Tilletia aegopogonis, a Homo-Heterothallic Bunt Fungus

Rubén Durán

Professor, Department of Plant Pathology, Washington State University, Pullman 99164.
This research was supported in part by National Science Foundation Grant DEB 75-23325 and the Washington State University College of Agriculture Research Center; it was designated Project 0140 and approved for publication as Scientific Paper 4966.
The author is indebted to Peter M. Gray for technical assistance.
Accepted for publication 4 December 1979.

ABSTRACT

DURÁN, R. 1980. *Tilletia aegopogonis*, a homo-heterothallic bunt fungus. Phytopathology 70:528-533.

When primary basidiosporic lines of *T. aegopogonis* were used to inoculate seedlings of *Aegopogon tenellus*, some unpaired lines of the fungus sporulated in the ovaries of the host. Such lines were self-fertile, heterokaryotic for mating type, and homothallic. Primary basidiospores from which they were derived invariably were multinucleate and did not fuse, but when they germinated some formed infection hyphae with conjugately associated nuclei, either in situ or after abstricting from promycelia. Such heterokaryotic lines readily dissociated in shake cultures and formed secondary mononucleate basidiospores of two mating types. Other unpaired primary lines, however, did not form infection hyphae or sporulate in the ovaries, and, hence, were self-sterile, homokaryotic for mating type, and were shown to be heterothallic by pathogenicity and incompatibility tests. Homokaryotic lines also dissociated in shake cultures and formed secondary mononucleate basidiospores of one or the other mating type, but not of both. When secondary mononucleate lines of either source or primary homokaryotic lines of different mating types were paired, they formed infection hyphae and sporulated in the ovaries. Homothallism was attributed to the presence of nuclei of both mating types in some primary basidiospores, heterothallism to the presence of only one type in others. Nuclei in promycelia outnumbered basidiospores produced by about 5:1. The multinucleate condition of primary basidiospores was thus a normal and presumably inevitable consequence. Basidia averaged 32.00 nuclei, 6.54 basidiospores, and the latter 4.89 nuclei. Migration of mating type nuclei to the basidiospores appeared to be nonrandom, since homokaryotic basidiospores were isolated more frequently than could be accounted for by Chi-square tests.

The progression of haplo-, dikaryo-, and diplophases in basidiomycetes has long implied changes in ploidy during the life cycles. However, these changes often have only been inferred because they frequently are difficult to demonstrate cytologically. In the smut fungi, for example, categorical evidence of karyogamy and meiosis (chromosome counts) has yet to be demonstrated with convincing photomicrographs. Yet there is little doubt that changes in ploidy occur during most smut fungus life cycles (7,8). Thus, the diplophase results when conjugately associated nuclei of parasitic mycelia fuse during sporogenesis or teliospore maturation; meiosis occurs when diploid teliospores germinate; basidiospores initiate the haplophase; and the parasitic dikaryophase forms when haploid basidiospores or somatic mycelia fuse either in or out of host tissue.

In the smut fungi, the inherent difficulties encountered in demonstrating chromosomes have made it necessary to infer changes in ploidy mostly by combined studies of nuclear behavior in basidia and pathogenicity of monosporidial lines (8). This approach has served to relate nuclear behavior to heterothallism and pathogenicity in many species of smut fungi (7,8) including the *Tilletia* spp. that attack wheat. For *Tilletia*, the concept of nuclear behavior in the basidium as it relates to heterothallism and pathogenicity largely has stemmed from studies of *T. caries*, *T. foetida*, and *T. controversa*. Consequently, there has been a tendency to portray basidial ontogeny and the attendant nuclear cycles of these species as typical of *Tilletia* as a whole (11). This stereotype has persisted, even though 28 yr ago Holton (10) showed the nuclear cycle of *T. elymi*, which is parasitic on *Elymus* spp., to be fundamentally different from the nuclear cycles of the *Tilletia* spp. on wheat. The significance of Holton's report was in calling attention to the multinucleate basidiospores of the *Elymus* bunt fungus which did not fuse, unlike those of the wheat bunt fungi which have a single nucleus and invariably fuse.

Shortly after Holton's report was published, Siang (14) reported similar observations in *T. cerebrina*, which is parasitic on *Deschampsia* spp. and other grasses. The multinucleate basidiospores, both of *T. cerebrina* and *T. elymi*, and the lack of basidiosporic fusions, prompted Holton (10) and Siang (14) to infer that both species were homothallic. However, neither demonstrated that unpaired monosporidial lines could complete the life cycle, and, hence, whether these species are homothallic remains an unanswered question. Nevertheless, both papers received serious attention because each implied apparent exceptions to heterothallism in the smut fungi (8).

In 1970, I described *Tilletia aegopogonis* (4) as an ovariicolous bunt fungus (Fig. 1), and, later, germination of teliospores (5) collected on *Aegopogon tenellus* in Creel, Chih., Méx. (altitude 2,300 m). Like those of T. elymi (10) and *T. cerebrina* (14), its primary basidiospores were shown in preliminary studies to be multinucleate (Fig. 2). They also formed in small numbers (commonly five to seven) and did not fuse. Moreover, because basidiospores multiplied rapidly and dissociated in shake culture, and the host is a short-lived annual, *T. aegopogonis* appeared to be an excellent model for a combined study of cytology, incompatibility, and pathogenicity.

The following is an account of basidial ontogeny and nuclear behavior of *T. aegopogonis* and their relation to pathogenicity of primary and secondary monosporidial lines. In planning the experiments and interpreting their results, it was assumed that teliospores were diploid, that meiosis occurred when the teliospores germinated, that nuclei in promycelia and basidiospores were haploid during basidial development, and that karyogamy occurred in the host prior to teliospore formation.

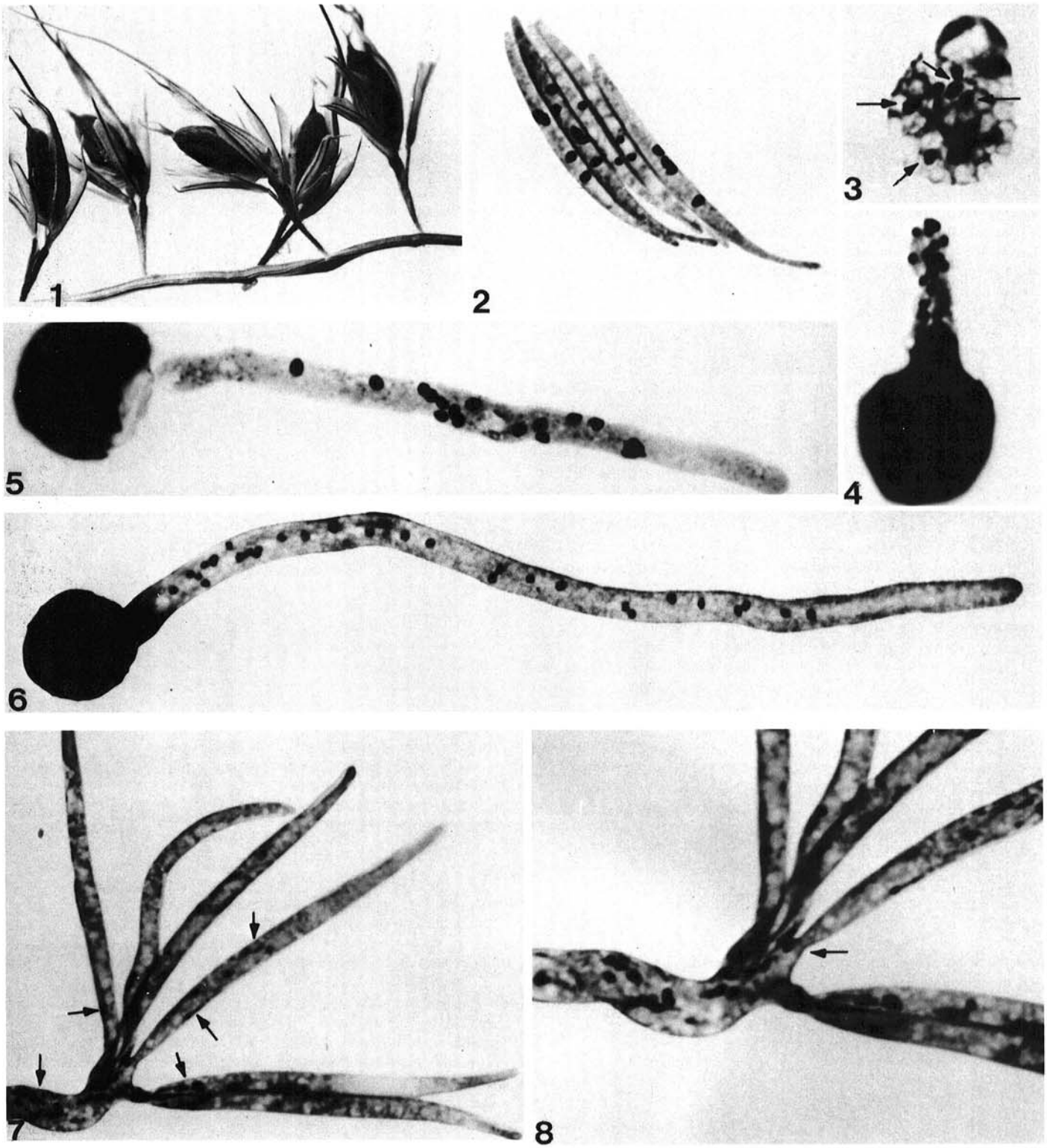
MATERIALS AND METHODS

Cytological studies. Basidia in different developmental stages, basidiospores of primary and secondary lines from shake cultures, and infection hyphae were killed and fixed. The nuclear condition was shown in the following manner. Blocks of 2% water agar (WA) on which teliospores germinated, or on which propagules of the fungus from shake cultures were placed, were inverted onto microscope slides, flooded with a 1:40 mixture of Haupt's adhesive and 4% formalin (2) and placed for 24–48 hr on a slide warmer adjusted to 40–45 C. Thereafter, the dried preparations were, i) hydrated for 5–8 min in water maintained at 60 C, ii) immersed in 60 C 5 N HCl until the hydrated agar dissolved, iii) rinsed in four

0031-949X/80/06052806/$03.00/0
©1980 The American Phytopathological Society

changes of deionized water, iv) rinsed in four changes of phosphate buffer, pH 6.5, v) stained 15 min in one part Giemsa stock solution (15) and 25 parts phosphate buffer and, vi) rinsed in phosphate buffer and dipped in a dilute solution of Tween-20. An acetone:xylene series was used to differentiate nuclei and to dehydrate the preparations, followed by mounting in synthetic resin.

Isolation and culture of monosporidial lines. Teliospores of the fungus were streaked on 2% WA and incubated at 23 C. After they germinated, single primary basidiospores were removed from promycelia with a Chambers micromanipulator and isolated to 1



Figs. 1–8. *Tilletia aegopogonis.* **1,** Bunt sori in the ovaries of a raceme of *Aegopogon tenellus* (× 15). **2,** Primary basidiospores with 2–5 nuclei. Note the absence of fusions (× 880). (3–4) Squashes of germinating teliospores in 13–16 nucleate stages. **3,** Discreet nuclei (arrows) and others superimposed near the bottom of the teliospore (× 1,150). **4,** Nuclei forcibly extruded from the teliospore (× 1,260). **5,** Promycelium with 16 nuclei which have migrated from the teliospore (× 1,000). **6,** Promycelium with 32 nuclei, some juxtaposed in pairs, suggestive of synchronous divisions (× 860). (7–8) Immature basidium in early stages of basidiospore formation. A continuum of nuclei extends from the promycelium into the basidiospores which have not yet formed basal septa. **7,** Nuclei (arrows) much condensed or tenuous, some still in the promycelium (× 1,200). **8,** The same basidium except enlarged. Nuclei are on both sides of the open isthmus (arrow) which connects the promycelium and basidiospore (× 1,800). (All nuclei were stained with Giemsa).

cm^2 blocks of potato sucrose agar (PSA). Basidiospores which subsequently germinated were transferred to PSA slants for permanent growth. Stock cultures of primary lines consisted of pedigreed sets of basidiospores isolated from single basidia and others isolated from randomly selected basidia.

Stock cultures of secondary mononucleate lines were established following nuclear dissociation of primary lines in shake cultures. Single basidiospores (samples of which were shown with Giemsa staining to be mononucleate) were isolated from these and used to start shake cultures of secondary lines. To enhance the likelihood of establishing stock cultures of purely mononucleate lines, this procedure was repeated two or three times with all mononucleate lines brought into shake culture. This technique provided a means of working with mononucleate haploid lines derived from single secondary basidiospores.

To provide adequate quantities of basidiospores for the experiments, all monosporidial lines were subcultured in shake-culture flasks containing 50.0 ml of potato-sucrose broth (made with 0.5 g of peptone and 20 g of sucrose per liter of solution). The flasks were inoculated with the fungus from the stock cultures and then placed on a rotary shaker. During 7–10 days of continuous shaking at 24–28 C, secondary mononucleate basidiospores developed in profusion. To harvest them, basidiospores were pelleted by centrifugation, rinsed twice, and suspended in sterile distilled water.

Inoculations. Seeds of *A. tenellus* were surface sterilized with 2% sodium hypochlorite and subsequently germinated between moist filter papers. Seedlings in the coleoptile stage (1 day old) were inoculated by submersion in paired and unpaired suspensions of basidiospores and then placed under partial vacuum for 15–20 min. Seedlings were inoculated with both primary and secondary basidiosporic lines. Inoculated seedlings were incubated at room temperature for 6 days and then grown to maturity in a greenhouse maintained at 16–25 C. To induce heading, inoculated and uninoculated check plants were covered during late afternoons with black plastic sheets starting 30 days after transplanting to provide a photoperiod of 10 hr of light and 14 hr of darkness.

Incompatibility. Secondary basidiospores, formed by primary basidiosporic lines in shake cultures, were used to study incompatibility. All pairings were made on PSA and incubated at 23 C. Teliospores from artificially bunted plants (lines 1 × 2, Table 1) provided one source of lines, and teliospores from a field collection of the fungus provided the other. In the first experiment, secondary basidiospores of 57 primary lines were paired in groups of five in all possible combinations; 36 were unpaired; and 26 were paired with two "tester lines." In the second experiment, 36 lines were paired with "tester lines"; and two pedigreed sets of basidiospores were paired in all combinations.

Sterile 5-mm diameter wire loops, which delivered about 1,000 basidiospores when filled, were used to pair the basidiosporic suspensions on agar. All pairings consisted of secondary basidiospores which originated from shake cultures of specific primary lines. The presence of infection hyphae along the margins of the developing colonies, which formed after 5–7 days at 23 C, served to identify compatible pairings. Portions of the margins of some compatible colonies, where decumbent infection hyphae characteristically formed, were stained to show the nuclear condition. Also, some lines shown by the tests to be compatible subsequently were paired and tested for pathogenicity. The same procedures were followed with some unpaired lines which formed infection hyphae.

TABLE 1. Evidence of homothallism among pedigreed lines of *Tilletia aegopogonis* based on sporulation of paired and unpaired lines in the ovaries of *Aegopogon tenellus*

Monosporidial[a] lines	1	2	3	4	5
1	S[b]	S	S	S	S
2		N[c]	S	S	S
3			S	S	S
4				S	S
5					S

[a] Complete set of primary lines from one basidium.
[b] S = sporulation in ovaries. Bunt averaged 7.83% following inoculation of 906 seedlings with self-fertile lines. Inoculations with all lines combined yielded 20.1% bunted plants (22/109). None of 27 uninoculated check seedlings were bunted at maturity. (Combined data from two experiments).
[c] N = self-sterile line. None of 146 inoculated seedlings were bunted.

RESULTS

Cytology. Teliospores that had barely begun to form promycelia showed 13–16 nuclei. In this stage, exact counts were difficult because nuclei mostly were superimposed on each other in the teliospores (Figs. 3,4). More elongated promycelia clearly showed 16 nuclei (Fig. 5) and those fully extended mostly showed about 32 (Fig. 6). Apparently, two postmeiotic divisions in the germinating teliospores resulted in migration of 16 nuclei which assumed a more or less linear position in the elongating promycelia. After another mitotic division (probably synchronous), the 32 nuclei migrated to the developing primary basidiospores. In immature basidia, the nuclei formed a continuum which extended from promycelia into the immature basidiospores before the latter formed basal septa (Figs. 7,8). Thus, basidiospores were distinctly multinucleate before they matured and abstricted from the promycelia or formed secondary basidiospores.

Counts of basidiospores and nuclei from 50 randomly selected basidia showed that basidia averaged about 32 nuclei (1,600/50) and 6.54 basidiospores (327/50). Primary basidiospores averaged 4.89 nuclei (1,600/327). (These data are of theoretical interest in explaining incompatibility genetics of the fungus [see Discussion]).

On WA, newly formed primary basidiospores frequently germinated directly and formed infection hyphae with conjugately associated nuclei, either before or after they abstricted from promycelia (Fig. 9). Secondary basidiospores produced in shake culture by some primary lines likewise formed infection hyphae when subcultured on PSA. Apparently, secondary basidiospores of different mating type, which formed when primary lines dissociated in shake cultures, simply reassociated on PSA. After 5–7 days, the infection hyphae were macroscopically visible and showed conjugately associated nuclei (Figs. 10,11). Some primary basidiospores, however, did not form infection hyphae, nor did their respective secondary basidiospores which formed in shake culture, presumably, because all were of one mating type (Fig. 12). On WA, primary basidiospores also germinated indirectly, forming secondary falcate ballistospores typical of *Tilletia* spp. (Fig. 13). Initially most had several nuclei, but because they germinated by budding or repetition, ultimately, only mononucleate ballistospores formed.

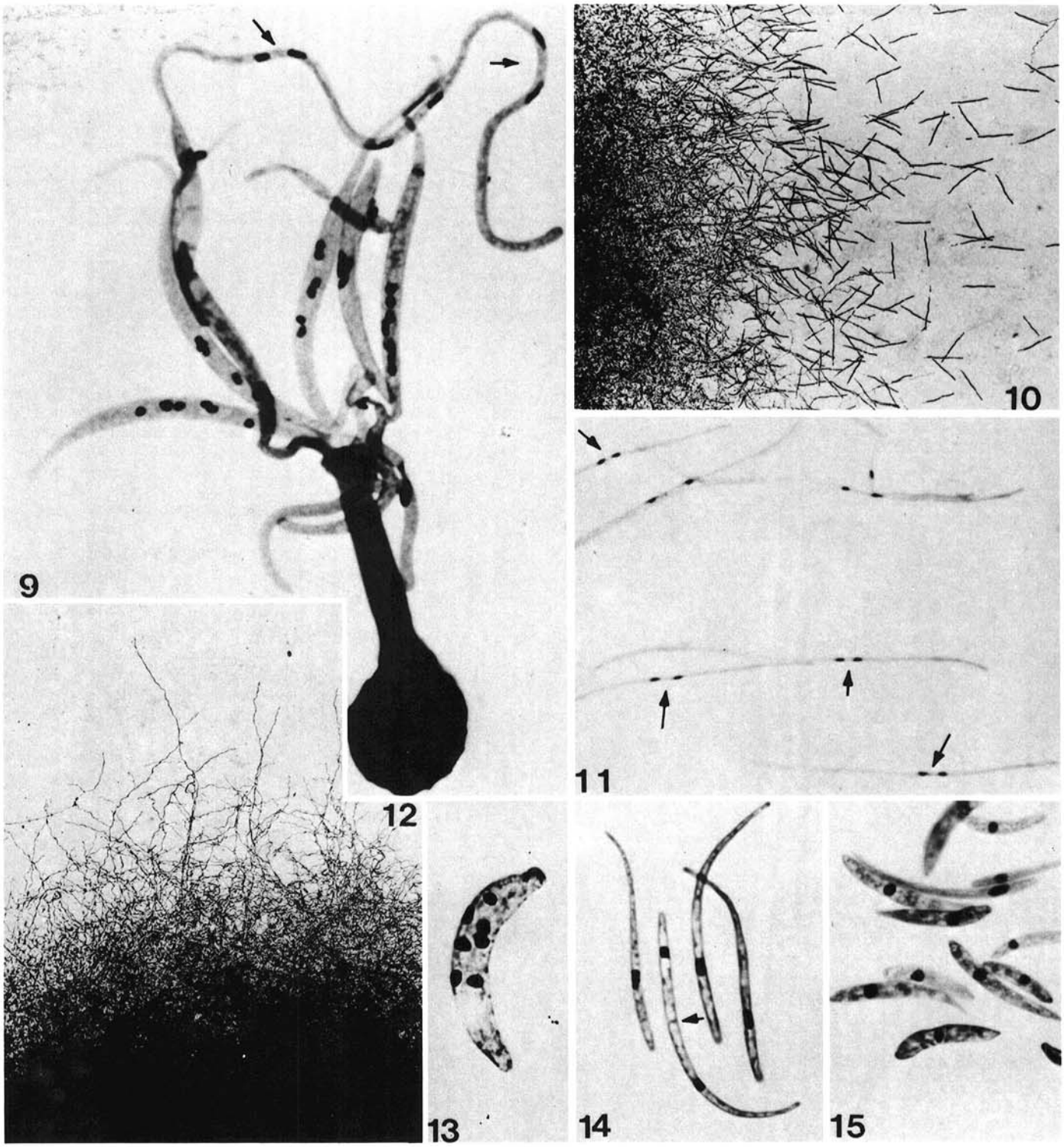
In shake culture, all primary lines budded rapidly, invariably dissociated, and always formed secondary basidiospores in profusion. Mostly, secondary basidiospores which formed in shake cultures were mononucleate and retained the elongated configuration of primary basidiospores. Occasionally, some showed a central septum with one nucleus in each cell. Falcate basidiospores, mostly with single nuclei, also formed in shake cultures (Figs. 14,15).

Pathogenicity. In one experiment, four of five pedigreed lines, which had been isolated from teliospores of spontaneously bunted plants, sporulated in the ovaries, whether used singly or in pairs to inoculate seedlings; these lines were self-fertile and homothallic (Table 1). Line 2, however, was consistently nonpathogenic, despite repeated inoculations. Nonpathogenicity was not attributed to attenuation in culture, since line 1 yielded 10/110 additional bunted plants after maintaining it on PSA for 6 mo.

Like line 2, some unpaired primary lines tested in other experiments also failed to sporulate in the ovaries, despite repeated inoculations. But certain pairs of these formed infection hyphae on PSA and also sporulated in the ovaries as well (Table 2). Thus, although they originated from multinucleate basidiospores, some unpaired primary lines consistently were self-sterile and obligately heterothallic.

Secondary mononucleate lines used as inocula provided additional evidence of obligate heterothallism; some paired lines formed infection hyphae and sporulated in the ovaries, whereas others did not (Table 3). The results also indicated the homo-heterothallic nature of the fungus, since mononucleate lines of two mating types were isolated from a primary line previously shown to be self-fertile.

Incompatibility. Unpaired secondary basidiospores from shake cultures of most primary lines formed masses of infection hyphae on PSA, which indicated that most primary basidiospores were



Figs. 9–15. *Tilletia aegopogonis.* **9,** Mature basidium. One basidiospore has germinated directly and formed an infection hypha with conjugately associated nuclei (arrows) (× 1,050). **10,** Portion of the margin of a colony formed by a self-fertile heterokaryotic line. The zig-zag infection hyphae shown also form when secondary basidiospores derived from homokaryotic lines of different mating types are paired on potato sucrose agar (× 80). **11,** Conjugately associated nuclei of infection hyphae (arrows) (× 660). **12,** Portion of the margin of a self-sterile homokaryotic line on potato sucrose agar (× 80). **13,** A typical secondary falcate ballistospore commonly formed by primary basidiospores when they germinate indirectly on water agar. In germinating by repetition, or budding, these dissociate and form mononucleate basidiospores on agar or shake culture media (× 1,000). (14–15) Secondary basidiospores formed in shake cultures. **14,** Note the mononucleate condition and elongated basidiosporic configurations. Occasionally, basidiospores have two nuclei separated by a central septum (arrow) (× 1,000). **15,** Falcate mononucleate basidiospores, (× 1,000). (All nuclei were stained with Giemsa).

TABLE 2. Evidence of heterothallism among primary monosporidial lines of *Tilletia aegopogonis* based on obligatory pairing of secondary basidiospores to form infection hyphae and sporulate in the ovaries of *Aegopogon tenellus*

	Primary lines[a]										
	27	28	31	32	33	34	37	38	67	68	69
Mating types[b]	+	−	−	+	+	+	−	+	+	+	±
+ 27	N[c]	S[d]	S	N	N	N	S	N	N	N	S
− 28		N	N	S	S	S	N	S	S	S	S
− 31			N	S	S	S	N	S	S	S	S
+ 32				N	N	N	S	N	N	N	S
+ 33					N	N	S	N	N	N	S
+ 34						N	S	N	N	N	S
− 37							N	S	S	S	S
+ 38								N	N	N	S
+ 67									N	N	S
+ 68										N	S
± 69											S

[a] Established from among 58 basidiosporic lines randomly isolated from a self-fertile primary line. At the time they were tested, all lines had dissociated in shake culture and consisted of secondary mononucleate basidiospores.
[b] Arbitrarily assigned after pairing lines on PSA in all combinations.
[c] N = self-sterile lines and incompatible combinations; no infection hyphae formed and no sporulation was observed in the ovaries.
[d] S = infection hyphae formed and sporulation in ovaries. Bunt averaged 4.5%, based on 4,904 seedlings inoculated with 10 self-sterile but cross fertile lines and line 69 which was self-fertile. Of 5,790 seedlings inoculated with incompatible lines, none was bunted, nor were 150 uninoculated checks. All lines combined yielded 1.8% bunted plants (3/169). The data are from two experiments.

TABLE 3. Evidence that primary lines of *Tilletia aegopogonis* dissociate and form basidiospores of two mating types based on obligatory pairing to form infection hyphae and sporulate in the ovaries of *Aegopogon tenellus*

	Secondary lines[a]				
	2	3	5	6	7
Mating types[b]	+	−	+	−	+
+ 2	N[c]	S[d]	N	S	N
− 3		N	S	N	S
+ 5			N	S	N
− 6				N	S
+ 7					N

[a] Mononucleate lines isolated from a self-fertile primary line which had dissociated in shake culture.
[b] Arbitrarily assigned after pairing lines on PSA in all combinations.
[c] N = self-sterile lines and incompatible combinations; no infection hyphae formed or sporulation in the ovaries.
[d] S = infection hyphae formed and sporulation in the ovaries.

heterokaryotic. Moreover, infection hyphae showed conjugately associated nuclei. Secondary basidiospores of some lines, however, did not form infection hyphae. However, lines of opposite mating type, when paired, also formed infection hyphae with conjugately associated nuclei which indicated that some primary lines were homokaryotic. (cf. Figs. 10–12).

The differential ability of primary lines to form infection hyphae generally proved to be a reliable basis for distinguishing between homo- and heterokaryotic lines. For example, when 11 primary lines of both types were paired in all possible combinations, homokaryotic lines showed a plus (+) and minus (−) relationship, whereas the heterokaryotic line formed infection hyphae, whether paired or unpaired (Table 2). Moreover, compatible pairings of homokaryotic lines and unpaired heterokaryotic lines subsequently sporulated in the ovaries, thus confirming results of the incompatibility tests.

Incompatibility of primary lines randomly isolated from teliospores of artificially bunted plants gave the following results: 57 lines, in groups of five, paired in all possible combinations yielded 42 heterokaryotic and 15 homokaryotic lines; 36 unpaired lines yielded 32 heterokaryotic and four homokaryotic lines; and 26 lines paired with two tester lines yielded seven heterokaryotic and 19 homokaryotic lines. Primary lines randomly isolated from teliospores of spontaneously bunted plants, also paired with the tester lines, yielded one heterokaryotic and 19 homokaryotic lines; for reasons that could not be determined, 16 lines did not react sufficiently clearly with either tester line to interpret results of the pairings. Finally, one pedigreed set of basidiospores yielded one homokaryotic and eight heterokaryotic lines and another two homokaryotic and six heterokaryotic lines.

DISCUSSION

Since the nuclear condition of basidiospores was shown to be typically multinucleate, it is not surprising that some were solopathogenic and self-fertile; therefore, it was concluded that some basidiosporic lines were homothallic. This conclusion is consistent with Ainsworth's definition (1) which states that homothallism is . . . "the condition in which sexual reproduction can occur without the interaction of two different thalli." But since some primary basidiospores were self-sterile but cross fertile and required pairing to complete the life cycle, the fungus also was shown to be heterothallic.

Obviously, the multinucleate basidiospores are an intrinsic character of the fungus, and should not be likened to the secondary homothallic lines of *Ustiligo maydis* which occasionally form when a plus (+) and a minus (−) nucleus adventitiously find their way into single basidiospores (3). Neither should homothallism, as described here, be likened to the single solopathogenic line of race T-16 (*T. caries*) produced by inbreeding it to establish pathogenic homozygosity (12), or to solopathogens resulting from interspecific crosses of some *Sphacelotheca* spp. (9). Common bunt fungi like *T. caries,* on the other hand, are widely held to be heterothallic because primary basidiospores contain single nuclei of plus (+) or minus (−) mating type and fusions are obligatory. Clearly, *T. aegopogonis* does not fit into these categories.

The reasons for concluding that *T. aegopogonis* is both homo- and heterothallic are compelling. For example, at least some primary lines of the fungus were self-fertile, but when these dissociated in shake cultures, secondary mononucleate basidiospores of two mating types were routinely isolated. Thus, the evidence is strong, albeit circumstantial, that some primary basidiospores contained haploid nuclei of both mating types, and, hence, were heterokaryotic. Other primary lines, however, were homokaryotic for one of the two mating types detected and required pairing to form infection hyphae and complete the life cycle.

Whether equal numbers of nuclei of both mating types migrated randomly from promycelia to basidiospores could not be definitely determined. As previously mentioned, basidia averaged about 32.00 nuclei and 6.54 basidiospores; the latter, in turn, averaged

4.89 nuclei. In applying chi-square tests to ratios of homokaryotic to heterokaryotic lines isolated, it was assumed that basidia contained equal numbers of nuclei of both mating types. Thus, according to the binomial expansion theory, if n = 4 (ie, if basidiospores averaged four nuclei of either type in all possible ratios), the probability of isolating homokaryotic basidiospores of either mating type would be 1/8. Since the average number of nuclei per basidiospore was about 4.89, the probabilities of isolating homokaryotic basidiospores was 1/16. According to chi-square values (assuming n = 5), more homokaryotic basidiospores were isolated in some experiments than could be accounted for by chance alone. Within the limits of sample size, therefore, it appeared that migration of the nuclei and subsequent ingress to the basidiospores was nonrandom. If more nuclei of one mating type than of the other were present in the basidia, the probabilities of isolating homokaryotic basidiospores of either mating type would be expected to increase in proportion to the skewness, but there was no evidence to substantiate this during the study.

Obviously, the low percentages of bunted plants which followed the inoculations indicate suboptimal inoculation and/or incubation procedures, but do not detract from the fact that, in some cases, infection resulted from monosporidial lines in about the same magnitude as when paired. The low bunt percentages following the inoculations may have resulted from culturing the fungus in a rich medium without starving it sufficiently prior to the inoculations. In shake culture media, basidiospores multiplied rapidly without producing dikaryotic elements. Perhaps basidiospores engorged with reserve products failed to produce sufficient infection hyphae for infection. This suggestion for explaining the low percentages of bunted plants following the inoculations generally is consistent with studies of some smut fungi in which infection hyphae are produced in optimum numbers in compatible lines are paired on non-nutrient media (7). Yet, secondary basidiospores taken from shake cultures of heterokaryotic lines produced abundant infection hyphae on PSA. Despite varying the inoculation procedures and incubation temperatures, low levels of infection persisted. Even when seeds were planted in soil (both of which were artificially and heavily infested with teliospores), only 43/3,984 plants were bunted at maturity.

I know of no other bunt fungus with multinucleate basidiospores which singly can complete the entire life cycle, although this capacity was implied by the researches of Holton (10) and Siang (14). The unequivocal capacity of some unpaired primary lines to complete the life cycle apparently is due to migration of nuclei of both mating types into the basidiospores following meiosis, thus precluding the necessity for fusions. The failure of other lines to produce a dikaryophase unless paired with other lines of different mating type, but not otherwise, is consistent with this explanation. Although homokaryotic primary basidiospores of different mating type logically would be expected to fuse, fusions between them were not seen. However, when secondary basidiospores from homokaryotic shake cultures of different mating type were paired on PSA, fusions were routinely observed.

Considering the multinucleate nature of the primary basidiospores, the lack of fusions between them, and the ability of some singly to produce the dikaryophase, it is not surprising that some were shown to be solopathogenic. In fact, the production of infection hyphae by single basidiospores during early phases of the study suggested solopathogenic potential.

It seems reasonable to assume that, had Holton (10) or Siang (14) attempted to prove that *T. elymi* or *T. cerebrina* were homothallic by demonstrating pathogenicity of single lines, they might have succeeded. In the future, these and other *Tilletia* spp. also may be proven to be homothallic by incompatibility and pathogenicity tests. In recent years, I have collected several *Tilletia* spp. (some of them as yet undescribed) with multinucleate basidiospores which also do not fuse. For example, nuclear behavior in the basidium of *Tilletia lycuroides,* which I recently described (6), is the same as that of *T. aegopogonis.* Pathogenicity tests currently are underway to determine if the multinucleate basidiospores of this species also are solopathogenic.

In axenic culture, the nuclear cycle of *T. aegopogonis* is similar to those of other smut fungi. Multinucleate phases dissociated in culture, the monokaryons reassociated and formed more dikaryons which likewise dissociated, and so on. Moreover, incompatibility was bipolar and biallelic when monokaryons were paired. Furthermore, in all cases tested, pairings shown to be compatible were subsequently shown to be pathogenic as well.

The invariable formation of supernumerary but functional nuclei in the basidia of *T. aegopogonis* proved to be among the most significant aspects of the nuclear cycle. Furthermore, this type of nuclear behavior points out the need to more clearly define homothallism in the smut fungi because the distribution of supernumerary nuclei to a small complement of basidiospores virtually assures formation of heterokaryotic lines.

Thus, according to Lemke (13) homothallism in basidiomycetes has been recognized as either homokaryotic (primary) or heterokaryotic (secondary). Terms to describe heterokaryotic homothallism in fungi largely have stemmed from studies of ascomycetes, particularly *Neurospora tetrasperma,* which normally produces four ascospores, each with a plus (+) and a minus (−) nucleus. These descriptors include: pseudoheterothallism, secondary homothallism, miktohaplonticism, facultative heterothallism, amphithallism, pseudomonothallism, and homoheteromixis. These terms, according to Ainsworth (1) more or less adequately describe homothallism in *T. aegopogonis,* but a more precise definition based on further studies is desirable for application to smut fungi with nuclear cycles like that of *T. aegopogonis.*

LITERATURE CITED

1. AINSWORTH, G. C. (ed.). 1968. Plant Pathologist's Pocketbook. Commonw. Mycol. Inst., Kew, Surrey, England. 267 pp.
2. BISSING, D. R. 1974. Haupt's gelatin adhesive mixed with formalin for affixing paraffin sections to slides. Stain Technol. 49:116-117.
3. CHRISTENSEN, J. J. 1963. Corn Smut Caused by *Ustilago maydis.* Phytopathological Monograph 2. Am. Phytopathol. Soc., St. Paul, MN. 41 pp.
4. DURAN, R. 1970. Hosts and distribution records of Mexican smut fungi. Mycologia 62:1094-1105.
5. DURAN, R. 1972. Aspects of teliospore germination in North American smut fungi. II. Can. J. Bot. 50:2569-2573.
6. DURÁN, R. 1979. *Tilletia lycuroides*: Biological implications of nuclear behavior in the basidium. Mycologia 71:449-455.
7. FISCHER, G. W. 1952. The Smut Fungi. A Guide to the Literature. Ronald Press, New York, NY. 387 pp.
8. FISCHER, G. W., and C. S. HOLTON. 1957. Biology and Control of the Smut Fungi. Ronald Press, New York, NY. 622 pp.
9. GOTH, R. W., J. B. ROWELL, and M. F. KERNKAMP. 1958. Behavior of solopathogens from interspecific crosses of some *Sphacelotheca* spp. Phytopathology 48:494-497.
10. HOLTON, C. S. 1952. *Tilletia elymi,* an apparently homothallic species. Phytopathology 42:635-636.
11. HOLTON, C. S., J. A. HOFFMANN, and R. DURÁN. 1968. Variation in the smut fungi. Annu. Rev. Phytopathol. 6:213-242.
12. KENDRICK, E. L. 1960. A solopathogen of *Tilletia caries.* Phytopathology 50:641.
13. LEMKE, P. A. 1969. A reevaluation of homothallism, heterothallism and the species concept in *Sistotrema brinkmanni.* Mycologia 61:57-76.
14. SIANG, W. N. 1954. Observations on *Tilletia cerebrina.* Mycologia 46:238-244.
15. WARD, E. W. B., and K. W. CIURYSEK. 1961. Somatic mitosis in a basidiomycete. Can. J. Bot. 39:1497-1503.