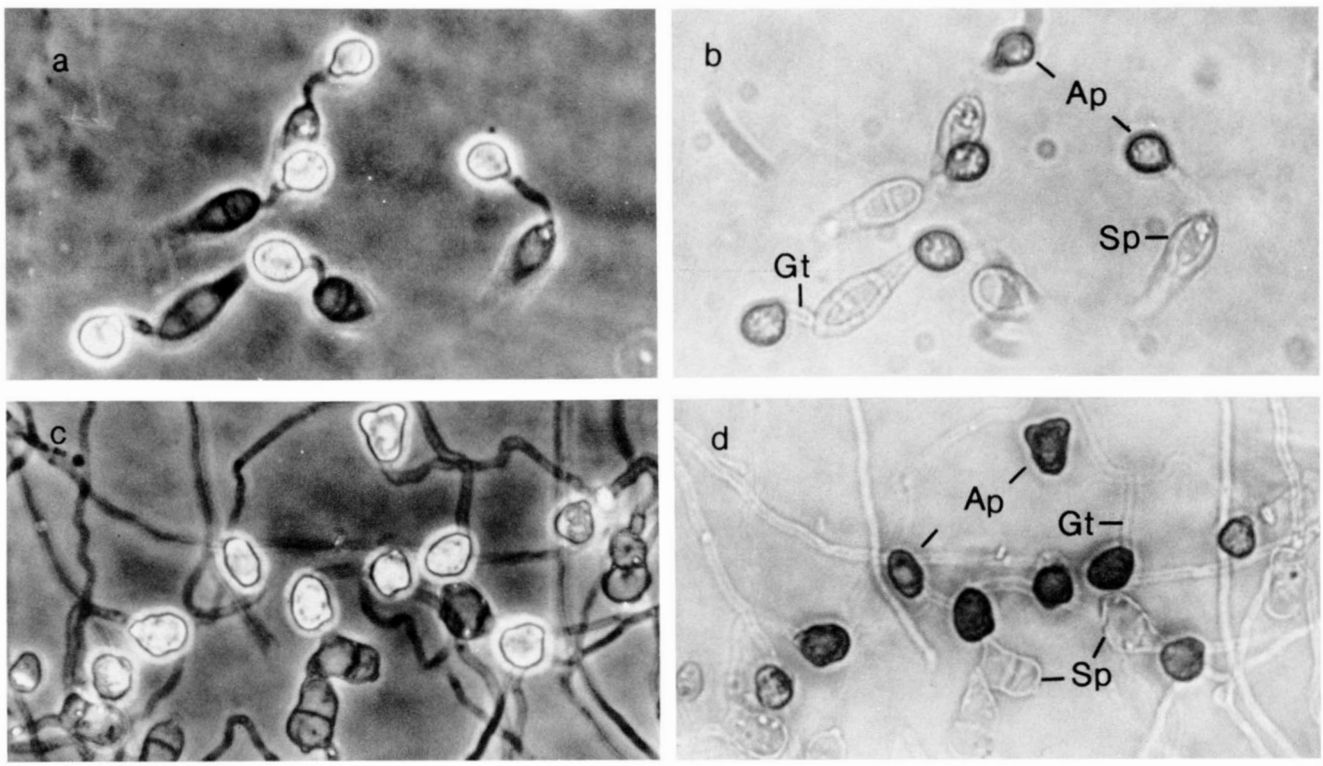

FIGURE 3.—Phase contrast light microscopy of wild-type and Smo$^-$ appressoria. Conidia from wild-type and Smo$^-$ strains were harvested, washed and placed on squares of Teflon film as outlined in the Materials and Methods. After 20-hr incubation at 23° the squares were inverted onto microscope slides and photographed immediately. (a and b) Wild-type appressoria formed on Teflon film. (c and d) Appressoria from a strain harboring the *smo1-3* mutation. Photographs a and c were taken using phase-contrast microscopy. Photographs b and d were taken using bright field microscopy. The use of bright field microscopy permits viewing appressoria as distinct from spores or hyphae because of the melanin in the appressorial wall. The Figure is labelled as follows: Sp (asexual spores), Ap (appressoria), Gt (germ tube). Abnormally shaped appressoria and unusually long germ tubes are formed by the Smo$^-$ strain (×640).

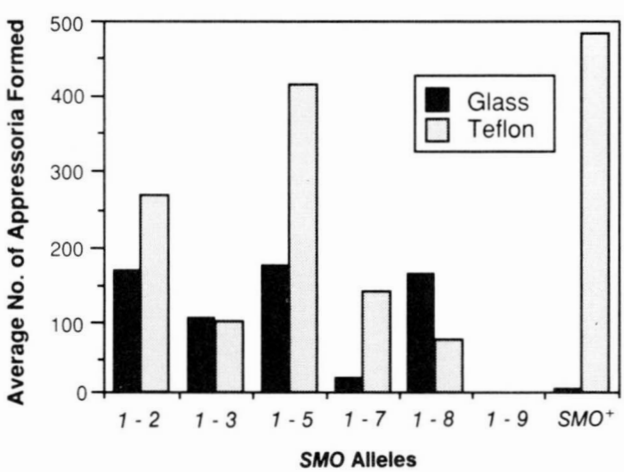

FIGURE 4.—Appressorium formation on glass or Teflon film surfaces by conidia from wild-type (Smo$^+$) and mutant (Smo$^-$) strains. Conidia were harvested, washed and deposited on surfaces as described in the MATERIALS AND METHODS. After 18–20 hr incubation at 23°, germinated conidia were inverted onto microscope slides and the average number of appressoria in five microscopic fields was determined at ×200. The Smo$^+$ strain (4091-5-8) forms many appressoria on Teflon but few appressoria on glass. Smo$^-$ strains vary in their ability to discriminate appropriate surfaces for appressorium formation.

detect quantitative differences between the ability of Smo$^+$ and Smo$^-$ strains to cause disease, a dilution series of conidia from several Smo$^-$ strains (*smo1-2, 1-9, 1-7, 1-3*) and a Smo$^+$ strain (4091-5-8) were used in infection assays. Smo$^-$ strains used in these assays were progeny derived from at least two backcrosses to 4091-5-8. Conidial concentrations ranging from 1×10^3 to 5×10^4/ml were used to inoculate weeping lovegrass plants in order to compare the minimal concentration of conidia from Smo$^+$ and Smo$^-$ strains required to cause disease symptoms. Under these conditions we could not detect any differences between the Smo$^-$ and Smo$^+$ strains. Conidia recovered from plants infected with Smo$^-$ strains retained the Smo$^-$ phenotype (data not shown). We conclude that alterations in appressorium formation in the Smo$^-$ strains tested do not deter the ability of these strains to cause disease symptoms on weeping lovegrass.

DISCUSSION

Mutations of the *SMO*$^+$ genetic locus permit conidia of *M. grisea* to assume a wide variety of unusual shapes (Figure 1). We have identified 13 independently derived *smo*$^-$ mutations, 12 of which cause severely

altered conidial shapes similar to those shown for strain CP750 in Figure 1, b and c. Smo$^-$ strains occasionally produce conidia that contain two apical cells, as well as conidia that appear to lack apical cells completely. We have also isolated a less severe *smo*$^-$ allele (*smo1-3*, Figure 1, d and e) that forms conidia that are rounded and enlarged. Strains containing *smo1-3* rarely produce conidia with two apical cells. Despite this alteration in conidial shape, conidia from all Smo$^-$ strains germinate normally, have high viability and differentiate from normal-looking conidiophores. Conidiophore density and colony morphology are indistinguishable from wild-type. We have also been unable to detect alterations in growth rate at various temperatures in Smo$^-$ strains.

Surprisingly, the effects of *smo* mutations are not confined to conidiation. Three major alterations occur in infection structure formation in Smo$^-$ strains. First, germinating conidia from Smo$^-$ strains make irregularly-shaped appressoria (Figure 3). Second, Smo$^-$ conidia do not immediately form appressoria on conducive surfaces after germination, but continue to extend their germ tubes, delaying the onset of appressorium formation. Third, Smo$^-$ strains form appressoria on glass surfaces, which are not conducive to the formation of appressoria in Smo$^+$ strains (Figure 4).

Two lines of evidence demonstrate that Smo$^-$ strains have a zygotic phenotype. First, when strains containing *smo* mutations are crossed to wild-type strains, there is a marked deficiency of the *smo*$^-$ allele among the recovered progeny. Second, when two Smo$^-$ strains are crossed, the asci appear rounded and stubby (Figure 2). Normal, slender asci are observed when Smo$^-$ strains are crossed to wild-type strains. The failure to detect recombination between *smo* alleles or zygotic complementation of the ascus shape phenotype strongly suggests that *smo* mutations are recessive and reside in the same gene. We have failed to detect linkage of *SMO* to *ALB* (albino pigmentation), *ben1-24* (resistance to benomyl), *MAT1* (mating type locus) or *arg3-12* (arginine requiring; data not shown). Linkage analysis in *M. grisea* is not sufficiently developed to allow assignment of *SMO* to one of the six *M. grisea* chromosomes.

Although the phenotype of Smo$^-$ strains is complex, a common feature of these mutations is alteration of the shape of certain cell types in *M. grisea*. These cell types are the conidia, appressoria and asci. Cell types that are exempt from shape alterations are vegetative hyphae and ascospores. Cells that become altered in shape do not necessarily lose their function. Smo$^-$ conidia germinate with wild-type kinetics, have high viability and are produced as abundantly as Smo$^+$ conidia. Despite alterations in shape, asci produced in Smo$^-$ × Smo$^-$ crosses contain eight normally shaped ascospores, and genetic markers such as *Mat1* and *ben1-24* segregate normally, indicating meiosis is not impaired in these crosses. Finally, appressoria produced in Smo$^-$ strains, although abnormally shaped, can infect weeping lovegrass plants and cause disease symptoms.

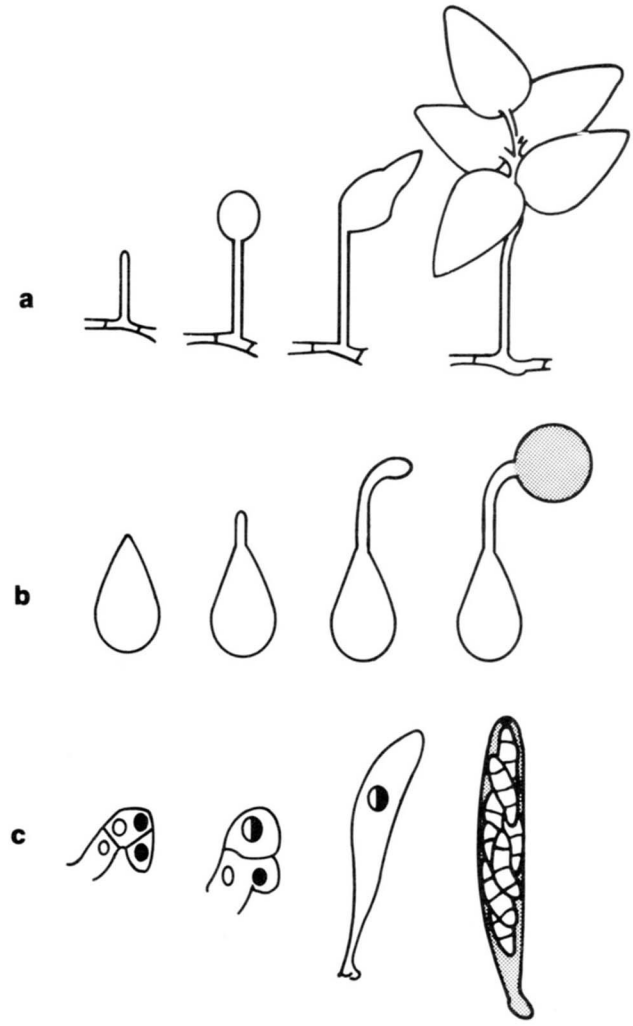

FIGURE 5.—Schematic representation of the developmental pathways of cell types affected by the *smo* mutations. (a) Conidiation. (b) Appressorium formation. (c) Ascus formation. The progenitor cell-type in each of these developmental pathways is a hyphal tip. The *smo* mutation may affect depolarization of hyphal tip growth to allow development of the differentiated cell types. Drawings are not to scale.

Figure 5 shows that the cell types affected by *smo* mutations are formed from growing hyphal tip cells. Conidia differentiate from an aerial hypha (SUBRAMANIAN, MANIBHUSHANRAO and RAJENDRAN 1978), and asci differentiate from the penultimate cell in a dikaryotic hyphal tip (YAEGASHI and HEBERT 1976; LEUNG and WILLIAMS 1987). Appressoria are formed from the hyphal tip of a germ tube. Unlike the asci, ascospores (normally shaped in Smo$^-$ × Smo$^-$ crosses) are not formed from a hyphal tip, but differentiate from within the cytoplasm of the ascus (LEUNG and

WILLIAMS 1987). We conclude that hyphal tip cells are a common progenitor of the cell types affected by *smo* mutations. During the formation of new cell types, polarized growth in hyphal progenitor cells must be reoriented to allow cell expansion and differentiation to give rise to cells with the correct dimensions and shape. A failure to depolarize hyphal tip growth efficiently could result in the formation of aberrant cell shapes. The *SMO* gene product may play a role in depolarizing hyphal tip growth to permit the differentiation of conidia, asci and infection structures. An alternative possibility is that the *SMO* gene product could function in cell wall or membrane biosynthesis. If this were so then the *SMO* gene product must be restricted to the cell types affected by the *smo* mutations, and not be involved in hyphal growth or ascospore development. We have recently observed that protoplasts formed from Smo$^-$ strains can efficiently regenerate walls and resume hyphal growth (J. E. HAMER, unpublished results). We are currently analyzing in more detail the process of conidiation in *M. grisea* to better understand the role of the *SMO* locus.

Developmental mutations that alter cell shape but not cell function are rare in fungi. In the related Ascomycete, *Neurospora crassa*, several mutations have been identified that alter the shape of the ascospores (SRB *et al.* 1973). In these cases all mutant ascospores have the same altered shape, whereas the *smo* mutation in *M. grisea* permits conidia to assume many different shapes (Figure 1). We have noted that mutations at the "peaked" (*pk*) genetic locus in Neurospora cause an ascus phenotype similar to that of Smo$^-$ strains of *M. grisea* (SRB *et al.* 1973). When two *pk*$^-$ strains are crossed the asci are stubby and rounded, but when a *pk*$^-$ strain is crossed with a *pk*$^+$ strain, normally shaped asci result. Allelic *pk* mutations fail to show zygotic complementation of the ascus phenotype. Dominant *pk* alleles alter ascus shape in heterozygous crosses. These results suggest that *pk* and *SMO* may have similar functions. However, unlike Smo$^-$ strains of *M. grisea*, *pk*$^-$ strains of *N. crassa* exhibit normally shaped conidia and a compact colony morphology.

We anticipated that mutants with phenotypes other than Smo$^-$ might be isolated using the nonstick enrichment. There may be several reasons why this procedure yielded only *smo* mutations. Smo$^-$ strains are delayed in appressorium formation because they grow extensively prior to forming appressoria. This property may contribute to Smo$^-$ strains being preferentially washed off the Teflon surface. Smo$^-$ strains grow at normal rates and produce abundant viable conidia. Mutants with defects in appressorium formation that also affect conidiation or vegetative growth would be out-competed by Smo$^-$ strains during growth and conidiation on oatmeal medium. Subsequent rounds of enrichment for strains failing to attach to Teflon plates would again select Smo$^-$ strains. The repeated isolation of Smo$^-$ strains in these enrichments may be a result of any or all of these factors. Alternatively, we are testing whether or not Smo$^-$ strains are defective in attachment to Teflon films.

Another reason for the repeated isolation of Smo$^-$ strains might be that the *SMO* locus is highly mutable. Several spontaneous *smo* mutants were obtained during the course of this study. Some of the spontaneous *smo* mutations apparently occurred simultaneously with other spontaneous mutations (Table 4). Genetic instability has been postulated to occur in *M. grisea*, particularly in genes responsible for cultivar specificity (see OU 1985). A genetic locus involved in pigment biosynthesis (*BUF*) has also been observed to mutate at a higher than expected frequency (B. VALENT, unpublished results). In the screen that yielded Smo$^-$ strains CP750 and CP751 we also obtained two Buf$^-$ mutants. We have not devised a method for measuring the frequency of *smo*$^-$ mutations, and thus we have no direct evidence to support the suggestion of high mutability at the *SMO*$^+$ locus. Spontaneous mutations to benomyl or methyl purine resistance occur at normal frequencies (3×10^{-7} per condium plated) in strains that have yielded spontaneous Smo$^-$ mutants (F. CHUMLEY, unpublished results). The molecular basis for the apparent mutability of some genetic loci in *M. grisea* is unknown. We have failed to observe wild-type conidia in preparations from Smo$^-$ strains, and all the *smo* alleles we have isolated appear stable. We are currently attempting to obtain revertants of the *smo* mutations.

It is difficult to discern a function for the *SMO*$^+$ locus solely from the phenotypes of the mutants that we have obtained. We do not understand why *smo* mutations failed to segregate through crosses efficiently. Attempts to detect chromosome rearrangements in Smo$^-$ strains, although not exhaustive, were unsuccessful. We also do not understand why Smo$^-$ strains make appressoria on surfaces that as a rule do not elicit appressorium formation in wild-type laboratory strains. If the *SMO*$^+$ gene product is an antagonist of polar growth, then signals required to trigger appressorium formation may not function efficiently in these strains. Based on studies with inhibitors, Hoch and co-workers have suggested that the cytoskeleton plays a role in the detection of appropriate surfaces for infection structure formation in rust fungi (HOCH *et al.* 1987). A cytoskeletal role for the *SMO*$^+$ gene product would be consistent with at least some of the phenotypes of the Smo$^-$ mutants. The phenotype of the Smo$^-$ mutants argues that the *SMO*$^+$ gene product plays a similar role at discrete stages in the life cycle and the infection cycle of *M. grisea*. Other gene prod-

ucts in *M. grisea* may have overlapping functions in unrelated cell types. The availability of a transformation system in *M. grisea* should facilitate the molecular cloning of the *SMO* locus and the characterization of the product(s) it encodes.

We wish to thank R. HOWARD for providing the DIC light micrographs of wild-type and Smo$^-$ conidia, communicating unpublished results and advising us on the use of bright field microscopy for observing appressoria. R. HOWARD and T. BOURETT also provided helpful advice on preparation of the photographic plates. We thank K. FELDMANN, M. ORBACH and J. SWEIGARD for their critical reading of the manuscript, and we also thank A. WALTER and L. FARRALL for excellent technical assistance.

Note added in proof: We have recently obtained a spontaneous Smo$^-$ mutant in a fertile *M. grisea* strain pathogenic on rice. The *smo* mutation in this strain segregates as a single-gene mutation and is allelic to the *smo* mutations reported in Table 2. Inoculation of two susceptible rice cultivars with progeny segregating for the *smo* mutation has demonstrated a pathogenicity defect cosegregating with the *smo* mutation. Rice plants infected with Smo$^-$ strains have fewer lesions and these lesions are reduced in size. We conclude that the *smo* mutation affects *M. grisea* pathogenicity on rice. Our inability to detect a pathogenicity defect on weeping lovegrass (see RESULTS) may be, in part, due to the small leaf area on this plant.

LITERATURE CITED

BARR, M. E., 1977 *Magnaporthe*, *Telimenella* and *Hyponectria* (Physosporellaceae). Mycologia **69:** 952–966.

CRAWFORD, M. S., F. G. CHUMLEY, C. G. WEAVER and B. VALENT, 1986 Characterization of the heterokaryotic and vegetative diploid phases of *Magnaporthe grisea*. Genetics **114:** 1111–1129.

CHIDA, T., and H. D. SISLER, 1987 Effect of inhibitors of melanin biosynthesis on appressorial penetration and reductive reactions in *Pyricularia oryzae* and *Pyricularia grisea*. Pestic. Biochem. Physiol. **29:** 244–245.

EMMETT, R. W., and D. G. PARBERRY, 1975 Appressoria. Annu. Rev. Phytopathol. **13:** 147–167.

HAMER, J. E., R. J. HOWARD, F. G. CHUMLEY and B. VALENT, 1988 A mechanism for surface attachment in spores of a plant pathogenic fungus. Science **239:** 288–290.

HEBERT, T. T., 1971 The perfect state of *Pyricularia grisea*. Phytopathology **61:** 83–87.

HOCH, H. C., and R. C. STAPLES, 1983 Visualization in situ by rhodamine-conjugated phalloin in the fungus *Uromyces phaseoli*. Eur. J. Cell Biol. **32:** 52–58.

HOCH, H. C., and R. C. STAPLES, 1987 Structural and chemical changes among rust fungi during appressorium development. Annu. Rev. Phytopathol. **25:** 231–247.

HOCH, H. C., R. C. STAPLES, B. WHITEHEAD, J. COMEAU and E. D. WOLF, 1987 Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. Science **235:** 1659–1662.

KATO, H., and T. YAMAGUCHI, 1982 The perfect state of *Pyricularia oryzae* Cav. from rice plants in culture. Ann. Phytopathol. Soc. Jpn. **48:** 607–612.

LATTERELL, F. M., 1975 Phenotypic stability of pathogenic races of *Pyricularia oryzae*, and its implication for breeding blast resistant rice varieties, pp 199–234 in *Proceedings of the Seminar on Horizontal Resistance to Blast Disease of Rice*. Columbia Series CE-No 9. Centro Internacional de Agricultura Tropical, Cali.

LEUNG, H., and P. H. WILLIAMS, 1987 Nuclear division and chromosome behavior during meiosis and ascosporogenesis in *Pyricularia oryzae*. Can. J. Bot. **65:** 112–123.

MORRIS, N. R., 1986 The molecular genetics of microtubule proteins in fungi. Exp. Mycol. **10:** 77–82.

NAGAKUBO, T., M. TAGA, M. TSUDA and A. UEYAMA, 1983 Genetic linkage relationships in *Pyricularia oryzae*. Mem. Coll. Agric. Kyoto Univ. **122:** 75–83.

OU, S. H., 1985 *Rice Diseases*, pp. 109–201. Commonwealth Agricultural Bureaux, Slough, U.K.

PARSONS, K. A., F. G. CHUMLEY and B. VALENT, 1987 Genetic transformation of the fungal pathogen responsible for rice blast disease. Proc. Natl. Acad. Sci. USA **84:** 4161–4165.

SRB, A. M., M. BASL, M. BORST and J. V. LEARY, 1973 Mutations in *Neurospora crassa* affecting ascus and ascospore development. J. Hered. **64:** 242–246.

STAPLES, R. C., 1985 The development of infection structures by the rusts and other fungi. Microbiol. Sci. **2:** 17–21.

STAPLES, R. C., and H. C. HOCH, 1987 Infection structures—form and function. Exp. Mycol. **11:** 163–169.

SUBRAMANIAN, C. V., K. MANIBHUSHANRAO and C. RAJENDRAN, 1978 Development of race ID-1 of *Pyricularia oryzae*. Phytopathol. Z. **91:** 340–345.

TAGA, M., H. NAKAGAWA, M. TSUDA and A. UEYAMA, 1979 Identification of three different loci controlling kasugamycin resistance in *Pyricularia oryzae*. Phytopathology **69:** 463–466.

UCHIYAMA, T., N. OGASAWARA, Y. NANBA and H. ITO, 1979 Conidial germination and appressorium formation of the plant pathogenic fungi on the cover-glass or cellophane coated with various lipid components of plant leaf waxes. Agric. Biol. Chem. **43:** 383–384.

VALENT, B., and F. G. CHUMLEY, 1987 Genetic analysis of host species specificity in *Magnaporthe grisea*. UCLA Symp. Mol. Cell. Biol. (New Series) **48:** 83–93.

VALENT, B., M. S. CRAWFORD, C. G. WEAVER and F. G. CHUMLEY, 1986 Genetic studies of fertility and pathogenicity in *Magnaporthe grisea* (*Pyricularia oryzae*). Iowa State J Res. **60:** 569–594.

WOLOSHUK, C. P., H. D. SISLER, M. C. TOKOUSBALIDE and S. R. DUTKY, 1980 Melanin biosynthesis in *Pyricularia oryzae*: site of action of tricyclazole inhibition and pathogenicity of melanin deficient mutants. Pestic. Biochem. Physiol. **14:** 256–264.

YAEGASHI, H., 1977 On the sexuality of the blast fungi, *Pyricularia* spp. Ann. Phytopathol. Soc. Jpn. **43:** 432–439.

YAEGASHI, H., and T. T. HEBERT, 1976 Perithecial development and nuclear behaviour in *Pyricularia*. Phytopathology **66:** 122–126.

YAMASAKI, Y., and H. NIIZEKI, 1965 Studies on variation of the rice blast fungus *Piricularia oryzae* Cav. I. Karyological and genetical studies on variation. Bull. Natl. Inst. Agric. Sci. Jpn. **13:** 231–273.

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