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Genetic and morphological characterization of a *Fusarium verticillioides* conidiation mutant

Anthony E. Glenn[1]

USDA, ARS, Russell Research Center, Toxicology and Mycotoxin Research Unit, Athens, Georgia 30604

Elizabeth A. Richardson

Plant Biology Department, University of Georgia, Athens, Georgia 30602

Charles W. Bacon

USDA, ARS, Russell Research Center, Toxicology and Mycotoxin Research Unit, Athens, Georgia 30604

Abstract: Enteroblastic phialidic conidiation by the corn pathogen *Fusarium verticillioides* (teleomorph *Gibberella moniliformis*) produces abundant, mostly single-celled microconidia in distinctive long chains. Because conidia might be critical for establishing in planta associations, we characterized a spontaneous *F. verticillioides* conidiation mutant in which phialides were incapable of enteroblastic conidiogenesis. Instead of producing a conidium, the phialide apex developed a determinate, slightly undulating, germ tube-like outgrowth, in which nuclei rarely were seen. Electron microscopy showed that the apical outgrowth possessed a thick, rough, highly fibrillar outer wall layer that was continuous with the thinner and smoother outer wall layer of the phialide. Both the inner wall layer and plasma membrane also were continuous between the apical outgrowth and phialide. The apical neck region of mutant phialides lacked both a thickened inner wall layer and a wall-building zone, which were critical for conidium initial formation. No indication of septum formation or separation of the apical outgrowth from mutant phialides was observed. These aberrations suggested the apical outgrowth was not a functional conidium of altered morphology. The mutation did not prevent perithecium development and ascosporogenesis. Genetic analyses indicated that a single locus, designated *FPH1* (*f*rustrated *ph*ialide), was responsible for the mutation. The conidiogenesis mutants were recovered only during certain sexual crosses involving wild-type conidiating parents, and then only in some perithecia, suggesting that mutation of *FPH1* might be meiotically induced, perhaps due to mispairing between homologous chromosomes and deletion of the gene from a chromosome.

Key words: conidia, conidium ontogeny, *Fusarium moniliforme,* hyphomycetes, meiosis, microconidia, microscopy, mitosis, phialide, SEM, TEM

INTRODUCTION

Fungi can produce a variety of propagules, including hyphal fragments, resistant structures such as sclerotia and rhizomorphs, and both sexual and asexual spores. All these structures facilitate survival and spatial distribution of fungi, yet asexual sporulation is clearly the most successful and impressive reproductive mechanism because of the large number of mitospores (conidia) that can be produced by a single colony. For example, a 2.5 cm diam colony of *Penicillium* can produce approximately 400 million conidia (Kendrick 2003).

Typical production of asexual spores often involves repetitive cycles of mitosis in a specialized conidiogenous cell followed by encapsulation of one daughter nucleus in an incipient spore that is released upon maturation. This process of clonal mitosporogenesis is exhibited by a large diversity of hyphomycete fungi (Hennebert and Sutton 1994, Kendrick 2003), including various animal and plant pathogens. Details of conidium development can vary significantly among mitosporic hyphomycetes. A common mechanism is enteroblastic phialidic conidiation, in which conidia are derived in a basipetal succession from a fixed, nonproliferating locus on a specialized conidiogenous cell called a phialide. Conidium ontogeny studies have shown that phialide cell-wall structure contributes inner wall material to a newly developing conidium, but all conidial outer wall material is deposited de novo (Hennebert and Sutton 1994, Tiedt and Jooste 1992). The reiterative basipetal process can lead to chains or heads of large numbers of conidia developing from a single phialide. Fungi such as *Aspergillus, Penicillium* and *Fusarium* produce enteroblastic phialidic conidia.

Conidiogenesis among hyphomycetes involves developmental processes derived during the evolution of filamentous ascomycetes (Berbee and Taylor 1993). A recent proposal has suggested that conidia, conidiogenous cells and conidiophores essentially

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[1] Corresponding author. E-mail: aglenn@saa.ars.usda.gov

are modified or condensed hyphal systems, such that ancestral processes analogous to hyphal branching have evolved into differentiated structures necessary for asexual spore formation and propagation (Kendrick 2003). These evolved structures and the conidiation process significantly affect fungal ecology and interspecies interactions. The abundant production of asexual spores and their importance for successful completion of life cycles clearly define mitosporogenesis as a fitness factor in terms of overall fungal survival, dispersal and evolution (Pringle and Taylor 2002).

Fusarium verticillioides (teleomorph *Gibberella moniliformis*) is a widely distributed mitosporic pathogen able to cause corn seedling blight, root rot, stalk rot and kernel or ear rot (Kommedahl and Windels 1981) but also can infect vegetative and reproductive tissues without any symptom development (Foley 1962, Bacon et al 1992, Bacon and Hinton 1996, Munkvold et al 1997a). Insect herbivory pressure and physiological stress facilitate disease development (Munkvold et al 1997a, Miller 2001). Indirect assessments have suggested that conidia are critical in the infection process of this fungus and might be necessary for systemic colonization of the corn plant (Bacon et al 1994, Bacon and Hinton 1996, Munkvold et al 1997b, Glenn et al 2001). Both symptomatic and asymptomatic kernel infections by *F. verticillioides* can result in decreased quality of corn and economic losses due to contamination by fumonisin B1, a mycotoxin causing severe species-specific diseases in some livestock and laboratory animals, including kidney and liver cancer in rodents and a postulated role in human esophageal cancer (Gelderblom et al 1991, Marasas et al 1988, Marasas 1996, Rheeder et al 1992, Voss et al 2001, Voss et al 2002). Reducing fumonisin contamination in corn will require greater understanding of how *F. verticillioides* infects and systemically colonizes corn tissues and an assessment of what role conidia might play in plant-fungal interactions.

The small, hyaline, mostly single-celled microconidia of *F. verticillioides* are produced in long catenate chains arising from morphologically simple phialides. The chains of conidia are well adapted for wind, rain and vectored dispersal (= xenospores). *F. verticillioides* is often the most common fungus reported from infected corn kernels and vegetative tissues (Desjardins et al 2000, Foley 1962, Kedera et al 1999, Kommedahl and Windels 1981, Nelson et al 1993), and efficient dispersal of microconidia undoubtedly facilitates such dominance in corn field environments. Sexual reproduction, although not common in nature (Leslie and Klein 1996), would be facilitated by microconidia serving as spermatia for fertilization of protoperithecia. Thus, microconidia are clearly important for survival, reproduction and dispersal of *F. verticillioides*.

We are aware of only a single report of aberrant conidiogenesis in a *Fusarium* species. Tiedt and Jooste (1988a) examined an isolate of *F. subglutinans* that exhibited abnormal acropetal conidium production. Ultrastructural analysis characterized the mutation as a reiterative developmental process of conidia acting as phialides to produce more conidia. Similar acropetal arrays of conidia were seen in an insertional mutant of *Magnaporthe grisea* (Lau and Hamer 1998). Normal *M. grisea* conidiation involves a sympodial pattern of sporulation on conidiophores. The head-to-tail arrangement of conidia was due to mutation of the *ACR1* locus, which encodes a regulator of conidiophore pattern formation (Lau and Hamer 1998, Nishimura et al 2000). Amino acid sequence comparison found that Acr1 is homologous to MedA, a developmental regulator of conidiation in *Aspergillus nidulans*. Mutation in the *medA* gene results in disruption of the precise spatial pattern of normal conidiophore development such that branching chains of metulae are produced, phialide differentiation is delayed and redifferentiation of sterigmata occurs to produce secondary conidiophores (Adams et al 1998). The majority of data concerning the regulation and developmental processes of conidiation stem from genetic examinations of *A. nidulans* mutants (Adams et al 1998). Regulation of conidiation in a *Fusarium* species started being examined only recently (Shim and Woloshuk 2001).

We characterize here a unique and distinct *F. verticillioides* conidiation mutation in which normal phialide development failed to elaborate a conidium. Instead of enteroblastic conidiation, the phialide apex developed a slightly undulating, germ tube-like outgrowth in which nuclei rarely were seen. The mutation occurred spontaneously during sexual crosses involving wild-type conidiating parents, with half of the collected progeny having normal conidiation and half exhibiting the mutation. Our goals were to characterize morphologically this conidiation mutation, including examination of the developmental aberration using electron microscopy, and to determine the number of genetic loci controlling the specific process of conidium initiation from a phialide.

MATERIALS AND METHODS

Fungal strains and genetic analyses.—Fungal strains examined in this study are detailed in TABLE I. Additional information on these and related strains can be found elsewhere (Glenn et al 2002). Strain MRC826 was the representative wild type exhibiting normal conidium development. Mutant strains AEG3-A3-5 and AEG3-A3-6 do not produce conidia

TABLE I. Strains of *Fusarium verticillioides* and characteristics examined in this study

Strain[a]	Other accessions	Source/origin	Mating type[b]	Female fertile	Conidiation phenotype
JFL-A00999	FGSC7603; FRC-M3703; NRRL20984	Corn; Indiana, U.S.A.	*MAT1-2*	Yes	Wild-type
MRC826	FRC-M1325; NRRL13447	Corn; South Africa	*MAT1-1*	Yes	Wild-type
NRRL25059	CBS624.87	Banana; Honduras	*MAT1-2*	No	Wild-type
AEG1-1-57	FGSC9463	MRC826 × NRRL25059	*MAT1-2*	No	Wild-type
AEG3-A3-1	FGSC9464	MRC826 × AEG1-1-57	*MAT1-2*	No	Wild-type
AEG3-A3-5	FGSC9468	MRC826 × AEG1-1-57	*MAT1-1*	Yes	Mutant
AEG3-A3-6	FGSC9469	MRC826 × AEG1-1-57	*MAT1-1*	Yes	Mutant

[a] See Glenn et al 2002 for more details on these strains. AEG = Anthony E. Glenn; CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; FGSC = Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, KS, U.S.A.; FRC = *Fusarium* Research Center, Pennsylvania State University, University Park, PA, U.S.A.; JFL = John F. Leslie, Department of Plant Pathology, Kansas State University, Manhattan, KS, U.S.A.; MRC = Medical Research Council, Tygerberg, South Africa; NRRL = Northern Regional Research Laboratory, NCAUR, USDA, ARS, Peoria, IL, U.S.A.

[b] Mating type determination was initially by genetic crosses with known tester strains but subsequently was confirmed by PCR amplification of mating type idiomorphs following the methods and designations of Yun et al (2000).

and were genetically identical twins isolated from a complete octad. Each strain was microscopically examined as detailed below. For long-term storage of strains, conidia or hyphae were frozen at −80 C in 15% glycerol. For routine culturing, strains were grown on potato-dextrose agar (PDA; Difco, Detroit, Michigan) or in potato-dextrose broth (PDB; Difco) and incubated at 25 C in the dark, with the addition of shaking (180 rpm) for PDB cultures.

Genetic crosses were performed and progeny were collected as detailed by Glenn et al (2002). In brief, a cirrhus of ascospores was collected from the top of a mature perithecium, suspended in sterile water and plated on 3% water agar. After overnight incubation at 25 C, germinating ascospores were transferred to PDA. For most crosses, the number of germinating ascospores collected per perithecium was limited to 24 to prevent bias for any one meiotic event and potentially violate independence assumptions (Leslie 1991). After 4–7 d growth on PDA, collected progeny were assessed microscopically for their conidiation phenotype. Phenotypic ratios of wild-type conidiating progeny to mutant nonconidiating progeny were determined, interpreted and tested using chi-square analyses.

Unordered octads were collected to phenotype progeny from single meiotic events and to further address the number and linkage of genetic loci involved in the conidiation mutation. A perithecium was collected from a cross, washed and broken open in a drop of sterile water on 3% water agar to expel the rosette of asci (Glenn et al 2002). Individual asci were pulled out of the water drop, and ascospores were separated. After overnight incubation at 25 C, individual germinating ascospores were transferred to PDA. Only octads with at least seven viable ascospores were phenotyped.

Light microscopy and fluorescent staining.—Observations were made using an Axioplan compound microscope (Zeiss, Thornwood, New York) equipped with an HBO 100W/2 mercury lamp illuminator for fluorescent stains. A similar microscope equipped for imaging using Nomarski DIC (differential interference contrast) also was used. For light microscopy, strains were grown routinely as stated above. For nuclear and cell wall staining, strain MRC826 was grown on PDA overlain with dialysis membrane (Spectra/Por membrane No. 132 675, Spectrum Medical Industries Inc., Los Angeles, California) that had been washed in distilled water and autoclaved. Sections of membrane (~0.25 cm^2) were cut from the colony within the outer 1–2 cm of growth using a sterile razor blade and carefully transferred through fixer and stain solution. Fixation and staining using fluorochromes Hoechst 33342 (Molecular Probes, Eugene, Oregon) and calcofluor white (Polysciences Inc., Warrington, Pennsylvania) were adapted from an available protocol (Momany 2001). Hyphal mats were removed from membranes before mounting on slides for microscopic observations using epi-fluorescence and a DAPI filter set.

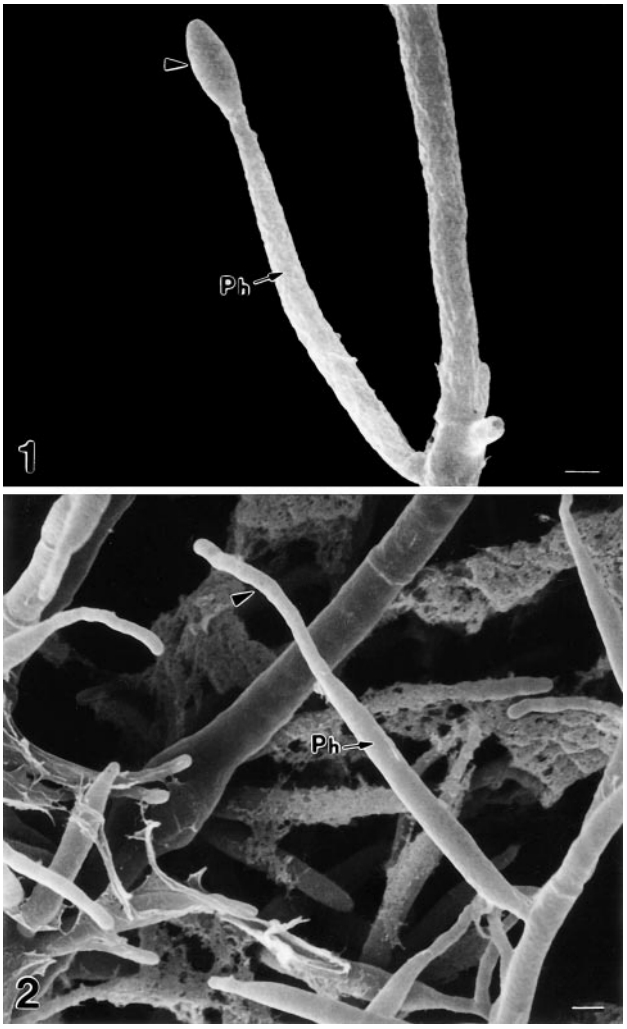
Growth on dialysis membrane was not necessary for strain AEG3-A3-6 because it produced only hyphal clumps in PDB cultures. Wild-type strains produced mostly conidia and little hyphae in PDB cultures. Aliquots (750 μL) of a PDB culture of AEG3-A3-6 were pelleted (5000 × *g* for 5 min), washed with sterile water and fixed and stained for fluorescent imaging. Images were taken with a Nikon Coolpix 995 (Melville, New York) digital camera mounted on the microscope by an ocular adapter (Optem Avimo Precision Instruments, Fairport, New York) and prepared for publication with Photoshop 5.5 software (Adobe, Mountain View, California).

Scanning and transmission electron microscopy.—Scanning electron microscopy (SEM) of the conidiation mutation (strain AEG3-A3-5) was performed using either cryo fixation or conventional chemical fixation. For cryo fixation, an agar cube (approximately 125 mm^3) was cut from a 7 d old colony, mounted onto a commercial cryo holder (Gatan Inc., Warrendale, Pennsylvania) and plunged into nitrogen slush (approximately −209 C) to avoid the Leidenfrost effect. The frozen sample was transferred quickly to an Alto

2500 cryo preparation unit (Gatan Inc.) attached to a LEO 982 field emission scanning electron microscope (LEO Electron Microscopy Inc., Thornwood, New York) and allowed to sublimate at vacuum of 2×10^{-6} torr and −80 C for 2 min to remove surface frost. The sample was cooled to −100 C before coating with AuPd to a thickness of 30 nm. Once coated, the sample was transferred to a −100 C cold stage in the microscope for viewing.

This procedure did not work well for wild-type strain MRC826, which instead was prepared for SEM according to the chemical fixation procedures of Enkerli et al (1997). Briefly, the fungus was grown on dialysis membrane as described above for light microscopy and sections of a 7 d old colony (3 mm^2) were cut with a razor blade and chemically fixed in a 1:1 solution of 5% glutaraldehyde and 0.1 M potassium phosphate buffer overnight at 4 C. Specimens were rinsed in buffer, postfixed in 2% Osmium tetroxide and 0.1 M potassium phosphate buffer for 2 h, rinsed in water and dehydrated through a graded ethanol series. Samples were dried in a critical-point dryer, mounted on stubs and coated with gold for observation in a JEOL 5800 scanning electron microscope operating at 15 kV.

Transmission electron microscopy (TEM) also was used to compare strains MRC826 and AEG3-A3-5 grown on dialysis membrane. Sections of a colony (3 mm^2) were plunged frozen in liquid propane and transferred to substitution fluid consisting of 2% Osmium tetroxide and 0.05% uranyl acetate in HPLC grade acetone chilled to −80 C (Hoch 1986). After 4 d at −80 C samples were slowly warmed (−20 C for 4 h, 4 C for 2 h, room temperature for 45 min) and gradually infiltrated with Araldite, Embed 812 (Electron Microscopy Sciences, Hatfield, Pennsylvania). Samples were flat embedded between Permanox slides and polymerized at 60 C for 48 h. A diamond knife was used to cut sections on a Reichert-Jung Ultracut E ultramicrotome. Sections were collected on slot grids, allowed to dry onto Formvar-coated aluminum racks (Rowley and Moran 1975) and poststained for 3 min each in aqueous uranyl acetate followed by lead citrate (Reynolds 1963). Sections were examined with a Zeiss EM 902A transmission electron microscope operating at 80 kV.



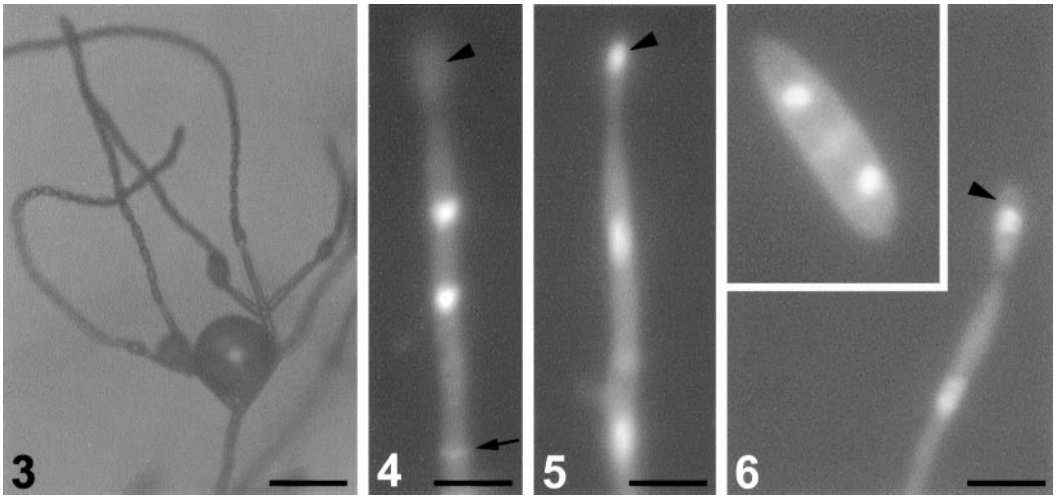
FIGS. 1–2. Scanning electron microscopy of wild-type and mutant *F. verticillioides* phialide development and conidiation. 1. Wild-type strain MRC826 showing the typical formation of a phialide (Ph) along a hypha with a conidium (arrowhead) developing from the phialide apex. 2. Mutant strain AEG3-A3-5 also had normal production of a phialide (Ph) along a hypha, but its phialide apex developed a determinate, germ tube-like outgrowth (arrowhead). Bars = 2 μm.

RESULTS

Origin and morphology of the mutation.—While performing a series of genetic crosses for a separate study of corn phytoanticipin detoxification by *F. verticillioides* (Glenn et al 2001, Glenn et al 2002), a subset of progeny were collected that did not produce conidia (TABLE I). Some progeny were morphologically normal and produced phialides and conidia (FIG. 1), while other strains produced phialides that did not elaborate conidia but instead developed aberrant apical outgrowths (FIG. 2).

Wild-type *F. verticillioides* produced elaborate, long, basipetal chains of conidia originating from phialides (FIG. 3). Observations were consistent with a developing conidium receiving its nucleus from the mitotic nucleus in the phialide (FIGS. 4–6). One of the resulting daughter nuclei migrated into the developing spore before its maturation and release from the phialide.

The mutant strains developed phialides that were morphologically normal except that conidium formation at the phialide apex did not occur. Instead the mutant phialides developed an outgrowth that resembled a germ tube (FIGS. 7, 8). This apical outgrowth appeared to be determinate and was slightly undulating, often crooked and sometimes branched (FIG. 9). The wall texture of the outgrowth appeared rough in comparison to the smoother texture of the



FIGS. 3–6. Wild-type *F. verticillioides* (strain MRC826) microconidiation and mitotic divisions. 3. Phialides producing long chains of conidia. 4. Hoechst and calcofluor staining of nuclei and cell walls, respectively, showing two postmitotic nuclei in a phialide. One of the daughter nuclei has not fully migrated to the conidium initial (arrowhead) at the phialide apex. Note septum at base of phialide (arrow). 5. A daughter nucleus has migrated fully to the immature conidium (arrowhead) at the apex of this phialide. 6. Phialide with an immature conidium (arrowhead), each with a distinct nucleus. Inset: A two-celled microconidium with two nuclei was cropped closer to the phialide without any magnification or reduction of the images. Bars = 20 μm in FIG. 3, 5 μm in FIGS. 4–6.

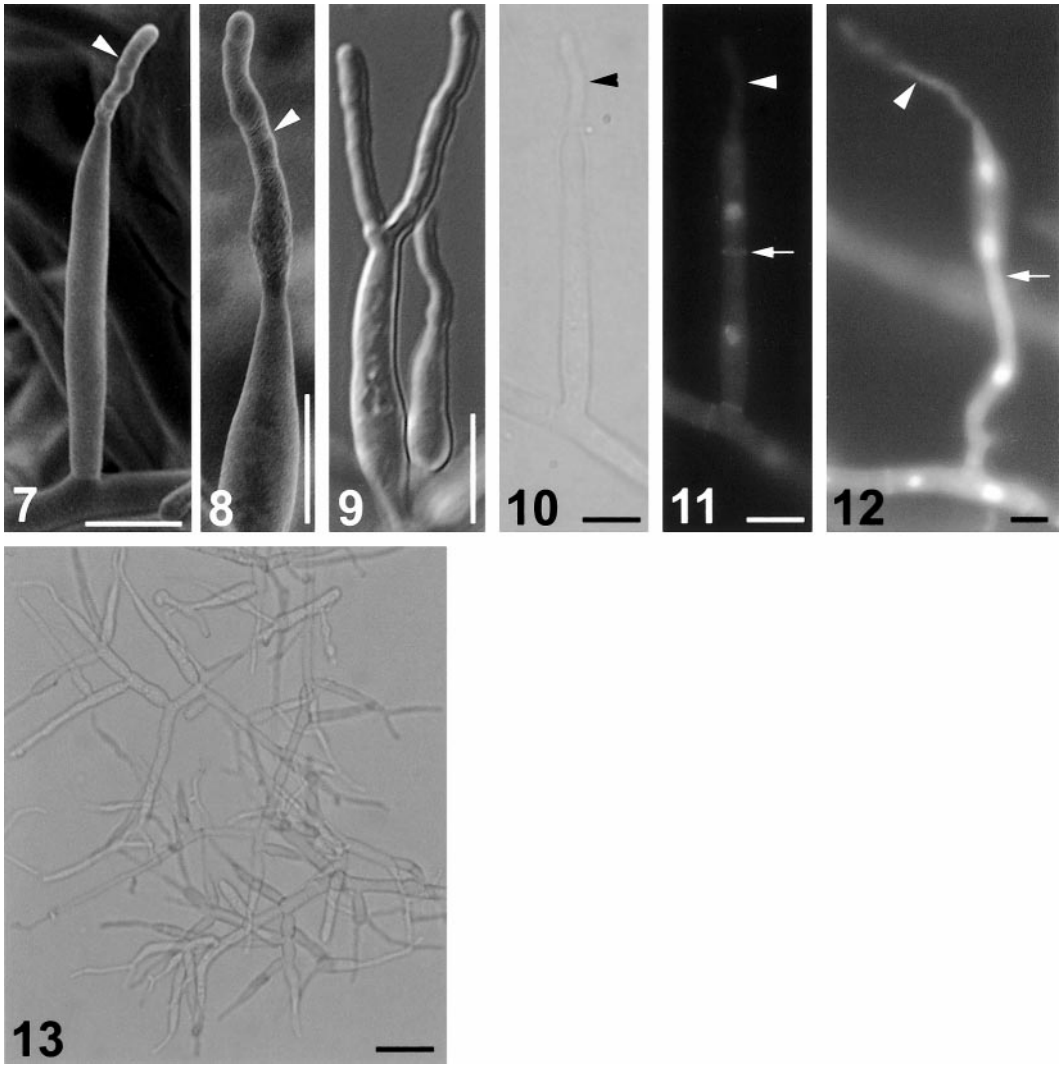
phialide wall (FIG. 8). Most mutant phialides contained a single nucleus, while the apical outgrowths were void of any nuclei (FIGS. 10, 11). A nucleus was observed in the apical outgrowth only on rare occasion. Two nuclei were seen only occasionally in phialides (FIG. 12), suggesting that mitosis generally was suppressed in phialides of these mutant strains. In addition, mutants possessed 1–3 nuclei in hyphal compartments as compared to the wild type, which maintained a single nucleus per compartment. In liquid culture mutants formed clumps consisting of short hyphal segments and bunched conidiophores and phialides (FIG. 13). Mutant phialides never were observed to release apical outgrowths due to septation and maturation as was seen with production of conidia by wild-type strains.

Wild-type phialides of strain MRC826 measured 18–30 × 3–4 μm (mean 23 × 3.4 μm). Single-celled microconidia were 4–10 × 3–5 μm (mean 6.8 × 3.3 μm). Two-celled microconidia were observed occasionally and measured 15 × 5 μm. Macroconidia were not produced in the cultures examined. Mutant phialides of strain AEG3-A3-6 tended to be more variable in length compared to wild type, measuring 8–32 × 3–4 μm (mean 20.3 × 3.5 μm). Apical outgrowths ranged in length depending upon their stage of growth (2–35 μm, mean 14.3 μm) and were approximately 1 μm wide.

Ultrastructural examination of mutant strain AEG3-A3-5 in comparison to wild-type strain MRC826 supported light microscopy observations that phialides were normal developmentally except for producing the apical outgrowths. In both strains, phialides developed along hyphae, possessed a nucleus and cellular organelles and had similar wall structure. The distinct developmental differences occurred at the phialide apex.

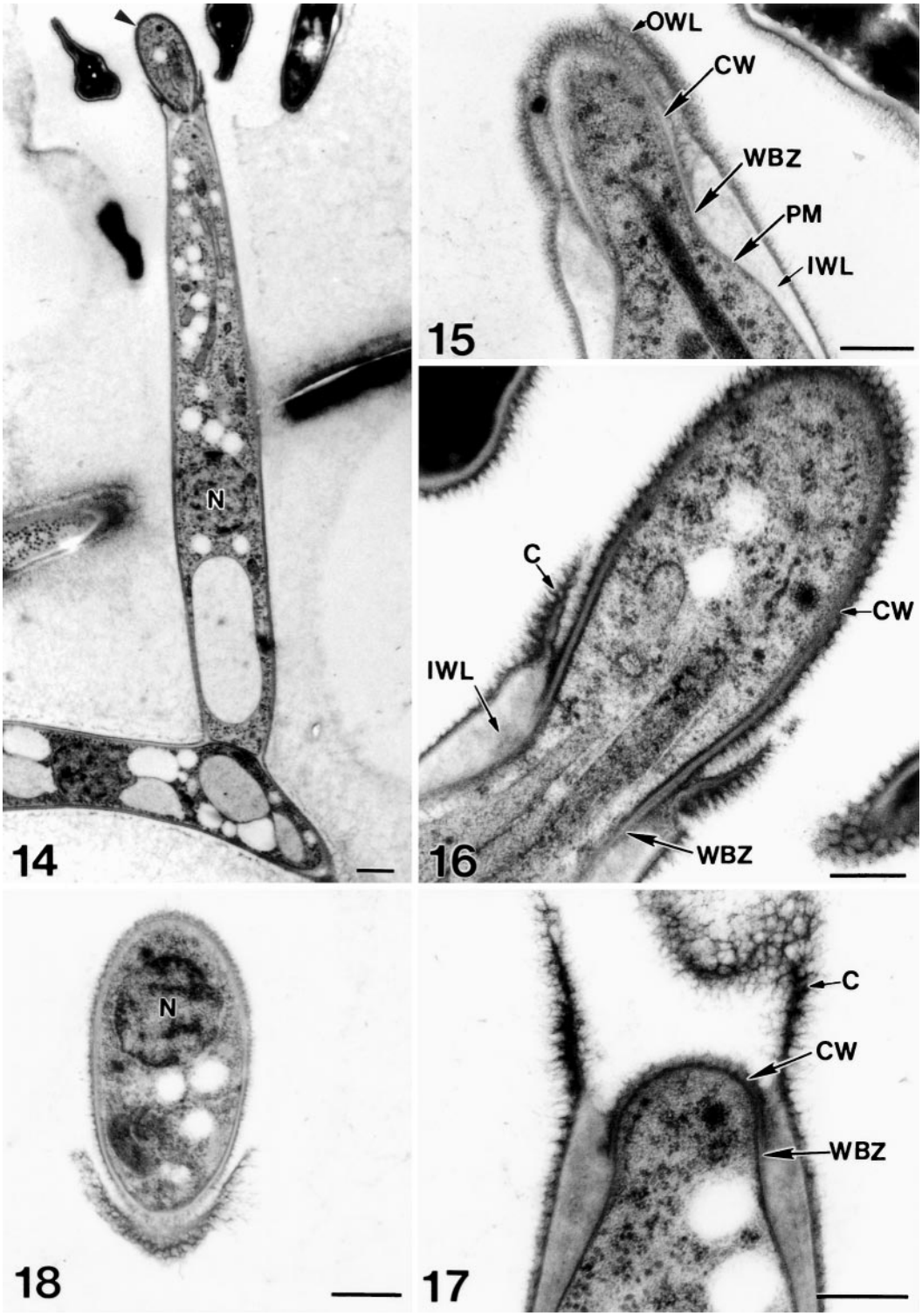
Wild-type enteroblastic conidiogenesis involved a zone at the phialide apex that was responsible for initiation and development of conidia (FIGS. 14–17). This wall-building zone in the phialide neck region was delimited by a thickening of the phialide inner wall layer (FIGS. 15, 16). The plasma membrane and inner wall layers were continuous between the phialide and the developing conidium. The conidial outer wall layer appeared to be derived de novo from the phialide inner wall layer in the wall-building zone (FIGS. 15–17). The first conidium initial produced by a phialide appeared to rupture the phialide outer wall layer as it matured (FIG. 15). Remnants of the outer wall remained at the phialide apex in the form of a distinctive collarette (FIGS. 16, 17). All subsequent conidia emerged from within the resulting collarette without proliferation of the phialide due to remnant wall deposition (FIG. 17). Microconidia were typically single-celled and contained a single nucleus (FIG. 18).

The conidiation mutant was defective in the formation of a wall-building zone, and the neck region at the phialide apex did not have a thickened inner wall layer (FIGS. 19, 20). The mutant phialide instead showed the distinctive outgrowth from the apex. The



FIGS. 7–13. Mutant *F. verticillioides* phialide development and mitotic divisions. 7–8. Scanning electron micrographs of cryo-fixed strain AEG3-A3-5 showing the undulating apical outgrowth (arrowhead) and its slightly roughened wall texture in comparison to the phialide wall. 9. Nomarski DIC image of strain AEG3-A3-6 showing branching that occasionally occurred at the phialide apex. 10–11. Light and fluorescent microscopy, respectively, of the same mutant phialide stained with Hoechst and calcofluor illustrating that the apical outgrowth (arrowhead) was void of any nuclei. The phialide nucleus was separated by a septum (arrow) from the nucleus in the subtending conidiophore. 12. Fluorescent microscopy showing two nuclei within a phialide but no nucleus in the apical outgrowth (arrowhead). Although not entirely clear in this plane of focus, the phialide nuclei were separated by a septum (arrow) from the nucleus in the subtending conidiophore. 13. Light microscopy of strain AEG3-A3-6 grown in liquid culture (PDB). Note the proliferation of phialides and lack of conidia. Bars = 5 μm in FIGS. 7–12, 20 μm in FIG. 13.

undulating nature of this outgrowth is evident in longitudinal section (FIG. 19). Separation of apical outgrowth from a phialide due to septum formation was not observed. The plasma membrane and the outer and inner wall layers all were continuous between the phialide and the apical outgrowth (FIG. 20). Though continuous, the structural nature of the apical outgrowth and phialide outer wall layers was different. The apical outgrowth had a distinctive thickened, fibrillar outer wall layer, which might have given it the rough texture noted earlier (FIG. 21). The phialide and hyphal outer wall layers of the mutant were developmentally normal like wild type and did not have the fibrillar texture (FIGS. 20, 22). The transition in the neck region from smooth phialide wall to fibrillar outgrowth wall (FIG. 20) was similar to the transition in outer wall structure seen in the neck region of wild-type phialides, where the apex and developing conidium had a slightly more fibrillar outer wall texture (FIGS. 15, 16).



FIGS. 14–18. Transmission electron micrographs illustrating wild-type *F. verticillioides* (strain MRC826) microconidiation. 14. Longitudinal section through a wild-type phialide showing a single nucleus (N) and immature conidium (arrowhead). Bar = 1 μm. 15. Section through the first conidium initial produced by this phialide. Distinct wall layers were evident, including the phialide outer wall layer (OWL) and inner wall layer (IWL), the latter of which was thickened in the neck region surrounding the wall-building zone (WBZ). The conidium wall (CW) was derived de novo from this zone. The conidium initial appeared to rupture the outer wall layer of the phialide. The inner wall layer and plasma membrane (PM) of the phialide were continuous with the conidium. Bar = 0.5 μm. 16. Slightly higher magnification of a phialide apex and immature conidium showing the wall-building zone (WBZ), conidium wall (CW), thickened inner wall layer (IWL) of the

Genetic analyses.—Conidiation mutats were observed among a subset of ascospore progeny collected from genetic crosses (TABLE II). Random ascospores collected from three perithecia produced in a cross between wild-type parents MRC826 and NRRL25059 all resulted in conidiating strains. Yet, half the progeny collected from two other perithecia from the same cross possessed the mutant conidiation phenotype. Wild-type conidiating strain AEG1-1-57, which was derived from this cross (TABLE I), was backcrossed with MRC826, and all random progeny collected from two perithecia were conidiating strains. However, one complete unordered octad was collected from a separate perithecium in this backcross and it possessed four mutant and four wild-type conidiation strains (TABLE II). One of the octad's mutant strains, AEG3-A3-6, was crossed with wild-type conidiating strains JFL-A00999 and AEG3-A3-1. All random ascospore progeny segregated 1:1 for the conidiation phenotypes. In addition, eight octads collected from the cross with strain AEG3-A3-1 all segregated 1:1 for conidiation.

The conidiation mutation exhibited by strain AEG3-A3-6 did not affect its ability to produce perithecia and serve as a female in genetic crosses (TABLES I and II). The mutant also served as a male in the cross with strain JFL-A00999 (TABLE II). The ability to fertilize protoperithecia was not restricted to wild-type conidia because hyphal structures from mutant strain AEG3-A3-6 apparently were capable of fertilization as well.

The 1:1 segregation data suggested a single locus was genetically responsible for the conidiation phenotypes. The locus was designated *FPH1* (*f*rustrated *ph*ialide), referring to the prolific development of phialides that were unable to produce conidia. We found that proper segregation of conidiation phenotypes was dependent on unbiased collection of germinating ascospores because two germination phenotypes were observed (data not shown). Some ascospores germinated to form initial hyphal growth along the surface of agar, while other ascospores germinated to form invasive germ tubes that penetrated into the agar where further hyphal development occurred. Strains having surface hyphal growth of germinating ascospores mostly had wild-type conidiation; likewise strains having invasive hyphal growth of germinating ascospores mostly had the mutant conidiation phenotype. Genetic segregation data on the ascospore germination phenotypes and the possible linkage with *FPH1* will be presented and discussed elsewhere.

The spontaneous conidiation mutation was found in some perithecia but not others, as noted above in the cross between MRC826 and NRRL25059 (TABLE II). We were unable to determine whether the mutation occurred before or after plasmogamy and heterokaryon formation because isolation of complete octads was difficult in the wild-type × wild-type crosses. In general complete octads were rare in these crosses. Only one such octad was collected from the cross between strains MRC826 and AEG1-1-57. The rarity of complete tetrads was reflective of the overall lower fertility observed in the crosses involving NRRL25059 and AEG1-1-57 as male parental strains.

DISCUSSION

We have characterized morphologically and genetically a unique conidiation mutation in *F. verticillioides* that occurred spontaneously during sexual crosses involving wild-type conidiating parents. To our knowledge this is the first conidiogenesis mutation described in this species and the first mutation in any phialidic hyphomycete having aberrant apical outgrowths as described here. Tiedt and Jooste (1988a) described a *F. subglutinans* conidiogenesis mutant in which a conidium emerging from a phialide itself functioned as a phialide to produce the next conidium. Despite the abnormal reiterative process in that mutant, enteroblastic development still occurred to produce a conidium. As we have shown, enteroblastic development and conidium formation were lacking in the *F. verticillioides fph1* mutant.

The predominant lack of nuclei in the apical outgrowth, its aberrant morphology, the lack of a wall-building zone at the phialide apex and the apparent lack of septum formation and release from the phialide all suggested the outgrowth would not be viable as a propagule and therefore would not be a conidium having altered morphology. Nuclear staining and TEM indicated that mutant phialides contained a nucleus, but apical outgrowths contained a nucleus only rarely. Two nuclei occasionally could be seen in mutant phialides, suggesting that mitosis had occurred but one daughter nucleus had not migrated into the

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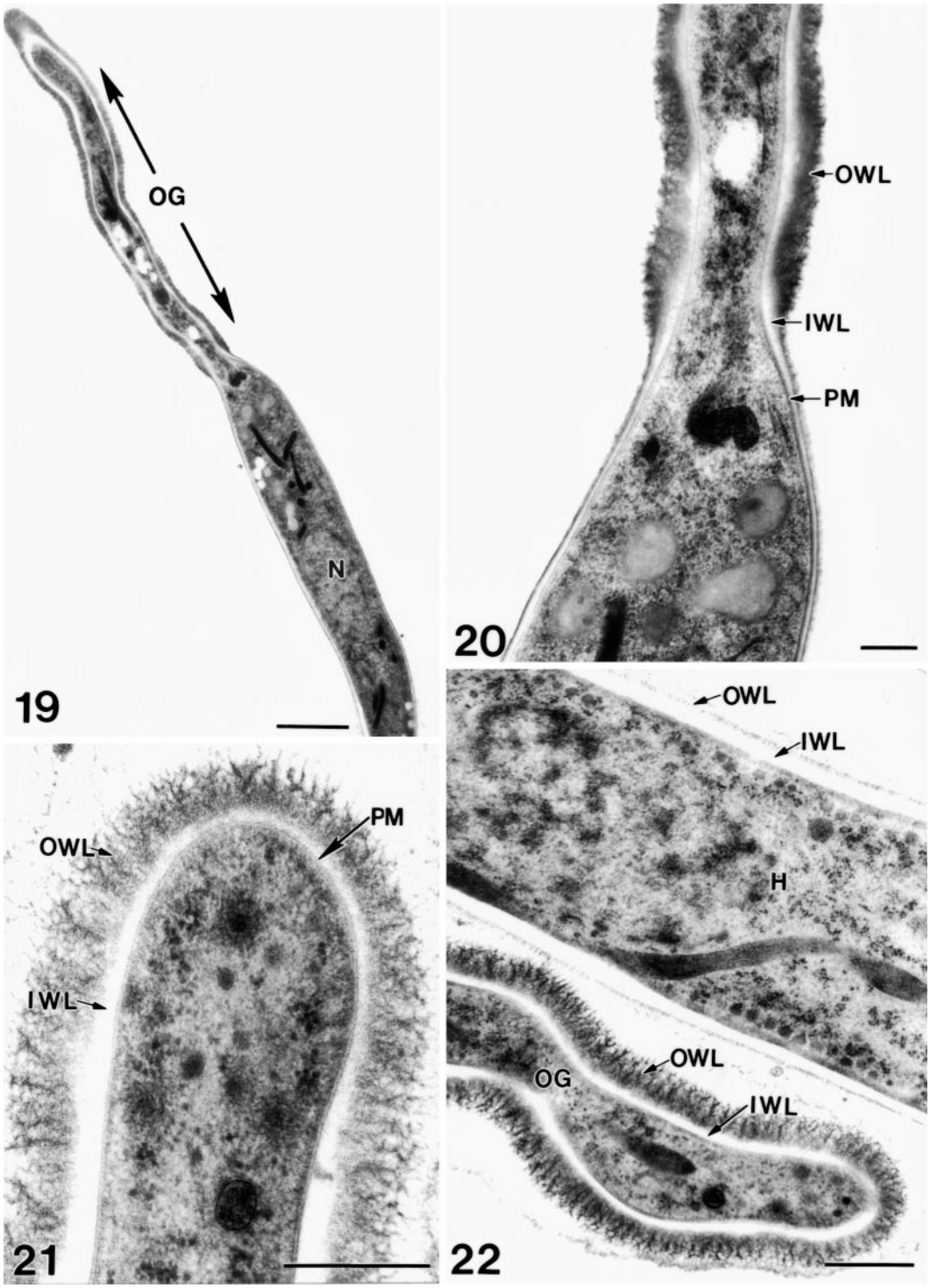
phialide apex, and a collarette (C) that was formed from remnants of the phialide outer wall layer that was ruptured by the first conidium initial. Bar = 0.25 μm. 17. A very early conidium initial distinctly showing the wall-building zone (WBZ) from which the conidium wall (CW) was being synthesized. The remnant nature of the collarette (C) was clearly evident. Bar = 0.5 μm. 18. A mature single-celled microconidium with a single nucleus (N). Bar = 0.5 μm.



FIGS. 19–22. Transmission electron micrographs illustrating mutant *F. verticillioides* (strain AEG3-A3-5) phialide development. 19. Longitudinal section through a phialide showing a single nucleus (N) and undulating outgrowth (OG) from the apex. Bar = 1 μm. 20. Higher magnification of the phialide apex illustrating that the outer wall layer (OWL), inner wall layer (IWL), and plasma membrane (PM) were continuous between the apical outgrowth and the phialide. The phialide clearly lacked a thickened inner wall layer and wall-building zone. The outer wall layer of the reinitiated growth was thick and more fibrillar compared to the thin, smooth outer layer of the phialide wall. Bar = 0.25 μm. 21. Higher magnification of the apical tip of the outgrowth illustrating the thickened, highly fibrillar outer wall layer (OWL), the inner wall layer (IWL), and the plasma membrane (PM). Bar = 0.25 μm. 22. The undulating apical outgrowth (OG) and an adjacent hypha (H) had distinct differences in outer wall layer (OWL) thickness and structure. Bar = 0.5 μm.

TABLE II. Sexual crosses and segregation of wild-type and mutant conidiation phenotypes

Strains crossed[a]	Conidiation phenotypes	Segregation of ascospore progeny (wild-type:mutant)		
		Random ascospores[b]	Predicted[c]	Unordered octads (# examined)
MRC826 × NRRL 25059	WT × WT	72:0[d]	1:0; $P = 1.0$	N.D.[e]
		22:26	1:1; $0.6 > P > 0.5$	
MRC826 × AEG1-1-57	WT × WT	48:0	1:0; $P = 1.0$	1:1 (1)
AEG3-A3-6 × JFL-A00999	Mutant × WT	10:14	1:1; $0.5 > P > 0.4$	N.D.
JFL-A00999 × AEG3-A3-6	WT × Mutant	24:24	1:1; $P = 1.0$	N.D.
AEG3-A3-6 × AEG3-A3-1	Mutant × WT	61:62	1:1; $1.0 > P > 0.9$	1:1 (8)

[a] Female strain is indicated first for each cross.

[b] In general only 24 random ascospore progeny were collected from a single perithecium.

[c] Predicted phenotypic ratio of random ascospore progeny collected from perithecia. Chi-square probability intervals (P) are indicated for each prediction.

[d] Collectively, three perithecia contained 72 progeny that were all wild-type conidiating strains. In contrast, half the progeny collected from two other perithecia from the same cross were conidiation mutants.

[e] N.D. = not determined.

apical outgrowth. The overwhelming lack of nuclei in the outgrowth, the predominance of a single nucleus in a phialide, and the manifestation of this mutant phenotype at a specific stage of development all suggested the mutation might result in disruption of mitosis in conjunction with the structural aberration. If this is the case, then the mutation might help define a self-surveillance mechanism that coordinates proper nuclear division and structural development during conidiogenesis and thus generally avoid generation of multinucleate phialides (Lew 2000).

Comparisons between the mutant and wild-type strains resulted in a detailed study of *F. verticillioides* microconidiation, and our observations support previous ultrastructural examinations of conidiogenesis in *Fusarium* species (Tiedt and Jooste 1988b, 1992). Wild-type enteroblastic conidium development appeared to involve the de novo synthesis and deposition of outer wall material on a conidium initial in a wall-building zone at the phialide apex where the phialide inner wall layer was thickened. Neither the first conidium initial nor any subsequent conidia appeared to derive any of their outer wall material from the phialide outer wall layer, as would happen during holoblastic conidiogenesis (Tiedt and Jooste 1988b, 1992, Hennebert and Sutton 1994). In contrast, the inner wall layer of the phialide and the developing conidium were continuous. The first conidium produced from a phialide appeared to rupture through the outer phialide wall layer to create the collarette that is distinctive for phialidic species such as *F. verticillioides*. All subsequent conidia were initiated from the wall-building zone just inside the collarette without proliferation or plugging of the phialide. Because this zone is below the wall material that it produces, no remnant conidial wall material was deposited during conidiogenesis. Release of a mature *Fusarium* microconidium from a phialide is suggested to occur through septum formation at the phialide apex followed by schizolysis (Tiedt and Jooste 1992). Although we examined only microconidiation due to our culture conditions, published observations of other *Fusarium* species suggest a similar developmental process occurs during macroconidiation (Marchant 1984, Tiedt and Jooste 1992).

The conidiation mutants lacked both the wall-building zone and the thickened inner wall layer at the phialide apex. Instead of elaborating conidia from the phialide apex, the mutant developed a germ tube-like outgrowth having polar growth that appeared to be determinate. In addition, both inner and outer wall layers were continuous between the phialide and the apical outgrowth. The outer wall of the aberrant growth was reminiscent of the slightly fibrillar texture seen on the outer wall of the wild-type phialide apex and conidium, but the mutant clearly had a more elaborate, thicker fibrillar outer wall layer. We did not observe any indication of septum formation and separation of the apical outgrowth from mutant phialides.

To our knowledge, this represents a morphological mutation involving both nuclear and unique structural perturbations to the conidiogenesis process not described previously in *Fusarium, Aspergillus* or any other phialidic species (Tiedt and Jooste 1988a, Adams et al 1998, Lau and Hamer 1998). Through this aberration, the mutation helps to define a precise temporal and spatial developmental process specific to the phialide apex, where wall structures differentiate to form a wall-building zone that facilitates co-

nidium initial formation and de novo deposition of a conidium outer wall layer. Despite the inability to form a phialide with a proper apex and wall-building zone, the polar nature of the outgrowth suggested apical dominance persisted as would be seen during normal conidium initial formation and development.

Segregation analyses indicated that a single locus, designated *FPH1,* was responsible for the conidiation phenotypes. We suggest that some aspect of sexual reproduction creates a genetic lesion responsible for the conidiation mutants, which were observed only among ascospore progeny from crosses involving wild-type parents. These conidiation mutants were not observed in axenic vegetative cultures of MRC826, NRRL25059 or any other conidiating strain. Given the morphological examinations, the underlying genetic lesion might result in loss of a regulator of cell wall differentiation localized to the phialide apex where the inner wall layer thickens and a wall-building zone forms. The aberrant outgrowth might represent loss of regulation of apical growth relative to proper development of the phialide apex and conidium initial formation.

The *BUF1* gene of *Magnaporthe grisea* is susceptible to high-frequency mutation when certain genetic crosses are performed (Farman 2002). The mutation event is dependent upon the combination of parental strains. Detailed analyses indicated the *BUF1* gene was deleted in *buf1* mutant progeny due to inheritance of a mutable chromosome from one of the parental strains. The mutable chromosome possessed numerous repetitive elements in the *BUF1* locus, which apparently contributed to deletion of that locus during meiotic pairing interactions between homologous chromosomes. Deletion occurred because the mutable *BUF1* locus remained unpaired in heteroallelic crosses. Mutations occurred with much less frequency in homoallelic crosses (0.3%) as compared to heteroallelic crosses (33.5%).

The production of mutant *buf1* progeny from crosses involving wild-type parents is similar to the situation we have described for *FPH1*. We do not have molecular data to determine if parental strains MRC826 or NRRL25059 possess a mutable *FPH1* locus due to some chromosomal aberration such as repetitive elements, but our observations do suggest that strain NRRL25059 might be contributing to the mutation. Strain MRC826 has been used consistently in genetic crosses with other *F. verticillioides* strains without any observed occurrence of the *fph1* conidiation mutation (personal observation). The *fph1* mutation was observed only among progeny from crosses involving either strain NRRL25059 or AEG1-1-57 (TABLE II). The latter strain might have inherited from NRRL25059 the *FPH1* locus contributing to the *fph1* genetic lesion (TABLE I). Through octad analyses Farman (2002) determined that deletion of the *BUF1* gene was occurring after plasmogamy and before the premeiotic round of DNA replication. We were unable to make such conclusions regarding the *FPH1* gene due to the lack of sufficient numbers of complete tetrads in our crosses involving wild-type parents (TABLE II).

The low fertility observed in crosses involving strain NRRL25059 was unfortunate. This strain, isolated from banana in Honduras, was identified as *F. verticillioides* by phylogenetic analyses (O'Donnell et al 1998). However, morphological examination of banana isolates from Mexico found consistent differences from the standard description of *F. verticillioides* (Hirata et al 2001). For example, the banana isolates produced 1(3)-septate microconidia more commonly than is typical for *F. verticillioides*. Recent phylogenetic analyses suggested a homogeneous clade of strains from banana were distinct from a more heterogeneous collection of *F. verticillioides* strains (Hirata et al 2001, Mulè et al 2003). Of interest, strain NRRL25059 was the only field isolate identified as *F. verticillioides* that could not metabolize preformed antimicrobial compounds produced by corn (Glenn et al 2001, Glenn et al 2002). This survey included nearly 60 *F. verticillioides* strains from a broad range of hosts and geographical locations but did not include any other isolates from banana in Mesoamerica. In addition, Central American banana isolates were reported to lack any production of fumonisin B1 (Mulè et al 2003). We found that strain NRRL25059 also did not produce fumonisin B1 and might be missing much of the fumonisin biosynthetic gene cluster (data not shown). Together, these observations suggest the banana isolates from Mexico and Central America might represent a phylogenetically distinct species with close relationship to *F. verticillioides*. Such a relationship would explain the ability of strain NRRL25059 to mate with *F. verticillioides* strain MRC826 but with reduced fertility. Mating of strains with interspecies genetic differences might also lead to unpaired loci that are subject to deletion during meiotic pairing interactions between homologous chromosomes, in a manner analogous to deletion of the *M. grisea BUF1* locus (Farman 2002). Thus, an interspecies mating scenario might explain why *fph1* conidiation mutants were recovered from crosses involving strains MRC826 and NRRL25059. Creation of genetic lesions due to aberrant pairing interactions of homologous chromosomes also would explain why some perithecia contained *fph1* mutant ascospore progeny while other perithecia from the same cross appeared to contain only *FPH1* conidiating progeny. The heteroallelic pairing interactions

leading to a genetic lesion would occur independently of each other.

The precise developmental nature of the conidiation mutation makes it particularly interesting in terms of identifying spatially and temporally expressed genes that are necessary for conidiogenesis after the formation of phialides, such as genes specifically involved in differentiation of the wall-building zone and conidium initial formation. Identification of such genes is of value given that propagation and dissemination are necessary for establishment and survival of hyphomycetous fungi such as *F. verticillioides,* in which enteroblastic phialidic conidiation is a highly effective mechanism for generating large numbers of dispersal propagules. As such, *F. verticillioides* conidiation may be described as a fitness factor contributing to its reproductive success and persistence in the environment (Pringle and Taylor 2002). Molecular cloning and characterization of the *FPH1* locus will be a component of future genetic studies. In addition, the mutation is being used to assess the impact of conidiation on infection and establishment of systemic in planta associations with corn.

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