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Morphological characterization of *Gibberella coronicola* sp. nov., obtained through mating experiments of *Fusarium pseudograminearum*

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Mating experiments were performed among 18 strains of *Fusarium pseudograminearum*, formerly recognized as the Group 1 population of *F. graminearum*. Heterothallic production of perithecia was observed in eight out of all 153 possible combinations. Mature asci and viable ascospores were recovered in seven of the eight combinations. Perithecia in the fertile pairings were subglobose to ovoid, dark, 120–370 μm diam and formed directly on the surface of rice stems placed on the culture media. Asci were unitunicate and 8-spored when mature. Mature ascospores were primarily hyaline, fusoid, straight or curved, with rounded ends and (1-)3-septate. Dimensions of the teleomorph obtained for *F. pseudograminearum* were different from those of the *G. zeae* teleomorph of *F. graminearum*. A new species of *Gibberella*, *G. coronicola*, is described and illustrated for the teleomorph of *F. pseudograminearum*. The Group 1 and Group 2 populations recognized previously within *F. graminearum* differ in their anamorphic and teleomorphic morphology, ecological habitats, pathogenicity, mode of sexual reproduction and phylogenetic relationships.

Key Words——crown rot; *Fusarium pseudograminearum*; *Gibberella coronicola*; mating experiment; teleomorph.

Two morphologically similar populations within *Fusarium graminearum* Schwabe were designated Group 1 and Group 2 by Burgess et al. (1975), both of which appear to have distinctive geographic distributions and induce different pathological symptoms on agriculturally important cereals. In a previous paper (Aoki and O'Donnell, 1999), we analyzed anamorphic morphological/phenotypic characters of 17 Group 1 and 15 Group 2 strains of *F. graminearum*, together with DNA sequence data from β-tubulin gene introns and exons to investigate their systematic and phylogenetic relationships. We concluded that the Group 1 strains represent a distinct species based on their anamorphic morphology and their phylogenetically distinct position within the β-tubulin gene tree. Therefore, we proposed the establishment of a new anamorphic species, *Fusarium pseudograminearum* O'Donnell & T. Aoki. Francis and Burgess (1977) and Burgess et al. (1988) reported that the key diagnostic feature for differentiating the two groups is that single-spored Group 2 strains (=*F. graminearum*) form homothallic perithecia of *Gibberella zeae* (Schw.) Petch abundantly on carnation leaf agar while those of Group 1 (=*F. pseudograminearum*) do not form perithecia in culture and are presumably heterothallic. In the present paper, we conducted mating experiments on strains of *F. pseudograminearum* to test for heterothallic production of perithecia. By comparing teleomorphic morphological characters obtained in mating experiments with those of *G. zeae*, the distinction of the new species, *G. coronicola*, is corroborated.

Materials and Methods

Strains examined and mating experiments Single conidial isolates obtained from 18 strains of *Fusarium pseudograminearum*, including those studied in the previous morphological and molecular analyses (Aoki and O'Donnell, 1999) and one additional strain (Table 1), were paired in all possible 153 combinations to test for the production of a teleomorph, i.e., perithecia with mature asci and viable ascospores. Isolates were first incubated for 1 wk on SNA (Nirenberg, 1990) at 20°C, after which an agar block containing mycelium was removed from the margin of each colony. Isolates were paired in all possible combinations by placing agar blocks from two different isolates 3 cm apart on the surface of an agar medium modified from Sachs' agar (Hsieh, et al., 1977), which contained 1 g $Ca(NO_3)_2$, 0.25 g K_2HPO_4, 0.25 g $MgSO_4 \cdot H_2O$, trace $FeCl_3$, 4 g $CaCO_3$, 2 g glucose, 0.2 g yeast extract (Difco Lab., Detroit, MI), 0.2 g KCl, and 20 g agar in 1 L of distilled water, in 9-cm Petri plates. Two pieces of autoclaved rice stem (5 cm long) and three pieces of sterile filter paper (ca. 2 × 3 cm) were placed near the inocula on the agar surface. Two agar blocks

Table 1. Strains of *Fusarium* species examined.

Species	NRRL No.	Geographic Origin	host/substrate	Equivalent No.	Source
Fusarium pseudograminearum	13821	Australia	wheat	FRC R-6710=MAFF 237829	FRC
Fusarium pseudograminearum	13822	Australia, Dalby, QLD	wheat crown	FRC R-6726=MAFF 237830	FRC
Fusarium pseudograminearum	26867	Australia, NSW	*Phalaris paradoxa*	F7040=MAFF 237831	L. W. Burgess
Fusarium pseudograminearum	28059	USA. Washington	wheat	FRC R-2616=MAFF 237832	FRC
Fusarium pseudograminearum	28060	USA, California	oat stem base	FRC R-5211=MAFF 237833	FRC
Fusarium pseudograminearum	28061	Australia, Darling Downs	wheat stem base	FRC R-5261=MAFF 237834	FRC
Fusarium pseudograminearum	28062[a)]	Australia, Darling Downs	barley crown	FRC R-5291=MAFF 237835	FRC
Fusarium pseudograminearum	28064	Australia, Milmerran, QLD	wheat stem base	FRC R-6729=MAFF 237836	FRC
Fusarium pseudograminearum	28065[b)]	South Africa	*Medicago* sp.	FRC R-6761=MAFF 237837	FRC
Fusarium pseudograminearum	28067	Australia, Wallahwelleh	wheat stem base	FRC R-6782=MAFF 237838	FRC
Fusarium pseudograminearum	28068	Australia, Morel, NSW	wheat head	FRC R-7527=MAFF 237839	FRC
Fusarium pseudograminearum	28069	Morocco, Settat	wheat root	FRC R-8636=MAFF 237840	FRC
Fusarium pseudograminearum	28331	USA, Washington	wheat root	FRC R-6563=MAFF 237841	FRC
Fusarium pseudograminearum	28333	South Africa, Oudtsboorn	*Medicago* pasture	FRC R-8057=MAFF 237843	FRC
Fusarium pseudograminearum	28334	South Africa, Swellendam	*Medicago truncatula*	FRC R-8064=MAFF 237844	FRC
Fusarium pseudograminearum	28337	Australia, Darling Downs	oat stem base	FRC R-5285=MAFF 237845	FRC
Fusarium pseudograminearum	28338	Australia, Breeza, NSW	uncultured pasture soil	FRC R-6215=MAFF 237846	FRC
Fusarium pseudograminearum	28431[b)]	Australia, Breeza, NSW	uncultured pasture soil	FRC R-6221=MAFF 237848	FRC
Fusarium graminearum	5883	USA, Ohio	corn	ATCC 46779=MAFF 237812	NRRL
Fusarium graminearum	26155	Canada, Ottawa	corn seed	DAOM 180378=MAFF 237814	G. A. Neish
Fusarium graminearum	26895	Finland, Espoo	barley root	92029=MAFF 237820	T. Yli-Mattila
Fusarium graminearum	26896	Finland, Jalasjärvi	barley stem base	29028=MAFF 237821	T. Yli-Mattila
Fusarium graminearum	26938	USA, North Dakota	barley seed	NDSU KB-172=MAFF 237822	B. Steffenson
Fusarium graminearum	26953	USA, North Dakota	barley seed	NDSU KB-582=MAFF 237823	B. Steffenson
Fusarium graminearum	28063	USA, Michigan	corn stalk	FRC R-6574=MAFF 237824	FRC

a) Ex-type strain of *F. pseudograminearum*.
b) Fertile combination of strains, from which the holotype of *G. coronicola* was obtained.

obtained from the same isolates were also paired (i.e., selfed) as a negative control. Paired cultures were placed under an alternating 12 h darkness/12 h BLB (Black Light Blue; near-UV, Toshiba FL20S BLB 20 W) light cycle at 20°C and incubated for ca. 3 mo. Mating experiments with strain NRRL 28065 were duplicated. Production of perithecia in culture was examined at a 2 wk interval. Homothallic production of a *G. zeae* teleomorph was also induced for seven single-conidial isolates of *F. graminearum* (Table 1) under the same conditions and the perithecia produced were examined for comparison. Stock cultures described here have been stored by lyophilization or cryogenically at ca. −175°C in the Agriculture Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL, USA and in the Genetic Resources Center, National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries (MAFF), Tsukuba, Japan.

Morphological examination *Gibberella* perithecia produced as a result of the mating experiments or by homothallic perithecial induction were examined morphologically under dissecting and compound microscopes. Measurements of perithecia, asci and ascospores were made for each of the fertile pairings. Colors cited are given according to the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). Length and width as well as length/width ratios were measured for ascospores produced from each of the fertile pairings mounted in water. At least 30 ascospores of each septation-type, i.e. 1- and 3-septate, were selected randomly and measured, except where stated otherwise. From the measurements, arithmetic means and ranges of their sizes, as well as their standard deviations (S.D.), were calculated for each of the strains/fertile combinations. On the basis of the morphological examination, a diagnosis and a description for a new species of *Gibberella* was prepared. A specimen obtained during this study, i.e., dried culture containing perithecia, was deposited in the herbarium of the U.S. National Fungus Collection (BPI), USDA/ARS, Beltsville, MD, USA, as the holotype of the new *Gibberella* species.

Results

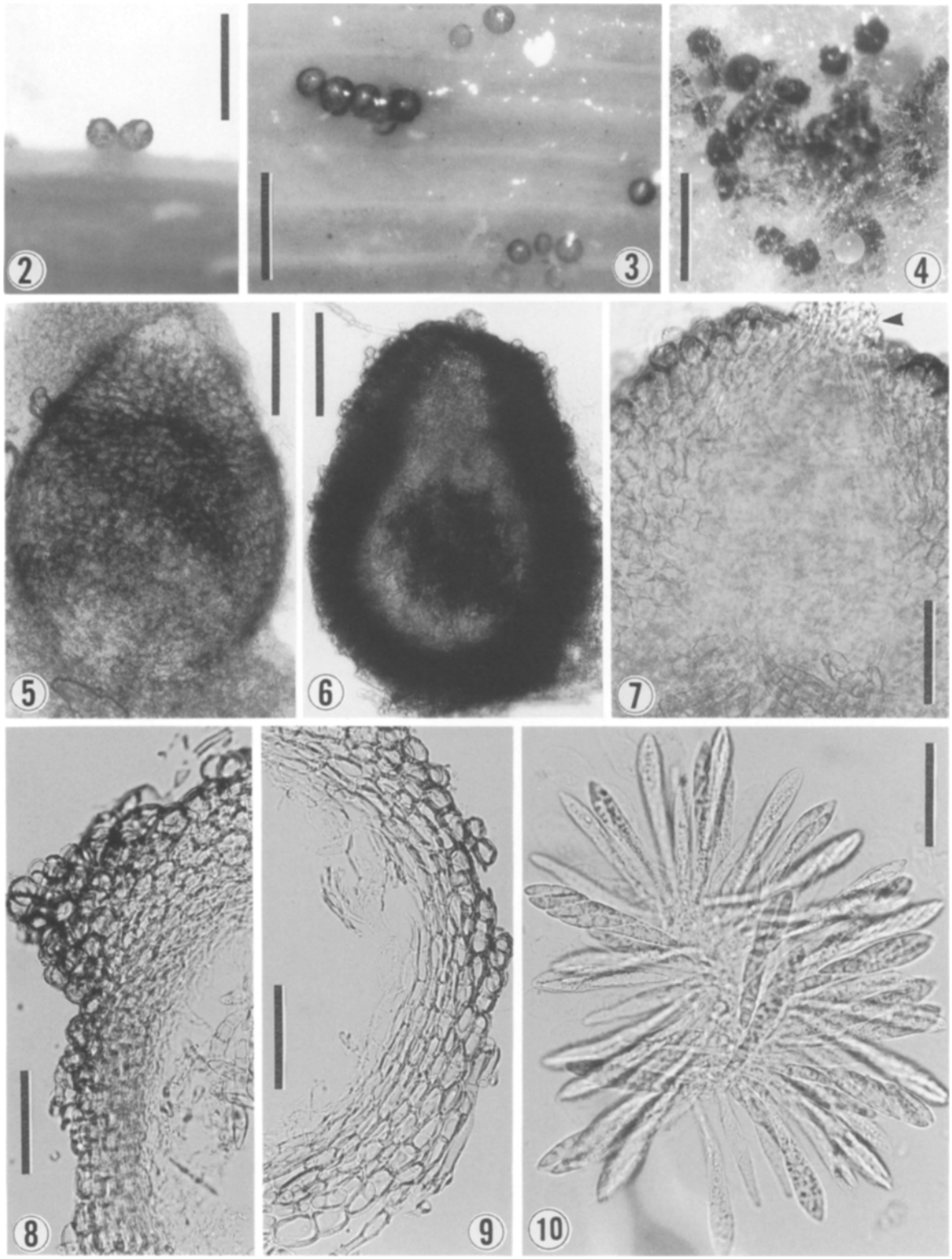
Heterothallic production of perithecia of *Fusarium pseudograminearum* in culture From the mating experiments, heterothallic production of perithecia was observed in eight out of all 153 possible combinations of the 18 *F. pseudograminearum* isolates (Fig. 1). In seven of the eight combinations, mature asci and ascospores were recovered. The mating experiments for strain NRRL 28065 were duplicated, and fertile perithecia were formed again in the same fertile combination, i.e., with NRRL 28431, as observed in the first trial. In the pairings of NRRL 28065 × NRRL 28069 in both trials, only immature perithecia without asci and ascospores were produced. These fertile combinations of strains, including those producing immature perithecia, were divided into three groups, which are outlined with solid rectangles in Fig. 1. Within the rectangles, fertile combinations often resulted from strains that originated from distant geographic areas, i.e., Australia vs. South Africa or Australia vs. North America, although an Australian strain, NRRL 28068 was compatible with two other Australian strains, i.e., NRRL 13822, NRRL 28337. No perithecia or primordia were observed in the other 135 combinations, as well as in the 18 selfed pairings. In many combinations, light brown stroma-like dense mycelial masses were produced in the agar medium. However, they did not appear to be correlated with perithecial production of the paired isolates. Fertile combinations of *F. pseudograminearum* isolates produced two to 37 subglobose to ovoid, dark perithecia, 120–370 μm diam directly on the surface of rice stems in culture, which were often aggregated and sometimes covered by aerial mycelium (Figs. 1–9). Asci in the crushed perithecia represented different stages of development (Figs. 10–12) and, when mature, were unitunicate and 8-spored. Mature ascospores were primarily hyaline, fusoid, straight or curved, with rounded ends, (1-)3-septate (Figs. 13–18). All of these structures were typical for a species of *Gibberella*. When mature ascospores were discharged onto the agar surface, they started germinating (Figs. 19, 20). Single ascospores were isolated, and germinated (Figs. 21–23) and 12 single-ascospore isolates were retained as progeny from the NRRL 28065 × NRRL 28431 pairing. All of these isolates produced a *F. pseudograminearum* anamorph. During mating experiments with the *F. pseudograminearum* isolates, abundant mycelia, sporodochia and sporodochial conidia were also formed on the agar surface and the rice straws. Seven single-conidial strains of *F. graminearum* also formed perithecia homothallically, as well as abundant mycelia and sporodochia.

Comparison of ascospore dimensions of the new *Gibberella* species and *G. zeae* Ascospores formed in the seven fertile pairings of *Fusarium pseudograminearum* (= *G. coronicola*) and in the homothallic perithecial production by the seven strains of *F. graminearum* (= *G. zeae*) were compared morphologically. Their dimensions ranged from 15.5–35.5 × 3.5–6.5 μm and 20.5–37 × 3.5–7 μm for 1- and 3-septate ascospores of *G. coronicola* and from 15–25 × 3–5 μm and 16–27 × 3.5–5 μm for 1- and 3-septate ascospores of *G. zeae*, respectively. Values for the length/width ratios ranged from 2.7–7.8 and 3.8–8.1 for 1- and 3-septate ascospores of *G. coronicola* and from 3.5–7.1 and 4.1–6.7 for 1- and 3-septate ascospores of *G. zeae*, respectively. Ascospores of *G. coronicola* were generally larger than those of *G. zeae*, although their ranges partly overlapped. A precise comparison of the length and width of 3-septate ascospores of both species is illustrated in Figs. 24 and 25. Ranges and means ± S.D. of length (Fig. 24) and width (Fig. 25) of ascospores are presented separately for individual fertile combinations and strains. Variation in the length and width of 3-septate ascospores for a strain/combination was greater in the new species than in *G. zeae*. Three-septate ascospores of the new *Gibbe-*

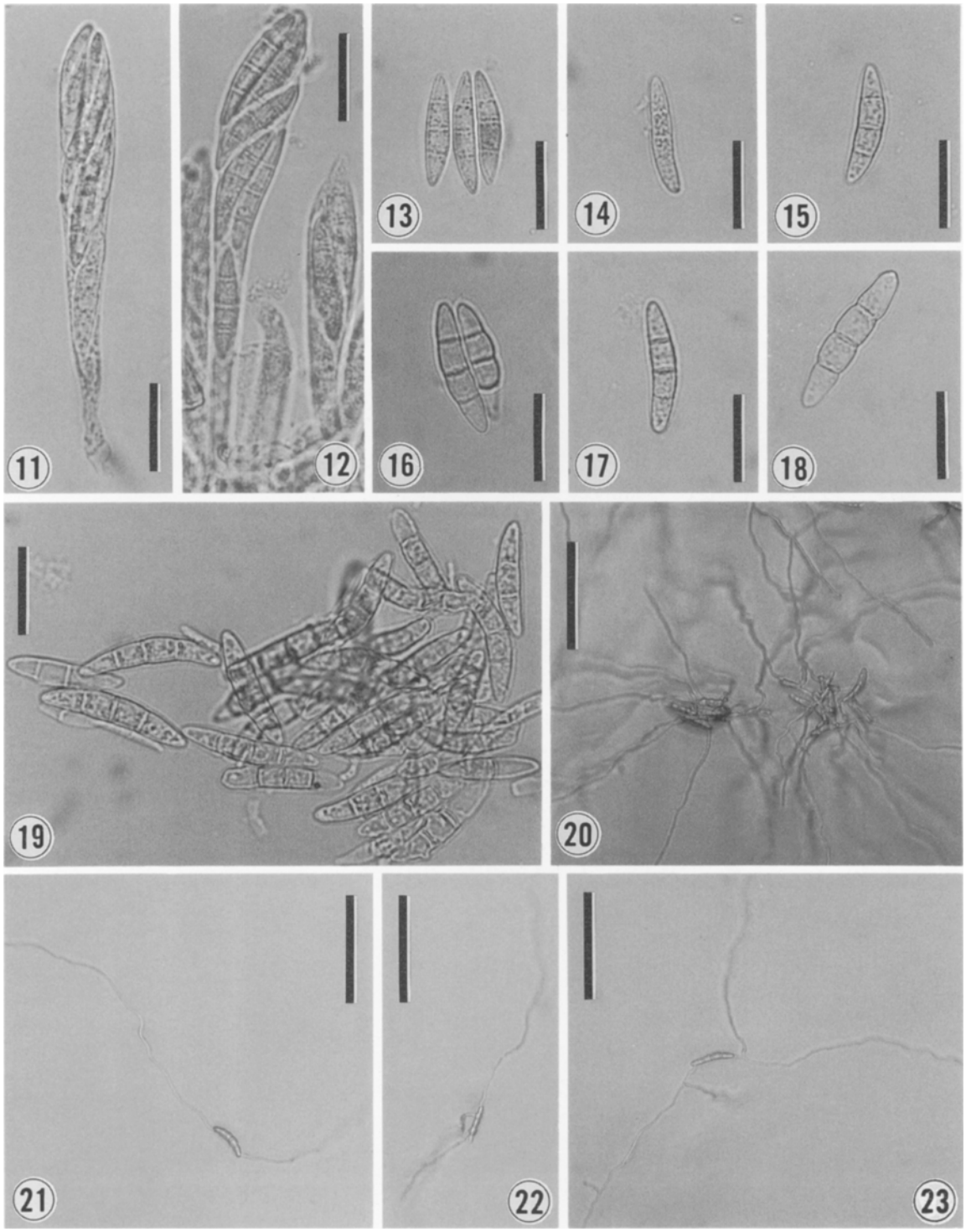
NRRL no.	13822	26867	28337	28338	28065	(28065)	13821	28060	28061	28062	28064	28067	28331	28333	28068	28334	28059	28069	28431	Origin of strains
13822	-	s		s	s				s	s		s		s			s	s		Australia, Dalby, QLD
26867	-	-	s	s	s	(s)	s		s	s	s	s	s						s	Australia, NSW
28337	-	-	-				s			s	s									Australia, Darling Downs
28338	-	-	-	-			s	s	s	s					s	s				Australia, Breeza, NSW
28065	-	-	-	-	-		s								s			s		South Africa
(28065)	(-)	(-)	(-)	(-)		(-)	(s)			(s)		(s)								(duplicated paring)
13821	-	-	-	-	-	(-)	-	s	s	s	s	s	s	s			s	s	s	Australia
28060	-	-	-	-	-	(-)	-	-					s						s	USA, California
28061	-	-	-	-	-	(-)	-	-	-					s	s	s				Australia, Darling Downs
28062	-	-	-	-	-	(-)	-	-	-	-	s		s		s	s	s		s	Australia, Darling Downs
28064	-	-	-	-	-	(-)	-	-	-	-	-				s	s	s			Australia, Milmerran, QLD
28067	-	-	-	-	-	(-)	-	-	-	-	-	-			s				s	Australia, Wallahwalleh
28331	-	-	-	-	-	(-)	-	-	-	-	-	-	-					s		USA, Washington
28333	-	-	-	-	-	(-)	-	-	-	-	-	-	-	-						South Africa, Oudtsboorn
28068	**2***	-	**39**	-	-	(-)	-	-	-	-	-	-	-	-	-	s				Australia, Morel, NSW
28334	**21**	**5**	**10**	-	-	(-)	-	-	-	-	-	-	-	-	-	-	s			South Africa, Swellendam
28059	-	-	-	**36**	-	(-)	-	-	-	-	-	-	-	-	-	-	-			USA, Washington
28069	-	-	-	-	**5i**	**(6i)**	-	-	-	-	-	-	-	-	-	-	-	-		Morocco, Settat
28431	-	-	-	-	**30**	**(26)**	-	-	-	-	-	-	-	-	-	-	-	-	-	Australia, Breeza, NSW
Origin of strains	Australia, Dalby, QLD	Australia, NSW	Australia, Darling Downs	Australia, Breeza, NSW	South Africa	(duplicated paring)	Australia	USA, California	Australia, Darling Downs	Australia, Darling Downs	Australia, Milmerran, QLD	Australia, Wallahwalleh	USA, Washington	South Africa, Oudtsboorn	Australia, Morel, NSW	South Africa, Swellendam	USA, Washington	Morocco, Settat	Australia, Breeza, NSW	

Fig. 1. Results of the mating experiment using 18 strains of *Fusarium pseudograminearum*. Pairings with NRRL 28065 were duplicated and are indicated in parentheses. Numbers in bold-face in the matrix show seven fertile combinations of strains and numbers of perithecia produced in individual pairings, which contained mature asci and ascospores. Symbols, "i" after the numbers indicates production of only immature perithecia without asci and ascospores, and "-" no perithecial production observed. Only eight 1-septate and ten 3-septate ascospores were recovered and measured from perithecia in the combination of NRRL 13822 × NRRL 28068, indicated by an asterisk, "*". Stroma-like hyphal masses were observed in the agar medium in many of the pairings, indicated by "s".

rella species were, however, consistently longer and wider, especially in the means and the means ± S.D. of the width, than those of obtained for *G. zeae* in this study. Mean values of the length of the 1- and 3-septate ascospores were plotted against those of the width for each of the strains/combinations in Fig. 26. Both *Gibberella* species could be differentiated clearly using the mean values of the width of ascospores. When the comparison was restricted to either 1-septate or 3-septate ascospores, arithmetic means of the length of ascospores could also be used to differentiate both species clearly. Mean sizes of ascospores for the individual strains/fertile combinations ranged from 21.8–24.0 × 5.1–5.4 μm and 25.0–29.7 × 5.2–5.8 μm for 1- and 3-septate spores of *G. coronicola* and from 17.9–20.4 × 4.0–4.2 μm and 20.5–23.2 × 4.0–4.5 μm for 1- and 3-septate spores of *G. zeae*, respectively. Dimensions of *G. coronicola* were also compared with those of other species and varieties of *Gibberella* described by Wollenweber and Reinking (1935), Booth (1971), Kuhlman (1982), Klittich et al. (1997) and Nirenberg and O'Donnell (1998). Ascospores of *G. coronicola* were longer than those of *G. acuminata* Wollenw., *G. avenacea* R. J. Cook, *G. baccata* (Wallr.) Sacc., *G. buxi* (Fuckel) G. Wint., *G. circinata* Nirenberg & O'Donnell, *G. cyanea* (Sollem.) Wollenw. (=*G. gordonia* C. Booth), *G. cyanogena* (Desm.) Sacc., *G. fujikuroi* (Sawada) Wollenw., *G. fujikuroi* var. *subglutinans* Edwards, *G. fujikuroi* var. *intermedia* Kuhlman, *G. heterochroma* Wollenw., *G. moniliformis* Wineland (≡*G. fujikuroi* var. *moniliformis*



Figs. 2–10. Perithecia of *Gibberella coronicola* formed in culture by the pairing of NRRL 28065 × NRRL 28431 of *Fusarium pseudograminearum*. 2–4. Young and mature perithecia formed on rice stems, some of which are covered by aerial mycelium (Fig. 4). 5. Perithecium in side view. 6. Vertical section of a perithecium. 7–9. Vertical sections of perithecia at a higher magnification; 7: upper part of a perithecium around ostiole, showing cylindrical periphyses protruding outwards and lining and plugging the ostiolar canal (arrowhead), 8: upper portion of lateral wall of a perithecium, showing outer and inner regions of the wall, 9: detail of lateral wall of a perithecium. 10. Developing asci and ascospores from a crushed perithecium. Scale bars: 1 mm in Figs. 2–4; 100 μm in Figs. 5, 6; 50 μm in Figs. 7–10.



Figs. 11–23. *Gibberella coronicola* formed in culture by the pairing of NRRL 28065 × NRRL 28431 of *Fusarium pseudograminearum*. 11, 12. Asci from crushed mature perithecia, showing development of 3-septate ascospores. 13–18. Three-septate ascospores from crushed mature perithecia. 19. Discharged ascospores. 20. Germination of ascospores after discharge. 21–23. Germination of single-spored ascospores. Scale bars: 20 μm in Figs. 11–19; 100 μm in Figs. 20–23.

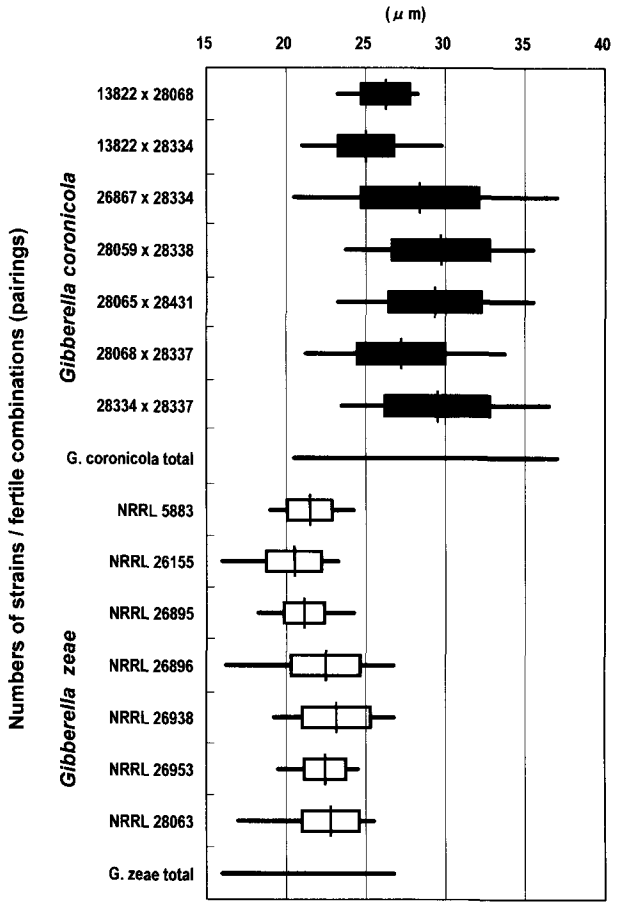


Fig. 24. Comparison of length of 3-septate ascospores of *Gibberella coronicola* and *G. zeae*. Total ranges (bars) and means±S.D. (rectangles) for individual strains/fertile combinations are shown.

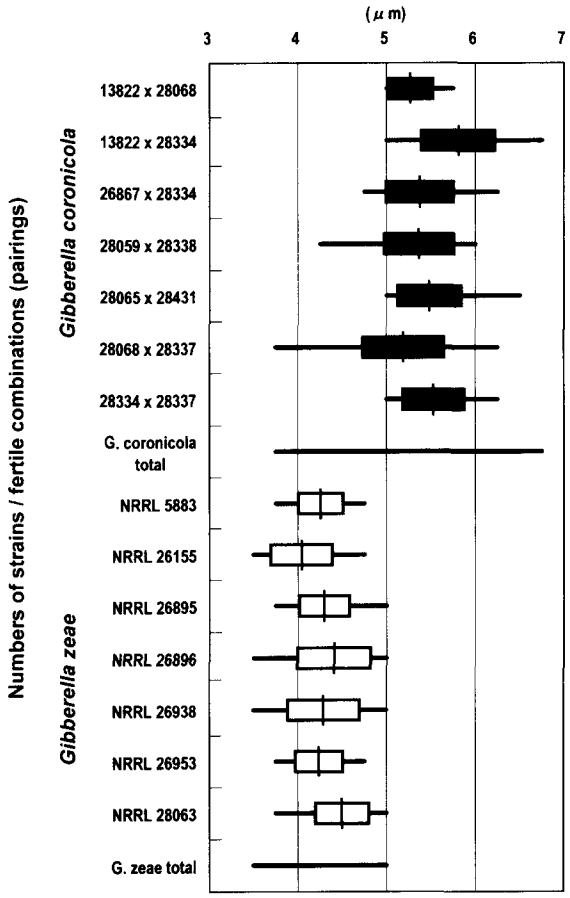


Fig. 25. Comparison of width of 3-septate ascospores of *Gibberella coronicola* and *G. zeae*. Total ranges (bars) and means±S.D. (rectangles) for individual strains/fertile combinations are shown.

(Wineland) Kuhlman), *G. pseudopulicaris* Wollenw., *G. stilboides* Gordon ex C. Booth, *G. thapsina* Klittich et al., and *G. xylarioides* Heim & Saccas. However, the ascospores of *G. intricans* Wollenw. [anamorph=*F. equiseti* (Corda) Sacc.], *G. pulicaris* (Fries) Sacc. [anamorph=*F. sambucinum* Fuckel], and *G. pulicaris* var. *minor* Wollenw. [anamorph=*F. sambucinum* var. *coeruleum* Wollenw.=*F. torulosum* (Berk. & Curtis) Nirenberg] were reported to have similar dimensions. One- and 3-septate ascospores measured 14–21×4.5–6 μm and 19–36×3.7–7 μm in *G. intricans*, and 15–27×5–7 μm and 17–40×4–9 μm in *G. pulicaris*, respectively, and 3-septate ascospores 17–33×3.7–7 μm in *G. pulicaris* var. *minor* (Wollenweber and Reinking, 1935). These values were similar to the dimensions of *G. coronicola*, however, its *F. pseudograminearum* anamorph is morphologically distinct from *F. equiseti*, *F. sambucinum* and *F. sambucinum* var. *coeruleum*. The width of ascospores of *G. coronicola* overlapped with many of the other species.

Description of the species Based on the morphology of perithecia, asci, ascospores formed in the seven fertile combinations of the isolates, and based on the anamorph, *Fusarium pseudograminearum*, a new species of *Gibberella* is recognized and described.

Gibberella coronicola T. Aoki & O'Donnell, sp. nov.
Figs. 2–23, 27

Anamorph: *Fusarium pseudograminearum* O'Donnell & T. Aoki, Mycologia **91**: 604–607, 1999.

Perithecia globosa, subglobosa vel late ellipsoidea, 140–400 μm alta 120–370 μm diam, non vel inconspicue papillata, plerumque superficialia vel nonnumquam immersa, non stromatica, brunneo-grisea, griseo-brunnea vel atra, ad basim brunnea, acido lactico rubescentia. Paries quasi levis vel modice verrucosus, 25–87.5 μm crassus, sursum crassior, e duobus stratis colore differentibus constans; cellulae verrucarum fere globosae, 3–7 μm diam, 1–3 μm crassitunicatae. Asci unitunicati, octospori, clavati, 79.5–106.5×10.5–17.5 μm, apice simplici, tenuitunicati, ascosporis in parte superiore saepe oblique dispositis. Ascosporae fusiformes, naviculares vel allantoideae, rectae vel modice curvatae, plerumque leves vel nonnumquam minime asperae, hyalinae vel pallide pigmentatae, plerumque 3-septatae et 20.5–37×3.5–7 μm, longit.: latitud. 3.8–8.1, maturae saepe constrictae ad septa, liberatae dilute brunnes-

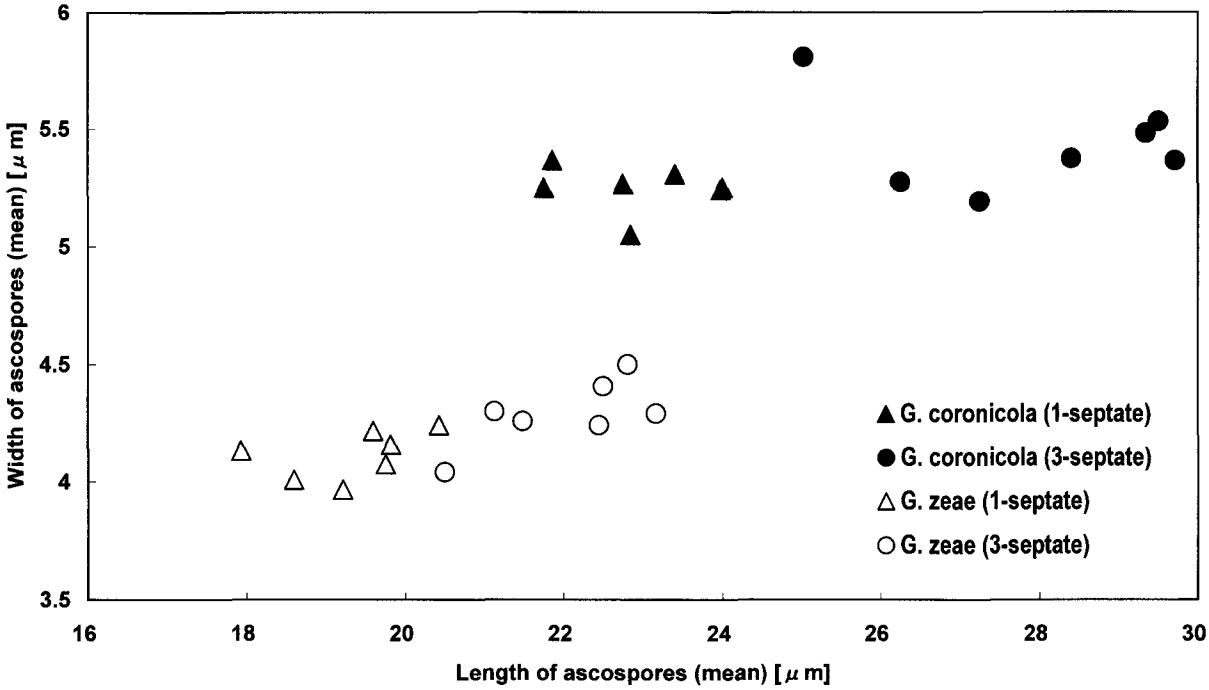


Fig. 26. Plots of arithmetic means of the length and width of 1- and 3-septate ascospores produced by *Gibberella coronicola* and *G. zeae*.

centes. Status anamorphicus *Fusarium pseudograminearum* O'Donnell et T. Aoki. Heterothallicus.

Holotypus: BPI 746116, perithecia exsiccata, in cultura ex NRRL 28065×NRRL 28431, in Herbario BPI, USA deposita.

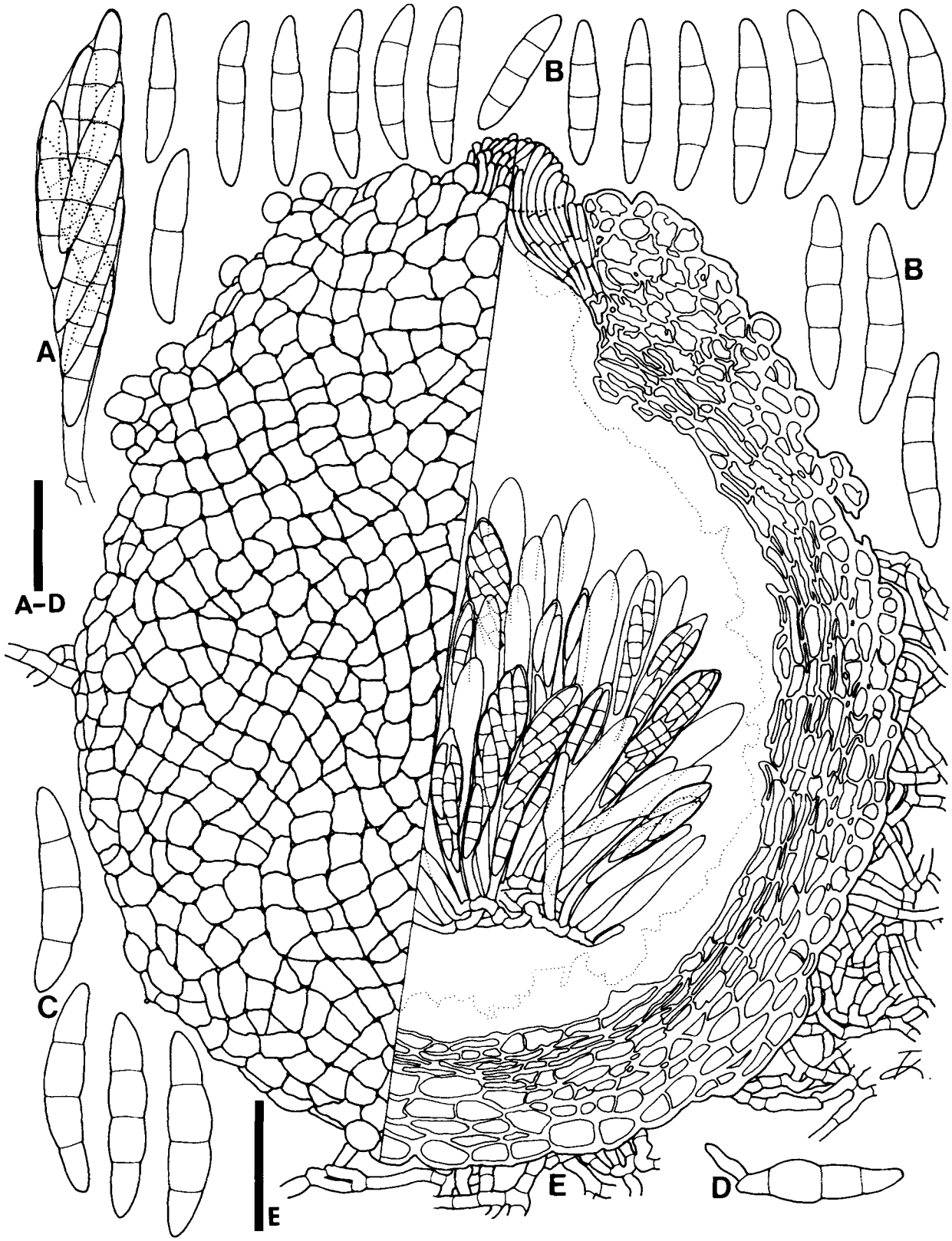
Perithecia solitary or in groups, globose, subglobose, broadly ellipsoidal, ovoid to obpyriform, 140–400 μm high × 120–370 μm diam. (range of the means: 225–319 × 193–260 μm), nonpapillate to slightly papillate, mostly superficial and surrounded by mycelia or sometimes immersed in the substratum, nonstromatic, brownish-grey, greyish-brown to black in aerial view under low magnification, greyish-green, olive, dark turquoise to dark green in water and in 3% KOH, brownish toward the base, turning red to dark red in undiluted (ca. 90%) lactic acid. Perithecial wall nearly smooth to somewhat warted, 25–87.5 μm thick, thicker towards the apex, comprising differently-pigmented outer and inner regions. Outer region 17.5–62.5 μm and 3–8 cells thick, cells globose, ellipsoidal or angular, sometimes compressed in section, 7.5–27.5 × 7.5–17.5 μm diam, cell walls 0.5–3 μm thick, pigmented dark turquoise to dark green in water and 3% KOH, turning red to dark red in undiluted (ca. 90%) lactic acid. Inner region 12.5–37.5 μm and 5–9 cells thick, cells compressed in section, 7.5–17.5 × 3.5–7.5 μm diam, thin-walled, non-pigmented or pigmented light brown to brownish grey. The outer and inner regions integrating, cells toward the exterior larger and thicker-walled. Cells of the warts nearly globose, 3–7 μm diam, walls 1–3 μm thick. Periphyses cylindrical, thin-walled, lining the ostiolar canal. Asci unitunicate, clavate, 79.5–106.5 × 10.5–17.5 μm, with a simple apex, thin-walled, sometimes with an obvious basal crozier remnant, containing 8 ascospores often arranged obliquely and helically toward the ascus apex. Ascospores fusiform, navicular to allantoid, straight or gently curved, mostly smooth or sometimes very minutely rough, hyaline to pale, (1-)3-septate, 1-septate ascospores 15.5–35.5 × 3.5–6.5 μm (range of the means: 21.8–24.0 × 5.1–5.4 μm), Length/Width (L/W) 2.7–7.8, 3-septate ascospores 20.5–37 × 3.5–7 μm (range of the means: 25.0–29.7 × 5.2–5.8 μm), L/W 3.8–8.1, often constricted at the septa when mature, becoming pale brown after discharge. Heterothallic.

Holotype: BPI 746116, deposited in the herbarium of BPI (U.S. National Fungus Collection, Beltsville, MD), USA, a dried culture containing perithecia from a cross of NRRL 28065 × NRRL 28431.

Etymology: *corona* (Lat. crown)+*-cola* (Lat. dweller), referring to the crown rot disease of cereals caused by the fungus.

Specimens examined: Cultures containing mature perithecia from crosses of NRRL 13822 × NRRL 28068, NRRL 13822 × NRRL 28334, NRRL 26867 × NRRL 28334, NRRL 28059 × NRRL 28338, NRRL 28065 × NRRL 28431, NRRL 28068 × NRRL 28337 and NRRL

Fig. 27. *Gibberella coronicola* formed in culture (from the pairing of NRRL 28065 × NRRL 28431 of *Fusarium pseudograminearum*). A. Ascus containing eight mature 4-celled ascospores. B, C. Ascospores. D. Germinating ascospore. E. Perithecium showing a view of its outer surface (left half) and in vertical section (right half), containing asci in the centrum. Scale bars: 20 μm for A–D, 50 μm for E.


A
B
B
A–D
C
E
E
D

28334 × NRRL 28337.

Discussion

In the present study, seven combinations of the pairings of single-conidial isolates of *Fusarium pseudograminearum* yielded mature perithecia of *Gibberella* containing viable ascospores. For strain NRRL 28065, pairings were duplicated and identical results were obtained, including production of immature perithecia (Fig. 1). The most striking result of the mating experiments was that dimensions of the asci and ascospores produced were found to be larger than those of *Gibberella zeae*. Seifert (1996) designated the lectotype of *G. zeae* from the Schweinitz collection in Kew and provided precise dimensions of the specimen, e.g. asci: 56–70 × 8–11.5 μm (mean, $\bar{x}$ = 64.3 × 9.9), ascospores: 19.5–29 × 3.5–4.5 μm (mean, $\bar{x}$ = 24.7 × 4.1), L/W = 4.7–8.3. In our study, homothallic production of *G. zeae* perithecia was also induced for seven strains of *F. graminearum*. Ascospores of this species ranged from 15–25 × 3–5 μm and 16–27 × 3.5-5 μm for 1- and 3-septate spores, (ranges of the means, $\bar{x}$ = 17.9–20.4 × 4.0–4.2 μm and 20.5–23.2 × 4.0–4.5 μm for 1- and 3-septate spores), L/W = 3.5–7.1 and 4.1–6.7 for 1- and 3-septate ascospores (Figs. 24-26). These values are clearly smaller than those of *G. coronicola* described in this study. Francis and Burgess (1977) characterized Group 1 and Group 2 populations of *F. graminearum*. In their study, pairing experiments were performed among isolates of Group 1 population of *F. graminearum* (=*F. pseudograminearum*) and perithecia were obtained for only two pairings. Their ability to cross was lost after 6 mo. Francis and Burgess (1977) stated that measurements of ascospores of Group 1 and those of Group 2 were within the range of 17.5–27.0 × 3.5–5.0 μm, which is in agreement with the values reported for *G. zeae* (Schw.) Petch by Petch (1936) and Purss (1969). However, they did not provide detailed measurements or descriptions of the perithecia, asci and ascospores, which they obtained for the Group 1 population. Our measurements of *G. coronicola*, however, are definitely larger than those given by Francis and Burgess (1977). Nelson et al. (1983) and Burgess et al. (1988, 1994) mentioned two populations within *F. graminearum*, but they commented less on the teleomorph formed by the Group 1 population, except stating that the latter population is apparently heterothallic.

Francis and Burgess (1977) studied differences in habitats of the two populations in Australia. They found that Group 1 strains were more frequently isolated from the crown regions of wheat plants while Group 2 strains were more frequently isolated from the aerial parts of maize. Strains of Group 1 have been described as soil-borne pathogens, causing crown rot and foot rot of wheat, barley, oats and *Medicago* spp. in Australia (Francis and Burgess, 1977; Wearing and Burgess, 1977; Burgess et al., 1987), Africa (Marasas et al., 1988; Van Wyk et al., 1988; Lamprecht et al., 1990) and the Pacific Northwest of the United States (Cook, 1980). Members of Group 2 typically cause ear rot of corn and head scab of wheat, oats and barley and have been shown to be seed-borne pathogens, especially in northern temperate regions of the Northern Hemisphere (McMullen et al., 1997). In our previous study (Aoki and O'Donnell, 1999), we proposed the establishment of a new anamorphic species, *F. pseudograminearum*, because the Group 1 strains represent a distinct species based on their anamorphic morphology and their phylogenetically distinct position within the β-tubulin gene tree. Our present study also clearly demonstrates that Group 1 strains, i.e., *F. pseudograminearum*, are heterothallic and are different from homothallic Group 2, *F. graminearum*. Further, the teleomorph obtained for *F. pseudograminearum*, as a result of mating experiments, is also different from the *G. zeae* teleomorph of *F. graminearum* in its morphology and dimensions. Considering all available data, it is clear that the Group 1 and Group 2 populations differ in their anamorphic and teleomorphic morphology, ecological habitats, pathogenicity, mode of sexual reproduction and phylogenetic relationships. We conclude that the two groups represent distinct species, although they have long been considered as two infraspecific populations within *F. graminearum* because of their morphological similarity. The present study illustrates the importance of combining precise morphological data and phylogenetic analyses to discover and characterize species of *Fusarium* (O'Donnell, 1996; Aoki and O'Donnell, 1999).

In our mating experiments (Fig. 1), ten out of 18 test strains mated and seven fertile pairs among all possible 153 combinations were found. Only about half of the strains examined were inter-fertile. These fertile pairs were classified into three inter-fertile strain groups. Strains that originated from different geographic regions often formed fertile pairs, i.e., Australia vs. South Africa or Australia vs. North America. However, two out of seven fertile combinations were crosses between Australian strains. In one pairing, NRRL 28065 (from South Africa) × NRRL 28069 (from Morocco, northern Africa), only 5 and 6 immature perithecia without asci and ascospores were formed. Francis and Burgess (1977) suggested that members of Group 1 are heterothallic and/or poorly fertile or infertile, after they conducted pairings experiments among ca. ten strains from Australia. Low incidence of fertile pairings in our study was also observed. While it is unclear why strains originating from geographically distant areas appear to be more fertile, further studies on *F. pseudograminearum*/*G. coronicola* should elucidate fundamental aspects of its heterothallic mating system.

Gibberella coronicola is differentiated primarily from the other species and varieties of *Gibberella* (see above) by the length of its ascospores. Ascospores of *G. coronicola* were longer than those of the other species based on its ranges or mean values, except for those of *G. intricans* Wollenw., *G. pulicaris* (Fries) Sacc. and *G. pulicaris* var. *minor* Wollenw. where ascospore width also exhibited overlapping values. Although ascospores of *G. coronicola* were similar in size to those of *G.*

intricans, *G. pulicaris* and *G. pulicaris* var. *minor*, *F. pseudograminearum* is morphologically distinct from their anamorphs.

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