Nitrate Reduction Mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*)

C. J. R. Klittich and J. F. Leslie*

Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506

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ABSTRACT

Twelve strains of *Fusarium moniliforme* were examined for their ability to sector spontaneously on toxic chlorate medium. All strains sectored frequently; 91% of over 1200 colonies examined formed chlorate-resistant, mutant sectors. Most of these mutants had lesions in the nitrate reduction pathway and were unable to utilize nitrate (*nit* mutants). *nit* mutations occurred in seven loci: a structural gene for nitrate reductase (*nit1*), a regulatory gene specific for the nitrate reduction pathway (*nit3*), and five genes controlling the production of a molybdenum-containing cofactor that is necessary for nitrate reductase activity (*nit2, nit4, nit5, nit6, nit7*). No mutations affecting nitrite reductase or a major nitrogen regulatory locus were found among over 1000 *nit* mutants. Mutations of *nit1* were recovered most frequently (39–66%, depending on the strain) followed by *nit3* mutations (23–42%). The frequency of isolation of each mutant type could be altered, however, by changing the source of nitrogen in the chlorate medium. We concluded that genetic control of nitrate reduction in *F. moniliforme* is similar to that in Aspergillus and Neurospora, but that the overall regulation of nitrogen metabolism may be different.

FUSARIUM moniliforme (Sheld.) emend. Snyd. and Hans. [sexual stage *Gibberella fujikuroi* (Sawada) Wr.] is a filamentous, ascomycetous fungus which is pathogenic to a variety of animals and plants (MARASAS, NELSON and TOUSSOUN 1984; NELSON, TOUSSOUN and COOK 1981). The fungus is heterothallic and can be readily crossed in the laboratory. Fusaria are often considered to be genetically unstable because they frequently produce sectors in culture which differ from the original colony in morphology, virulence, or other characteristics (BURNETT 1984; PUHALLA 1981). The cause of this variability and its importance to the fungus are unknown, but genetic plasticity might be advantageous for a plant-pathogenic fungus such as Fusarium. Survival often requires adaptation to drastic environmental changes, so a source of genetic variability is essential. For example, genetic instability could generate variants that are fungicide resistant, able to overcome host resistance, or tolerant to toxic wastes in the soil. A high mutation rate could be an important means of generating variability if sexual or parasexual recombination is rare. As a first step toward determining the genetic basis of instability in Fusarium, we have examined spontaneously occurring, nitrate-nonutilizing mutants of *F. moniliforme*.

Several strains of *F. moniliforme* produce spontaneous, chlorate-resistant sectors on medium containing chlorate, a toxic analog of nitrate (PUHALLA and SPIETH 1985). Most of these sectors are nitrate-nonutilizing mutants, or *nit* mutants. In Aspergillus and Neurospora, such mutants have lesions in either the structural or regulatory loci of the nitrate reduction pathway (MARZLUF 1981; ARST and SCAZZOCCHIO 1985). Our objectives were to determine if this high frequency of sectoring is typical of *F. moniliforme* strains, to determine the number of loci affected, and to determine if some *nit* loci are more susceptible to mutation than others. We tested 12 strains of *F. moniliforme* for ability to sector spontaneously to chlorate resistance on chlorate-containing medium, and determined the frequency of mutation at each locus. Mutants were characterized by their phenotype, complementation patterns, and meiotic recombination patterns.

MATERIALS AND METHODS

Strains: All strains of *F. moniliforme* used in this study (Table 1) belong to mating group A (HSIEH, SMITH and SNYDER 1977), which corresponds to the variety *moniliformis* of KUHLMAN (1982). Speciation in the *Liseola* section of *Fusarium* is unresolved, but all strains in this study belong to the same genetic species in that they are cross-fertile.

Strains were stored on sterile filter paper at 4° (CORRELL, PUHALLA and SCHNEIDER 1986). Prior to storage, mutants were purified by isolating and culturing a single, uninucleate microconidium. All cultures were incubated at 25° with a 12-hr day/ 12-hr night cycle. Light intensity was 150–350 foot-candles from cool-white fluorescent bulbs. Female-fertile *nit* mutants of both mating types have been deposited with the Fungal Genetics Stock Center, University of Kansas Medical Center, Kansas City, Kansas. Parental strains are available from the Fusarium Research Center, Department of Plant Pathology, Pennsylvania State University, University Park, Pennsylvania.

Media: Complete medium (CM) (CORRELL, KLITTICH and LESLIE 1987) was used for routine culturing. Minimal

* Corresponding author.

TABLE 1

Strains of *F. moniliforme* used in this study

Strain	VCG[a] Mating type	Original designation[b]	Source
102	A1/+	F80	Sorghum, California
124	A6/+	F152	Maize, California
133	A4/−	F166	Maize, California
148	A2/−	F223	Maize, California
149	A7/−	F237	Maize, California
150	A3/+	F288	Maize, California
171	A5/+	F594	Rice, Italy
185	A8/+	F791	Maize, Italy
408	A7/−	FSL 172	Maize, California
409	A1/+	FSL 173	Sorghum, California
410	A3/+	FSL 174	Maize, California
411	SI[c]/−	FSL 175	Maize, California

[a] Vegetative compatibility group; groups A1–A7 assigned by JOHN PUHALLA.

[b] "F" strains from J. E. PUHALLA, "FSL" strains from E. G. KUHLMAN (1982).

[c] SI = self-incompatible, cannot form a heterokaryon (CORRELL, KLITTICH, and LESLIE 1987).

medium (MM) (PUHALLA and SPIETH 1983) contains nitrate as the sole source of nitrogen and was used for complementation tests. Mutants were generated on chlorate medium (CLM) (PUHALLA and SPIETH 1985).

Carrot agar modified from PUHALLA and SPIETH (1985) was used for sexual crosses. Fresh carrots (400 g) were washed, diced, and autoclaved 10 min in 400 ml distilled water. The carrots and liquid were pureed, mixed with an additional 500 ml distilled water and 20 g agar, autoclaved an additional 30 min, and dispensed into 60 x 15 mm plastic Petri dishes at 15–17 ml per dish. Fertility was generally higher on carrot agar than on the commonly used V8 agar (KATHARIOU and SPIETH 1982). Fertility varied from strain to strain; strains 102 and 149 were highly fertile as either males or females, whereas strains 411 and 185 were irregularly fertile as males and infertile as females.

Isolation of chlorate-resistant mutants: A mycelial transfer (approximately a 2 mm^3 block) from CM was placed in the center of a CLM plate. Each 100 x 15 mm plate contained 20–25 ml CLM, and 60 x 15 mm plates contained 10–13 ml CLM. Sectoring frequencies were similar regardless of plate size. Each strain was transferred to 100 CLM plates. Growth was highly restricted (<1 cm per week). Fast-growing sectors developed after 4–14 days and were transferred to fresh CLM. Each colony was sampled only once. If less than 100 chlorate-resistant sectors were obtained from 100 CLM plates, additional plates were inoculated and sampled until at least 100 sectors were obtained. Chlorate-resistant mutants were transferred from CLM to CM and MM to check for ability to utilize nitrate, then from CM to MM and CLM to check stability on CM and confirm the phenotype. Colonies which were resistant to chlorate and unable to utilize nitrate were considered *nit* mutants. Genetic terminology follows the guidelines of YODER, VALENT and CHUMLEY (1986) for plant pathogenic fungi.

Analysis of *nit* mutants: *nit* mutants were categorized by their ability to complement other mutants from the same strain, by their growth on different nitrogen sources, and by segregation ratios resulting from crosses of different *nit* mutants.

Physiological complementation of *nit* mutants within a strain was tested by placing mycelial transfers 2–4 cm apart on MM. *nit* mutants grow at the same lineal rate as the wild type on MM, but colonies are very thin and sparse. Plates were incubated 5 to 14 days and scored periodically for dense, heterokaryotic growth where colonies intersected. Mutant pairs that did not form dense growth belonged to the same complementation group. Cross-feeding does not occur when *nit* mutants are separated by cellophane, indicating that wildtype growth is the result of hyphal fusion (PUHALLA and SPIETH 1985). To differentiate complementation groups, ten *nit* mutants from a strain were paired with one another in all possible combinations. Mutants belonging to the three most common complementation groups were then paired with the remaining *nit* mutants from that strain. Mutants which complemented all three testers were paired with each other in all possible combinations to identify the remaining complementation groups. Mutants which could not be positively assigned to a complementation group were identified by their growth on media containing different nitrogen sources.

Mutants from each complementation group were tested for their ability to utilize different nitrogen sources following the procedures described by CORRELL, KLITTICH and LESLIE (1987) for *Fusarium oxysporum*. Relative growth of the mutant on media containing different nitrogen sources indicated which metabolic function may have been affected by the mutation (Table 2).

Complementation groups were assigned to *nit* loci based on results from sexual crosses. Loci designated *nit1*, *nit2* and *nit3* have been mapped by PUHALLA and SPIETH (1985); strains with mutations in these loci were used as standards in initial crosses. To initiate a cross, the female parent was inoculated by mycelial transfer onto a 60-mm carrot agar plate, and the male parent was simultaneously inoculated onto a CM slant (8 ml medium in a 16 x 150 mm culture tube). After 7 days, conidia from the male parent were suspended in 3–5 ml of a 2.5% Tween 60 solution, and 1.0–1.5 ml of the suspension were gently spread over the surface of the female parent colony with a glass rod, thoroughly wetting the mycelium. Perithecia formed after approximately 7 days and exuded a cirrhus of ascospores 14–30 days after fertilization. Viability of the ascospores was best within 1–2 weeks of extrusion. Germination of ascospores was occasionally poor on water agar. Ascospores were therefore separated from each other and from conidia on a slab of VOGEL's (1964) minimal N medium using a Cailloux stage-mounted micromanipulator. Slabs gelled with 3% agar were formed in an agar-layer casting block (HAEFNER 1967). Ascospores which had germinated after 18 hr at 25° were cultured on CM, then transferred to MM to determine the frequency of prototrophic recombinants.

Generation of *nit* mutants on alternative nitrogen sources: The source of nitrogen in chlorate medium has been shown to affect the frequency of *nit* mutant types generated from *Aspergillus nidulans* (COVE 1976b). We therefore generated mutants on chlorate medium supplemented with different nitrogen sources in an attempt to recover additional types of *nit* mutants. The nitrogen sources tested are listed in Table 3. The media were identical to CLM except that asparagine was replaced with the alternative nitrogen source at 19.2 mM N (equivalent to 1.4 g/liter asparagine). All media were adjusted to pH 4.7 (the pH of CLM). Sixteen plates of each medium were inoculated with a mycelial transfer from strain 102. Fast-growing sectors were transferred to CLM, and chlorate-resistant sectors were screened for their ability to utilize different nitrogen sources (CORRELL, KLITTICH and LESLIE 1987).

TABLE 2

Identification of *F. moniliforme* mutants based on growth tests

Fusarium locus	Neurospora locus[a]	Aspergillus locus[a]	Function[a]	Growth on nitrogen sources[b]					Nitrite[c] excretion
				NH_4^+	NO_3^-	NO_2^-	HX	UA	
nit1	*nit-3*	*niaD*	Structural gene for nitrate reductase	+	−	+	+	+	NT
nit3	*nit-4*	*nirA*	Pathway-specific regulatory gene	+	−	−	+	+	−
nit2, *nit4*, *nit5*, *nit6*, *nit7*	*nit-1*, *nit-7*, *nit-8*, *nit-9*	*cnxABC*, *cnxE*, *cnxF*, *cnxG*, *cnxH*	Genes controlling production of a molybdenum-containing cofactor	+	−	+	−	+	NT
None isolated	*nit-2*	*areA*	Major nitrogen regulatory locus	+	−	−	−	−	NT
None isolated	*nit-6*	*niiA*	Structural gene for nitrite reductase	+	−	−	+	+	++
			Wild-type	+	+	+	+	+	+

[a] See MARZLUF (1981) for full discussion.
[b] From GARRETT and AMY (1978) and TOMSETT and GARRETT (1980): HX = hypoxanthine, UA = uric acid, (−) = thin growth, (+) = wild-type growth.
[c] From COVE (1976b): (−) = no nitrite excretion, (+) = slight nitrite excretion, (++) = abundant nitrite excretion, NT = not tested.

TABLE 3

Phenotypes of *nit* mutants from *F. moniliforme* produced on chlorate media with different nitrogen sources

Nitrogen source[a]	Total *nit* mutants	Frequency (%)		
		nit1[b]	nit3	MoCo
L-Asparagine	16	69	19	13
L-Aspartic acid	16	50	31	19
Ammonium	11	45	36	18
L-Glutamine	17	71	24	6
L-Glutamic acid	12	75	8	17
Hypoxanthine	18	67	17	17
Nitrate	11	64	18	18
L-Ornithine	12	83	17	0
L-Proline	17	65	24	12
L-Threonine	15	40	13	47
L-Tryptophane	14	36	43	21
Urea	1	0	0	100
Uric acid	15	33	47	20

[a] Nitrogen, 19.2 mM (equivalent to 1.4 g/liter asparagine).
[b] nit1, lack of nitrate reductase production; nit3, lack of nitrate and nitrite reductase production; MoCo, lack of molybdenum-containing cofactor production.

RESULTS

Generation of mutants: All 12 strains of *F. moniliforme* spontaneously produced chlorate-resistant sectors at a high frequency on CLM (Table 4). Most strains produced spontaneous sectors on over 95% of the CLM plates. Strains 171 and 408, however, sectored at a lower frequency (54 or 55% of CLM plates), and were less restricted in growth on CLM compared with the other strains. Most chlorate-resistant sectors (66–82%, depending on the strain) were *nit* mutants, *i.e.*, unable to utilize nitrate (Table 4). The remaining sectors were either unstable in phenotype when scored twice on minimal medium or could utilize nitrate. Nitrate-utilizing sectors either were heterokaryons containing *nit* mutants or contained a different type of mutant which could both utilize nitrate and grow on chlorate medium (*crn* mutants). Genetic analysis of *crn* mutants will be described elsewhere. Sectors of altered color or morphology also appeared occasionally, particularly in strains 171 and 408, but these sectors were usually not chlorate-resistant.

Approximately 14% of all colonies on CLM produced two or more chlorate-resistant sectors. Seventy colonies (6%) produced more than one sector containing identical *nit* mutants; these sectors could have arisen from the same mutation event. Thirty-four colonies (3%) produced multiple sectors containing different *nit* mutants; these sectors must have arisen from multiple mutation events. One colony produced a *nit4* sector and a heterokaryotic sector consisting of a *crn* mutant and a *nit3* mutant, indicating that three mutation events had occurred. The frequency of multiple sectors varied from one strain to another; strain 149 had the highest frequency (25% of the colonies produced two or more sectors) and strain 409 had the lowest frequency (4%).

To determine if the frequent recovery of spontaneous *nit* mutants reflects an inherently high frequency of nuclei carrying *nit* mutations, spores from colonies growing on CM were tested for chlorate resistance. Germinating, uninucleate microconidia from strains 102 and 148 were placed on CLM. None of the 52 conidia was resistant to chlorate. Forty spores were unable to form colonies and 12 spores formed highly restricted colonies. Eleven of the re-

TABLE 4

Frequency of isolation of spontaneous, chlorate-resistant sectors from *F. moniliforme*

Strain	Percent plates with sectors	Total sectors[a]	Percent *nit* mutants[b]
102	100	129	81
124	96	105	82
133	95	102	82
148	99	130	77
149	96	107	68
150	96	104	80
171	54	105	73
185	97	128	78
408	55	90	66
409	98	104	68
410	98	96	71
411	96	118	81

[a] Sectors developed spontaneously on a minimal medium amended with 1.5% $KClO_3$ (CLM). Nitrogen sources were $NaNO_3$ and L-asparagine.

[b] Percentage of chlorate-resistant sectors that were unable to utilize nitrate as a nitrogen source (*nit* mutants).

TABLE 5

Segregation of progeny in crosses between *nit* mutants of *F. moniliforme*

Parent strain[ab]	Tester *nit1*	*nit2*	*nit3*	*nit4*	*nit5*	*nit6*	*nit7*	Locus
124-3	0/43							*nit1*
124-65		0/31		5/31	5/26			*nit2*
124-1			0/42					*nit3*
148-6				0/24	6/27		6/31	*nit4*[c]
148-27				0/36	8/36		3/26	*nit4*[c]
185-53		8/35		5/31	0/39	4/22		*nit5*
185-62		6/31		4/28	0/27	6/22	12/33	*nit5*
133-15		13/36		7/36	10/32	0/28	9/33	*nit6*
150-52		6/36		8/36	8/34	2/29	0/34	*nit7*
150-75		7/62		11/36	8/34	2/26	0/34	*nit7*

[a] Strains designated by parent and isolation number, *e.g.* 124-3 is the third *nit* mutant from strain 124.

[b] Mutants with a common parent can form a heterokaryon which utilizes nitrate.

[c] *nit4* mutants from strain 148 fell into three complementation groups. Crossing data for representatives of two of these groups are shown. The third group contains the *nit4* standard tester.

stricted colonies later produced sectors of chlorate-resistant growth.

Identification of mutants: *nit* mutants were divided into complementation groups by testing mutants in pairs on minimal medium. A mutant from each group was tested for growth on different nitrogen sources to identify the point in the nitrate reductase pathway affected by the lesion. Mutants (Table 2) were found in loci presumably coding for the nitrate reductase enzyme (*nit1*), for a product regulating induction of the nitrate reductase pathway (*nit3*), and for a molybdenum-containing cofactor (*nit2*, *nit4*, *nit5*, *nit6*, and *nit7*). No mutants of the nitrite reductase structural locus or of a major nitrogen regulatory locus were recovered.

Complementing pairs of mutants differed in the speed and vigor of heterokaryon formation. Mutants deficient in the molybdenum cofactor rapidly formed robust, heterokaryotic growth with mutants from other complementation groups. Pairings between *nit1* mutants and *nit3* mutants, on the other hand, developed heterokaryotic growth more slowly. The *nit1*, *nit3*, *nit4*, *nit5*, and *nit7* loci all contained more than one complementation group.

Strain 411 differed from the other strains in that *nit* mutants with different phenotypes were unable to form dense, heterokaryotic growth. Thus, *nit* mutants from strain 411 could not be divided into complementation groups. These mutants were categorized by their ability to utilize different nitrogen sources and by crossing them with *nit* mutant testers. This "self-incompatibility" phenomenon has also been observed in *F. oxysporum* (CORRELL, KLITTICH and LESLIE 1987).

TABLE 6

Frequency of isolation (in percent) of *nit* mutants from 12 strains of *F. moniliforme*[a]

Strain	Location of mutation *nit1*[b]	*nit2*[c]	*nit3*[d]	*nit4*[c]	*nit5*[c]	*nit6*[c]	*nit7*[c]	Total No. mutants characterized
102	49	4	34	15	1	0	0	104
124	47	4	37	9	0	3	1	81
133	50	4	23	17	0	3	4	78
148	46	0	28	25	0	0	1	76
149	39	5	36	16	3	2	0	67
150	54	1	37	3	1	0	4	76
171	64	0	28	5	0	3	0	76
185	50	0	39	4	7	0	0	104
408	42	0	37	19	0	2	0	52
409	42	0	35	12	0	3	8	65
410	53	1	42	4	0	0	0	74
411	66	1	27	5	1	0	0	82

[a] *nit* mutants occurred spontaneously on CLM medium (contains nitrate and asparagine as nitrogen sources, plus 1.5% $KClO_3$).

[b] Nitrate reductase structural gene.

[c] Genes controlling production of a molybdenum-containing cofactor.

[d] Pathway-specific regulatory gene.

nit mutants with the same phenotype from strains of opposite mating type were crossed to determine the number of loci represented. If no recombinants were obtained in a cross, the mutants were considered allelic. Typical data are shown in Table 5. Crosses with each mutant were made only until the locus was identified.

Not all *nit* mutants were isolated with equal frequency (Table 6). *nit1* mutants were most frequently isolated, constituting from 42–66% of all *nit* mutants,

depending on the strain. *nit3* mutants comprised 23–42% of the *nit* mutants, and molybdenum cofactor mutants collectively made up 5% to 27% of the *nit* mutants. Mutants of *nit4* were isolated twice as often as mutants of the remaining four molybdenum cofactor genes combined.

Alternative nitrogen sources: Chlorate-resistant sectors were isolated from media supplemented with chlorate and various nitrogen sources with the goal of obtaining *nit* mutants at additional loci. All of the *nit* mutant types generated, however, had also been isolated from CLM containing asparagine (Table 3). No mutants typical of a major nitrogen regulatory locus were isolated. The frequency of isolation of different *nit* mutant types did vary with nitrogen source, however (Table 3). The highest proportion of *nit1* mutants was obtained from chlorate media containing glutamine, glutamic acid, or ornithine, and high frequencies of *nit3* mutants (over 40%) were recovered from uric acid and tryptophan-containing chlorate media. The highest proportion of molybdenum cofactor mutants (over 40%) was obtained from threonine-chlorate medium.

DISCUSSION

Genetic instability: Although the genus *Fusarium* is often considered to be genetically unstable in culture, genetic examination of this instability has been minimal (BURNETT 1984; PUHALLA 1981). We found that *F. moniliforme* was unstable when grown on medium containing chlorate, a toxic analog of nitrate. All 12 strains examined sectored frequently and spontaneously to chlorate resistance. Most of these sectors were mutants with lesions in the nitrate reduction pathway. Although some variation in overall sectoring frequency between strains was observed, instability on chlorate medium appears to be a general characteristic of strains of *F. moniliforme*. Recently C. J. R. KLITTICH, J. C. CORRELL and J. F. LESLIE (unpublished data) found that 94% of wild-collected *F. moniliforme* isolates sector when cultured on chlorate medium.

The cause of instability in *F. moniliforme* is unknown. No chlorate-resistant isolates were found among 52 uninucleate conidia. Using these data, we calculate that no more than 5% of the nuclei in the colony carry a *nit* mutation prior to culture on chlorate medium. Thus, the high sectoring frequency does not result from an inherently high frequency of chlorate-resistant nuclei in the parental culture. These data do not allow us to determine if the chlorate medium induces mutations or if it merely selects for preexisting mutations. The behavior of the colonies that resulted from 12 of these conidia suggests that culturing *F. moniliforme* on chlorate medium can result in genetic instability. These colonies, since they originated from a single conidium, should be homokaryotic, yet 11 of 12 sectored to chlorate resistance. These mutations must have occurred while the colony was growing on the chlorate medium, although the medium itself need not be the cause of the mutation. We cannot estimate mutation rate directly because we cannot determine the number of nuclei in a colony. Some isolates of *F. moniliforme* (C. J. R. KLITTICH, J. C. CORRELL and J. F. LESLIE, unpublished data) do not sector under these conditions, so it is likely that some process other than background mutation is responsible for the chlorate-resistant sectors that we have observed.

Instability in *F. moniliforme* might be associated with a transposable element. Transposon movement has been associated with high mutation frequencies in a number of eukaryotic organisms, including yeast (ROEDER *et al.* 1980), *Drosophila* (GREEN 1980; ENGELS 1983), and maize (LILLIS and FREELING 1986). Instability in *F. moniliforme* may also be associated with environmental stress. Frequent sectoring was observed on toxic chlorate medium, but was not observed on complete medium. Resistant sectors also form frequently when *F. moniliforme* is cultured on medium containing selenate, a toxic analog of sulfate (CORRELL and LESLIE 1987). Environmental stress has been shown to induce heritable changes in phenotype in several organisms, notably flax (CULLIS 1986). Stress has also been shown to increase the frequency of transposon movement; *Ty* element transposition in yeast increases 100-fold at cold temperatures (PAQUIN and WILLIAMSON 1984), and *Ac* elements in maize are activated by chromosome breakage (MCCLINTOCK 1984). Parasites such as *F. moniliforme* are often exposed to strong selection pressures. Genetic mechanisms which increase variability could enhance rapid adaptation of the pathogen population to host resistance, fungicides, and extreme environmental changes. The movement of transposable elements, perhaps triggered by environmental stress such as growth on a toxic medium, could cause the high frequency of spontaneous mutation observed in *F. moniliforme*. Further research is necessary to determine the molecular mechanism of instability.

Nitrate metabolism: The *nit* loci identified in this study are similar in number and phenotype to genes identified in *A. nidulans* and *Neurospora crassa* (MARZLUF 1981; COVE 1976a; Table 2). In each organism, the nitrate reductase enzyme is encoded by a single locus, and a single locus also regulates this pathway. In all three organisms, multiple loci control production of a molybdenum-containing cofactor; four loci in Neurospora (TOMSETT and GARRETT 1980), and five loci each in Aspergillus (MARZLUF 1981) and *F. moniliforme*. Both the *cnxABC* locus of Aspergillus and

the *nit-9* locus of Neurospora are complex; each contains three complementation groups (MARZLUF 1981). The *nit4* locus in *F. moniliforme* contained up to three complementation groups and may be analogous to *nit-9* and *cnxABC*.

No locus resembling either a gene for nitrite reductase, analogous to *nit-6* in Neurospora and *niiA* in Aspergillus, or a major nitrogen regulatory gene, analogous to *nit-2* in Neurospora and *areA* in Aspergillus, was identified. We did not expect to isolate nitrite reductase mutants, because these mutants are chlorate-sensitive in Neurospora and Aspergillus (TOMSETT and GARRETT 1980; COVE 1976a). The absence of a mutant in a major nitrogen regulatory locus is unexpected, however. In Neurospora, mutants in the *nit-2* locus can constitute 12–15% of *nit* mutants generated on chlorate medium (MARZLUF, PERRINE and NAHM 1985; PERRINE and MARZLUF 1986), and some *areA* mutants of Aspergillus are chlorate-resistant (ARST and COVE 1973).

To increase the likelihood of obtaining a mutation of the major nitrogen regulatory locus, we generated mutants on chlorate media containing one of 13 nitrogen sources. No mutants analogous to *nit-2* or *areA* were found. Similar results have also been obtained with *F. oxysporum* (CORRELL, KLITTICH and LESLIE 1987). The absence of this mutant class suggests that mutations at this locus are either lethal or sensitive to chlorate. Chlorate sensitivity seems a likely explanation, as a mutant from *F. graminearum* which may have a lesion in the major nitrogen regulatory locus is chlorate-sensitive (LESLIE 1987). If mutations in the general nitrogen regulatory locus are generally chlorate-sensitive in the Fusaria, overall regulation of nitrogen metabolism may differ from that in Aspergillus and Neurospora.

***nit* mutant frequency.** *nit* loci differed in their susceptibility to mutation in these experiments; mutants of *nit1* were most frequently recovered, followed by *nit3* and *nit4* mutants. Mutants of the nitrate reductase locus are also the most frequently isolated *nit* mutants in Aspergillus (COVE 1976b) and Neurospora (TOMSETT and GARRETT 1980; PERRINE and MARZLUF 1986). Mutants of the pathway-specific regulatory locus are frequently isolated in Aspergillus (COVE 1976b) as well as in *F. moniliforme*, but infrequently isolated in Neurospora (TOMSETT and GARRETT 1980; MARZLUF, PERRINE and NAHM 1985). Differences between loci in susceptibility to mutation could be related to the physical size of the gene, with larger genes representing a larger target. Alternatively, some loci may contain sequences which are mutational "hot spots," rendering them more susceptible to mutation.

COVE (1976b) has demonstrated that the nitrogen source can significantly bias the relative frequency of *nit* mutant types generated on chlorate medium. We also found that nitrogen source biased the type of *nit* mutants generated. A biasing of *nit* mutant frequencies by nutrient source has also been observed with *Fusarium oxysporum*; on chlorate-amended potato dextrose agar, 82–95% (depending upon the strain) of the *nit* mutants were nitrate reductase mutants, compared with 59–66% of the *nit* mutants generated on a minimal chlorate medium (CORRELL, KLITTICH, and LESLIE 1987).

***nit* mutants for vegetative compatibility tests.** Spontaneous *nit* mutants are useful as auxotrophic markers for forcing heterokaryons to test isolates for vegetative compatibility. This technique has been described in detail for *F. oxysporum* (CORRELL, KLITTICH and LESLIE 1987). Different isolates are vegetatively compatible (*i.e.*, capable of forming a heterokaryon) only if alleles at all vegetative compatibility loci are identical. Vegetative compatibility groups (VCGs) are ideal markers for population studies because they occur naturally, are easy to score using spontaneous *nit* mutants, and should be selectively neutral. SIDHU (1986) used *nit* mutants to categorize 60 isolates of *F. moniliforme* into 13 VCGs. A third of these isolates could not be classified because SIDHU was unable to obtain complementing *nit* mutants from them. Our study of the genetics of nitrate metabolism suggests some guidelines for the use of *nit* mutants to examine VCGs in *F. moniliforme*. For example, we found that 1 out of the 12 strains examined was incapable of forming a heterokaryon. Self-incompatible isolates, such as strain 411, are a potential source of confusion in VCG tests, and may have contributed to SIDHU's inability to classify 22 of his *F. moniliforme* isolates. We also found that *nit* mutant types differ in their suitability for VCG standards. Mutants deficient in the molybdenum-containing cofactor are infrequently isolated and readily form complementing heterokaryons, making them good choices for standards in vegetative compatibility tests. *nit1* and *nit3* mutants, on the other hand, may form weak, slow-growing heterokaryons, giving ambiguous results. Thus, the phenotypes of *nit* mutants should be determined before standards are selected.

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