# A Genetic System for Trichothecene Toxin Production in *Gibberella pulicaris* (*Fusarium sambucinum*)

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## ABSTRACT

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Trichothecene-toxin-producing isolates of *Gibberella pulicaris* (anamorph: *Fusarium sambucinum*) were mated on water agar containing steam-sterilized mulberry (*Morus alba*) twigs. Mature ascospores developed at 15 C following several weeks of incubation under an alternating 12 hr/25 C light and 12 hr/20 C dark schedule. Segregation patterns among random ascospore progeny from parents differing in diacetoxyscirpenol production and other traits were examined. Protoperithecium formation and red pigmentation were monogenically controlled. Preliminary analyses of progeny from crosses among toxin-producing and nonproducing isolates indicated that ability to produce toxin is controlled by genes at several loci. We conclude that trichothecene toxin biosynthesis in *G. pulicaris* is amenable to classical genetic analysis. This is the first documented genetic system in a trichothecene-producing fungus.

*Additional key word*: *Fusarium sulphureum*.

Many species of *Fusarium* produce trichothecenes, a family of bicyclic sesquiterpene toxins that have been implicated in mycotoxicoses of humans and animals (11). Genetic manipulation should be a useful technique for studying the regulation of toxin production and for determining the relationship of toxin production to plant pathogenicity. However, although genetic systems have been developed in some *Fusarium* species (7–9,15,18), the association of such a genetic system with trichothecene toxin production has been elusive. Genetically characterized isolates of the heterothallic species *Nectria haematococca* (*F. solani*), *Gibberella fujikuroi* (*F. moniliforme*), *G. baccata* (*F. lateritium*), and *G. tricincta* (*F. tricinctum*) do not include any documented trichothecene producers (2,11,12). Conversely, many trichothecene-producing species, such as *F. sporotrichioides* and *F. poae*, produce no known teleomorph (2,11,12). In other species, such as *G. zeae* (*F. graminearum*), all documented toxin-producing strains are homothallic (11).

Heterothallic bipolar teleomorphs of the trichothecene-producing species *G. acuminata* (*F. acuminatum*) and *G. pulicaris* (*F. sambucinum*) have been reported (1,5,6), but toxin production by these heterothallic strains has not been examined. Although *G. pulicaris* was first described more than 100 years ago, little work has been published on the genetics of this species. We examined isolates of *G. pulicaris* (Fries) Sacc. (*F. sambucinum* Fuckel) for the ability to produce trichothecene toxins and to participate in the formation of fertile crosses.

In this paper we report the association of a heterothallic teleomorph with trichothecene toxin production in *G. pulicaris*. We describe conditions for obtaining mature ascospores of *G. pulicaris* in the laboratory and present preliminary random ascospore analyses of the inheritance of toxin production and other traits in this species.

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## MATERIALS AND METHODS

**Source of isolates.** The six isolates used in this study were obtained from the Fusarium Research Center Collection, Pennsylvania State University, and were reisolated from single conidia prior to use. Cultures were grown routinely on V-8 agar medium slants (17) on an alternating 12 hr/25 C light and 12 hr/20 C dark schedule. For long-term storage, strains were maintained on V-8 agar slants at 4 C, as conidial suspensions in 10–15% v/v glycerol at −90 C, or as lyophilized conidial suspensions in the Northern Regional Research Laboratory collection, Peoria, IL.

**Media.** Media used for crosses, with and without 2- to 3-cm pieces of propylene-oxide-sterilized wheat straw, were water agar, potato-dextrose agar (12), potato-carrot agar (9), and V-8 juice agar. An additional medium was water agar with a 2- to 3-cm twig of mulberry (*Morus alba* L.) presterilized by intermittent steaming for 3 days. All crosses were grown for 3–5 wk on 8-ml agar slants in test tubes in an incubator on an alternating 12 hr/25 C light and 12 hr/20 C dark schedule under 20-W General Electric cool white fluorescent tubes. Cultures were then transferred to a 15 C incubator with low-level continuous or intermittent light.

**Crosses.** For initial crosses, slants of a variety of media were coinoculated with mycelia from V-8 medium plates of the two strains to be tested. For subsequent crosses, the recipient strain was grown on mulberry twig agar under the 25 C/20 C conditions for 3–6 wk, then washed with a water suspension of conidia from a V-8 agar slant of the donor strain. These washed cultures were then transferred to 15 C. All crosses were examined weekly for the presence of protoperithecia and mature perithecia with ascospore masses extruded from the ostioles. A cross was scored as infertile if mature perithecia did not form after four or more attempts.

**Ascospore isolation.** To minimize contamination with conidia, the ascospore mass, extruded from the ostiole of the perithecium, was removed with sterile forceps. The spore mass was then suspended in 0.5 ml of sterile water and checked microscopically (Zeiss microscope at ×250) for contamination with conidia. Ascospore suspensions were poured onto a thin layer of water agar in a petri dish. Ascospores were then identified under a Wild stereomicroscope at ×100 magnification and each ascospore was transferred to an individual slant.

**Scoring cultural traits.** Cultural characteristics such as pigmentation were based on 10- to 14-day-old cultures on potato-dextrose agar (12). Protoperithecium formation was evaluated in 3- to 6-wk-old cultures grown on mulberry twig agar. The production of protoperithecia, the female gametangia in *Fusarium* species (16), is used as a criterion of femaleness in this study. Compatibility means that a culture is of the appropriate mating type and sex to cross with another culture. Fertility means the production of mature ascospores in a cross. Viability means the ability of an ascospore to germinate. Genotypes, phenotypes, and mating types are designated according to accepted genetic nomenclature (23).

**Analysis of toxin production.** The ability of the six toxigenic *G. pulicaris* isolates and of the 40 random ascospore progeny to produce trichothecenes in liquid shake culture was examined. R-5389, R-5390, R-5455, and R-6112 are known to synthesize the trichothecene diacetoxyscirpenol (DAS) under other growth conditions; the toxins produced by R-6354 and R-6380 have not been previously identified (11). Inocula were prepared from strains grown on V-8 agar plates for 6 days. Conidia, washed from the surface of the plate with sterile water, were used immediately to inoculate YEPD-5G medium (0.1% yeast extract, 0.1% peptone, and 5.0% glucose) (19) at $1.0 \times 10^5$ conidia per milliliter in an Erlenmeyer flask fitted with a morton closure (Bellco Glass Inc., Vineland, NJ). Cultures were incubated for 7 days at 28 C on a rotary shaker at 200 rpm. Growth was measured by dry weight determination of mycelia collected on preweighed GFA (Whatman) filters. All samples were dried at 80 C for 24 hr before being weighed.

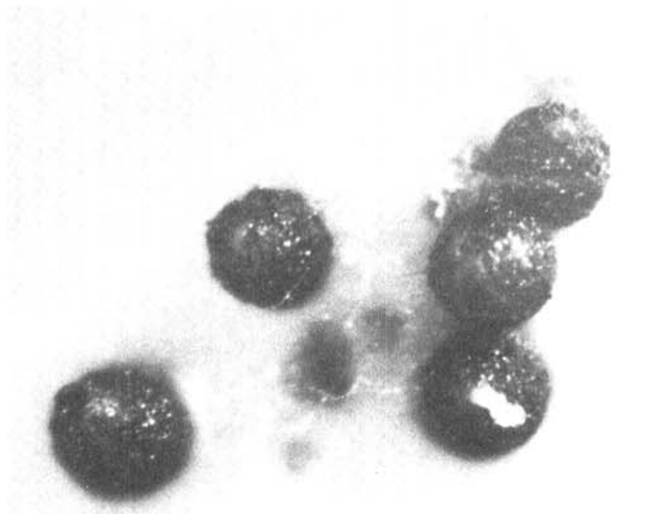

**Fig. 1.** Mature perithecia of *Gibberella pulicaris.* Average perithecium diameter is 400 $\mu$m.

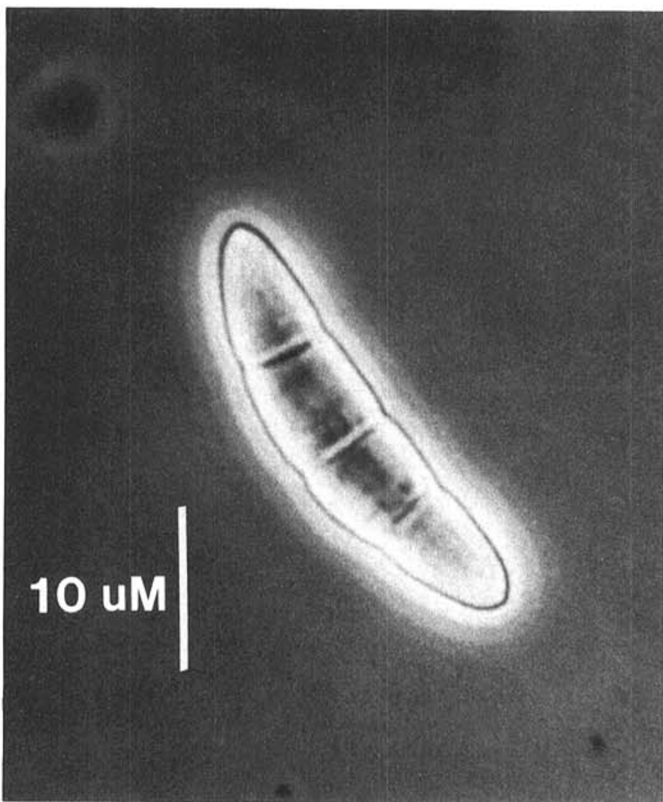


**Fig. 2.** Ascospore of *Gibberella pulicaris.*

Cultures were assayed for DAS, T-2 toxin, and other trichothecene toxins by gas liquid chromatography (F. VanMiddlesworth, *unpublished*). Following extraction of the cultures with two volumes of ethyl acetate, the ethyl acetate layer was passed through a charcoal column (Romer Labs, Inc., Washington, MO), pooled with two subsequent ethyl acetate column washes (10 ml each), and evaporated in vacuo; the residue was resuspended in toluene:acetone:methanol (2:1:1). Aliquots were first evaporated to dryness under nitrogen at 80 C for 1 hr, then reacted with 100 $\mu$l TriSil/TBT (Pierce Co., Rockford, IL) at 80 C for 1 hr and finally brought to 1 ml with hexane. Measurements were made by flame ionization detection on a Spectra-Physics gas chromatograph (Model SP7100). Toxin concentrations were calculated from standard curves generated for each analysis by chromatography of known concentrations of T-2 toxin and DAS standards obtained from Sigma. To assess the limits of variability in the measurement of DAS levels in the liquid shake cultures, DAS production was measured in 25-ml triplicate cultures of the two toxin-producing parents R-6380 and R-5389. The limits of variability for these two strains were $\pm 18\%$ and $\pm 14\%$, respectively (data not shown). These results suggest that the level of variation present in the studies with the $F_1$ progeny cannot be accounted for by experimental variability in the production and analysis of DAS. Results on the ability of the $F_1$ progeny to synthesize other trichothecenes will be reported elsewhere.

**Photography.** Photographs of ascospores suspended in 0.1% gelatin were taken on a Zeiss microscope at ×500 (×40 objective and a ×12.5 eyepiece). Photomicrographs of perithecia were made at a magnification of 62.5 on a Wild stereoscope equipped with a Nikon camera (M-35S). Kodak Panatomic X film was used in both cases.

## RESULTS

**Production of perithecia.** Protoperithecia developed in some strains on V-8 agar, potato-dextrose agar, potato-carrot agar, and on water agar containing mulberry twigs or wheat straw. However, mature perithecia with extruded ascospores were produced only in crosses on mulberry twigs or, rarely, on wheat straw and only after incubation at 15 C. Compatible pairs that had been incubated for up to 3 mo on an alternating 12 hr/25 C light and 12 hr/20 C dark schedule without producing mature ascospores produced them in 2–3 wk when transferred to 15 C. When compatible strains were inoculated simultaneously in the same slant, often few protoperithecia matured, although they were abundant. Mature perithecia were more abundant (often up to 100 per twig) if one strain was grown until it had developed protoperithecia and was then washed with conidia from a second strain.

Mature perithecia developed in groups or singly on the surface of mulberry twigs after incubation at 15 C. Perithecia were deep violet-black and typical of the genus *Gibberella* (1) (Fig. 1). Ascospores were released as a pale-gold mass from the ostiole of mature perithecia. The ascospores were, as described by Booth (1), hyaline, uniform, often curved, and slightly constricted at the three septa (Fig. 2). Eighty to ninety percent of several dozen asci observed in crosses 278 and 287 contained seven or eight ascospores. The high frequency of asci containing eight ascospores and the high percentage of ascospore germination (95% in cross 278 and 76% in cross 287) allowed the use of random ascospores for

determining segregation ratios in the preliminary studies reported here.

**Inheritance of mating type and sex.** All six of the field isolates tested crossed successfully with one or more of the other isolates, and none of them was self-fertile (Fig. 3). Fertile isolates segregated into two compatibility or mating type groups designated Mat-1 and Mat-2 (Table 1). All six isolates were able to function as males in reciprocal crosses with one or more of the other isolates (Fig. 3). Isolates R-5390, R-5455, and R-6380 also produced protoperithecia and are therefore hermaphrodites.

The inheritance of femaleness was studied by examination of the progeny from two of the above crosses (Table 2). All progeny of cross 287 (R-6380 × R-5455), between two hermaphroditic parents, were able to produce protoperithecia. In cross 278, between a male (R-5389) and a hermaphrodite (R-6380), 34 of the progeny produced protoperithecia and 46 did not (Table 2). These data fit a 1:1 segregation ratio indicating that femaleness, as defined by protoperithecium formation, segregated as a single gene, designated *Fmn*.

Inheritance of mating type was investigated by backcross analysis of the progeny of crosses 278 and 287. The first backcross involved 80 progeny of cross 278 (R-6380 × R-5389). All progeny were tested as females against R-5389 and as both males and females against R-6380. If mating type were controlled by alternate alleles at a single gene locus, *MAT*1, such that R-6380 is *MAT*1-1 and R-5389 is *MAT*1-2, and if *MAT*1 and *Fmn* were unlinked, then half of the progeny (those having *MAT*1-2) would be expected to cross with R-6380, the hermaphroditic parent of the opposite mating type. Only one-fourth of the progeny (those with the putative *MAT*1-1, $Fmn^+$ genotype) would be expected to cross with the other parent, R-5389, which does not form protoperithecia and is of the opposite mating type. The other fourth of the progeny (those with the putative *MAT*1-1, $Fmn^-$ genotype) would be infertile with both R-6380 and R-5389 because of mating incompatibility with the former and sexual incompatibility with the latter (Table 3). Twenty-nine of an expected 40 progeny crossed with R-6380. Eleven of an expected 20 progeny crossed with the male parent, R-5389. Because in both cases the observed fertility was less frequent than expected, our interpretation of these data involves the hypothesis that R-5389 carries a gene, $Efx^+$, that results in infertility when it is associated with the $Fmn^+$ allele or an R-6380 specific allele of a gene closely linked to *Fmn* (*Efx* is not linked to *Fmn*). This model predicts that 10 of the progeny would form fertile crosses with R-5389 and that 30 would form fertile crosses with R-6380 (we obtained 11 and 29, respectively). In addition, of those 30 that crossed with R-6380, 10 would be $Fmn^+$ and 20 would be $Fmn^-$ (we obtained 7 and 22, respectively). Thus our data are compatible with this model and are consistent with the hypothesis that mating type is controlled by a single gene, *MAT*1, with two alleles and that it is unlinked to the *Fmn* locus controlling femaleness.

Data from the second backcross are also consistent with 1:1 segregation of mating type, although once again a portion of the progeny displays some infertility. Sixty viable progeny were tested as both males and females against both parents. Fifty percent of the

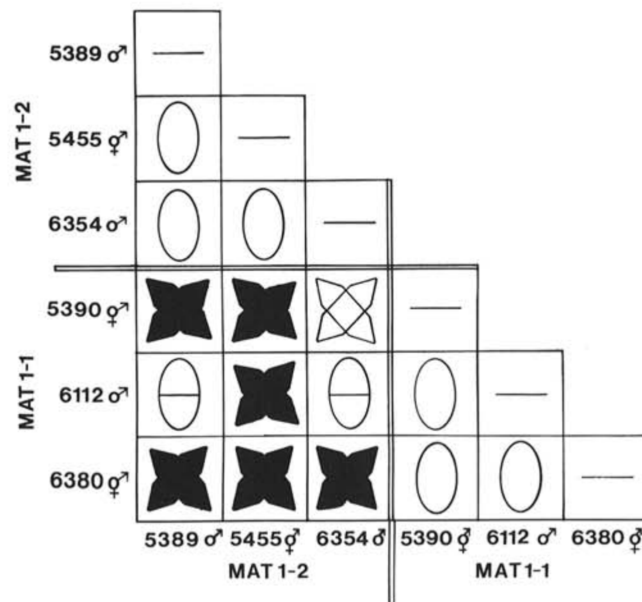


**Fig. 3.** Results of crosses between field isolates of *Gibberella pulicaris*. Isolates that did not produce perithecia when selfed are indicated by dash; crosses infertile as result of mating type incompatibility are indicated by open circle; crosses infertile as result of sexual incompatibility are indicated by barred circle; crosses infertile for unknown reasons are indicated by open star; and fertile crosses are indicated by closed star. For clarity, prefix R is omitted from isolate numbers.

TABLE 1. Characteristics of *Gibberella pulicaris* isolates used in this study

| Isolate | Origin[a] | Host[a] | Mating type | Sex[b] | Predominant colony color[c] | Diacetoxyscirpenol (mg/L)[d] | Identification sensu Booth[a] |
|---|---|---|---|---|---|---|---|
| R-5389 | Iran | Potato | Mat-2 | $Mln^+$ $Fmn^-$ | Yellow | 18 | *Fusarium sulphureum* |
| R-5390 | Iran | Potato | Mat-1 | $Mln^+$ $Fmn^+$ | Yellow | 88 | *F. sulphureum* |
| R-5455 | Minn. USA | Corn | Mat-2 | $Mln^+$ $Fmn^+$ | Red | <1 | *F. sambucinum* |
| R-6112 | Unknown | Unknown | Mat-1 | $Mln^+$ $Fmn^-$ | Yellow | 15 | ... |
| R-6354 | Canada | Corn | Mat-2 | $Mln^+$ $Fmn^-$ | Red | 76 | *F. sambucinum* |
| R-6380 | Germany | Potato | Mat-1 | $Mln^+$ $Fmn^+$ | Yellow | 40 | *F. sulphureum* |

[a]From Marasas et al (11), pp. 139-143.
[b]Femaleness ($Fmn^+$) is indicated by production of protoperithecia.
[c]On potato-dextrose agar.
[d]Based on 150-ml liquid cultures in 300-ml Erlenmeyer flasks after 5 days of incubation; results are from single representative experiment.

TABLE 2. Inheritance of femaleness in random ascospore progeny of *Gibberella pulicaris*

| Cross | Parents | | Number of progeny: Protoperithecial ($Fmn^+$) | Number of progeny: Nonprotoperithecial ($Fmn^-$) | Ascospore germination (%) |
|---|---|---|---|---|---|
| 278 | R-6380 | Protoperithecial Mat-1 $Fmn^+$ | | | |
| | × | | 34[a] | 46[a] | 95 |
| | R-5389 | Nonprotoperithecial Mat-2 $Fmn^-$ | | | |
| 287 | R-6380 | Protoperithecial Mat-1 $Fmn^+$ | | | |
| | × | | 60 | 0 | 76 |
| | R-5455 | Protoperithecial Mat-2 $Fmn^+$ | | | |

[a]$\chi^2 = 0.8$, $P = 0.37$.

progeny crossed with R-5455, indicating that they were Mat-1. Twenty-five percent of the progeny crossed with R-6380 and are therefore Mat-2. Twenty-five percent of the progeny failed to cross with either parent, even after repeated attempts. This pattern of fertility agrees with the hypothesis that R-6380 is *MAT*1-1 and R-5455 is *MAT*1-2 and that they also have different alleles, + and −, respectively, for a gene, *Efy*, that affects fertility. It is suggested that strains with the putative *MAT*1-2, *Efy*$^+$ genotype are infertile in crosses to either R-6380 or R-5455 because of association of the *Efy*$^+$ allele from R-6380 with the *MAT*1-2 allele or a linked allele from R-5455 (Table 4). Twenty-five percent of the progeny from cross 28 are expected to be of this recombinant genotype and hence would account for the 25% infertile progeny observed.

Finally, although all the progeny from cross 287, between two hermaphrodite parents, should function sexually as hermaphrodites, an unexpected pattern of sexual function was observed. Of the 30 fertile Mat-1 progeny, 7 behaved as hermaphrodites in backcrosses with R-5455 whereas 17 functioned as females only and 6 functioned as males only. Similarly, of the 15 fertile Mat-2 progeny, 4 behaved as hermaphrodites in backcrosses with R-6380 whereas 3 functioned as females only and 8 functioned as males only. The basis for this sexual dysfunction is not known.

**Inheritance of red pigmentation.** The six *G. pulicaris* isolates used in this study were morphologically diverse (Table 1). On potato-dextrose agar, four produced only a diffusible sulphur yellow color and two produced an additional nondiffusible deep carmine red. In cross 287, red pigmentation segregated as a single pair of alleles (Table 5).

Furthermore, pigmentation appears to segregate independently of mating type (Table 5). Pigmentation displays a 1:1 segregation pattern with respect to the hypothesized *MAT*1-1 allele; i.e., 50% of the Mat-1 progeny are red. Likewise, nearly equal numbers of Red$^+$ and Red$^-$ fertile progeny are Mat-2. In addition, if the infertile progeny are the result of the hypothesized *MAT*1-2 *Efy*$^+$ genotype, then 1/4 (14 of 60) are *MAT*1-2 Red$^+$, 1/4 (15 of 60) are *MAT*1-2 Red$^-$, 1/4 (16 of 60) are *MAT*1-1 Red$^+$, and 1/4 (15 of 60) are *MAT*1-1 Red$^-$. These results fit a 1:1:1:1 segregation pattern ($\chi^2 = .133$, $P = 0.98$) expected for two unlinked genes in a standard dihybrid cross. Based on the above data, the gene for red pigmentation is not linked to the mating type locus.

**Inheritance of DAS production.** Gas chromatograph analyses of culture extracts revealed that all isolates except R-5455 produce significant levels of DAS in liquid culture (Table 1). Inheritance of DAS production was determined in the F$_1$ progeny from crosses 278 and 287. In both crosses, 20 progeny were selected for analysis. An equal number from each identifiable segregating class was chosen according to mating type and sex or color. Cross 287 was between a DAS producer (R-6380) and a nonproducer (R-5455). Although R-5455 produces a trace amount of DAS, it will be referred to as a nonproducer relative to the other five isolates. Diacetoxyscirpenol production in the 20 progeny from this cross did not yield a distinct 1:1 segregation ratio, but, rather, showed a continuous range of intermediate levels (Fig. 4). This pattern of inheritance is indicative of allelic differences between several genes involved in the genetic control of DAS biosynthesis. The pattern of inheritance of DAS production appeared to be equally complex in the cross between two toxin-producing strains R-6380 and R-5389 (Fig. 5). A transgressive segregation inheritance pattern for DAS production was observed among the 20 ascospore progeny. All produced DAS, but in amounts intermediate to, greater than, or lesser than the parents. Polygenic traits display this pattern of inheritance. These results also suggest that DAS production is controlled by multiple unlinked loci.

## DISCUSSION

The primary goal of this study was the preliminary characterization of a genetic system for the study of trichothecene biosynthesis. The first requirement was to find laboratory conditions for the production of abundant fertile perithecia in *G. pulicaris*. Gordon and others (3,6) have reported the production of fertile perithecia of this species in the laboratory under unspecified conditions. Although Booth (1) observed perithecia in matings on wheat straw or potato-carrot agar at 20–25 C, we and other workers (E. B. Lawrence and P. E. Nelson, *personal communication*) have not observed mature perithecia under these conditions. However, the novel method reported here using mulberry twigs and cold treatment supported the rapid formation of abundant, highly fertile perithecia. This method was based on prior observations on the effects of cold treatment (6) and of woody substrates (1,14) on perithecium formation in *Fusarium*. In general, the six field isolates examined proved to be fertile, although some infertile crosses and crosses with low fertility were observed. Most of the infertile crosses among the field isolates proved to be the result of mating type or sexual incompatibilities. Unexplained infertility occurred in only one cross.

To verify that *G. pulicaris* is amenable to genetic analysis, the inheritance of mating type, femaleness, and pigmentation was investigated. Previously, Gordon (5,6) reported the species to be

TABLE 3. Hypothetical segregation patterns for mating type, female, and *Efx* loci among F$_1$ progeny of cross 278: R-6380 (*MAT*1-1 *Fmn*$^+$ *Efx*$^-$) × R-5389 (*MAT*1-2 *Fmn*$^-$ *Efx*$^+$)

| F$_1$ genotype | No. of progeny expected by independent segregation | No. expected[a] to be fertile in backcross to | | No. predicted[a,b] to be fertile in backcross to | |
|---|---|---|---|---|---|
| | | R-5389 | R-6380 | R-5389 | R-6380 |
| *MAT*1-1 *Fmn*$^+$ *Efx*$^-$ | 10 | 10 | 0 | 10 | 0 |
| *MAT*1-1 *Fmn*$^+$ *Efx*$^+$ | 10 | 10 | 0 | 0 | 0 |
| *MAT*1-1 *Fmn*$^-$ *Efx*$^-$ | 10 | 0 | 0 | 0 | 0 |
| *MAT*1-1 *Fmn*$^-$ *Efx*$^+$ | 10 | 0 | 0 | 0 | 0 |
| *MAT*1-2 *Fmn*$^+$ *Efx*$^-$ | 10 | 0 | 10 | 0 | 10 |
| *MAT*1-2 *Fmn*$^+$ *Efx*$^+$ | 10 | 0 | 10 | 0 | 0 |
| *MAT*1-2 *Fmn*$^-$ *Efx*$^-$ | 10 | 0 | 10 | 0 | 10 |
| *MAT*1-2 *Fmn*$^-$ *Efx*$^+$ | 10 | 0 | 10 | 0 | 10 |
| Total | 80 | 20 | 40 | 10 | 30 |

[a] *MAT*1-1 *Fmn*$^-$ strains are sexually incompatible with R-5389 (*MAT*1-2 *Fmn*$^-$1) because both are female deficient.
[b] Fertility according to our hypothesis, which suggests that *Fmn*$^+$ *Efx*$^+$ strains are infertile with R-6380 and R-5389.

TABLE 4. Hypothetical segregation pattern for mating type and *Efy* loci in cross 287: R-6380 (*MAT*1-1 *Efy*$^+$) × R-5455 (*MAT*1-2 *Efy*$^-$)

| F$_1$ genotype | No. of progeny expected by independent segregation | No. expected to be fertile in backcross to | | No. predicted[a] to be fertile in backcross to | |
|---|---|---|---|---|---|
| | | R-5455 | R-6380 | R-5455 | R-6380 |
| *MAT*1-1 *Efy*$^+$ | 15 | 15 | 0 | 15 | 0 |
| *MAT*1-1 *Efy*$^-$ | 15 | 15 | 0 | 15 | 0 |
| *MAT*1-2 *Efy*$^+$ | 15 | 0 | 15 | 0 | 0 |
| *MAT*1-2 *Efy*$^-$ | 15 | 0 | 15 | 0 | 15 |
| Total | 60 | 30 | 30 | 30 | 15 |

[a] It is hypothesized that strains with putative *MAT*1-2 *Efy*$^+$ genotype are infertile with R-6380 and R-5455.

TABLE 5. Segregation of red pigmentation, mating type, and *Efy* in random ascospore progeny of cross 287: R-6380 (Mat-1 *Efy*$^+$ Red$^-$) × R-5455 (Mat-2 *Efy*$^-$ Red$^+$)

| F$_1$ observed phenotype | | Number of progeny[a] | Putative genotype |
|---|---|---|---|
| Mat-1 | Red$^+$ | 16[b] | *MAT*1-1 *Efy*$^+$ *Red*$^+$<br>*MAT*1-1 *Efy*$^-$ *Red*$^+$ |
| Mat-1 | Red$^-$ | 15[b] | *MAT*1-1 *Efy*$^+$ *Red*$^-$<br>*MAT*1-1 *Efy*$^-$ *Red*$^-$ |
| Mat-2 | Red$^+$ | 6 | *MAT*1-2 *Efy*$^-$ *Red*$^+$ |
| Mat-2 | Red$^-$ | 9 | *MAT*1-2 *Efy*$^-$ *Red*$^-$ |
| Infertile | Red$^+$ | 8 | *MAT*1-2 *Efy*$^+$ *Red*$^+$ |
| Infertile | Red$^-$ | 6 | *MAT*1-2 *Efy*$^+$ *Red*$^-$ |

[a] Thirty of the progeny were Red$^+$ and 30 were Red$^-$. $\chi^2 = 0$, $P = 1.0$.
[b] Distinction cannot be made between *Efy*$^+$ and *Efy*$^-$ genotypes in Mat-1 progeny under these test conditions.

bisexual, hermaphroditic, and self-sterile. El-Ani (3) found that a mutant male-only strain made no protoperithecia and, when crossed with a hermaphroditic strain, produced hermaphroditic and male progeny in the ratio of 1:1. In our study, mating type appeared to segregate as a single pair of alleles (Tables 3 and 4). In addition, cross 278 between hermaphroditic and male parents resulted in a 1:1 ratio of the female sexual type in the progeny (Table 2). In cross 278, the loci for mating type and femaleness appeared to segregate independently (Table 3). These preliminary results suggest that mating type and femaleness as defined by protoperithecium formation in *G. pulicaris*, as in other *Fusarium* teleomorphs (6), can be monogenically controlled and unlinked. In cross 287, red pigmentation was inherited as a single gene and appeared to be unlinked to mating type. Inheritance of predominant colony color is also controlled by a single gene in *G. fujikuroi* (15) and *G. baccata* (10).

The major goal of the present study involved a preliminary investigation of the pattern of inheritance of DAS production. Our results suggest that multiple, unlinked loci are involved in the genetic control of DAS biosynthesis (Figs. 4 and 5). These findings are consistent with the fact that DAS biosynthesis involves a complex series of reactions. The data are also consistent with results obtained in the genetic analysis of a variety of toxins produced in other fungi via complex biosynthetic pathways. Studies with field isolates of *Cochliobolus heterostrophus* and other fungi have shown that production of other types of toxins can be controlled by a lesion in only one of the several required genes (13,22) or by the coexistence of lesions of allelic differences in several of the genes (21).

Although taxonomy is not the main focus of this study, the existence of a heterothallic teleomorph in *G. pulicaris* has direct bearing on controversial questions in taxonomy. Historically, speciation in *Fusarium* has been based on anamorph morphological characters, such as the shape of macroconidia and the branching of conidiophores. Wollenweber and Reinking (20) recognized five forms and two varieties within the species *sambucinum*, treated *F. sulphureum* Schlecht. as a taxonomic synonym of *F. sambucinum* f. 6 Wollenweber, and considered *G. cyanogena* to be synonym of *G. pulicaris*. However, subsequent investigators (1,4) have considered the variety *sulphureum* to be a distinct species, in part because of its lack of the carmine red pigmentation typical of *sambucinum*. Gordon (5) and others (1,4) have considered *G. cyanogena* to be the teleomorph of *F. sulphureum* sensu Booth. In contrast, Nelson and co-workers (12) have recently included *F. sulphureum* in a broader species concept of *F. sambucinum* based mainly on macroconidial shape. Gordon (5) was unable to obtain perithecia by mating isolates of *F. sambucinum* with ascospore cultures of *G. cyanogena*. He considered this genetic incompatibility to be evidence that these two species are distinct. In contrast, we have repeatedly obtained fertile perithecia in crosses between red pigmented *F. sambucinum* sensu Booth isolates (e.g., R-5455) and yellow pigmented *F. sulphureum* sensu Booth isolates (e.g., R-6380). Differences in experimental procedure and the particular isolates tested may

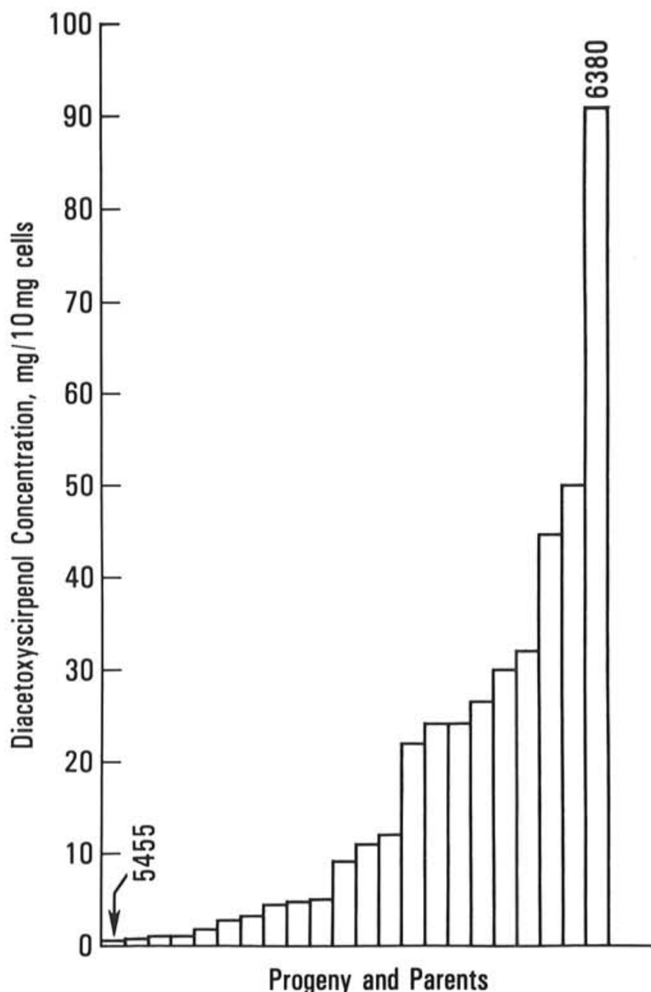


**Fig. 4.** Diacetoxyscirpenol (DAS) production by 20 random ascospore progeny of cross 278. The 25-ml cultures in 50-ml Erlenmeyer flasks were incubated for 7 days and analyzed for DAS as described in Materials and Methods. Parents are R-6380 (DAS producer) and R-5455 (nonproducer). Results shown are from one simultaneous test of all progeny and parents.

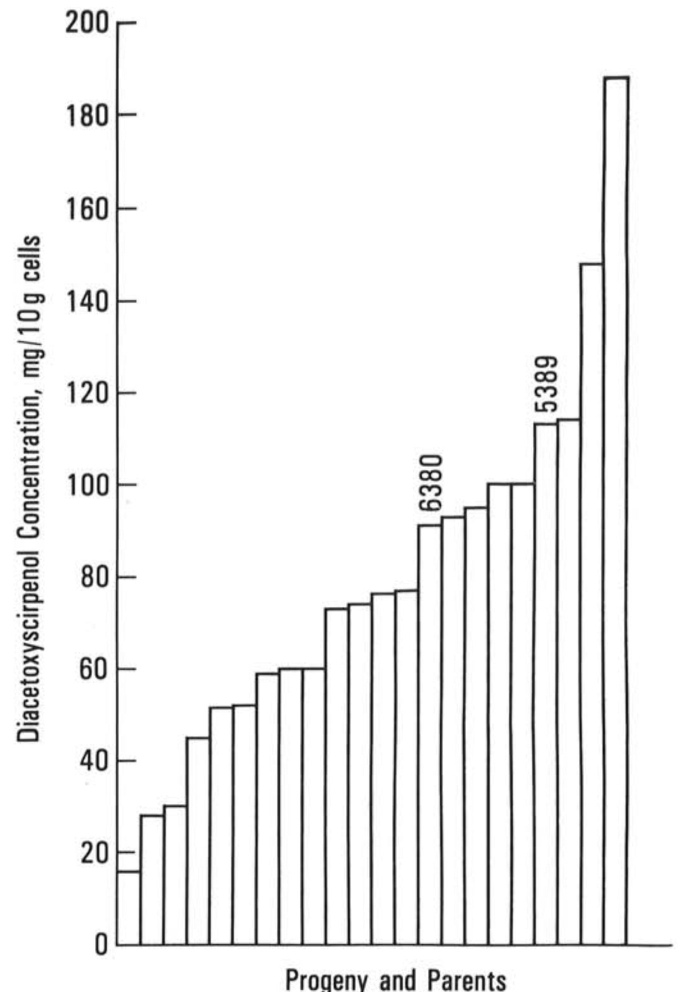


**Fig. 5.** Diacetoxyscirpenol (DAS) production by 20 random ascospore progeny of cross 287. The 25-ml cultures in 50-ml Erlenmeyer flasks were incubated for 7 days and analyzed for DAS as described in Materials and Methods. Parents are R-6380 and R-5389 (both DAS producers). Results shown are from one simultaneous test of all progeny and parents.

account for these discrepancies. We conclude that sexual compatibility supports a wider species concept of *F. sambucinum* as presented by Nelson and co-workers (12).

The results reported here indicate that *G. pulicaris* can be used for genetic investigations of trichothecene toxin biosynthesis. Our intent is to find monogenic lesions in the trichothecene toxin biosynthetic pathway by attempting the appropriate crosses. The relationship between trichothecene production and plant pathogenicity should also be amenable to genetic analysis by using crosses between toxin-producing and nonproducing strains of *G. pulicaris.*

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