REPRODUCTION AND BREEDING IN A DIOECIOUS BLUEGRASS, *Poa confinis* VASEY

by

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ABSTRACT

Dune bluegrass, Poa confinis Vasey, is a dioecious grass native to the Pacific Coast of North America. It has a very restricted habitat, being confined almost entirely to the semi-stabilized, porous sand areas of the coast. Herbarium studies, and first-hand observations of a number of P. confinis sites indicate that it is a very uniform species.

Under cultivation on The University of British Columbia farm P. confinis grows vigorously and forms a thick sod. Its fine-leaved growth, rhizomatous root system and rapidly-spreading habit, together with the sandy nature of its native habitat, all suggest that it may find use as a turf species for sandy golf courses and similar areas along the coast.

A survey of the literature on reproduction in the bluegrasses reveals that apomixis is widespread in Poa, and that apospory or diplospory followed by pseudogamous embryo development is the usual form of apomictic reproduction. Breeding procedures with apomictic bluegrasses muct be considerably modified, but the standard techniques of improvement are still theoretically available to the bluegrass breeder.

A cytological study of embryo sac development

in pistillate plants indicates that it follows the "normal" scheme, and that reproduction is sexual. Each ovule contains a single EMC which undergoes a regular meiotic division, giving rise to a triad or tetrad of megaspores, one of which forms the haploid embryo sac. Somatic chromosome numbers of 2n = 42 are found in two EMCs in diakinesis. The presence of twin embryo sacs is observed in two ovules. Very marked antipodal development, with an increase in size and number of cells, and in the number of nuclei per cell, is characteristic of the mature female gametophyte.

Microsporogenesis in the staminate plants also appears "normal". Studies of anthers in pistillate florets and ovules in staminate florets show that their development proceeds normally up to a certain point and then breaks down.

Embryo and endosperm development can be seen in sections of ovules prepared after the pollination of P. confinis by P. pratensis. The embryos are believed to be the product of true hybridization. Seed forms after all the crossings among P. confinis, P. macrantha, P. pratensis and P. compressa using the first two species as female parents, but the seed invariably shrivels shortly before reaching maturity.

The excision of hybrid embryos from seeds formed after some of the interspecific crosses, and the culture

of these embryos on agar media have been successfully carried out. Tissues of putative *macrantha* x *confinis* hybrids are growing, and showing some differentiation.

The results indicate that hybrids between *P. confinis* and other bluegrasses can probably be obtained through embryo culture techniques.

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I INTRODUCTION

Many grasses need only to be given study to demonstrate their usefulness to man. Although grass is one of the world's greatest natural resources, until relatively recent times very little has been done to conserve, improve, replenish or in many instances even make any use of more than a small proportion of the total number of grass species. The cereals and sugar-supplying species have been cultivated and improved by man for centuries, and the cultivated and range forage grasses are now receiving considerable attention. Nonetheless, the potentialities of a vast number of the 4,000 or more grass species are virtually unknown.

There are some species of *Poa*, the bluegrasses, among these little-known grasses, although the members of this genus are probably more widely known than those belonging to many other grass genera. Part of their recognition is due to the particular importance of a few of the individual *Poa* species, especially *P. pratensis**, Kentucky bluegrass, but many other bluegrasses are also of great value. The challenge of the difficult taxonomy

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* Full botanical nomenclature for all species named appears in Appendix.

of Poa has resulted in much study on the individual species, subspecies and forms in attempts to determine relationships and evolutionary patterns within the genus. The genus is of added interest because of its cosmopolitan character.

Although primarily grasses of the temperate regions of the world, bluegrasses are found on every continent and in a wide variety of habitats. Different bluegrasses thrive in arctic, antarctic, and alpine habitats, in woodlands, meadows, marshes and arid plains, and on rocky seashores and coastal dunes. The variety of form within Poa is also considerable: annuals and perennials, rhizomatous, stoloniferous, and bunchgrass types, some as tall as a man and others only a few inches in height--all are found among the bluegrasses.

Among the Poa species about which very little is known is Poa confinis or dune bluegrass, a small, dioecious species native to the sandy meadows and dune areas of the Pacific Coast of North America. Established in trial plots on The University of British Columbia farm several years ago, a biotype of P. confinis originating from Vancouver Island has shown several characteristics which merit particular attention. Its very fine-leaved growth and its strong, rapidly-spreading rhizomatous root system, together with the nature of its native habitat, have all suggested that it might prove of value as a turf

species for sandy golf courses, parks and similar locations along the coast. A further possibility is that some of the more desirable features of P. confinis might be combined, through interspecific hybridization, with those of other bluegrasses, particularly P. pratensis and P. compressa.

The fact that dune bluegrass is a dioecious species is of added interest, as scant attention seems to have been paid to dioecism in grasses. No less than eight North American bluegrasses are dioecious, yet little is known of the reproductive features or other characteristics of most of them.

The discovery during the last two decades of the widespread occurrence of apomictic seed formation among the Poa species has aroused a great deal of interest among plant breeders and is proving to be of great significance. New opportunities for intra- and interspecific hybridization have been opened up, and the development of almost limitless numbers of new Poa forms now seems potentially possible. But the presence of apomixis greatly modifies the standard plant breeding procedures; for this reason it is essential to establish the method of reproduction in plants with which breeding work is being contemplated and which are related to known apomictic forms. With this object in mind, a detailed cytological study of

reproduction in P. confinis was undertaken. This study was complemented by attempts at hybridization between P. confinis and other Poa species.

II THE SYSTEMATICS OF THE BLUEGRASSES, WITH PARTICULAR REFERENCE TO *Poa confinis*

A. Tribal, generic, and specific relationships

The tribe Festuceae, to which the genus *Poa* belongs, ranks with the tribes Hordeae, Aveneae, Oryzeae and Andropogoneae as a most important economic group of the Gramineae. *Festuca*, *Bromus*, *Dactylis*, *Eragrostis* and *Koeleria* are others of importance among the eighty-three genera within the tribe (89).

The tribe is distinguished from the other ten tribes recognized by Hackel (49) and Rendle (89) by the following combined characters:

> Culms herbaceous, annual; leaf blade sessile, not jointed to the sheath; the spikelets upon distinct pedicels and arranged in panicles or racemes; spikelets containing two or more florets; the flowering glumes generally longer than the empty glumes, and unawned or with a straight awn from the point; the rachilla jointed above the empty glumes, which remain after the fruiting glumes have fallen; distinct internodes produced between the florets.

The genus *Poa* itself is described by Hitchcock (54):

> "Spikelets 2- to several-flowered, the rachilla disarticulating above the glumes and between the florets, the uppermost floret reduced or rudimentary; glumes acute, keeled, somewhat unequal, the first usually 1-nerved, the second usually 3-nerved; lemmas somewhat keeled, acute or acutish, rarely obtuse, awnless, membranaceous, often somewhat scarious at the summit, 5-nerved (intermediate nerves, that is,

> the pair between the keel and the marginal nerves, rarely obsolete), the nerves sometimes pubescent. Low or rather tall slender annuals or usually perennials with spikelets in open or contracted panicles, the relatively narrow blades flat, folded, or involute, ending in a boat-shaped tip."

Hitchcock (54) divided the 60-odd Poa species found in the United States into seven sections: the Annuae, Pratenses, Palustres, Alpinae, Epiles, Scabrellae and Nevadenses. These separations he based principally on single characters.

The species within the section Pratenses were defined by Hitchcock (54) as perennial plants from creeping rhizomes, having compressed spikelets, with the glumes and lemmas being keeled. The sixteen species which he placed in this section include six dioecious species: Poa confinis, P. macrantha, P. arachnifera, P. Douglasii, P. rhizomata and P. atropurpurea. The remaining species are P. pratensis, P. compressa, P. curta, P. nervosa, P. Kelloggii, P. laxiflora, P. cuspidata, P. arida, P. glaucifolia and P. arctica. Hitchcock (54) did not actually describe P. rhizomata and P. atropurpurea as dioecious species, but Marsh (68) describes them as such in a more recent taxonomic treatment. Grun (42, 43) has also found that P. nervosa has only abortive anthers over almost its entire range.

P. confinis, P. macrantha, and P. Douglasii are all Pacific Coast sand dune species and have been generally considered to be closely related by other taxonomists (33, 14), since Hitchcock (54) first placed them together within the Pratenses. However, Hartung (53) and Clausen et al. (22) indicate that the so-called "rhizomes" of P. macrantha and P. Douglasii are actually long aerial runners or whip-like stems which bend over and root at the nodes. These have the appearance of rhizomes when buried under sand. According to Hartung (53) these aerial runners are not found in any other North American Poa species.

Beetle (14) considers all three of these dune species to be closely related to P. eminens of coastal northeastern Asia and arctic America and P. labradorica of the coast of Labrador. Marsh (68) says that Poa confinis "should not be confused with any other species" and that its closest relative seems to be P. atropurpurea, a species with a very restricted range in Bear Valley in the San Bernadino Mountains, Southern California.

Marsh (68) has proposed a division Dioecia to include all the completely dioecious species in the United States and southern Canada. In this group he places P. confinis, P. macrantha, P. Douglasii, P. atropurpurea, P. Piperi, P. rhizomata, P. Pringlei, and P. arachnifera.

The description of P. confinis given by Marsh (68) is:

"Perennial; with creeping rhizomes, often 1 m. or more long. Culms 10-35 (usually 15-20) cm. tall, erect, rarely somewhat spreading, glabrous, smooth or striate, leafy to above the middle or nearly to the inflorescence in many small plants. Sheaths 1-3 (usually 2) per culm, glabrous, usually striate; green, tawny or purplish in color; shorter than internodes; closed 1/3 - 1/2 their length. Culm blades 0.5-2.5 cm. long, 1-2 mm. wide, involute, glabrous on dorsal surface, glabrous to scabrous or minutely pubescent on ventral surface. Innovation blades varying in length to as much as 20 cm., often as long or longer than the culms, folded or involute, usually more scabrous or pubescent on ventral surface. Ligules up to 1.5 (2.2) mm. long, obtuse to acute, scabrous on dorsal surface. Panicles erect, contracted, 1-7 (usually 3-4) cm. long, up to 2 (usually 1) cm. wide. Rachis glabrous, its branches usually sparsely scabrous. Plants completely dioecious. Spikelets 2- to 5-flowered, 3-6 (8) mm. long, 2-4 mm. wide. First glume 1- or 3-nerved; keeled, acute 1/2 - 2/3 the length of the 1st floret, about 2/3 the length of the 2nd glume, glabrous or slightly scabrous on keel near tip. Second glume usually about 3 mm. long, 3-nerved, keeled, broadly acute to obtuse, wider than 1st glume, 2/3 - 3/4 the length of 2nd floret, scabrous on upper 1/2 - 1/4 of keel. Lemmas 2-3.5 (4.5) mm. long, acute to obtuse, keeled, glabrous to sparsely and minutely scabrous all over the back, occasionally scantly pubescent on the back, usually a few (often copious) cobwebby hairs present at base of lower florets. Paleas 7/8 as long as lemmas, or in upper florets as long as the lemmas, scabrous on keels. Rachillas glabrous to minutely pubescent. Anthers 1.5-2 mm. long."

The only recorded chromosome count for Poa confinis is that made by Hartung (53), who found 2n = 42, the hexaploid number for Poa.

B. A study of prescribed herbarium specimens of *Poa* species

An examination was made of a number of herbarium specimens of *Poa confinis* in order to check several characteristics of the species against those presented by Marsh (68) (above), and to note any variations in characters that might be related to geographic distribution. Differences were also noted between *P. confinis* and several other *Poa* species, particularly *P. abbreviata*, an arctic species (11, 41, 104) which resembles *P. confinis* quite closely in outward appearance.

The specimens of *P. confinis* examined were borrowed from the University of California, the National Museum of Canada, and The University of British Columbia. These specimens represented collections made in British Columbia, Washington, Oregon and California.

The numbers of the herbarium sheets were:

University of California Herbarium numbers: 563639, 580408, 926146, 801585, 759245, 759092, 759091, 525623, 183532, 60817, 525631, 525624, 187226, 249669, 570515, 570516, 121967, 128074, 603582, 586098.

National Museum of Canada Herbarium numbers: 34712, 34711, 34710, 34709, 34708, 34707, 34706, 161661, 161769.

The University of British Columbia Herbarium number: 20,211.

The following characteristics were noted: Presence or absence of rhizomes; leaf length in relation to culm length; culm length; panicle length; spikelet length; number of florets per spikelet; length of 1st and 2nd glumes, and number of nerves; scabrousness of keel of 1st glume; length of lemma of 1st floret and number of nerves; prominence of web at base of lemma; sex of plant.

In nearly all features the specimens examined were essentially the same as described by Marsh (68), although minor differences were noted.

All plants had strongly developed rhizomes. Leaf length in relation to culm length, culm length itself, and panicle length were much as described by Marsh, although the panicles were more often 3 cms. or less in length, somewhat shorter than his figure. Slight differences in several other features were noted: the first glume was never found to be 1-nerved, and was occasionally faintly 5-nerved; the second glume was also occasionally 5-nerved, but usually 3-nerved like the first glume. The scabrousness of the keel of the first glume was somewhat variable, but was usually quite evident at the tip of the

glume. The web at the base of the lemma seemed a very variable character, in agreement with Marsh's description; in a few cases it was almost entirely lacking, in others it was very pronounced, but intermediate types predominated. Of the 31 specimens examined 18 were pistillate plants and 13 were staminate; in almost all florets examined the rudimentary sex organs of the opposite sex could be seen, and there was no instance observed of the strong development of both sets of sex organs in a single floret or a single plant.

On the whole, the plants examined appeared to be remarkably uniform, and the variations that did exist in some characters were totally unrelated to the north-south distribution of the species along the Pacific Coast.

The specimens of P. abbreviata examined differed markedly from P. confinis in several features. All were hermaphroditic and none had rhizomes. The equal length of the 1st and 2nd glumes, pubescence of the lemmas, and copious web of all plants studied were distinctive. The plants were generally less than 10 cms. tall. All the specimens of P. abbreviata represented collections made in arctic regions.

No other coastal species examined bore any close resemblance to P. confinis.

III THE DISTRIBUTION AND ECOLOGY OF *Poa confinis*, AND SOME GENERAL COMMENTS ON ITS INTRODUCTION TO CULTIVATION

A. Distribution

Poa confinis is limited entirely to the Pacific Coast of North America (54). Although it is listed in a number of floras as being found as far north as Alaska (54, 57, 104), no specimens have been seen by the writer from farther north than Ucluelet, on the West Coast of Vancouver Island, and Comox, on the eastern side. Hultén, in his flora of Alaska and the Yukon (56) says that he did not see any Alaskan specimens of *P. confinis*. Collections from the northern coastal regions of British Columbia are rather infrequent, and the species may exist considerably farther north than Vancouver Island. However, at the moment the northernmost limit of *P. confinis* remains unknown.

Howell (55) has found *P. confinis* as far south as Point Reyes Peninsula, California, just north of the Golden Gate, although he states that on recent occasions he has been unable to find it again in this location. It certainly occurs frequently farther north in Mendocino and Humboldt Counties, California, and many collections have also been made along the Oregon and Washington coasts.

Collections in British Columbia have been made at a number of points, including the west and east coasts of Vancouver Island, the Gulf Islands and Boundary Bay.

B. Ecology

The restricted habitat of Poa confinis is one of its most distinctive features, making it an extremely interesting subject for ecological study. Throughout its distribution range it is confined almost completely to sand dunes, sandy meadows, spits and flats along the ocean coast. Practically every herbarium sheet of dune bluegrass examined has borne a note emphasizing the sandy nature of its collection site, and certainly all sites seen at first hand have been predominantly sandy areas.

The ecology of coastal dune areas, particularly of Great Britain, has been thoroughly examined by Salisbury (91), and the writer is not equipped to extend the knowledge of dune species and their environment. However, many of the sites visited merit some descriptive study because of their rather specialized nature and because the total area of such localities in the world is probably not very great. The descriptions provided here should be regarded as casual observations rather than attempts to present accurate ecological data.

Poa confinis was found at the following locations during the course of the study: on Vancouver Island at Whiffin Spit, Sooke Harbour; Witty's Lagoon, Metchosin; Island View Beach, Saanichton; Englishman's River "delta," Parksville; and French Creek, Qualicum. It was also located on the north-west spit on James Island, just off Sidney, Vancouver Island. In the United States *P. confinis* was found on the western Washington coast at several places around Gray's Harbour and Point Chehalis, and also at Point Roberts, Boundary Bay. Some of these sites were studied in somewhat more detail than others.

At Parksville, *P. confinis* grows both on the raised beach or flat "delta" area of Englishman's River, in very sandy soil, and also in almost pure sand just above the high water mark (Figs. 1, 2 and 3).

Figure 1: Parksville, V. I. The dried grass on the bank above the littoral area is mostly *P. confinis* and the taller *P. pratensis*.

In the former area, behind the littoral area itself, the *P. confinis* grows in close association with *P. pratensis* and *P. compressa* and a number of other grasses and broad-leaved species under a scattered cover of low-growing, shrubby *Rosa gymnocarpa*.

Figure 2: Parksville, V. I. Scattered tufts of *P. confinis* can be seen in the open sand in the foreground, just above the upper limit of the driftwood area. The shrub is *Grindelia squarrosa*.

Figure 3: Parksville, V. I. Close-up of the *P. confinis* which can be seen in the foreground in Figure 2.

In this area the dune bluegrass is a common though not dominant species and does not appear to be competing strongly with the associated plants. On the low bank just above the littoral area (Fig. 1) the P. confinis is more evident and seems to be effectively holding the sandy soil along parts of the bank edge, in conjunction with the taller P. pratensis. All the dune bluegrass is characterized by a strong rhizome system which connects the more or less scattered tufts of grass and acts as a sand-binding agent. On the beach itself, just behind the driftwood logs at the high tide line (Figs. 2 and 3), the tufts are somewhat more scattered, although still connected by the rhizomatous stems.

At French Creek, Qualicum, only a few scattered plants of dune bluegrass occur in the raised beach sand area close to a short road leading to the shore. This area is considerably disturbed. A few Canada bluegrass plants are also scattered in the beach sand.

At Witty's Lagoon P. confinis grows on the sandy spit which separates the lagoon from the ocean. It is found on the exposed areas of sand among the broom (Cytisus) bushes which dominate much of the central ridge of the spit. The dune bluegrass is absent from the areas where a number of grasses (including P. pratensis and P. compressa) and broad-leaved plants have formed a more or less

solid cover over the sandy soil. The whole area is subject to heavy grazing by cattle, sheep and horses.

On James Island and Island View Beach Spit P. confinis is again found in open, porous sand just behind the littoral area. The larger P. macrantha is well established on Island View Beach Spit, along with the P. confinis, P. pratensis, and other plants. A small area of P. macrantha is also present beside the roadway on the north-west spit, James Island, in open sand.

On the large, drifting expanse of sand dunes at Twin Harbours State Park, by Gray's Harbour, Washington, P. macrantha and a coarse Ammophila species are the dominant plants (Fig. 4).

Figure 4: Dune area of Twin Harbours State Park, Gray's Harbour, Wash., where P. macrantha and a species of Ammophila are the dominant grasses.

Poa confinis is not found here on the open, drifting sand, but occurs in small patches in the more stabilized area which shows in the foreground in Fig. 4. A closeup of this well-stabilized portion of the sand dunes (Fig. 5), shows mostly the coarse bunches of P. macrantha, with the low-growing broad-leaved cover in between the bunches.

Figure 5: Closeup of portion of foreground in Fig. 4 showing large tufts of P. macrantha. Some P. confinis is also found in this stabilized area.

Poa confinis is also found in several places behind the actual dune or beach area, in sandy semi-stabilized banks along the roadways and in one instance in a flat, sandy vacant lot (Fig. 6). However, none of these locations are more than a few hundred yards from the dune area. P. confinis and P. pratensis occur in close

association on one sandy bank just behind the dunes, close to the Point Chehalis lighthouse.

Figure 6: Small tufts of P. confinis growing in a sandy vacant lot near Point Chehalis, Gray's Harbour.

The only site visited when P. confinis plants were in flower was at Point Roberts, Boundary Bay. Here, large areas of P. confinis are found on the slopes of the sand banks at the back of the raised beach area. On these sloping banks the dune bluegrass receives little competition from other plants and sometimes forms extensive patches twenty to thirty feet in diameter. The plants in these patches are connected by the characteristic rhizome system, and each patch seems to be composed of either all female or all male plants.

At none of the sites visited during the study is there any sign of seedling plants of dune bluegrass.

As perhaps the specific epithet confinis implies, dune bluegrass exists in a very circumscribed habitat. The general impression gained from the study of the various sites is that P. confinis is adapted particularly to the semi-stabilized, porous sand areas which often lie behind the littoral zone along the Pacific Coast. It is not often found in the more seaward locations--the pebble or sand beaches which must often be subject to wind-blown salt spray--where the Lathyrus (beach pea), Glaux and Elymus species frequently flourish. Nor is P. confinis ever seen on the muddy flats or saltmarsh areas where the Salicornia, Distichlis, Atriplex and Puccinellia halophytes thrive. It does sometimes occur on the more stabilized sandy flats where a heavier cover of other grasses and legumes has formed, but even in these areas it does not seem to be well-adapted. The narrow confines of the dune bluegrass habitat are rather unique, as almost all the other species of higher plants with which P. confinis can be found associated possess much wider ranges of adaptation.

C. Introduction to cultivation

The P. confinis material on which most of the cytological and hybridizational studies were carried out came originally from Whiffin Spit, Sooke Harbour, V. I., where Dr. V. C. Brink made a collection of a few plants in the summer of 1948. These plants were grown in a single row on the University of British Columbia farm and showed great vigour and spreading capacity. Unfortunately, detailed notes were not kept on the grass at this time, and it is not known whether any male plants were among the original group. However, a number of these plants gave a heavy seed set following their establishment on the farm. This seed was used to sow several metre square plots in a bluegrass nursery close to the original row of plants.

The growth and vigour of the P. confinis plants in the metre square plots was again good. However, no note was taken of their composition with respect to the proportion of male and female plants, although it is known that a good seed set was obtained on at least some of the plants.

When the bluegrass nursery had to be abandoned, the dune bluegrass was perpetuated through the establishment of a new plot by clonal propagation from the metre

square plots. Rhizomes were spread and buried under a layer of soil in a 6 x 12 foot plot. As far as is now known, the rhizomes used for the establishment of this new plot were taken from a fairly large portion of the metre square plantings. Therefore, the plants in the 6 x 12 foot plot are presumed to have been reasonably representative of the earlier material established from seed. However, when these plants first flowered after the writer undertook the dune bluegrass study, in May 1954, it was found that the entire plot contained only female plants. This was confirmed when the plants again flowered in May 1955.

P. confinis has done exceptionally well on the University farm, both in the solid plantings and in single row plantings. The fine-leaved growth is very attractive, and the plants in the single-row spacings, particularly, have shown an extremely rapid spread by means of the creeping rhizomes. The solid planting in the 6 x 12 foot plot shows a very thick, rhizomatous sod formation.

The dune bluegrass on the University farm did not begin growth in the spring until the middle of April in the two years during which the writer made observations. However, the inflorescences appeared very rapidly after growth began, and little vegetative growth preceded the

emergence of the panicles. In the first year of observation (1954) the panicles emerged on May 2nd, and flowered about two weeks later; the following year flowering took place almost two weeks later, due to the late spring.

In both years there were very few other bluegrasses flowering as early as P. confinis, except annual bluegrass, P. annua. In May 1955 a row of P. alpina plants flowered at approximately the same time as P. confinis, but at several hundred feet distant from the nearest plants of the latter species. Kentucky bluegrass in the nursery, and growing in nearby uncultivated areas, flowered a few days after P. confinis had finished flowering; Canada bluegrass flowered considerably later.

The writer has not found any seeds formed on the dune bluegrass plants growing on the University farm. Bagged plants have set no seed.

A number of seed collections were made from P. confinis plants and from other bluegrasses at the various sites along the coast which were visited during the study. Seed from these collections was sowed in flats of sand in the greenhouse in December 1954. There was no obvious sign of any hybrids among the thousands of seedlings from the various collections, although this feature could not be checked too closely and all seedlings could not be saved. In February, seedlings from each collection

were transferred to very light, sterilized soil in a greenhouse bench. Most of these seedlings were planted in spaced rows in the University nursery in May.

Poa confinis from the following places is now represented in the nursery: Whiffin Spit (original material); Island View Beach Spit; Witty's Lagoon; Parksville; Twin Harbours State Park; Point Roberts.

IV REPRODUCTION IN THE BLUEGRASSES

A. A general statement on literature review

The literature on apomictic reproduction in both the lower and higher plants has reached substantial proportions since the first observation of seed formation without fertilization was made in the middle of the last century (44). A comprehensive collation of observations was not undertaken until 1930, when Rosenberg (90) published a summary of the literature on apomixis. Stebbins (96), for the higher plants, extended and brought up to date the Rosenberg summary, and emphasized that the apomictic processes were more than "freak" phenomena. Gradually, the very general occurrence and significance of apomixis was realized, and in some respects an expression of this was the publication of Gustafsson's (44, 45, 46) monograph on apomixis in the angiosperms. Most of the more recent findings have been reviewed by Nygren (83).

A large literature on reproduction in Poa has developed because of the common occurrence of apomixis in this genus, and because of the wide distribution and economic importance of the Poa species. Much of this literature has been reviewed here; nonetheless, many of the papers read have not been cited, while a few papers

listed are citations of other authors which the writer was unable to read.

B. The terminology of the apomictic phenomena

One of the difficult problems confronting the student of apomixis is that of terminology. As Gustafsson (44) has pointed out, the terminology may appear unnecessarily complicated because of the number of terms that have been used in descriptions of apomictic phenomena by various authors. Furthermore, there have been differences of opinion over the correct usage of some of these terms. The complexity of the phenomena themselves, their radical departure from those encountered in sexually-reproducing plants, and the failure of early workers to distinguish clearly between the sporophytic and gametophytic generations in some apomictic species have also been advanced as reasons for the difficulties over terminology (44, 97). A certain stabilization of terminology seems to be recognizable now in the literature, although different systems and definitions of terms still occur.

The system of terminology given by Gustafsson (44, 48) is the one used here and the one now adopted by the majority of writers. This system has been based, in turn, on that of Winkler (106, 108, 109) and Edman (35). Winkler emphasized particularly that two of the most significant points in the normal sexual cycle, meiosis and

fertilization, are completely separated by the gametophyte generation, and are therefore likely influenced by entirely different environmental and genetic factors (107). As summarized by Stebbins (97), "Every harmonious apomictic cycle must therefore provide either a single substitute for both of these processes or a separate substitute for each of them, with coordination of these two substitutes."

All plants may first of all be grouped into two main classes according to their mode of reproduction. Those in which sexual differentiation and fertilization have not yet been evolved can be described as amictic. Those which have differentiated sexually may be termed mictic or amphimictic. Within the latter group there is a further subgroup of plants whose reproduction does not involve fertilization and are termed apomictic. Thus, ". . . amixis is a primitive stage, apomixis a derived stage in which the fertilizing mechanism once acquired has again been lost." (44).

According to these definitions, vegetative reproduction by means of bulbils, bulbs, rhizomes, etc. can be considered as apomictic reproduction when the sexual process is non-functional or greatly suppressed. Thus, vivipary, in which vegetative propagules arise in the inflorescences of plants, replacing or reducing staminal and ovular development, is sometimes included as

a form of apomixis. However, apomixis has commonly come to have a more restricted meaning, synonymous with agamospermy, or seed formation without fertilization.

The normal sexual cycle in higher plants includes an alternation of a sporophytic and a much reduced gametophytic generation. In certain agamospermous species the gametophytic generation is omitted completely and the embryo develops directly from somatic cells in the ovule of the parent sporophyte as integumentary or nucellar outgrowths. This type of agamospermy is termed adventitious embryony, and like vegetative reproduction it is an example of a single process substituting for both meiosis and fertilization in the life cycle.

In those agamospermous species which still maintain a complete alternation of generations the gametophyte can originate in one of two fundamental ways. The gametophyte can develop by diplospory from a true archesporial cell (macrospore mother cell), or it can develop by apospory from a somatic cell of the nucellus or chalaza. Whichever of these two general schemes of development is followed, the gametophyte receives the unreduced chromosome number. The mature embryo sac is usually in all other respects perfectly normal, with three antipodals, two polar nuclei, two synergids and an egg cell. However, as happens in some sexual species, exceptions may occur in the number of cells present in the mature embryo sac.

Diplosporous and aposporous gametophyte development can be quite easily differentiated cytologically in species having a unicellular archespore, as in Poa. In species with a multicellular archespore, which may develop a number of EMCs*, it is often hard to determine whether the embryo sac initial cell actually originates in archesporial or somatic tissue. Transitions ". . . between ordinary EMCs, potential EMCs and somatic cells . . . ." also seem to occur, complicating the situation (44).

Fagerlind's (38, 39) terminology concerning diplospory and apospory should be mentioned here because it is still followed by some authors. This scheme is based on the character of the first nuclear division in the embryo sac initial cell as well as on the origin of this cell. The development of the apomictic gametophyte is subdivided into (a) Diplospory (b) Semi-apospory (c) Generative apospory and (d) Somatic apospory. In diplospory a meiotic division in the EMC is followed by the formation of a restitution nucleus; in semi-apospory the EMC division is pseudohomeotypic**; in generative

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* EMC = embryo sac mother cell, PMC = pollen mother cell.

** In a pseudohomeotypic division, according to Gustafsson (47), who proposed the term, the EMC undergoes the start of a meiotic prophase but bivalents are not formed. Instead, the chromosomes are gathered as univalents at the central plate and split into chromatids. In this way, the division is completed mitotically and two identical daughter cells are formed.

apospory the EMC division is mitotic. The net result of any of these modifications of normal EMC behaviour is the formation of an embryo sac with the somatic chromosome number. The three terms are thus collectively equivalent to diplospory in Gustafsson's (44, 48) sense of the word. In somatic apospory the embryo sac initial cell is not an EMC, and is therefore the same as Gustafsson's (44, 48) apospory.

Meiosis having been circumvented by diplosporous or aposporous embryo sac development, the subsequent formation of an embryo without fertilization can be achieved in one of two ways. Such an embryo can develop from the egg cell, a process termed parthenogenesis, or from one of the vegetative cells of the embryo sac by apogamety. Although common in the pteridophytes, apogamety is apparently rare in higher plants (44, 98).

In some plants developing apomictic embryos, pollination is still required for seed development, usually because a fertilization of the fusion nucleus is necessary to produce the endosperm, even though the other half of double fertilization is omitted. In other plants pollination per se may merely provide a stimulation for apomictic development. Pseudogamy is a collective term employed for any apomictic seed formation for which pollination is still necessary. In other apomicts the

seed formation may be autonomous (44).

In sexually-reproducing plants an egg cell sometimes develops without fertilization, forming an embryo with the reduced chromosome number (i.e. a haploid). This has been termed haplo-parthenogenesis to distinguish it from parthenogenesis or diplo-parthenogenesis as it occurs in diplosporous or aposporous apomicts. Haploid plants produced by haplo-parthenogenesis will deviate morphologically from the parent plant and may show a wide range of variation, in contrast to the uniformity of progenies arising by adventitious embryony, diplospory or apospory.

The terminology on apomictic seed formation can be adequately summarized by stating that there are three principal processes concerned (44):

(1) Diplospory, followed by parthenogenesis or apogamety.
(2) Apospory, followed by parthenogenesis or apogamety.
(3) Adventitious embryony.

Stebbins (97) has suggested gametophytic apomixis as a collective term for the first two types of agamospermy, which emphasizes the fact that a gametophytic generation is still maintained in the life cycle.

C. Reproduction in individual *Poa* species

Poa pratensis (Kentucky bluegrass)

Being the most important and widespread member of its genus, *Poa pratensis* has received more attention than any other *Poa* species. It is an extremely complex and polymorphous species, and with chromosome numbers ranging in aneuploid series from about 2n = 38 - 124 (83). Consequently, the taxonomic treatment of *Poa pratensis* is made difficult. Certain divisions within the species are generally recognized and some taxonomists define these as subspecies and others (65) define them as separate species. The delegation of these taxa to subspecific rank, as followed by Nygren (83), is recognized here simply for convenience in discussion.

Reproduction has been studied in many wild and cultivated biotypes of *P. pratensis* from all over the world, mostly belonging to the subspecies *eupratensis* which embraces the majority of cultivated forms (84). This is the subspecies generally referred to merely as *P. pratensis*, a convention followed in this paper. Very few investigators have referred specifically to the other three subspecies, *alpigena*, *angustifolia* and *irrigata*; a few of the findings in these last subspecies will be mentioned separately later.

Müntzing (69) first reported the presence of agamospermy in the genus, concluding that this was the method of seed formation in certain Swedish biotypes of P. pratensis. He gave as criteria of apomixis:

(1) Extreme uniformity within a biotype.

(2) A constant aneuploid chromosome number for the biotype.

(3) Good seed production, even in plants evidencing great meiotic irregularities.

Rancken (87) used these same criteria in establishing the occurrence of apomictic seed production in Finnish strains of Kentucky bluegrass. Confirmation of apomixis in many biotypes of this species has been made by others, including Åkerberg (3, 6, 7), who was also the first to show that pollination is necessary for seed development in several apomictic strains (7). He found that P. alpina pollen was almost as effective as pratensis pollen in initiating seed development in these apomicts and that the seed thus formed gave rise to plants identical with the maternal pratensis parent. P. glauca and P. compressa pollen were somewhat less effective, but where seed did form this also gave rise to matroclinous offspring. Nilsson (80), Müntzing (71), Åkerberg (1) and Engelbert (37) confirmed this pseudogamous development of seed primordia into seed. Engelbert (37) studied pollen

tube growth on stigmas of P. pratensis and concluded that merely the growth of pollen tubes down the styles acted as a stimulant to seed formation. Åkerberg (7) found four sexual pratensis biotypes among those he investigated.

The uniformity of single plant progenies collected in Maryland was noted by Kemp (58), and in biotypes from Eastern Canada by Armstrong (12). Tinney and Aamodt (101) first used the progeny test as a means of identifying apomictic strains of Kentucky bluegrass. They grew populations of plants originating from open-pollinated seed collected from single plants, and rated the plants for uniformity. Of 102 progenies from single plant clones from widely scattered areas in Europe and North America only two failed to show extreme uniformity, and 48 contained no variants at all. Morphological constancy in pratensis progenies is held by all writers to be proof of apomictic reproduction; conversely, lack of uniformity or the presence of aberrant individuals in otherwise uniform progenies is considered as evidence of sexual reproduction in the parents (15, 16, 17, 21, 25, 26, 27, 80, 85, 72, 93, 94, 100).

Andersen (10) followed the development of the female gametophyte in P. pratensis but saw nothing unusual and concluded that reproduction was normal, although she observed no fertilization of the egg cell. Nishimura (82) observed the formation of embryos of sporophytic

origin by adventitious embryony in conjunction with the development of the normal embryo from the fertilization process. In most cases the normal embryo was supplanted by one or more adventive embryos. Nishimura (82) considered this development to be the result of galls caused by insect attacks. There appears to be no other record of any instances of adventitious embryony occurring in the genus Poa, and Nygren (84) says that Nishimure "misinterpreted" the embryological evidence.

Armstrong (12) attempted to explain the constancy of an aneuploid chromosome number in pratensis biotypes by postulating that only gametes with a certain chromosome number could function in fertilization. The possibility of such a scheme had been previously rejected by Müntzing (69). Åkerberg (1) suggested that the cytological evidence presented in Armstrong's paper did not rule out the possibility of apomictic reproduction.

The first investigator to outline the cytological basis of agamospermy in Kentucky bluegrass was Åkerberg (1). He observed the presence of somatic cells of the nucellus developing as embryo sac initials, thus producing diploid embryo sacs by apospory. Tinney (100) first detailed these developments thoroughly in Wisconsin biotypes. He found that the EMC usually underwent a somewhat irregular meiotic division, giving rise to a triad

or a complete tetrad of macrospores, all of which disintegrated. An aposporous cell of the nucellus or chalaza took over the function of a macrospore and formed a diploid embryo sac. As a rule, he found only one embryo sac per ovule, although he found two in several instances. These he attributed to independent development from separate aposporous cells. The egg cell of the diploid embryo sac functioned parthenogenetically to form a proembryo even before anthesis occurred. He was unable to determine whether endosperm development was associated with pollination.

A detailed study of apospory was also carried out by Kiellander (63). Unlike Tinney (100) and Åkerberg (5) he found two or three aposporous embryo sacs per ovule to be the commonest number, with four or five and even up to seven fairly frequent. Through competition between embryo sacs usually only one or two developed to maturity. The organization of a legitimate (sexual) embryo sac rarely occurred.

Åkerberg (4, 5) and Nielsen (78) have also shown that the embryo develops by parthenogenesis, as established by Tinney (100). Nielsen (78) found 8- to 16-celled proembryos a day before flowering. Åkerberg (5) found that embryo formation occurs regularly even in emasculated florets of apomictic P. pratensis but that seed formation

failed because no endosperm formed. Endosperm development was induced by pollination. In one instance, the first ever recorded in a Poa apomict, Åkerberg (5) was able to show that the central fusion nucleus was fertilized before dividing, forming pentaploid* endosperm. He thus concluded that autonomous embryo development (parthenogenesis) with induced endosperm formation (probably through fertilization) is a general phenomenon in apomictic types of P. pratensis.

Polyembryony, the formation of two or more embryos in a single ovule, is of fairly common occurrence in Kentucky bluegrass. The origins of twin and triplet seedlings have been studied by Andersen (10), Armstrong (12), Åkerberg (1), Tinney (100), Christoff (20), Müntzing (70), Brittingham (16) and Nielsen (75, 79). Armstrong (12) noted differences between apomictic strains in their capacities to form two or more embryos within one seed. Both Åkerberg (1) and Müntzing (70) found the frequency of twins to be lower in sexual than in apomictic biotypes

* The terms "haploid," "diploid," "triploid," "pentaploid," etc. are used in a loose sense in this paper to refer to the chromosome complement present. Since the basic genome number in Poa is 7, a true diploid would have a somatic count of 2n = 14, a triploid 2n = 21 etc. But the extreme range of chromosome numbers in P. pratensis (and other Poa species), and the occurrence of many aneuploid numbers, prevent the effective use of a stricter terminology.

of P. pratensis. This they assumed to be in agreement with the embryological data on Kentucky bluegrass, as normally only one EMC is found and only one macrospore functions in the ovules of the sexual biotypes. In apomictic biotypes, on the other hand, there is a tendency for several aposporous cells to develop in a single ovule, and there is also a tendency for both sexual and aposporous embryo sacs to develop simultaneously in the same ovule. Brittingham's (16) analysis of the progenies of 115 open-pollinated Kentucky bluegrass plants indicated a significant (although only barely so) negative correlation between polyembryony and variability (variability being a criterion of sexual reproduction). Nielsen (79) has summarized his own findings and those of previous authors in respect of the origin of twin and triplet plants in this species. Development of embryos from two or more sexual embryo sacs arising from the same or different EMCs is possible; development from two members of the egg apparatus of a sexual or aposporous sac (e.g. normal and synergid embryos) is probably uncommon; development from a sexual and an aposporous sac, or from two or more aposporous sacs seems to be the most common cause of polyembryony. Haploid-diploid, diploid-diploid, and diploid-triploid relationships have been found in twins

depending upon the origin of the embryos and whether fertilization was effected before their development (79).

The formation of haploid plants by haploparthenogenesis occurs often in Poa pratensis (8).

Åkerberg (1), Brittingham (17), and Tinney and Aamodt (101), among others, have reported different degrees of apomictic seed formation in the biotypes with which they worked, based on the frequency of morphological aberrants. Although apomictic, semi-apomictic and sexual types all occur within the species, the apomictic types are the most frequent (8). The frequency of sexual Kentucky bluegrass biotypes in nature is quite low (6).

Most of the findings in the subspecies eupratensis seem to be applicable to the other three subspecies. Apospory occurs in subsp. alpigena (84), subsp. angustifolia (4) and subsp. irrigata (65). A viviparous form of subsp. alpigena occurs in northern Scandanavia and has been investigated by Nygren (84). It produces good pollen, and aposporous cells are developed in the ovules; however, these cells rarely produce mature embryo sacs and the viviparous propagules almost invariably function in the reproduction of the plants, although it may be possible for some seed to form. The Icelandic form of subsp. irrigata studied by Löve (65) apparently never forms sexual embryo sacs,

although hybridization is still possible through the fertilization of the diploid, aposporous egg cell.

Poa compressa (Canada bluegrass)

Poa compressa is found almost throughout the North American continent, having much the same range as P. pratensis, although it is characteristically found on somewhat poorer soils than the latter species. Like Kentucky bluegrass, it is a rhizomatous perennial used primarily as a pasture species. It also finds some usefulness as a lawn grass. It is of less importance in Europe than on this continent (40, 54, 92).

Canada bluegrass has nothing like the variability in morphology and chromosome number of Kentucky bluegrass. The range in 2n chromosome number is almost a euploid series--numbers of 35, 42, 49, 50 and 56 being known (83).

Andersen (10) investigated female gametophyte development in P. compressa but concluded that reproduction was sexual. Apomixis in this species was first demonstrated by Brittingham (15), who pollinated heat-emasculated florets of P. compressa with P. pratensis pollen. He obtained only one hybrid among the progeny, the remainder being maternal-type plants developed through pseudogamy. Christoff (19) showed that aposporous

development was involved, as in P. pratensis, and, through chromosome counts in endosperm tissue, proved that the fusion nucleus requires fertilization for apomictic seed formation.

Poa arctica (Arctic bluegrass)

Poa arctica, like P. pratensis, is a form complex, with a range of 2n chromosome numbers running from about 38 to 100 (83). As its name implies, it has a circumboreal and alpine distribution. On this continent and in Europe it is found usually above timberline in mountainous regions (54). It is a rhizomatous perennial.

It has been divided by some of the Scandanavian taxonomists into a number of subspecies (83, 84); however, some of the earlier studies on reproduction in P. arctica were carried out before such divisions of the species were defined. Flovik (41) concluded that seed formation was probably apomictic in the forms which he examined, as he noted many meiotic irregularities in both EMCs and PMCs, yet seed setting was very good and the percentage of good pollen high. On the basis of her emasculation, interspecific cross-pollination and pollen germination studies on western Greenland P. arctica plants, Engelbert (37) concluded that seed formation was pseudogamous. She also showed that no endosperm developed until emasculated florets were pollinated, although embryo formation was

autonomous (36, 37). Although she made no chromosome counts she said that germination of the pollen on the stigmas stimulated the development of endosperm, the pollen tube functioning as an activator and "not in a genetic capacity" (37).

Nygren (84) has found that the subspecies caespitans, depauperata, elongata and microglumis are all aposporous. In caespitans no pollen is formed (74, 84), and it is considered by Nygren to be a non-pseudogamous apomict. Some sexual as well as aposporous embryo sacs arise, and the fusion nuclei of both types are occasionally fertilized by foreign Poa pollen, as evidenced by the occurrence of both triploid and pentaploid endosperm; however, most counts in endosperm nuclei reveal tetraploid numbers, indicating that the majority of embryo sacs are aposporous and that no fertilization is required in these for endosperm formation. Nygren (84) cites this as a "rare and interesting case."

In the subspecies depauperata and elongata the presence of only aposporous embryo sacs, together with the very early autonomous division of the egg cells in these, and the low percentage of good pollen produced, exclude most chances of fertilization. "This mechanism explains why the two actual subspecies have not been split up in a manifold of forms as is the rule in Poa arctica."

(84). In the subspecies microglumis, on the other hand, the aposporous egg cell sometimes divides late, the time of division apparently varying according to the point when the polar nuclei fuse. This, together with a high percentage of good pollen formation, indicates that microglumis can probably be involved fairly readily as either the female or male parent in hybridizations with other Poa populations (84).

The subspecies stricta is viviparous, and has been investigated by Flovik (41), Nannfeldt (74) and Nygren (84). It is obligately viviporous, and is not able to produce either pollen or mature female gametophytes.

Poa alpina (Alpine bluegrass)

Poa alpina, although not cultivated, has some value as a range species in alpine meadows and arctic regions of North America and northern Europe (54).

Sexual, apomictic (agamospermous), and viviparous biotypes of P. alpina exist. A series of 2n chromosome numbers ranging from 14 to 57 are found in these strains (83).

At the same time as he discovered apomixis in Swedish P. pratensis biotypes, Müntzing (69) also determined apomixis in Swedish P. alpina forms, using the same criteria of apomictic seed formation. On the other hand, he found certain Swiss P. alpina types to be sexual. Pseudogamous seed formation was shown by Engelbert (37), who

concluded that stimulation of both endosperm and embryo development was involved, without any fertilization of either egg or fusion nucleus.

Müntzing (70) established that diplospory was the basis of apomictic seed formation, the EMC developing through successive mitotic divisions directly into a morphologically normal, although diploid, embryo sac (i.e. generative apospory under Fagerlind's terminology). Håkansson (51) studied the embryology of both sexual and apomictic strains of P. alpina in more detail and confirmed Muntzing's findings. He also showed fertilization of the fusion nucleus in two apomictic strains, resulting in the formation of pentaploid endosperm, in contradiction to Engelbert's (37) conclusions. An example of apogamety was also found in which a diploid, synergid cell in an apomictic embryo sac developed into an embryo. The production of haploid plants among sexual strains indicated the autonomous development of egg cells in some sexual embryo sacs. In a later paper (52), Håkansson reported the development of both haploid and diploid embryo sacs in apomictic strains. In the diploid sacs the egg cell usually divided autonomously, while fertilization was always necessary before the fusion nucleus could divide in both sexual or apomictic embryo sacs, the endosperm then being triploid or pentaploid, respectively.

Flovik (41) and Nygren (84) have studied viviporous P.alpina forms and found that in spite of many meiotic irregularities in the PMCs a high percentage of good pollen is formed. On the female side, the beginning of diplosporous embryo sac development occurs, but there is no chance for seed to form as the viviporous propagules develop at an early stage in the spikelets and carry on all reproduction (84).

Poa palustris (Fowl bluegrass)

Poa palustris is widespread across the North American continent at low and medium altitudes from Newfoundland to California and as far south as New Mexico. It is occasionally cultivated in meadow mixtures but seldom important agriculturally on this continent (54). In northern Sweden, however, P. palustris is an important cultivated hay and pasture species (81).

Kiellander (60) found both haploid and diploid embryo sacs in the material he examined. He showed this to be due to diplospory through a mitotic division of the EMC directly into an embryo sac, and in one particular plant he found that the EMC divided about as often mitotically as meiotically (62). He concluded that pseudogamy was not very probable in this species, as he saw no pollen tubes in the slide preparations until the egg-cells and endosperm nuclei had divided several times (60).

However, Åkerberg (7) demonstrated pseudogamous development which Kiellander (62) said ". . . must be explained by the pollen tube inducing the development of the embryo at some distance from the egg cell." No further embryological work appears to have been carried on with this species.

Poa glauca

Poa glauca is a tufted perennial, closely related to Poa alpina, which is found in arctic regions and on alpine summits on this continent as far south as New Hampshire and Vermont (54).

Flovik (41) found meiosis to be very irregular in the PMCs of the arctic material he examined; however, the pollen produced was good and the progeny did not show varying chromosome numbers, indicating that apomictic seed formation was probable. Kiellander (cited in 66) made embryological studies and found it to be diplosporous. As in the other diplosporous bluegrasses the diplospory took the form of mitotic divisions in the EMCs. An aneuploid series of chromosome numbers is known for the species.

Poa nemoralis

Poa nemoralis, another tufted perennial, has been introduced onto this continent from Europe and is occasional in meadows across the northern United States (54). It is used to some extent in Sweden as a lawn grass for shaded areas (81). The only reported embryological study is that of

Kiellander (cited in 66), who found it to be diplosporous, with the EMC division again mitotic.

Poa nervosa (Wheeler bluegrass)

Poa nervosa is a rhizomatous species, allied to Kentucky bluegrass, which is found at medium altitudes in western Canada and the United States (54).

Grun (43) found that, "Barring rare exceptions, throughout its range in California and most of its range in Oregon, Washington, Montana, Idaho, Colorado, Utah and Nevada it is female, its flowers containing either no anthers or only aborted anthers." Nevertheless, P. nervosa does contain variant forms. Embryological examination has shown that this grass reproduces apomictically by diplospory, the EMC division being mitotic (42). When P. nervosa plants were put in pollen-proof cages prior to blowing ". . . the seed set was nevertheless normal and the germination of the seeds high," despite the complete absence of pollen (42). The endosperm formed is 4n, resulting from the fusion of a pair of 2n polar nuclei. P. nervosa and P. arctica subsp. caespitans are the only bluegrasses in which this completely autonomous development of both endosperm and embryo has definitely been established. Variation in P. nervosa is believed due to some formation of sexual embryo sacs with chance crossing by pollen from other Poa species (42).

Poa jemtlandica

Poa jemtlandica, believed to be the natural hybrid between P. alpina and P. laxa subsp. flexuosa, is a completely viviparous species having a restricted distribution in central Scandanavia and Scotland (73, 83, 84, 95). According to Nygren (84) it is ". . . absolutely uniform in its whole distribution sphere." It produces little or no viable pollen, and female gametophyte development always fails completely, so that it is probably quite isolated from other Poa populations (84). Although female gametophyte formation is never completed, the EMC development is apparently quite normal up until the time the ovules abort and are superseded by the viviparous propagules (84).

Poa herjedalica

Poa herjedalica is described as the natural hybrid between viviparous P. alpina and P. pratensis subsp. alpigena, and, like P. jemtlandica, is viviparous (73, 83, 84, 95). However, it has a much wider distribution in the Scandanavian mountains than P. jemtlandica, is not found in Scotland, and has a wide range of morphological types forming an aneuploid series (83, 84). All strains examined by Nygren (84) formed good pollen, although in varying percentages, and the species is therefore not isolated from other bluegrasses as is P. jemtlandica.

Although female gametophytes never mature, the presence of aposporous cells in the ovular tissues of several P. herjedalica strains has been reported by Nygren (84).

Poa bulbosa (Bulbous bluegrass)

Poa bulbosa is a perennial species introduced into America. In Sweden and the United States it is completely viviporous, producing bulbils in the florets; stamens or seeds are not formed (44, 59). In England, on the other hand, a sexual form of P. bulbosa is indigenous, and the viviparous form introduced (44). The sexual form has 2n = 28, while the viviparous forms have 2n = 42 or 2n = 56.

Poa ampla (Big bluegrass)

Poa ampla is an important perennial bunchgrass which is widespread on western rangelands of North America and which has been cultivated to a limited extent (40, 54). It has been utilized extensively in the Carnegie Institution of Washington bluegrass breeding program, and a number of the most successful interspecific hybrid strains developed in this program involve P. ampla as one of the parents (21, 83). P. ampla has aposporous seed development (83, 85). In two strains of big bluegrass it has been found that the diploid egg cell remains undivided in the mature embryo sac and can easily be fertilized by pollen grains from the same or other P. ampla plants, or

from other Poa species (85). Nygren (85) states that there was ". . . no difficulty in obtaining hybrids when either of these two ampla strains was used as the female parent."

Poa scabrella (Pine bluegrass)

Poa scabrella is another perennial bunchgrass, found in the north-western States and California at low and medium altitudes (54). It is of some significance as a range species (92).

It is reported by Nygren (83) to be aposporous, although no further information has been published. P. scabrella has been used successfully in a large number of crosses with P. pratensis, P. ampla, P. compressa and several other species in the Carnegie Institution of Washington program (24, 28, 29, 83, 102). Clausen et al. (22) report the use of a "partially sexual" strain in crosses with P. arachnifera.

Poa arachnifera (Texas bluegrass)

Poa arachnifera is a rhizomatous species, valuable as a source of palatable winter forage in the southern Great Plains region of the United States, where it is native (40, 64, 92). So far, however, it has not been widely cultivated because of a webby lemma, ". . . which makes harvesting, processing, or seeding of this species impossible with ordinary machinery." (64).

Texas bluegrass is completely dioecious, and one

of the only dioecious Poa species whose reproduction has been studied. It is sexual, and has been used successfully both as a male and female parent in crosses in the bluegrass breeding program of the Carnegie Institution of Washington (22, 102). It is referred to as viviparous in the table of apomictic plants presented by Nygren (83), although the writer has been unable to find any other record of it being anything but completely sexual. An unusual feature of eight hybrids produced in the Carnegie program from the cross arachnifera x pratensis is that all were morphologically indistinguishable from P. arachnifera, although seven out of eight were shown from chromosome counts to be derived from the fusion of a reduced egg nucleus with a reduced pollen nucleus (102). The origin of the remaining hybrid is not explained.

Despite the apparently sexual mode of reproduction, chromosome numbersof 2n = 42, 54 to 56, 58 and 63 have been reported (18, 53, 22).

Other bluegrasses

A number of bluegrasses which are possibly apomictic, but on which no detailed studies have been made, have been used in the Carnegie Institution of Washington bluegrass breeding program. Clausen et al. (27) suggest that Poa nevadensis is probably apomictic. Successful hybrids have been obtained using P. Canbyi and P. gracillima

but it is not known whether these species are apomictic or sexual. At least one strain of P. caespitosa from New Zealand is sexual and has been hybridized with P. pratensis, P. ampla, P. arachnifera and P. compressa (22, 103).

Several other bluegrasses are known to be sexually reproducing. P. trivialis, which is cultivated to a small extent in Europe, is entirely sexual, and is also one of the few diploid species in the genus (2n = 14) (2, 6, 81). P. laxa subsp. flexuosa is of some interest because it has been found to be sexual and almost completely self-fertilizing. The early development of both embryo sacs and pollen is a "severe obstacle" to cross-fertilization, and self-pollination is already finished when the florets open (84).

V BREEDING IN THE BLUEGRASSES

A. Intraspecific improvement

Müntzing's initial report on apomixis in Poa has been termed by Atwood (13), ". . . a turning point in the cytogenetic and breeding investigations with bluegrass." This is certainly true in respect of interspecific hybridization in the genus, a phase of breeding in which advantage has been taken of the apomictic means of reproduction to perpetuate species hybrids without the loss of desirable combinations through segregation (21). However, apart from improvement through individual plant selection (below) there appear to have been no great strides in intraspecific improvement, despite the fact that the same methods are available to the breeder as have been utilized in interspecific hybridization work (17, 86). Brittingham (17) concluded from his study of reproduction in P. pratensis biotypes that the standard techniques of ". . . selection, strain building, and intra- and interspecific hybridization are theoretically applicable to breeding problems in Kentucky bluegrass."

Single plant selection, or the selection of groups of individual plants, has undoubtedly been the most significant form of improvement, even before the discovery of apomixis in Poa (81, 86). Among apomictic bluegrasses,

which includes the majority of species of agricultural importance, individual plant selection is a sound practice. The most desirable plant types can be perpetuated indefinitely by apomictically-formed seed. Some of the earlier workers assumed that improvement in Poa is limited to individual plant selection. Nilsson-Leissner and Nilsson (81), for instance, stated that because ". . . crosses can be made only in exceptional cases . . . combination breeding is therefore of small importance."

Müntzing (70) successfully produced a number of hybrids between predominantly sexual and predominantly apomictic strains of P. alpina, and found that the hybrids were mostly the result of the fertilization of a reduced egg cell by a reduced male gamete. A few, however, arose from the fusion of an unreduced egg and a reduced male gamete. All the F_1 plants were sexual, as were all the F_2 progenies, leading Müntzing (70) to conclude that, "Apomixis cannot be due to a single gene but rather to special constellations of genes brought about by natural selection." The results from other studies corroborate this view, although as yet there is really very little known of the inheritance of apomixis in Poa. (29). From Müntzing's study (70) it appears that the establishment of apomictic hybrid strains of a species is dependent upon the use of apomictic plants as both male and female parents

in a cross. The techniques of intraspecific hybridization must therefore be essentially like those of interspecific hybridization in Poa, described in the following section.

B. Interspecific hybridization

The first recorded artificial hybridization between Poa species seems to be that made by George W. Oliver in 1908. He crossed a female Poa arachnifera plant with a P. pratensis plant and obtained a highly variable F_1 progeny (105). No further work was done at this time. A repetition of the same cross by E. Marion Brown of the United States Department of Agriculture also gave a variable F_1, and included plants showing greater heat- and drought-resistance and productivity than P. pratensis (105).

Åkerberg (3) produced a progeny of 17 plants from a semi-sterile P. pratensis plant pollinated with P. alpina pollen. Only one of the plants was a hybrid, the remainder being maternal types produced apomictically through pseudogamous development. The hybrid arose from the fertilization of a reduced egg cell by a reduced male gamete. In other hybridizations between the same species Åkerberg obtained hybrids both from the union of reduced and of unreduced egg-nuclei with reduced pollen nuclei, the majority being of the former type (1, 4, 7). These

observations were based on chromosome counts in the parents and hybrids.

Hybrids between P. pratensis and P. alpina showed much more rapid germination than P. pratensis ". . . a character which it would be highly desirable to be able to transmit to the latter, the slow germination of which is frequently a cause of poor establishment." (6). These hybrids also reached the panicle-bearing stage more quickly. A hybrid from the cross P. pratensis x P. glauca was obtained by Åkerberg (4). Kiellander (61) also found one hybrid in the cross P. palustris x P. compressa.

Brittingham (15) produced a single hybrid between a plant of an apomictic strain of P. compressa as the female parent and a P. pratensis plant as pollen parent. On the basis of pollen grain size comparisons in parents and hybrid it was concluded that the hybrid arose from the fusion of an unreduced compressa egg nucleus and a reduced pratensis pollen nucleus. (Müntzing (70) first showed the positive correlation between cell size and chromosome number for P. pratensis). The hybrid appeared to have several desirable qualities, including good vigour, a good spreading habit and larger and heavier (although fewer) panicles than either parent. Seed set was high. The leaves of the hybrid were relatively free from leaf spot and rust, which were severe on the parent plants.

It also seemed somewhat less susceptible to mildew.

Åkerberg and Bingefors (8) record that, "Crosses between sexual pratensis and alpina give normal hybrids and can be easily produced."

Some of the difficulties involved in both intra- and interspecific hybridization between apomictic forms are brought out by Nygren (86), although his comments apply particularly to Poa pratensis. The best available plants will be the ones sought by the breeder as potential parents in a cross, but unfortunately these will often be the most uniform strains as a result of improvement through selection. For P. pratensis, "Embryologically this means that they do not only have a strong tendency to aposporous embryo sac formation, but also that the egg cell in these aposporous sacs divides in such an early stage that a fertilization is more or less excluded. This makes the breeding work very difficult, if such a good strain is wanted as mother in the combination." (86). If the other strain or species to be used in the cross has a tendency to a later egg-cell division it can be used as the female parent; however, this may also be disadvantageous if unreduced egg-cells tend to be fertilized in the formation of hybrids. Thus, if the better strain is used as a male parent, because of its uniformity, it may only contribute

approximately one third of the total chromosome complement of the hybrid. The disadvantage of such an inheritance pattern is obvious; degeneration of the better strain, rather than improvement, may result. Nygren (86) suggests that, "In order to estimate the chances to obtain a special hybrid combination an embryological investigation ought to be made on the planned mother strain." In this way a study of the relation between the number of sexual and apomictic embryo sacs can be made, and the time of division of the egg nuclei in these can be observed. The importance of such a study is also emphasized by Gustafsson's conclusion (44) that ". . . hybrid formation . . . increases in the measure that reduced embryo-sacs arise or that the first division of the egg-cell begins late."

One of the most ambitious and successful breeding programs with the genus Poa in North America has been carried on by the Carnegie Institution of Washington workers at Stanford, California, in cooperation with the Soil Conservation Service of the United States Department of Agriculture. This program, started in 1942-1943, arose out of the growing realization of the depletion of the Western rangelands and of the possibility of restoring to them some of their former productivity with the aid of new, improved bluegrass forms (25, 26).

The bluegrasses, because of the extent to which polyploidy has arisen in them, and because of their predominantly apomictic means of reproduction, were seen to have unique possibilities. The farsighted members of the Carnegie group saw that the bluegrasses are among the groups of plants which have reached an evolutionary level in which ". . . the genome, rather than the gene, becomes the basic evolutionary unit." (25).

One of the basic principles behind the program has been to combine the best strains available of species belonging to different sections of the genus. These remotely related species have also been chosen for their contrasting regions of adaptation. This has meant crossing forms from high altitudes with those from low altitudes, crossing coastal types with interior types, high latitude forms with low latitude forms and so on (25, 26). The reason for such a course is that, as pointed out by Clausen et al. (29), "The probabilities of success in such an endeavour are enhanced by crossing forms that do not occur together in the wild; in those that occur together hybridization and natural selection have presumably already sorted out the best combinations." By combining the genomes of forms from opposite extremes of environment the Carnegie workers have found it possible to produce hybrids adaptable to intermediate conditions.

Other particular considerations have been made in the selection of types to be crossed. One of these has been to combine the greater drought resistance of the bunchgrass with the superior soil-holding capacity of the rhizomatous type and to ". . . utilize the better storage facilities of the rhizome grass as an additional protection against overgrazing and fire." (26). Other objectives have been the lengthening of the season of use by combining winter- and spring-active bunchgrasses with summer-active rhizome grasses, and to breed for more disease-resistant and winter-hardy forms. In many of the hybridizations it has been possible to aim for several objectives at once.

Because the parental species are predominantly apomictic types the percentage of hybrids to be expected in any cross is very low. For this reason a mass-pollination technique is employed whereby the plants to be crossed are grown in pots sunk side by side in the ground. A pollen-tight tent cage, designed for outdoor pollination, is placed over two plants. Pollen is collected daily in parchment bags placed over the inflorescences and reciprocal pollinations made by exchanging the bags and shaking the pollen over the respective inflorescences. (26). The seed from crosses is sown in the greenhouse and the seedlings pricked out into flats and spaced carefully so that

they can be examined individually. The hybrids can be detected when they are two to three months old, in about the 8- to 12-leaf stage. The non-hybrids are then eliminated (27). The frequency of the hybrids varies between about 0.2 and 5 per cent in different combinations. In 1952, Clausen (21) stated that the Carnegie program had resulted in the production of some 700 F_1 hybrids out of about 80,000 seedlings in the 70 attempted crossings up to that time.

Some combinations have been much more successful than others, and many of these have been made several times, using different races. The most successful crosses have been those involving P. ampla, P. scabrella, P. pratensis, P. compressa and P. arida, which ". . . appear to have the gene sets (genomes) from which new forms can most successfully be synthesized." (29). Despite many attempts, no hybrids have been obtained using P. pratensis as the female parent, in fact, very few have been obtained using any member of the Pratenses as the maternal parent.

Chromosome counts of the parental strains and the hybrids have revealed that most of the hybrids have resulted from the fusion of a reduced egg with a reduced pollen nucleus, while a smaller number have arisen from the fusion of an unreduced egg with a reduced pollen

nucleus (32, 83). One particular P. ampla strain used as a female parent produced hybrids from reduced eggs only when pollinated by one strain of P. pratensis, but from unreduced eggs only when pollinated by a second pratensis strain (32).

Normally, in crossing widely separated natural plant forms, the balanced gene systems of the parents are disturbed, and the segregation products which arise in the F_2 and subsequent generations are too ill-adapted to be of any value. But as has been mentioned previously, where apomictic reproduction prevails it is possible to obtain non-segregating hybrids which can be perpetuated indefinitely without further genetic change. The hybrid vigour characteristic of many first generation hybrids can therefore be maintained (25, 26, 27, 28). In practice, Clausen and his co-workers have found that about two thirds of the interspecific hybrids between predominantly apomictic strains reproduce sexually, and only one third apomictically (28, 29, 30). This characteristic of the hybrids was unexpected, but has since proved to be of immense value in the breeding program because the sexually-reproducing hybrids tend to segregate into a number of F_2 lines about half of which are apomictic (83). Thus, the sexual mode of reproduction in the hybrids permits the release of a

certain amount of variability, yet segregation stops in those F_2 lines in which apomixis is reinstated. Selection among these apomictic F_2 lines gives the breeder the opportunity of choosing constant lines that still retain much of the initial vigour of the F_1 hybrid and that show the other desirable characteristics being sought. The segregation into numerous F_2 lines also increases the chances of finding new, desirable strains because, as already noted, the initial number of hybrids formed is so low.

The Poa program is now under completion by the Carnegie group, and is being carried on by other institutions and government agencies (23, 24). Some 45 stabilized hybrid lines have been produced, of which as many as 10 to 15 may prove to be of immediate value in the western States (23). Some quadruple hybrids (e.g. arida-ampla x ampla-pratensis) have also been synthesized and may be used (102). The testing of hybrid lines is greatly facilitated by the apomictic reproduction, because seed can be used to propagate strains without genetic change. Many lines have been on test under varying climatic conditions in different areas throughout the United States and in a number of European countries; some may be utilizable in these areas (31).

As Clausen et al. (23) point out, "In Poa the potentialities for producing a great range of new apomictic

strains through intersectional hybridization are almost unlimited." More of such breeding work in the bluegrasses will undoubtedly be carried on. Furthermore, the breeding principles that have been developed may also be applicable to other groups of economic plants (23, 24).

VI STUDIES OF REPRODUCTION IN Poa confinis

A. Attempts to induce flowering under artificial conditions

1. Introduction

One of the difficulties throughout most of the study was the inability to induce flowering in P. confinis plants grown in the greenhouse. This meant that there was a lack of fresh material for cytological study during much of the course of the investigation. Furthermore, the hybridization experiments were considerably delayed. For this reason, a series of attempts were made to induce flowering in P. confinis plants, not as an experiment in the induction of flowering as such, but merely to provide material for the cytological and hybridizational work.

2. Materials and methods

A number of plants of the Whiffin Spit biotype were taken from the 6 x 12 foot University farm plot and potted in sandy loam soil in the greenhouse in September 1953 at the start of the study. They were supplied with complete fertilizer and kept under approximately a 14-hour day during the winter months with the aid of electric lights. During the summer months they were left under the prevailing day-length conditions. These plants were maintained through December 1954.

In October 1954 a series of treatments were started on P. confinis plants brought in at that time from the plot of Whiffin Spit material and placed in large porcelain pots. Six plants were used for each of nine treatments, and within each treatment three plants were potted in pure beach sand and three plants in fairly sandy orchard soil.

The nine treatments were as follows:

(1) "Drought": the plants were given enough water merely to keep them alive.

(2) Carbohydrate: sucrose was supplied at .5 grams per pot per week.

(3) Nitrogen: ammonium nitrate (33 per cent nitrogen) was supplied at .6 grams per pot per month.

(4) Salt: sodium chloride was supplied at .5 grams per pot per month.

(5) Complete fertilizer: 10-20-10 was supplied at 1 gram per pot per month.

(6) Cold shock: plants were given three weeks' treatment at 32° F.

(7) Cold shock: plants were given eight weeks' treatment at 32° F.

(8) Continuous light: artificial light was supplied by a 500-watt bulb, three feet above the plants.

(9) Control: no treatment.

These treatments were continued for just over five months, during which time no artificial light was used (except for the continuous light treatment).

A number of P. confinis plants brought from Gray's Harbour, Washington, on November 5, 1954, were potted in sand in the greenhouse after their collection. Several plants from the University plot were also brought into the greenhouse and potted in sand in early December 1954. No artificial light was supplied for either of these two groups of plants.

In the middle of February 1955 three plants from the University were potted in sand in the greenhouse and put under the light conditions prevailing in early May (i.e. approximately a 13-hour day-length). At two-week intervals other P. confinis plants from the farm plot were brought in and placed under the same conditions; this was continued until the middle of April.

During all these various attempts to induce flowering in the greenhouse there was often considerable fluctuation in temperature; no records of the temperature were kept. All plants, except those under the "drought" conditions were watered regularly. Aphids were particularly troublesome on the dune bluegrass plants, and regular fumigation was necessary.

3. Results

None of the plants in the group initially brought into the greenhouse flowered. Nearly all plants showed a fairly vigorous vegetative growth during the entire fifteen months that they were kept in the greenhouse.

All the nine treatments started in October 1954 were unsuccessful in inducing flowering in any of the plants. One striking feature noted was that in all the treatments the plants grown in beach sand maintained a far more healthy and vigorous vegetative growth than those grown in soil. Growth of the plants in soil was almost uniformly poor. Plants in both sand and soil were very spindly under continuous light. All plants in the two cold temperature treatments kept in relatively good condition, although the plants were somewhat etiolated after the longer treatment. Nevertheless, they all recovered quickly and resumed normal vegetative growth when returned to the greenhouse. There was very little growth by the plants kept under drought conditions. There were no visible differences between the plants of the carbohydrate, salt, nitrogen, complete fertilizer and control treatments --again, vegetative growth was good in sand and poor in soil.

The plants taken into the greenhouse from Gray's Harbour and from the University plot in early November and

early December, respectively, did not show any signs of growth for almost two weeks. After this time, however, they began vegetative growth but did not flower.

Almost all the P. confinis plants brought into the greenhouse during February, March and April 1955, produced inflorescences within two weeks. They flowered about two weeks after the first appearance of the panicles. On all these plants flowering was very sparse, and from a large section of the rhizomatous sod only one or two inflorescences would develop. Two or three plants did not flower at all. This paucity of inflorescences is in direct contrast to the profuse flowering of the plants grown in the field.

The material which flowered in the greenhouse was used partly for cytological study and partly for the hybridization experiments.

B. Cytology and histology

1. Megasporogenesis in pistillate flowers

(a) Materials and methods

Most of the material used in the cytological and histological studies was obtained from the plot of P. confinis on the University farm when the plants flowered in May 1954. Some additional study of particular

stages of development was made on the plants of this same Whiffin Spit biotype which were induced to flower in the greenhouse in March 1955. As previously recorded, the entire plot was composed of pistillate plants.

Fixations were started as soon as the first panicles were observed emerging from the sheath on May 2nd. Whole inflorescences were taken at random from throughout the plot and placed in Rollin-Carnoy killing and fixing solution* for 6 to 10 hours, the longer times being used for the later stages. Seventy per cent alcohol was used for rinsing off the inflorescences after fixation and for storing them. Material not imbedded immediately was kept in the 70 per cent alcohol in the refrigerator.

Collections were made daily until anthesis occurred about fourteen days after the first appearance of the panicles. These collections were taken in both evenings and mornings, although more frequently in the former. Several additional collections were made subsequent to anthesis.

Spikelets from the fixed material were dissected from the outer glumes, without the aid of a lens or microscope, and imbedded intact. The spikelets were dehydrated

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* 85 cc. 70 per cent ethyl alcohol: 5 cc. glacial acetic acid: 5 cc. commercial formalin.

in alcohol in the usual manner, and a small amount of Erythrosin B was added at the 95 per cent alcohol level to lend a pink tinge to them, thus improving their visibility when imbedded. They were cleared in xylol and imbedded in tissue mat or Parowax.

The spikelets were cut on the microtome at varying angles at all stages, and at thicknesses varying from 8 to 22 microns, depending on their maturity. Gum arabic was used as an adhesive, and a 1 per cent solution of potassium dichromate used to straighten the ribbons.

After mordanting in 4 per cent iron-alum solution the sections were stained in Harris' hematoxylin for $2\frac{1}{2}$ to 4 hours, destained in 2 per cent iron-alum under the microscope and counterstained with light green.

All drawings were made with the aid of a camera lucida.

(b) Observations

At the time of emergence of the panicle from the sheath the single ovule in each of the basal florets of the spikelets is already well developed. An inner and outer integument, each composed of two layers of cells, surround the nucellus, although at this stage the integuments have not yet grown up around the nucellus to form the micropyle (Fig. 7, A). The nucellus contains a single,

often somewhat wedge-shaped EMC, with a large nucleus typically closer to the micropylar end of the cell, but sometimes centrally placed or near the chalazal end.

The chromatin material in the nucleus in the majority of EMCs at this time shows a characteristic synizesis--a stage of the meiotic prophase in which the chromatin forms a spireme and is aggregated towards one side of the nucleus (Figs. 7, A and B). The thread-like organization of the spireme is usually evident in synizesis. A single, large nucleolus is also present, although in one EMC a second, smaller nucleolus can be seen.

The EMC appears to undergo a normal first meiotic division. Paired chromosomes at zygotene are evident in a number of slides (Fig. 7, C). Fig. 8, A shows a pachytene or diplotene stage. Later stages in diakinesis have invariably shown all chromosomes to be associated in pairs, as far as can be determined (Figs. 7, D and E; Fig. 8, B). The two cells drawn in Figs. 7, D and E are the only EMCs in which an almost positive count can be obtained; both cells contain 21 pairs of chromosomes. In Fig. 7, D the large nucleolus is still organized, and one bivalent is closely associated with it; in Fig. 7, E the nucleolus has disappeared and the 21 bivalents are more closely grouped. Satellites, or trabants, are observable on

Figure 7: Megasporogenesis in Poa confinis

A. Ovule at time of emergence of panicle; single EMC in meiotic prophase (synizesis); the inner and outer integuments developing around the nucellus.

B. Synizesis in EMC (Prophase I).

C. Zygotene in EMC (Prophase I).

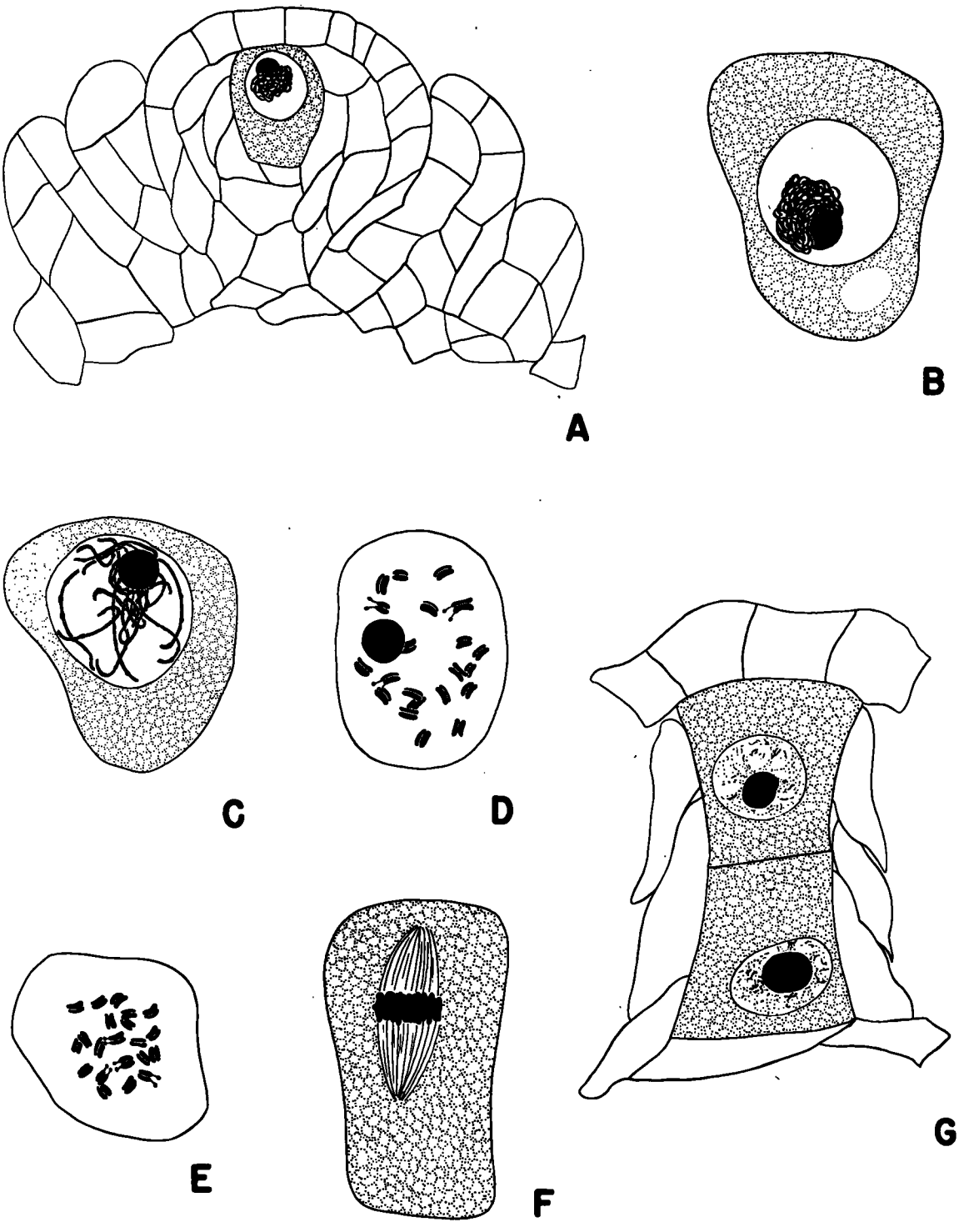
D. and E. Diakinesis in EMC (Prophase I); 21 bivalents present. Note the trabants (satellites) on several pairs of chromosomes in both cells. (D constructed from two adjacent serial sections.)

F. Metaphase I in EMC.

G. Dyad cells in interkinesis.

(all x 1760)

The micropylar end is to the top of the page in all drawings.


A
B
C
D
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F
G

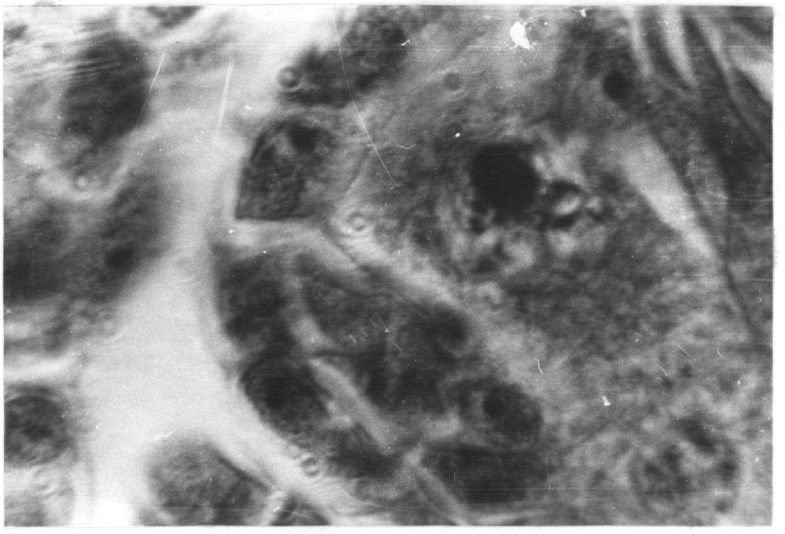
Figure 8: Megasporogenesis in Poa confinis

A. Diplotene in EMC (Prophase I).

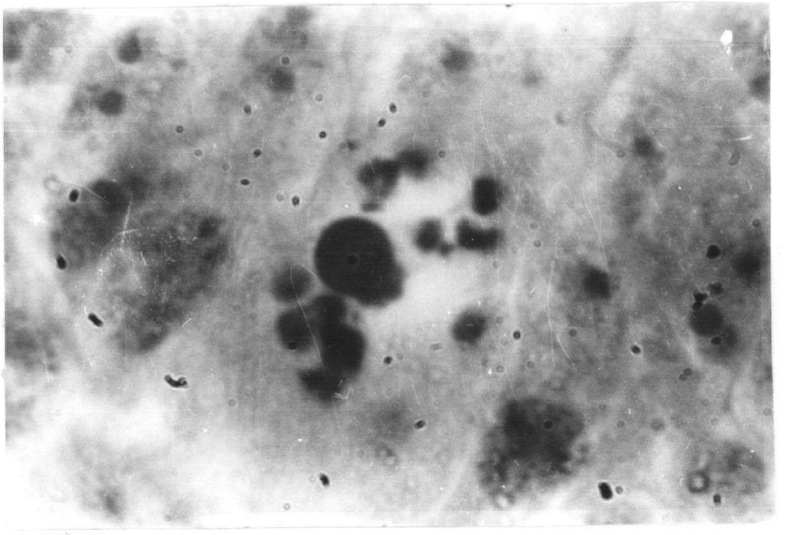
B. Diakinesis in EMC (Prophase I).

C. Metaphase I in EMC.

(all x 2640)



A



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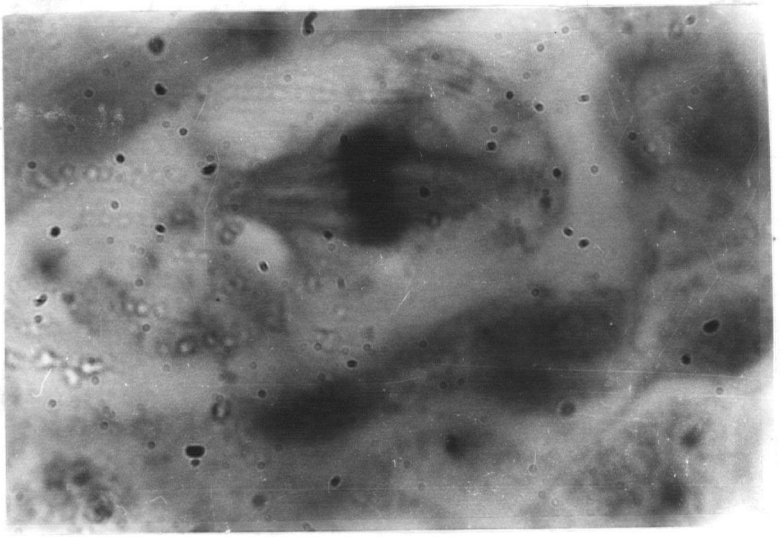


C

several pairs of chromosomes in these and other EMCs at diakinesis.

An elongation of the EMC accompanies the organization of the spindle figure and the alignment of the bivalents at the metaphase plate (Fig. 7, E; Fig. 8, C). Although good sections at anaphase and telophase of the heterotypic division are difficult to find, the separation of the homologous pairs appears normal, with no evidence of univalents or other irregularities. The first meiotic division always takes place parallel to the long axis of the ovule. Dyad cells with nuclei in interkinesis are illustrated in Fig. 7, G, in which a reorganization of the nucleolus in each cell appears to have taken place.

The second meiotic division evidently follows very rapidly upon the first. A typical triad stage is shown in Fig. 9, A, in which the division in the micropylar cell of the dyad has been preceded by the division in the chalazal cell. The micropylar dyad cell often divides in a plane at right angles to the long axis of the ovule, forming a T-shaped tetrad (Fig. 9, B), but more frequently in a plane parallel to the long axis of the ovule (Figs. 9, C, D and E). Delayed second division of the micropylar dyad cell is common, and in some cases the division fails altogether (Fig. 9, F). The division

Figure 9: Megasporogenesis in Poa confinis

A. Triad.

B. Tetrad; division in the chalazal dyad cell has preceded that in the micropylar cell; note the T-shaped form of the tetrad.

C. Linear tetrad; delayed disintegration of the three upper non-functional spores of the tetrad; the chalazal megaspore has already divided to form a 2-celled embryo sac.

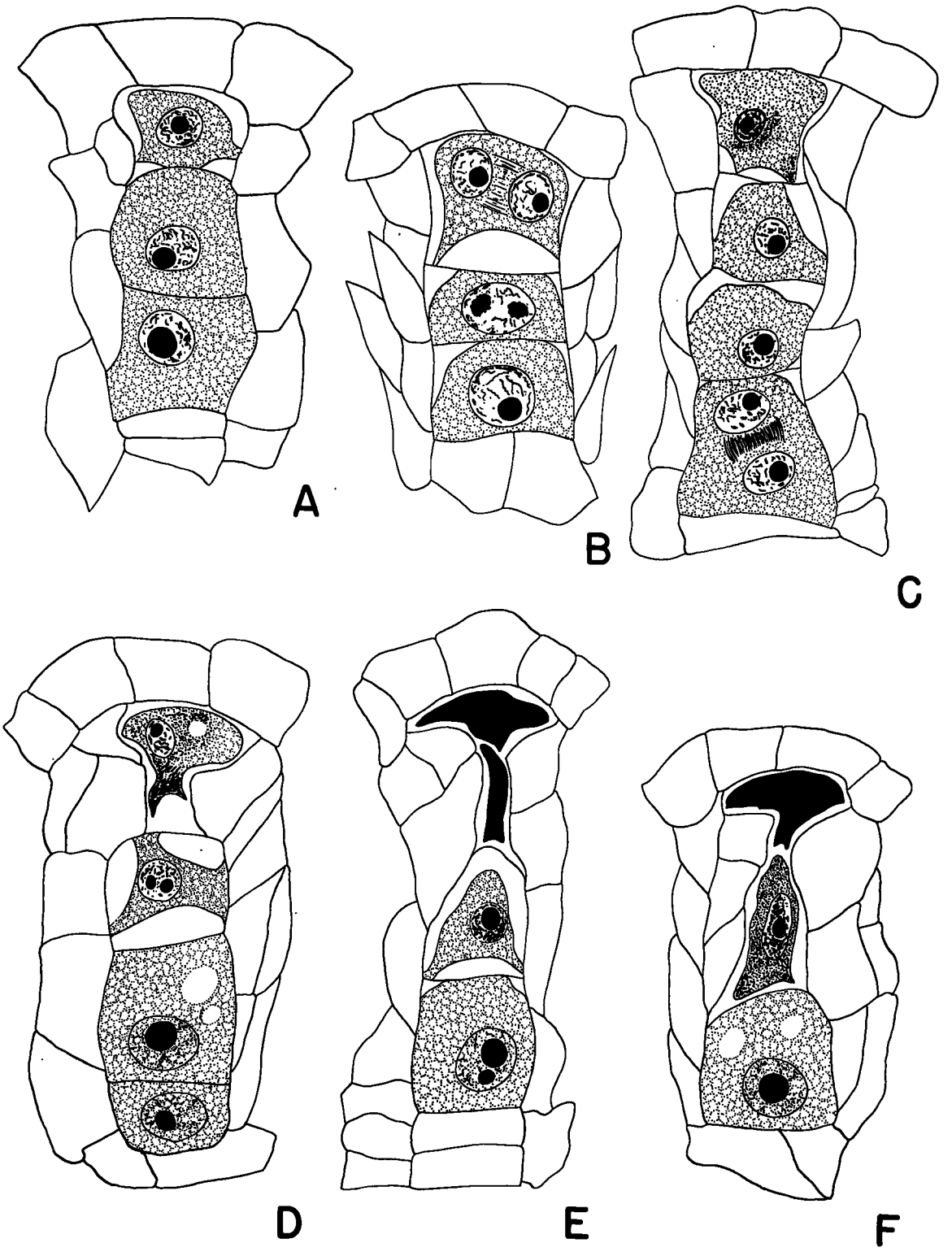
D. Linear tetrad; the second megaspore from the chalazal end is functioning as the embryo sac initial.

E. "Umbrella" stage following tetrad formation; the chalazal megaspore functioning as the embryo sac initial.

F. "Umbrella" stage following triad formation; the chalazal cell is the functional megaspore.

(all x 1520)

The micropylar end is to the top of the page in all drawings.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of the chalazal dyad cell is invariably in the plane of the longitudinal axis of the ovule. Complete tetrad stages are shown in Figs. 9, C and D. In Fig. 9, C the basal cell of the tetrad is functioning as the embryo sac initial cell and is already dividing before the disintegration of the upper three megaspores. In Fig. 9, D the second megaspore from the chalazal end is probably functioning as the embryo sac initial in preference to the basal megaspore.

Typical "umbrella" stages, seen in a very large number of slide preparations, are illustrated in Figs. 9, E, F and 10, A and B. The flattening of the micropylar cell of the spore triad or tetrad, together with the disintegration of the lower, non-functional megaspores, gives the umbrella- or mushroom-like appearance. In Fig. 9, E the two micropylar spores have already disintegrated, and the second megaspore from the chalazal end also shows signs of breaking down. Fig. 9, F is a similar stage, except that the second division in the upper dyad cell has not been completed, and only a triad has formed. The second megaspore from the chalazal end of the ovule is seen disintegrating above the embryo sac initial cell in Fig. 10, C.

Figure 10: One-celled embryo sacs in Poa confinis

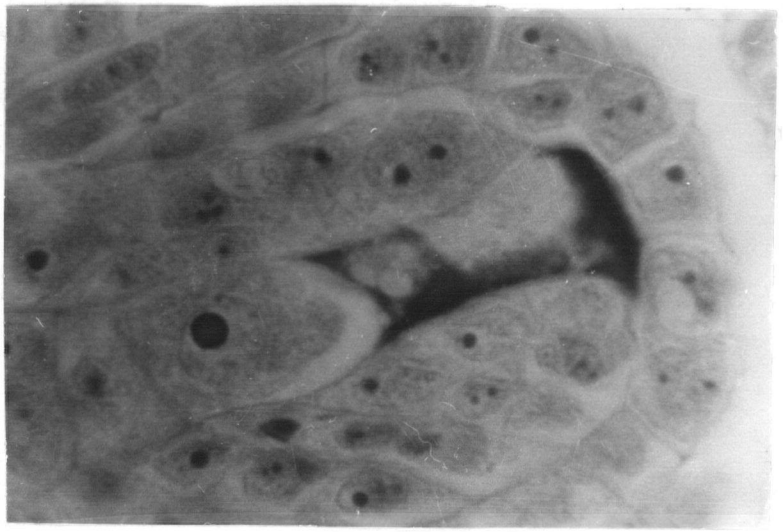
A. and B. "Umbrella" stages; the chalazal megaspore is the embryo sac initial cell in each case.

(x 1075)

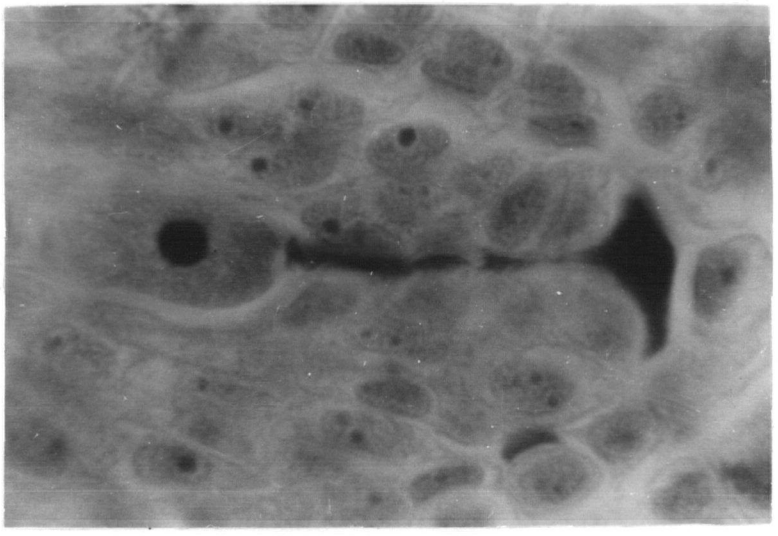
C. The chalazal megaspore is functional; the lower member of the line of disintegrating megaspores is visible on the right.

(x 1610)

The micropylar end is to the right hand side in all three photographs.



A



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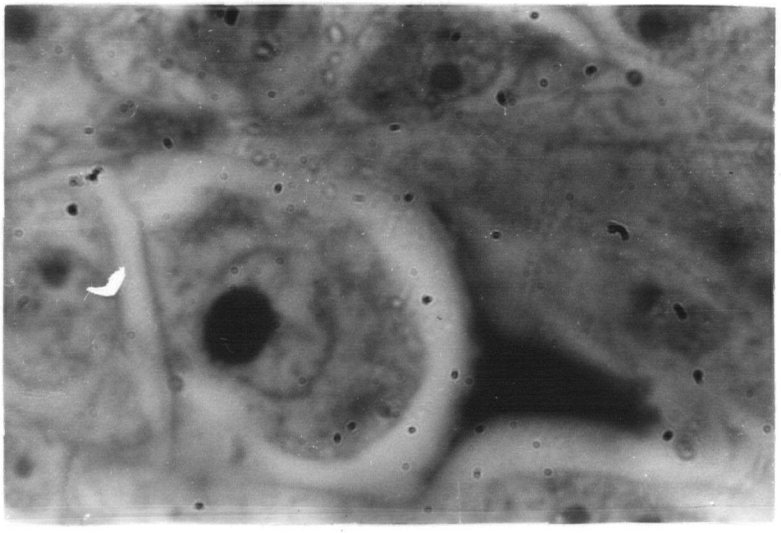


C

Complete disintegration of the non-functional megaspores is accompanied by the increased vacuolation and growth of the functional megaspore, or uninucleate embryo sac. The first mitotic division in the embryo sac may take place either parallel to or across the longitudinal axis (Figs. 11, A and B). Fig. 11, A shows a late anaphase - early telophase stage, and Fig. 11, B a late telophase stage in which the nuclear membranes have formed around the chromosomes. The remains of the spore tetrad are evident at the micropylar end of both embryo sacs. Two-celled embryo sacs are seen in Figs. 11, C and 12, A. In the former, the three non-functional megaspores are still discernible, although usually by this stage it is difficult to pick them out individually from the cell debris.

In only two instances can more than one embryo sac be seen in an ovule. One of these instances is illustrated in Fig. 11, D, which is taken from a transverse section of a pair of two-celled embryo sacs lying side by side in the nucellar cavity. The first mitotic division in the embryo sac on the right has just been completed. One of the two telophase figures in this sac can be seen, while the second is in a neighboring serial section. In the second example of polyembryony found a pair of four-

Figure 11: Embryo sac development in Poa confinis

A. First division in the embryo sac--late anaphase - early telophase; disintegrated megaspores at micropylar end.

B. Two-celled embryo sac--the division just completed; disintegrated megaspores evident.

C. Two-celled embryo sac; the three disintegrated megaspores clearly distinguishable.

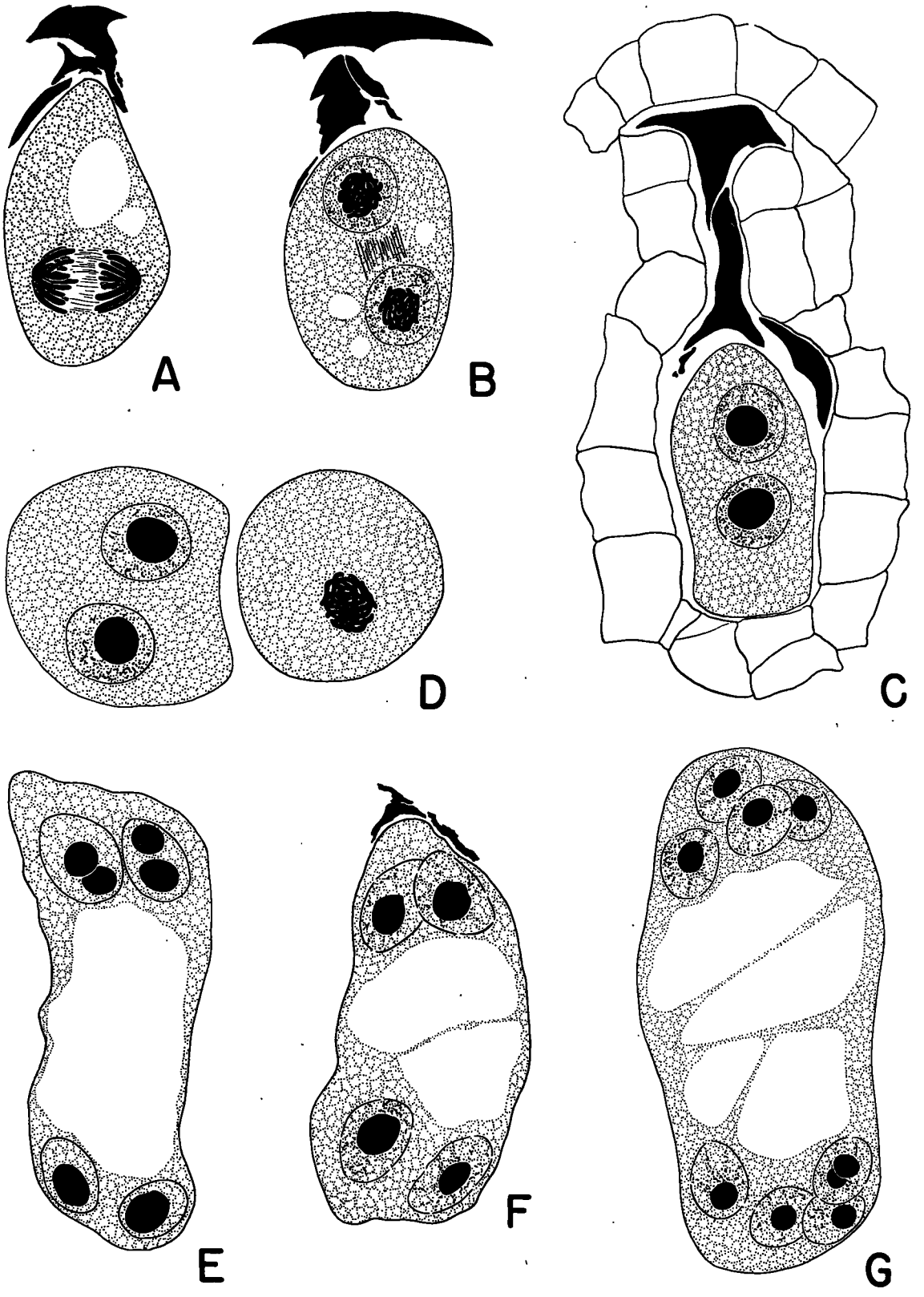
D. Polyembryony; two embryo sacs lying side by side in the nucellar chamber (transverse section).

E. and F. Four-celled embryo sacs.

G. Eight-celled embryo sac (constructed from several serial sections).

(all x 1370)

The micropylar end is to the top of the page in all drawings.


A
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G

celled embryo sacs are placed side by side in the ovule.

Typical four-celled embryo sacs show the vacuolation of the center of the sac, where the cytoplasm forms a thin, peripheral layer against the wall (Figs. 11, E and F). The number of nucleoli in the cells of the embryo sac seems quite variable, although one is most usual. Two, or occasionally three, may be found, and there is no tendency for cells with more than one nucleolus to be found more often at one end of the embryo sac than the other.

Development of an eight-celled embryo sac, two to three days before anthesis, is accompanied by further vacuolation and increase in size (Fig. 11, G). A polar nucleus from each group of four cells at either end of the embryo sac migrates toward the center of the sac where they fuse to form a single cell (the fusion nucleus) (Fig. 13, A) or remain closely associated but unfused (Fig. 13, B). At the micropylar end of the embryo sac the three cells remaining there become distinguishable as an egg and a pair of synergids (Fig. 12, B; Figs. 13, A and B). In Fig. 13, B the egg cell is larger than the synergids and contains two nucleoli.

Marked antipodal cell development is evident in all embryo sacs, and begins just prior to anthesis. This

takes the form not only of an increase in the number of antipodals (Figs. 13, A and B) but later also a huge increase in their size and in the number of nuclei per antipodal cell (Figs. 12, C; Fig. 13, C). Division figures can be seen in two of the three original antipodal cells in Fig. 13, A. In Fig. 13, B four antipodal cells are present. At least eight or ten antipodals are seen in almost every embryo sac at anthesis, and as many as twenty can be counted in several instances. A characteristic channelling or furrowing of the dense, granular cytoplasm appears to divide off the multinucleate antipodals (Fig. 12, C and Fig. 13, C). In most embryo sacs the antipodal cells fill up as much as two thirds of the sac, although there does not appear to be any compression of the underlying fusion nucleus and egg apparatus.

Florets sectioned five days after the beginning of anthesis appear much the same as florets at anthesis. In none of the embryo sacs examined was any division of either egg cell or fusion nucleus seen. In the small number of florets examined seven days after anthesis several of the embryo sacs are presumably aborted, taking only a heavy stain all over, through which it is impossible to discern any cellular detail.

Figure 12: Embryo sac development in Poa confinis

A. Two-celled embryo sac.

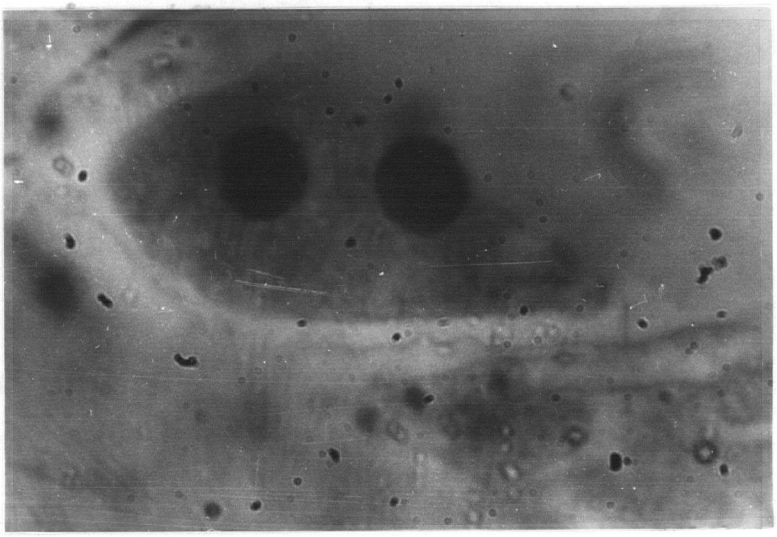
(x 1610)

B. Micropylar end of mature embryo sac, showing egg cell and two synergids.

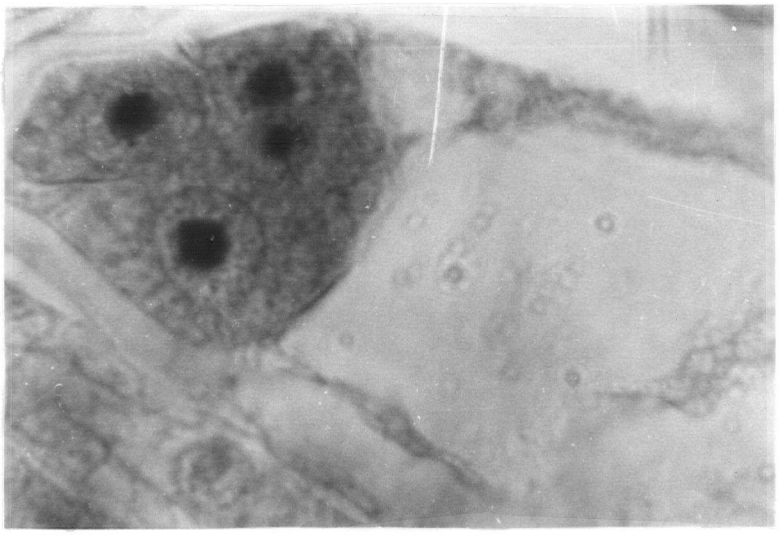
(x 2150)

C. Antipodal cell development in mature embryo sac; note furrowing of the cytoplasm between the multinucleate antipodals.

(x 1075)



A



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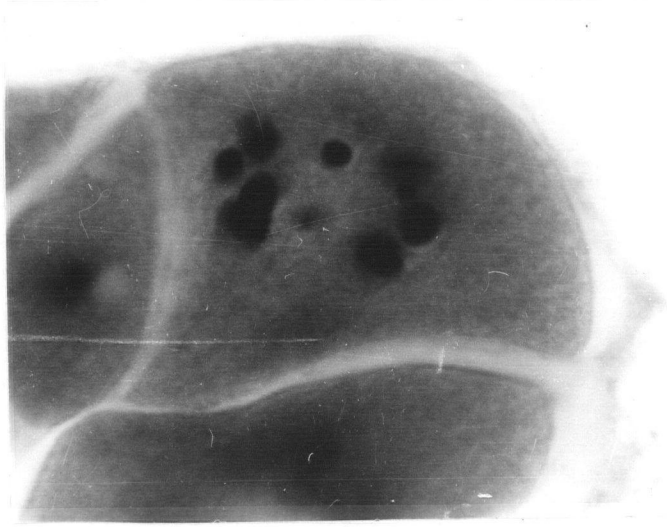


C

Figure 13: A, B, C, and D - Female gametophyte development in Poa confinis.

E and F - Embryo and endosperm formation following pollination of pistillate Poa confinis floret with Poa pratensis pollen.

A and B. Embryo sacs a few days prior to anthesis; A shows an egg and synergids, fusion nucleus and divisions in antipodal cells; in B the egg is well differentiated from the two synergids, the polar nuclei have not yet fused, and four antipodals are present.

(Both constructed from serial sections; x 1610)

C. Extreme antipodal cell development; note the channelling between the cells, and the numerous nuclei per cell.

(x 815)

D. Mature embryo sac several days after the start of anthesis; the large egg lies between two pear-shaped synergids, the polar nuclei are still unfused, and the antipodal development is strong.

(Constructed from two adjacent sections; x 520)

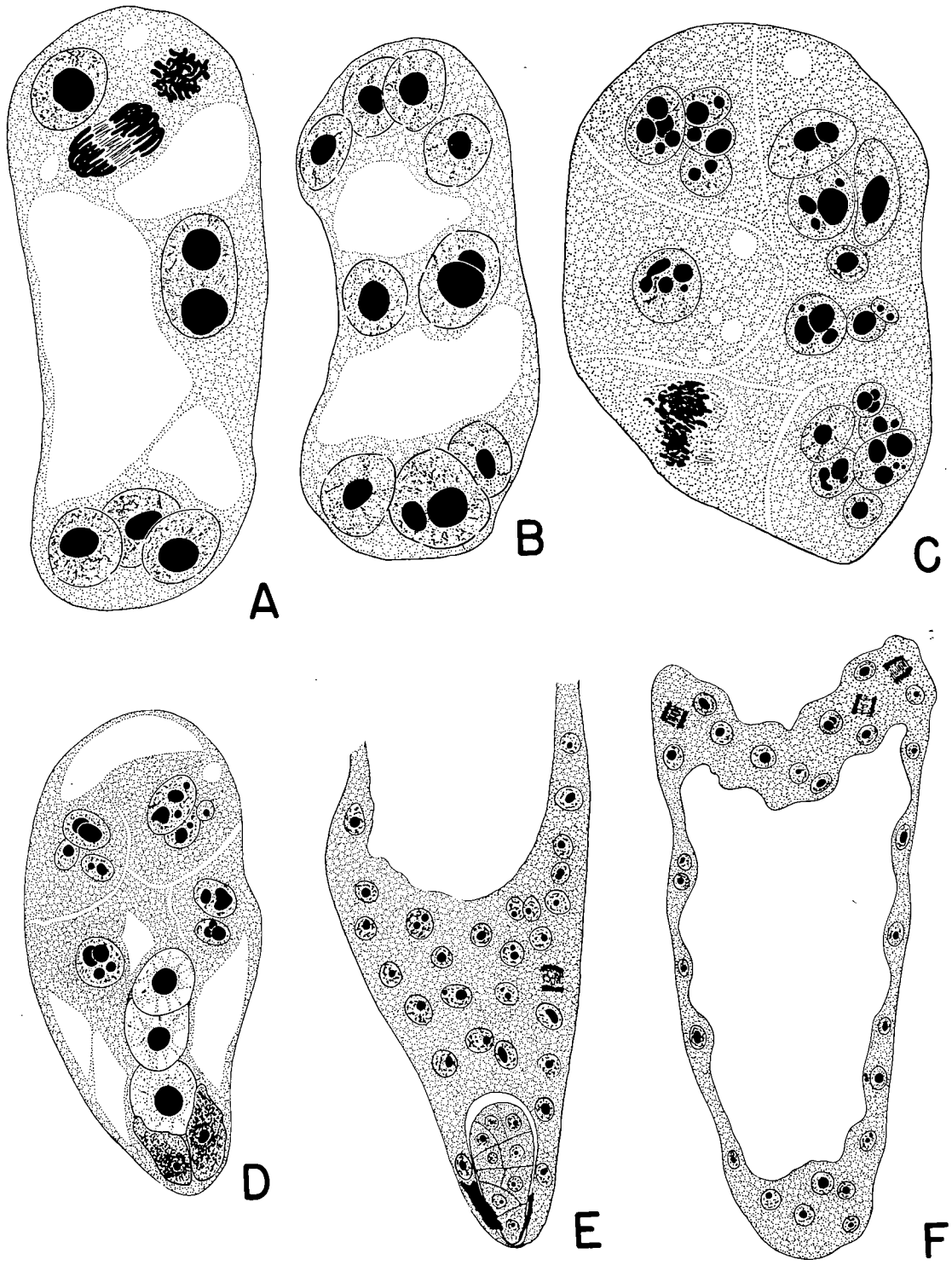
E. Young embryo developing after pollination with pratensis pollen; a multi-celled endosperm has already formed and surrounds the embryo; the two dark bodies on either side of the embryo are the disintegrated synergids.

(x 370)

F. Neighboring section through same ovule as E; peripheral layer of endosperm forms laterally; note division figures in several nuclei.

(x 370)

The micropylar end is to the bottom of the page in all drawings.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Chromosome counts cannot even be approximated in any of the cells of the embryo sacs seen throughout the various stages of development.

Chromosome counts in the cells of the somatic tissues surrounding the developing gametophyte are also very difficult, as the cells are normally small and crowded, with the chromosomes hard to distinguish even in good polar views of metaphase plates. Several very rough counts in nucellar cells indicate no more than that the somatic chromosome number of 42 is possible, but the most accurate count obtained is about 40 ± 5.

2. Embryo and endosperm development following pollination of Poa confinis by Poa pratensis

(a) Materials and methods

Because no seed was set in the Whiffin Spit material in the University plots in May 1954 no cytological study beyond the stage of the mature female gametophyte could be made. However, in April 1955, when flowering was induced in P. confinis plants in the greenhouse, further cytological study was made following pollinations made with P. pratensis pollen.

The P. confinis spikelets were fixed at a series of time intervals following pollination. Due to the

sparse flowering only three or four spikelets could be used for each of the treatments.

The fixations were made at the following times:

(1) At the time of pollination

(2) 6 hours after pollination

(3) 11 hours after pollination

(4) 16 hours after pollination

(5) 25 hours after pollination

(6) 50 hours after pollination

(7) 122 hours after pollination

The fixation, staining and other features of the permanent slide preparations were exactly the same as for the study of megasporogenesis. All sections were cut at a thickness of 24 microns.

(b) Observations

At the time of pollination with pratensis pollen the mature female gametophyte of P. confinis shows the strongly developed antipodal structure and a normal fusion nucleus and egg apparatus. In Fig. 13, D, however, the polar nuclei have not yet actually formed a single nucleus. Two somewhat pear-shaped synergid cells lie rather below the large egg cell.

Six hours after pollination the pratensis pollen grains can be seen germinating on the stigmas of the

confinis florets. In the 11- and 16-hour stages a careful search for pollen tubes in the stylar and nucellar tissues reveals several probable examples, but their presence is very hard to confirm.

One slide at the 16-hour stage shows the initial division of the endosperm nucleus taking place, while the egg cell is undivided. At 25 hours after pollination an endosperm of about eight cells can be seen in one ovule, and in another an endosperm of six cells. In both these the endosperm is developing around the antipodal mass, and the egg cell remains undivided. A third floret at the 25-hour stage shows what is almost certainly the terminal end of a pollen tube lying next to the egg cell. The actual stage of fertilization is either lacking or else cannot be interpreted accurately in any of the slides examined.

At 50 hours after pollination three ovules appear to contain two-celled embryos, although the disintegration of the synergids and what are possibly the remains of the pollen tubes make it difficult to interpret the slides at this stage. The endosperm has about twenty-four cells in one of these ovules, and is forming a peripheral layer against the wall of the nucellar chamber, and surrounding the antipodal cells, which are only just

showing signs of disintegration. The two-celled embryo in this ovule is buried in the endosperm at the micropylar end of the nucellar cavity.

At 122 hours after pollination, eight- to sixteen-celled embryos are present in half a dozen observable instances. Fig. 13, E shows an approximately eight-celled embryo surrounded by endosperm tissue. The dark bodies on either side of the basal end of the embryo are probably the two disintegrated synergid cells. Fig. 13, F, from a neighboring section through the same ovule, shows the peripheral endosperm formation. The antipodal cells have been ingested by the endosperm tissue. Several telophase figures are observable in the endosperm in Figs. 13, E and F, and the endosperm still shows free nuclear development at this time.

The histological study was not carried beyond this stage.

3. Anther development in pistillate flowers

(a) Materials and methods

Anther development in the pistillate flowers of P. confinis was studied concurrently with the study of female gametophyte development, and no slides were prepared specifically for the purpose.

(b) Observations

In the florets of the young spikelets there is little differentiation of staminal tissues at the time the panicle first emerges from the sheath. In the uppermost floret the filament is barely evident, and the epidermal layer of cells of the anther surrounds a homogeneous mass of cells in which even the provascular strand is not differentiated.

Successive stages of development can be seen in the stamens from the uppermost to the lowermost florets. A provascular strand is developed and the filament lengthened. An area of sporogenous tissue becomes differentiated in each of the four anther-lobes which form. In the primary florets of all but the youngest spikelets (i.e. those on the lower branches of the panicle) the anthers show a single epidermal layer, a tapetal layer surrounding the sporogenous cells of each anther-lobe, and a central vascular strand surrounded by parenchyma tissue (Fig. 14, A). The thin-walled archesporial cells at this stage are little different from the surrounding cells. Their cytoplasm is thin and lightly-staining; their nuclei are small and characteristically contain one to three nucleoli. These cells enlarge somewhat as development proceeds but otherwise undergo only slight change in appearance until about the two- or four-celled stage of the embryo sac in

Figure 14: Anther development in pistillate plants of Poa confinis

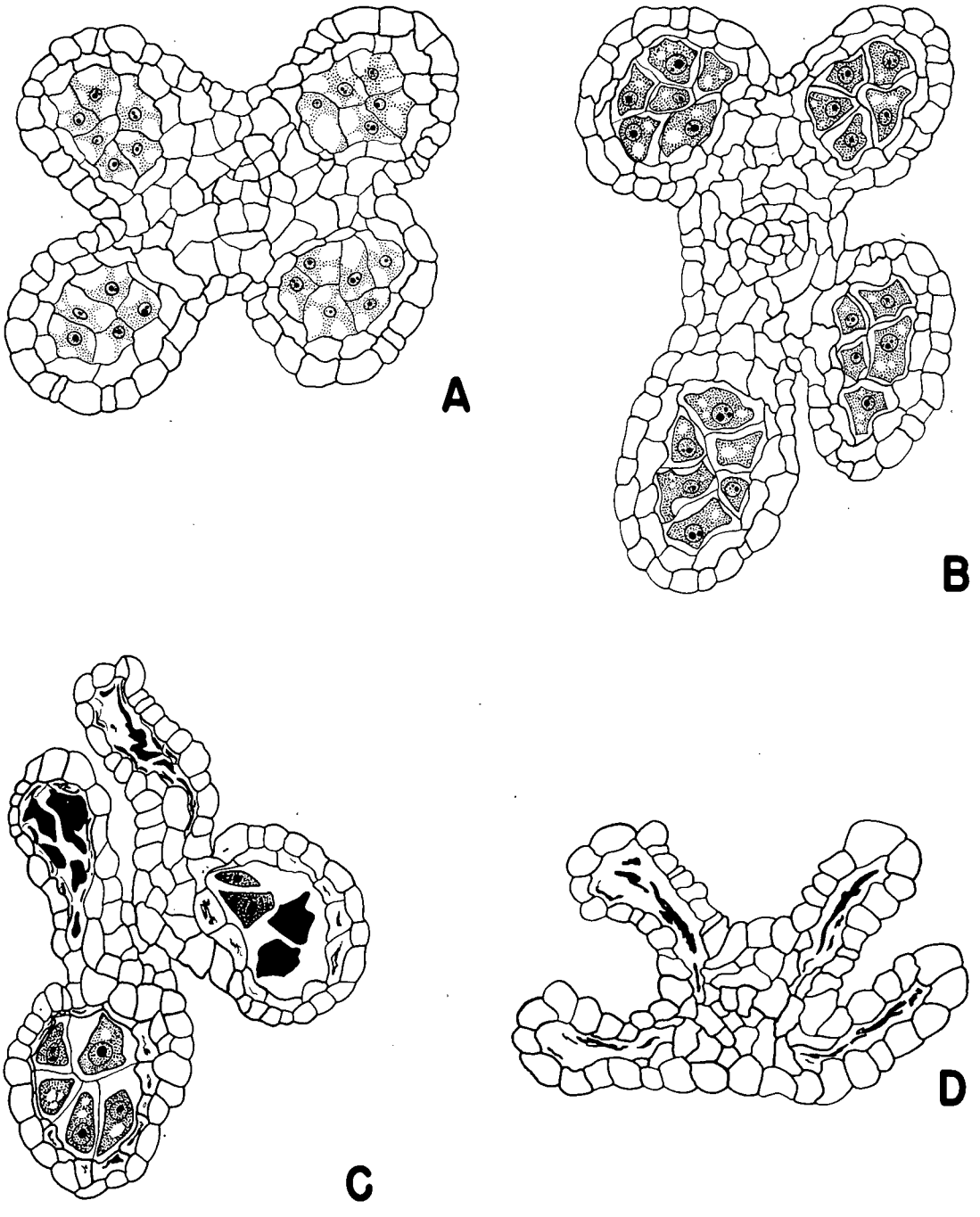
A. Transverse section of anther in primary floret at time of emergence of panicle; sporogenous cells developing in four lobes of the anther.

B. Later stage showing vacuolation and retardation of cytoplasmic membranes from cell walls in the potential PMCs.

C. Disintegration of the potential PMCs and the surrounding tapetal layer.

D. Complete disintegration of all the inner tissue at the time of flowering.

(all x 520)


A
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the accompanying pistil. At this time the archesporial cells begin to take the hematoxylin stain more heavily. They become vacuolated, the nuclei enlarge, and the cytoplasmic membrane starts to pull away from the cell wall (Fig. 14, B).

Complete disintegration of all the potential PMCs follows rapidly, accompanied by the disintegration of the tapetal layer, which shows practically no differentiation throughout the development of the anther (Fig. 14, C). By the time anthesis occurs, most of the cellular contents of both sporogenous and tapetal regions have been digested by the surrounding epidermal tissue (Fig. 14, D). The coalescence of the sporangia into two spore chambers or pollen sacs is not completed, the four sporangia remaining separated, as shown in Figs. 14, C and D.

In no instance were any but mitotic figures seen in the anthers, and even these rather infrequently.

The timing of the foregoing sequence of events in relation to female gametophyte development seems to be somewhat variable. Complete disintegration of the anther contents has been seen occasionally as early as the "umbrella" stage in the accompanying pistil, and sometimes not until after anthesis. Disintegration appeared to take place somewhat earlier in the pistillate plants which flowered in the greenhouse than in those which flowered in the University farm plot.

4. Microsporogenesis in staminate flowers

(a) Materials and methods

Microsporogenesis was studied in the staminate P. confinis plants which were collected at Point Roberts on April 17th and April 23, 1955, and grown in beach sand in the greenhouse. At the time of collection the panicles were already showing, and some fixations were made at the site at Point Roberts on the second date of collection. However, most of the stages of development were found in the fixations that were made after the plants were transferred to the greenhouse.

Permanent slides were made in the same way as for the study of female gametophyte development. All sections were cut at thicknesses of 8 to 14 microns.

Additional examination of the earlier stages of development was made through the preparation of acetocarmine anther squashes. Whole spikelets were placed in Carnoy's Fluid No. 1* for 1½ to 2 hours and then washed first in 70 per cent alcohol and then in distilled water. The anthers were dissected out of all the florets in a spikelet and placed in a drop of acetocarmine on a slide for about one minute. The anthers were then squashed under a cover slip and the excess stain removed with paper

* Three parts absolute alcohol: one part glacial acetic acid.

towelling. After the slide was warmed briefly over a flame the edges of the cover slip were sealed with nail polish.

(b) Observations

Anthers showing almost all stages of a normal microsporogenesis can be seen in the permanent and temporary slides.

PMCs in synizesis fill the anther sacs in the earliest stages found (Fig. 15, A). A single, large nucleolus is seen in each cell. At this time the surrounding cells of the anther are already well compressed by the growth of the archesporial cells, and it is difficult to see clearly the number of outer cell layers. However, a single layer of darkly-staining tapetal cells is always present, lining the sporangial cavity and surrounding the PMCs. One or two layers of compressed cells appear to lie between the tapetum and the epidermis, but the number of layers is usually not distinct.

Several zygotene stages can be seen in the PMCs in anthers treated by the acetocarmine squash technique (Fig. 15, B). PMCs at diakinesis show only bivalents, as far as can be seen, although no accurate counts are obtainable. In the cell at the upper right in Fig. 15, C the bivalents are separating at anaphase I. In this and

Figure 15: Microsporogenesis in staminate plants of Poa confinis

A. Synizesis in PMCs.

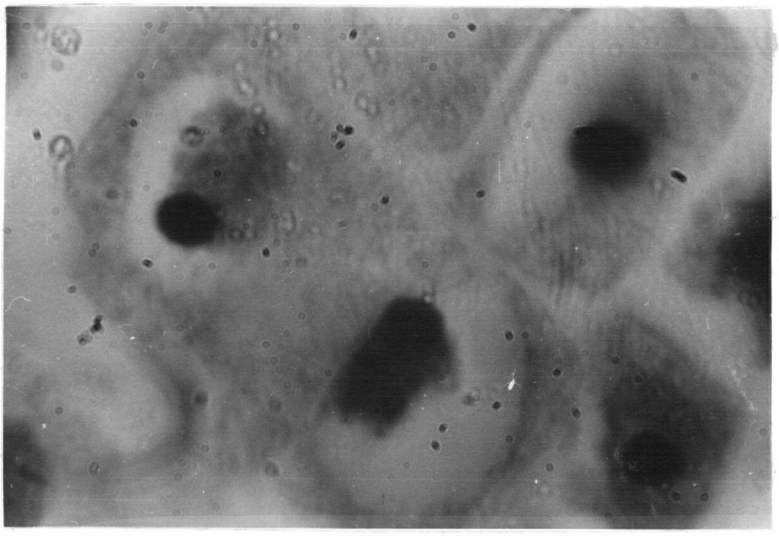
(x 1610)

B. Zygotene in PMC.

(x 3220)

C. Metaphase I in PMC at left; anaphase I in PMC at upper right.

(x 3220)



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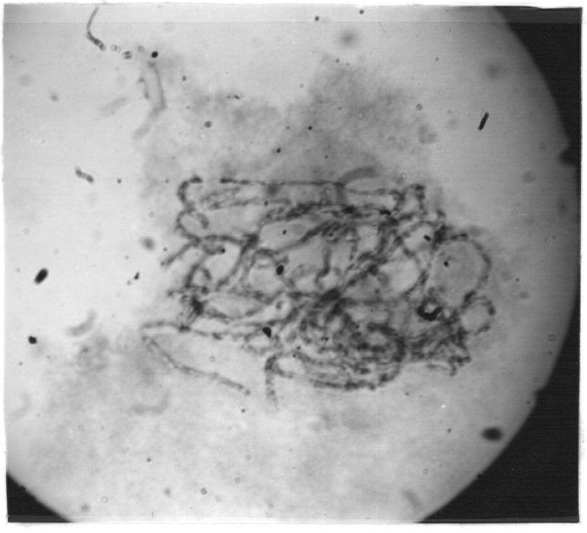

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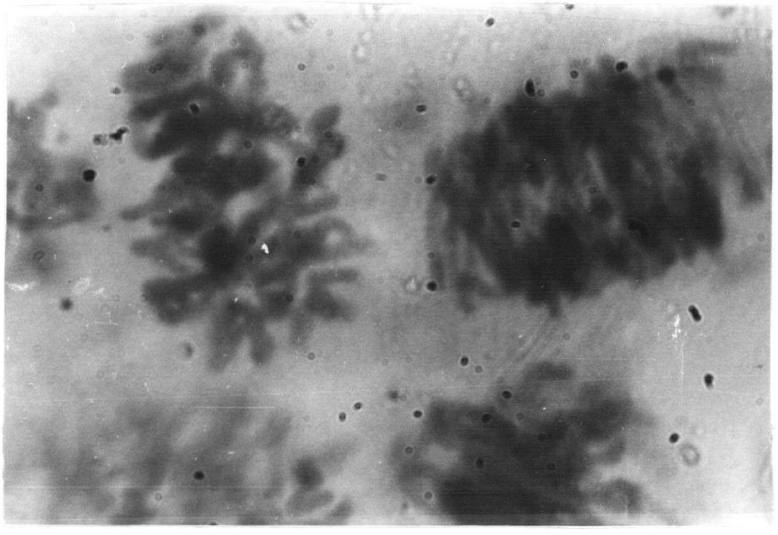


C

other cells at anaphase and telophase (Fig. 16, A), no meiotic irregularities can be discerned. Several chromosome counts in PMCs at first division metaphase and anaphase show numbers approximating 2n = 40. No exact counts are possible in the cells examined.

Dyad formation is followed rapidly by the second division and the formation of the spore tetrads (Fig. 16, B). By this stage the tapetal layer has been almost completely digested by the archesporial cells, and shows as a line of disintegrated cells. In Fig. 16, C several microspores can be seen lying against the anther wall, a day before anthesis. The layer of dark tissue directly beneath them is the tapetum.

The percentage of good pollen produced in the staminate confinis plants is evidently high, judging from the staining properties of the pollen grains and the low percentage of shrunken grains.

5. Ovule development in staminate flowers

(a) Materials and methods

Ovule development in staminate flowers was examined in the permanent slides prepared for the study of microsporogenesis; as in the study of anther development in the pistillate plants, no slides were prepared especially for this purpose.

Figure 16: Microsporogenesis in staminate plants of Poa confinis

A. Telophase I in PMCs.

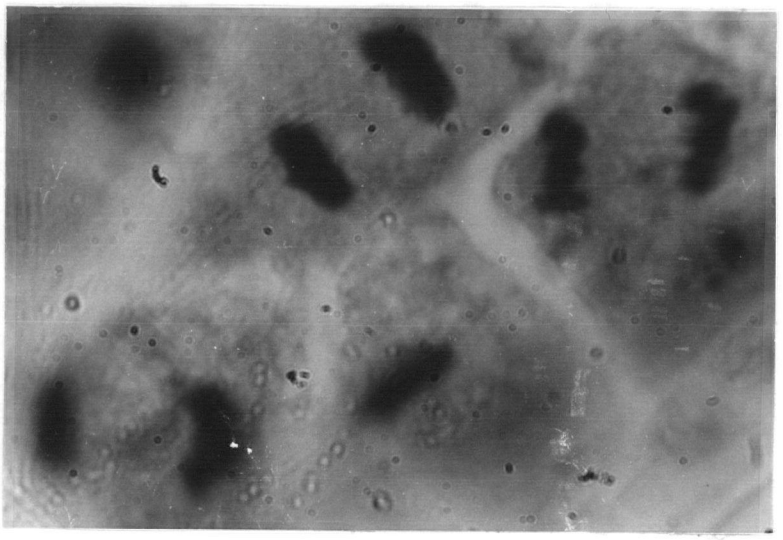
(x 1610)

B. Spore tetrads.

(x 1300)

C. Microspores, one day before anthesis; note the digested tapetal layer just below the microspores.

(x 1610)



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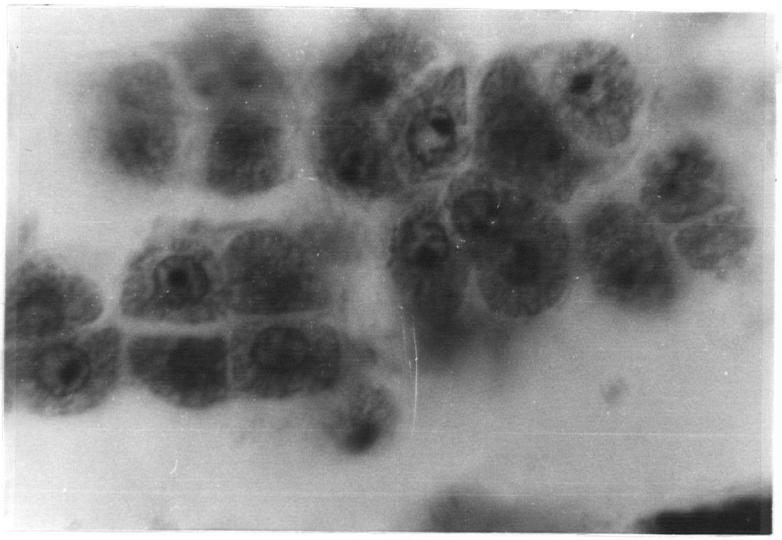

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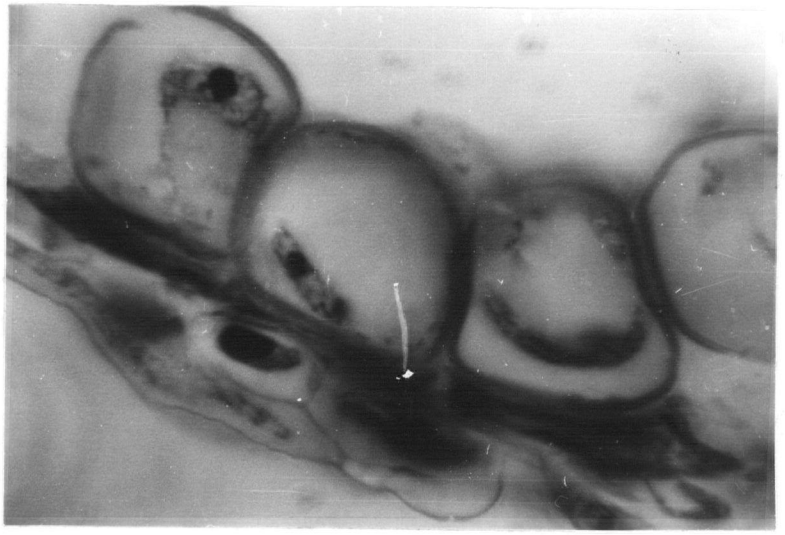

C

(b) Observations

Due to the relatively small number of slides prepared, only a few stages of development can be seen. A number of EMCs, typical "umbrella" stages, and several early embryo sacs are seen in some ovules in the male florets. One anomaly observed is the presence of two EMCs, both in synizesis, in one ovule. Development on the female side is well behind that in the anthers, and in several florets in which the anthers are almost ripe the embryo sacs are only two-celled. No embryo sacs can be seen which are beyond the two-celled stage. Although the developmental picture in the ovules, as far as it progresses, appears essentially like that in the pistillate plants, in all but the very young pistils the styles and stigmas are very much shrivelled.

C. Hybridization techniques and attendant macroscopic observations

1. Techniques used to secure crosses

Plants of P. confinis, P. pratensis, P. compressa, P. macrantha and P. annua were brought in from the field at various dates during February, March and April of 1955. Except for the P. annua, which was brought in as a slab of sod in a wooden flat, all plants were potted in

beach sand and put under 14-hour day length conditions with the aid of artificial light.

The sources of these five species were as follows:

(1) Poa confinis: Pistillate plants were obtained from the original Whiffin Spit biotype on the University farm, and also from the Point Roberts material collected on April 17th and April 23rd. All the staminate plants used were brought from Point Roberts.

(2) Poa pratensis: The plants used belonged to a strain obtained from an abandoned grass nursery site on the University farm. The origin of this strain is unknown.

(3) Poa compressa: These plants were also from a strain of unknown derivation, obtained from the same abandoned nursery as the P. pratensis plants.

(4) Poa macrantha: These plants were from a number collected on the sand dunes at Twin Harbours State Park, Washington, in November 1954. They had been in the University plots until they were brought into the greenhouse in March. All plants brought in proved to be pistillate.

(5) Poa annua: A sod composed chiefly of P. annua was dug up from a waste site close to the University greenhouses.

The first series of pollinations were made from April 13th to 15th between pistillate P. confinis plants (Whiffin Spit) and P. pratensis plants brought in together on March 15th. A total of seven P. confinis inflorescences were pollinated. Two of the plants used in these crossings are seen in Fig. 17.

Figure 17: Plants used in the attempted cross P. confinis x P. pratensis. Note the sparse flowering of the pistillate confinis parent.

A second series of pollinations were made from May 1st to 11th, involving all five Poa species. In this series all the P. confinis used was from the material collected at Point Roberts.

The following crossings were attempted*:

(1) P. confinis x P. pratensis (16)

(2) P. confinis x P. annua (3)

(3) P. confinis x P. compressa (8)

(4) P. macrantha x P. pratensis (1)

(5) P. macrantha x P. compressa (2)

(6) P. macrantha x P. confinis (2)

(7) P. macrantha x P. annua (1)

Pollination of two P. confinis inflorescences with confinis pollen was also carried out. Two panicles each of P. confinis and P. macrantha were kept bagged without any pollination. A start was made to emasculate P. compressa inflorescences by the hot water treatment developed by Stephens and Quinby (99), but there proved to be very few staminate confinis plants available for reciprocal crosses and the emasculations were not carried on. There was just enough confinis pollen for the four pollinations which were made.

The P. confinis plants used as male and female parents and the P. macranths plants used as female parents were bagged with glassine bags one to several days before anthesis. P. annua inflorescences were also bagged prior to their shedding pollen. The pratensis and compressa

* Numbers in brackets refer to number of inflorescences pollinated.

panicles were not bagged, but the two species were kept in separate sections of the greenhouse during the few days that their flowering times coincided.

Most pollinations were carried out between 7:00 and 8:00 a.m., as it was found that the P. pratensis and P. compressa plants, particularly, shed their pollen very early, and there was often very little pollen left later in the day after the greenhouse ventilators had been opened up. The still air of the early morning hours also reduced the amount of foreign pollen that might have been floating around.

Pollen was collected from the plants of the species being used as the male parent by tapping their panicles over a clean glass microscope slide. The glassine bags were then removed from the panicles of the female parent or parents and the pollen on the slide dusted lightly over the stigmas with the aid of a fine camel-hair brush. The glassine bags were then replaced. Pollinations were usually made on at least three consecutive mornings, and occasionally five or six, as the flowering of the confinis and macrantha plants progresses downward from the top spikelets of the panicles and often takes several days to be completed. The glassine bags were removed three or four days after the last pollinations had been made.

2. Observations following pollinations

Seed began to form after all of the attempted interspecific crosses except the two involving P. annua; however, none of the seed from these crosses reached maturity without shrivelling. A single, shrivelled seed found on the P. macrantha inflorescence pollinated with annua pollen may have been the result of accidental pollination by one of the other Poa species.

Healthy seed formed on the two P. confinis inflorescences pollinated with confinis pollen. No seed formed on either the P. confinis or P. macrantha pistillate inflorescences which were bagged and left unpollinated.

The seed which formed after the interspecific crossings appeared to fill out and develop quite normally until about three weeks after pollination. A few seeds from the crosses confinis x pratensis and confinis x compressa were compared under the binocular microscope, at a magnification of x 40, with several seeds from the confinis x confinis pollination two weeks after the pollinations were made: no visible differences could be detected between these seeds.

The number of seeds per spikelet which began to form after the interspecific crosses, in which P. confinis was the female parent, was only slightly less than the number found on P. confinis plants collected in nature.

In nature, two seeds per spikelet seem to form regularly; after the crosses in the greenhouse the average was slightly over 1½ seeds per spikelet. Dissection of the spikelets containing the shrivelled seed revealed that almost all the primary florets bore seeds, but only about 50 per cent of the secondary florets contained a seed. This accounts for the difference in the percentage of seed developing in nature and the percentage developing after the crosses. There did not appear to be any difference between the percentage of seed forming after the cross confinis x pratensis and the cross confinis x compressa. In the crosses where P. macrantha was the female parent about two seeds per spikelet formed, although not enough inflorescences were pollinated to make very accurate estimates. Comparable figures from nature were not available for P. macrantha.

All seeds which were not used for embryo excision (below) were placed on moist filter paper in Petri dishes, but not a single seed germinated out of the several hundred obtained from the various interspecific crosses. The shrivelled seeds absorbed moisture and looked plump and healthy after about three days in the Petri dishes, but closer examination revealed that the maternal tissues of the seed formed a hollow shell, devoid of normal endosperm tissue. Apparently, most of the seeds

had already been infected with mould organisms at the time they were placed in the Petri dishes. Very few seeds were not mouldy after a week.

The seeds from the cross macrantha x confinis were somewhat plumper than those from any of the other crosses, although still being slightly shrivelled. Unlike any of the seeds from the other interspecific crosses, a number of those from the macrantha x confinis cross still contained fairly large amounts of endosperm when placed in the Petri plate. However, none of these seeds germinated.

At the time of writing (two weeks after the germination tests were started), none of the seeds from the confinis x confinis pollinations have germinated, although most of the seeds have not yet been touched by mould growths.

3. Embryo culture

(a) Materials and methods

After the seed from the first group of crosses shrivelled it was decided to try some embryo excision before the seed from the later crosses also began to shrivel.

As far as possible, only seed that had not begun to shrivel was used. The seeds were first soaked in .1

per cent mercury bichloride solution for twenty minutes and then rinsed in distilled water for five minutes. Each seed was placed immediately on the flamed glass plate of the stage of a Reichert binocular dissection microscope. A retort ring at a height of two inches above the stage of the microscope enabled the hands to be kept steady during the excision procedure.* The embryos were excised with the aid of two very sharp-pointed, flamed scalpels under a magnification of x 40. Each embryo was dissected out with as little associated tissue as possible adhering to it.

The embryos were cultured on media used by Randolph and Cox (88) for growing Iris embryos. Sodium hexametaphosphate (Calgon) is the source of phosphorus in this 0.7 per cent agar medium containing 2 per cent cane sugar, together with other mineral salts. This was prepared in 125 cc. Erlenmeyer flasks, and several embryos were placed together in each flask. The constant temperature room in which the embryo cultures were placed was kept at 25° C.

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* This idea was suggested to the writer by Engelbert (37), who used a retort ring for steadying her hands while emasculating Poa florets. The inflorescence of the plant being emasculated was put through the retort ring.

(b) Observations and results

Embryos were successfully excised from seeds which formed after the following crosses:

	No. Embryos Excised
P. confinis x *P. pratensis*	(5)
P. confinis x *P. compressa*	(10)
P. macrantha x *P. compressa*	(7)
P. macrantha x *P. confinis*	(7)

In some of the seeds at the time of excision the beginning of endosperm failure could be seen, as evidenced by a brown colour of the outer endosperm tissue and the retraction of the endosperm from the inner wall of the seed coat.

Some of the embryos were undoubtedly excised a few days too late and were probably already contaminated with fungus spores. There was very little differentiation of embryonic tissues noticeable in any of the seeds which were dissected, except one or two of those from the cross *macrantha* x *confinis*.

At the time of writing (eighteen days after the excisions were made and the cultures started) six of the embryos from the *macrantha* x *confinis* cross are still alive and growing well. There are signs of root growth and tissue differentiation in some of them and all have enlarged

considerably. Only three other embryos have not been contaminated by bacterial or fungus growths. Two of these are from the cross confinis x compressa and the third from the cross macrantha x compressa. These three embryos appear to be still alive, but as yet there is little or no sign of growth in them.

VII DISCUSSION

On the basis of both the herbarium and field studies it seems evident that P. confinis is a rather distinct species, a conclusion already drawn by Marsh (68). Although it grows in many locations in close association with P. pratensis, P. compressa, P. macrantha and probably other Poa species, there is no obvious indication that hybrids are formed between these species and P. confinis in nature. However, it should be emphasized that this conclusion is based on somewhat limited observations. The different flowering times of P. confinis and some of the other bluegrasses probably reduces the opportunity for interspecific crossing to occur; furthermore, as suggested by the results from the hybridization attempts, any seed that does form after interspecific crossing involving P. confinis is probably inviable.

The extreme uniformity of dune bluegrass throughout its range of upward of 600 miles along the Pacific Coast is very noticeable; as previously noted, any variability that does exist in a few characters seems to bear no relationship to the geographic distribution of the species. The fact that P. confinis probably rarely reproduces by seed undoubtedly contributes to the lack of variation; many of the biotypes have possibly existed for

hundreds of years, maintaining themselves entirely by means of the rhizomatous root system. Evidence of this is particularly striking at Point Roberts, where the fairly extensive, individual clans of the grass on the sand banks are composed of either all pistillate or all staminate plants. Quite likely all the plants within each group are members of a single clone.

Both staminate and pistillate clones seem to exist at most localities, although this feature has not been checked closely. At any rate, the situation in P. confinis certainly does not parallel that already mentioned (page 47) in P. nervosa, which has only plants with abortive anthers over most of its range.

The problems concerned with dioecism in plants --its sporadic occurrence throughout the plant kingdom, its possible advantages, and other features--are of considerable interest. Although the present study has not contributed much to the general knowledge of dioecism, the mere fact that P. confinis is a dioecious species is of some special interest.

Exactly what benefits dioecism confers upon dune bluegrass, growing as it does in such a restricted habitat, is hard to imagine. It is interesting that the two other Poa species which are confined solely to the sandy areas

of the Pacific Coast of North America, P. Douglasii and P. macrantha, are also dioecious. The writer has little to suggest beyond Darwin's (34) idea that under severe conditions the separation of the sexes may avoid the strain on the reproductive powers of a plant which develops both male and female organs simultaneously. A further possibility is that the separation of the sexes may allow the accumulation of greater amounts of hidden variability within the species than would be possible if the plants were hermaphroditic. Presumably, in hermaphroditic plants, mutations affect, and are transmitted by both male and female elements alike. In a dioecious species, on the other hand, different mutations will accumulate independently of each other in the plants of the two sexes. In this way a greater amount of hidden variability is available for release following crossing. In a species such as dune bluegrass, which probably rarely reproduces by seed, "flexibility" is sacrificed somewhat for "immediate fitness" (97). But dioecism may provide a means of maintaining greater flexibility or capacity for variation through the independent accumulation of mutations in male and in female plants.

The adaptation of P. confinis to sandy areas of a particular type is in itself rather interesting, and there is much room for conjecture about the plant's ecology.

Such factors as soil aeration and plant competition may have much to do with the apparently narrow range of adaptation of dune bluegrass. It is rather surprising that it has grown so well on a soil with fine aggregates at the University farm, where it shows itself capable of spreading rapidly and forming a thick sod, maintaining a vigorous leaf growth and flowering profusely. On the other hand, the plants growing in soil in the greenhouse did not do as well as those in sand.

The sand dunes and beach flats where P. confinis thrives are obviously not as bare and unsuitable for plant growth as one might initially suppose, although P. confinis certainly exists under more stringent conditions than most plant species. A certain amount of organic matter is always available from the decay of driftwood and from what is blown by the wind. As Salisbury (91) has pointed out, too, ". . . dune soils are normally not saline despite their proximity to the sea." because leaching is rapid. Since most of the Pacific Coast receives a fairly heavy rainfall, heavy leaching must be expected. Furthermore, the moisture-holding capacity of the sand or sandy soil must be low.

The experiments on the induction of flowering in P. confinis mostly point out the need for further study

on this aspect of the problem. The fact that P. confinis plants brought into the greenhouse from the field in December did not flower, whereas those brought in during February (and later) did flower, suggests the need for a definite period of dormancy and/or low temperature before flowering can be initiated. Further experiments using cold treatments might enable flowering to be initiated even earlier in the winter or perhaps at other times of the year. The lack of control over the temperatures in the greenhouse makes flowering experiments somewhat difficult. The light conditions in the greenhouses at The University of British Columbia are usually poor during the winter, and trouble is apparently often encountered in inducing plants to flower in the greenhouses at this time. Flowering is frequently sparse if it does occur at all.

Almost all the cytological details of both male and female gametophyte development seem to indicate that P. confinis reproduces sexually, and all the slides examined have been interpreted in the light of this final conclusion.

A careful study of all stages of female gametophyte development has failed to reveal any irregularities that might lead to apomictic embryo sac and seed formation. It should be emphasized that the regular behaviour of the

EMC at meiosis is not in itself evidence of sexual reproduction, although it does preclude the possibility of any form of diplospory occurring. However, in some aposporous biotypes of other Poa species the EMC meiosis is quite normal (44, 68, 84, 100). The subsequent disintegration of the meiotic products in such plants, accompanied by the development of the aposporous embryo sac or sacs, is therefore apparently not related to the regularity or irregularity of the EMC meiosis. On this basis it is possible to assume that P. confinis might be aposporous --the embryo sacs developing from somatic cells and thus being diploid. It becomes very important, therefore, to establish the origin of the embryo sac initial cell that can be seen below the disintegrating megaspores in the characteristic "umbrella" stages. Unfortunately, no chromosome counts have been obtainable in any initial cell, or in any later embryo sac stages; however, there are other indications that the embryo sac initial, or one-celled embryo sac, actually is a haploid megaspore derived from the normal meiosis.

The fact that the initial cell is always found directly in line with the disintegrating cells of the triad or tetrad, in the longitudinal axis of the ovule, is not suggestive of aposporous development as it occurs in other typical aposporous apomicts. In most aposporous

Poa species, for instance, the somatic initial cells do not usually have such a definite and constant point of origin, and commonly arise at various places in the nucellus or chalaza (1, 5, 63, 84, 100). A second observation which lessens the likelihood of aposporous development is the appearance in early stages of only one embryo sac initial in an ovule, and in later stages of only a single embryo sac (with two exceptions, as previously mentioned). This, again, is unlike the commonly encountered situation in other aposporous apomicts, where several somatic cells often develop simultaneously and compete with each other, or where a sexual embryo sac and one or more aposporous embryo sacs compete with each other. As Åkerberg (1) first proposed for P. pratensis, there should consequently be a positive association between polyembryony and apospory. On the other hand, there is no a priori reason for assuming that the development of a single aposporous cell, always at the basal end of the line of megaspores, is not theoretically possible in P. confinis.

A further probable indication of sexual embryo sac development is that four disintegrating megaspores have never been seen in any ovule. Admittedly, there are a great many slides in which the number of disintegrating megaspores is difficult or impossible to count, but some

slides definitely showing only two or three such cells can be seen. This suggests that one of the megaspores, rather than a somatic cell, always functions as the embryo sac initial. Where only two disintegrated cells appear the second meiotic division in one of the dyad cells has likely failed; where three disintegrated cells appear the second division has presumably been completed. In either case, a functional megaspore completes the triad or tetrad. The interpretations of the rather critical stages at this period of development have all been based on the foregoing presumptions. The fairly frequent occurrence of triads in P. confinis is also found in other Poa species, and may be taken as a normal phenomenon. Tinney (100) and Åkerberg (4), working with apomictic strains of P. pratensis, found that the formation of a triad was more frequent than the formation of a complete tetrad. This was due to the partial or complete failure of the second division in the micropylar dyad cell. Håkansson (50) noted the same thing in sexual strains of P. alpina.

The origins of the twin embryo sacs seen in two slides are unknown. Although the presence of more than one EMC in a single ovule has never actually been shown in the pistillate plants, two EMCs do occur in one of the non-functional pistils of a male floret, as previously

noted. Therefore, there is good reason to believe that two EMCs could occasionally develop also in the ovule of a pistillate floret, giving rise to two spore triads or tetrads and two functional megaspores. A second possible source of twin embryo sacs is the simultaneous development of two megaspores derived from a single EMC. That it is not exclusively the chalazal megaspore which is functional is evident from Fig. 9, D, which shows the second megaspore from the chalazal end developing as the embryo sac initial. It also seems possible, then, that both a chalazal megaspore and one of the other spores could be functional. This may actually be occurring in Fig. 9, D, although the basal megaspore is not as well developed as its neighbor.

The division and growth of the antipodal cells in the later stages of embryo sac development is certainly remarkable, but probably has little or no bearing on the problem of determining the method of reproduction in P. confinis. According to Andersen (10) "The Gramineae are conspicuous for their strongly developed antipodals." and Maheshwari (67) also states that the presence of many antipodals is common in the grasses. A number of workers have reported the occurrence of unusual antipodal behaviour in Poa species. Andersen (10) found antipodals of great size in P. compressa and P. pratensis biotypes

which she studied. They had dense protoplasm, and persisted until late into endosperm formation. Tinney (100) reported the presence of up to five or six antipodals in some of his apomictic pratensis biotypes, while Nielsen (71) noted the enlargement and the densely-staining property of the antipodals in sexual plants of the same species. Åkerberg (5), investigating apomictic strains of Kentucky bluegrass, found the antipodal cells almost filled the whole of the nucellar chamber, and Håkansson (51) observed a similar phenomenon in sexual strains of P. alpina, particularly in unfertilized embryo sacs. The writer is unaware of any explanation for excessive antipodal development; possibly such tissue may function as a temporary source of nutrition for the egg cell and fusion nucleus when pollination and fertilization are delayed for some reason.

There is no sign of parthenogenetic embryo development in P. confinis, even in florets which have been kept unpollinated for some days after anthesis. In other apomictic Poa species, as has already been seen, precocious embryo formation is quite common, and is not contingent upon pollination, even though endosperm formation usually requires pollination and (probably) the fertilization of the fusion nucleus.

The lack of material and shortage of time did

not permit a very extensive examination of microsporogenesis to be made, although it was studied closely enough for the writer to conclude that it is quite regular. Nielsen (77) says that "Among the grasses most of the apomictic species examined have exhibited a considerable amount of meiotic disturbance, particularly during microsporogenesis." Once again, although the evidence from the study of microsporogenesis is not in itself conclusive, it provides a further indication that reproduction in P. confinis is probably sexual.

The cytological study of embryo and endosperm development following the pollination of female P. confinis florets with pratensis pollen is of considerable importance. The evidence certainly points to the formation of true hybrid embryos between the two species, although the possibility that the pratensis pollen merely stimulates the development of haploid (or diploid) egg cells into embryos cannot be entirely ruled out. The period of delay between pollination and both endosperm and embryo formation is certainly long enough for double fertilization to take place. On the other hand, the fertilization of the fusion nucleus only may be involved. Unfortunately, because of the small size of the ovules, P. confinis is particularly unfavourable for the observation of fertilization.

The fact that embryos and seeds form in all the

crosses involving P. confinis and P. macrantha (except those with P. annua), and that in all cases the seeds subsequently shrivel at about the same stage of maturity, shows that whatever the explanation of embryo and endosperm formation may be it is probably the same in all cases. The production of apparently normal seed following the artificial pollination of female P. confinis florets with confinis pollen is also important; it shows almost definitely that the shrivelling of the seed from the interspecific crosses is not due merely to environmental conditions in the greenhouse, aphid infestations, etc. The formation of apparently normal seed, followed by the abortion of the seed prior to its maturation, is a fairly common development after certain interspecific crosses in other genera (67). From the observations made during the embryo excision it appears that the seed abortion following the interspecific crossings is due to some form of endosperm failure in the later stages of development. There has been considerable study carried out on the development and role of the endosperm, its physiological relationship, and other features. Further histological study of the endosperm failure following the crossings made in the present study might be worthwhile.

The very brief attempts at embryo excision and culture show that there is hope of obtaining hybrid plants

between P. confinis and other Poa species by this method (assuming that the embryos which form are actually interspecific hybrid embryos, and not haploids). Chromosome counts in these putative hybrids, if they can be raised to a sufficiently advanced stage, should provide the final proof of the nature of their origin. It is obvious that more work needs to be done on embryo culture; the writer has only been able to show that it is definitely practical. Since a method for inducing flowering in P. confinis and other bluegrasses at the same time is now known, and the embryo excision and culture shown to be fairly easy, the culture of quite a large number of embryos should not be a very long or difficult task.

The formation of a higher percentage of healthier-looking seed from the cross P. macrantha x P. confinis, together with the success of the embryo culture following this cross, is possible evidence of a closer relationship between these two species than between any other two species entering into the crosses.

On the basis of the results from the cytological and hybridizational work it is obvious that any breeding work of the type carried out in the Carnegie Institution of Washington program will not be applicable to P. confinis. Any hybrids between P. confinis and other species would

presumably reproduce sexually and would not give rise to apomictic lines in later generations, even if the other parent was apomictic. This conclusion is based on the fact that so far apomictic hybrid lines have only been obtainable following crossings in which both parental strains or species are themselves predominantly apomictic. Through backcrossing of hybrids with apomicts as recurrent parents it might be possible to obtain apomictic hybrid lines eventually. However, this reasoning is very speculative and probably very impractical. The chances of obtaining economically worthwhile hybrid lines in this manner are certainly rather remote, and the economic use of dune bluegrass *per se* is much more likely. Nevertheless, further attempts at interspecific hybridization involving *P. confinis* should be carried out if possible; any hybrids will be of considerable scientific interest even if they are of no practical value.

Several aspects of the *P. confinis* study which might have been investigated are worth mentioning as lines of further possible work. One of these is a study of the range of chromosome numbers in dune bluegrass. Despite the uniformity of the species throughout its distribution range, differences in chromosome numbers may exist in biotypes from separate localities. The fact that the only previously recorded chromosome count (by Hartung (53) made

on a female plant from Tillamook Bay, Oregon) and that determined by the writer are both the same (2n = 42) does not mean that other euploid or aneuploid numbers may not exist.

Another problem which has not been covered is that of the ratio of male to female plants in the progenies of P. confinis plants. If P. confinis reproduces sexually the sex ratio should be approximately 1:1 in seedling progenies (assuming that other factors, such as unequal viability of male and female seedlings, do not enter into the picture). If it reproduces predominantly by apomixis (which, as we have now seen, is very improbable), the sex ratio would be considerably modified. Because apomixis implies the production of progeny identical with the maternal parent one would expect all apomictically formed seed to give rise to female plants. Male plants could presumably only arise from seed formed sexually. This question of dioecism and apomixis has not been discussed earlier because it seems to have no direct bearing on the study of dune bluegrass, but it should be mentioned that a number of dioecious plants in several genera are also apomictic (45). However, the writer is not aware of any papers on dioecious apomicts in which the problem of the sex ratio and apomixis is fully discussed.

The small size and lack of heteromorphism of the chromosomes of P. confinis makes any detection of sex chromosomes very unlikely. In any event, Allen (9) points out that "A visible difference between the chromosomes of a pair does not necessarily accompany dioecism." As yet, there are apparently no effective staining techniques which enable the inert, heterochromatic regions of sex chromosomes to be picked out.

A number of P. confinis plants from several biotypes have been grown from seed and are now established in the University nursery. These will be examined for the determination of sex ratios when they flower.

Further study of dune bluegrass under cultivation needs to be made; at the moment, the potentialities of the species have still not been fully investigated. The development of more trial plots and their maintenance under conditions of standard turf management should be attempted. The opportunity for adequate testing of different turf species and strains now seems to be possible following the formation of the University of British Columbia President's Committee on Sports Turf Research. A fine turf nursery has been established on the University farm, and funds are available for its upkeep. The establishment of P. confinis on a sandy area of the Point Grey Golf and Country Club is also contemplated.

VIII SUMMARY

Dune bluegrass, Poa confinis Vasey, is a dioecious grass native to the Pacific Coast of North America. It has a very restricted habitat, being confined almost entirely to the semi-stabilized, porous sand areas of the coast. Herbarium studies, and first-hand observations of a number of P. confinis sites indicate that it is a rather distinct species.

Under cultivation on The University of British Columbia farm P. confinis grows vigorously and forms a thick sod. Its fine-leaved growth, rhizomatous root system and rapidly-spreading habit, together with the sandy nature of its native habitat, all suggest that it may find use as a turf species for sandy golf courses and similar areas along the coast.

A survey of the literature on reproduction in the bluegrasses reveals that apomixis is widespread in Poa, and that apospory or diplospory followed by pseudogamous embryo development is the usual form of apomictic reproduction. Breeding procedures with apomictic bluegrasses must be considerably modified, but the standard techniques of improvement are still theoretically available to the bluegrass breeder.

A cytological study of embryo sac development

in pistillate plants indicates that it follows the "normal" scheme, and that reproduction is sexual. Each ovule contains a single EMC which undergoes a regular meiotic division, giving rise to a triad or tetrad of megaspores, one of which forms the haploid embryo sac. Somatic chromosome numbers of 2n = 42 are found in two EMCs in diakinesis. The presence of twin embryo sacs is observed in two ovules. Very marked antipodal development, with an increase in size and number of cells, and in the number of nuclei per cell, is characteristic of the mature female gametophyte.

Microsporogenesis in the staminate plants also appears "normal." Studies of anthers in pistillate florets and ovules in staminate florets show that their development proceeds normally up to a certain point and then breaks down.

Embryo and endosperm development can be seen in sections of ovules prepared after the pollination of P. confinis by P. pratensis. The embryos are believed to be the product of true hybridization. Seed forms after all the crossings among P. confinis, P. macrantha, P. pratensis and P. compressa using the first two species as female parents, but the seed invariably shrivels shortly before reaching maturity.

The excision of hybrid embryos from seeds formed after some of the interspecific crosses, and the culture

of these embryos on agar media have been successfully carried out. Tissues of putative *macrantha* x *confinis* hybrids are growing, and showing some differentiation.

The results indicate that hybrids between *P. confinis* and other bluegrasses can probably be obtained through embryo culture techniques. These would be of considerable scientific interest; at the moment, however, their practical value, if any, is not known.

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X APPENDIX

Full botanical nomenclature for all plant species and subspecies mentioned in the text:

Grindelia squarrosa (Pursh) Dunal.

Poa abbreviata R. Br.

" alpina L.

" ampla Merr.

" annua L.

" arachnifera Torr.

" arctica R. Br.

subsp. caespitans (Simm.) Nannf.

" depauperata (Fr.) Nannf.

" elongata (Bl.) Nannf.

" microglumis Nannf.

" stricta (Lindeb.) Nannf.

" arida Vasey

" atropurpurea Scribn.

" bulbosa L.

" caespitosa Hk.

" Canbyi (Scribn.) Piper

" compressa L.

" confinis Vasey

" curta Rydb.

" cuspidata Nutt.

" Douglasii Nees.

Poa eminens Presl.

" glauca Vahl.

" glaucifolia Scribn. and Will.

" gracillima Vasey

" herjedalica H. Smith=(P. alpina L. var. vivipara L. x P. pratensis L. subsp. alpigena (Fr.) Hiit.)

" jemtlandica (Almqu.) Richt.=(P. alpina L. var vivipara L. x P. laxa Hke. subsp. flexuosa (Sm.) Hyl.)

" Kelloggii Vasey

" labradorica Steud.

" laxa Hke.

" subsp. flexuosa (Sm.) Hyl.

Poa laxiflora Buckl.

" macrantha Vasey

" nemoralis L.

" nervosa (Hook.) Vasey

" nevadensis Vasey

" palustris L.

" Piperi Hitch.

" pratensis L.

subsp. eupratensis Hiit.

" angustifolia (L.) Lindb. Fil.

" irrigata (Lindm.) Lindb. Fil.

" alpigena (Fr.) Hiit.

Poa Pringlei Scribn.

" rhizomata Hitch.

" scrabrella (Thurb.) Benth.

" trivialis L.

Rosa gymnocarpa Nutt.