Polyploid Stenotaphrum Germplasm: Resistance to the Polyploid Damaging Population Southern Chinch Bug

Philip Busey*

ABSTRACT

'Floratam' St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] has been widely used in lawns because of its resistance to the southern chinch bug (*Blissus insularis* Barber). The southern chinch bug adapted genetically to Floratam and seriously damaged this cultivar within 12 yr after release. The Floratam-adapted southern chinch bug has been described as the polyploid damaging population (PDP), because polyploid germplasm had previously shown resistance. This study examined polyploid germplasm for resistance to the PDP southern chinch bug. *Stenotaphrum* clones were bioassayed by confining stolon cuttings with PDP southern chinch bugs from different regions. Resistant clones were detected based on reduced southern chinch bug longevity, oviposition rate, and excrement production, compared with Floratam. The most resistant clones were PI 365031, a pembagrass [*S. dimidiatum* (L.) Brongn.], and FX-2, FX-10, and FX-33 (intercrosses of polyploid *S. secundatum* from Africa). They supported low PDP southern chinch bug oviposition (≤5 eggs female^{-1} wk^{-1} and ≤25 eggs lifetime^{-1}). Resistance of FX-33 was verified against PDP southern chinch bugs (representing laboratory colonies and field recollections) in mated pairs and congregations, and against southern chinch bugs that had received different prior food sources. Both FX-33 and FX-10 typified the African polyploid St. Augustinegrass germplasm by having $2n=30$ chromosomes, bivalent pairing at metaphase I, good crossed seed set, and abundant laminar hairs.

THE GRASS *Stenotaphrum* is widely distributed in coastal areas of the Pacific and Indian Oceans. Of seven species recognized (Sauer, 1972), only *S. secundatum*, St. Augustinegrass, is naturalized in the New World. An aggressive, stoloniferous perennial, it is important as a subtropical turf and pasture grass, and as a ground cover under coconut, *Cocos nucifera* L. (Smith and Whiteman, 1983). As is common among the Paniceae (Gould, 1968), most chromosome counts in St. Augustinegrass are multiples of $x = 9$, including triploids ($2n=27$) and tetraploids ($2n=36$) (Long and Bashaw, 1961). Floratam St. Augustinegrass has about $2n=32$ (Busey, 1979), which is anomalous, and it has poor seed set. Pembagrass, *S. dimidiatum* is widely used as a turf and pasture grass in the tropics of the Old World. This species is the wild relative closest to *S. secundatum* in ecological habitat and taxonomy. Chromosome numbers reported for *S. dimidiatum* are $2n=48$ from Malagasy (Sauer, 1972), which is anomalous for the genus, and $2n=36$ from Sri Lanka (Gould and Soderstrom, 1974).

St. Augustinegrass is the most widely used turfgrass in Florida. Extension agents recognize the chinch bug, *Blissus* spp., as its most serious insect pest (Busey and Coy, 1988). The southern chinch bug, *B. insularis*, is generally associated with St. Augustinegrass (Leonard, 1966). Because of its genetic resistance to the southern chinch bug (Reinert and Dudeck, 1974), Floratam St. Augustinegrass was released in 1973 (Horn et al., 1973). By 1981 this asexually reproduced cultivar represented 77% of commercial sod sampled in South Florida (Busey, 1986). The resistance of Floratam has been described as antibiosis, because of the high mortality and reduced oviposition of southern chinch bugs confined on Floratam (Reinert and Dudeck, 1974), but its basis in morphology or chemistry is unknown. Siblings of Floratam, FA-118 and FA-108 ('Floralawn'), are resistant (Reinert and Dudeck, 1974), and so are gamma-ray-induced mutants from Floratam (Reinert et al., 1981). In St. Augustinegrass, only polyploid clones are resistant (Reinert et al., 1986). Resistance occurs also in the related *S. dimidiatum*.

Recently, a polyploid damaging population (PDP) of southern chinch bug population was discovered that kills Floratam St. Augustinegrass (Busey and Center, 1987). The host adaptation of the PDP southern chinch bug is genetic, based on its transmission to reciprocal crosses involving different southern chinch bug populations (Busey, 1990). Because it is propagated asexually, Floratam retains its resistance to standard (STD) southern chinch bugs (Busey and Center, 1987), but not to the adapted PDP southern chinch bug. The problem of PDP southern chinch bugs has been reported in lawns and in sod farms, in both muck and sand soil, and in most counties of Florida (Busey and Coy, 1988). Floralawn St. Augustinegrass, released in 1986 (Dudeck et al., 1986), is also susceptible to the PDP southern chinch bug (Busey and Center, 1987). The objective of this study was to find St. Augustinegrass germplasm resistant to the PDP southern chinch bug.

MATERIALS AND METHODS

The *Stenotaphrum* lines were asexually reproduced clones. One clone, PI 365031, from the Republic of South Africa, was *S. dimidiatum* (L.) Brongn; other clones were *S. secundatum*. Floratam was obtained from foundation stock at the Fort Lauderdale Res. and Education Ctr., in Davie, Broward County, FL. 'Bitterblue' and 'Florida Common' were accessions representing trade types (Busey, 1986) known to be susceptible to STD southern chinch bugs. Polyploid African introductions PI 290888, 293666, 300127, and 300130 were used as parents; they came from W.R. Langford (USDA-ARS, Regional Plant Introduction Stn., Experiment, GA) and had been collected in 1963 and 1964 by W.W. Huffine and A.J. Oakes. Intercrosses were made among African germplasm, from 1980 to 1983. Inflorescences were emasculated, crossed, and covered with bags for 1 d. Crossed seed set was calculated by dividing the number of seed produced by the number of emasculated, pollinated florets. Progeny were obtained in 1983 from an open-pollinated plant of Floratam that had been grown in a greenhouse at Fort Lauderdale, and that also contained African germ-

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plasm. FX-332 was a diploid susceptible to the STD southern chinch bug (Busey, unpublished data), and FX-703 was a lawn collection identified as Floratam.

Four resistance bioassays were performed. There were four sources of southern chinch bugs: (1) PDP-1, an Orange County, Florida, PDP lawn strain that was found damaging Floratam and had been maintained as a laboratory colony since its collection in November 1985; (2) PDP-2, a Hendry County, Florida, PDP sod strain that was found damaging Floratam and had been maintained as a laboratory colony since its collection in October 1985; (3) PDP-Field, a Hendry County, Florida, PDP sod strain that was recollected in October 1987; and (4) STD, a Fort Lauderdale standard (non-Floratam-damaging) laboratory strain. Except for PDP-Field southern chinch bugs, these were the same sources used by Busey and Center (1987) and had been maintained on Floratam (PDP southern chinch bugs) or Florida Common (STD southern chinch bugs) for at least 1 yr.

Adult insects were confined on terminal stolon cuttings of *Stenotaphrum* clones. For at least the first 83 d in all bioassays, cuttings were obtained from plots in a uniformly managed field at the Fort Lauderdale Res. and Education Ctr. Cuttings were about 100 mm long and had two to four nodes. Leaves and roots were removed, except that roots on the lowermost node were not removed and sheaths on the uppermost node were cut back, but not removed. The rooted end of each cutting was placed in a water-filled glass tube (10 by 75 mm) to which it was tightly secured with a band of Parafilm (American Can Co., Greenwich, CT) wrapping. Once each week, stolon cuttings were changed, the number of eggs counted, and survival of the original males and females noted. Because bug survival was observed only once each week, longevities may have underestimated longevity in confinement by up to 7 d. Except as indicated, southern chinch bugs were maintained in confinement for their lifetimes (as long as 243 d). For bioassays involving southern chinch bug pairs, one tube-and-cutting unit was placed in a glass culture tube (25 by 150 mm) in which one male and one female southern chinch bug were confined. Culture tubes were covered with a double layer of Kimwipes (Kimberly-Clark Corp., Roswell, GA), fastened to the tube rims with either rubber cement or a band of Parafilm. For bioassays involving southern chinch bug congregations, four tube-and-cutting units were placed in a plastic bin (70 by 125 by 55 mm deep) covered with Kimwipes. Various numbers of males and females were placed in each bin.

Except as indicated below, experimental designs were randomized complete block. The experimental unit was the unit of southern chinch bug confinement, either culture tube (southern chinch bug pairs) or bin (southern chinch bug congregations). Longevity and cumulative (lifetime) oviposition were analyzed by the GLM procedure (SAS Institute, 1988), and clone means were compared by the Waller-Duncan k-ratio t-test, using the pooled-error mean square. Oviposition was analyzed and means were compared using log-transformed values [$\ln(x + 0.5)$], but original values are reported. Except as indicated, oviposition rate (eggs female^{-1} wk^{-1}) was an average of full weeks of female survival. Two sources of PDP southern chinch bug (PDP-1 and PDP-2) were pooled for means presentations, because there was no grass clone × southern chinch bug source interaction ($P > 0.05$).

The first resistance bioassay was a preliminary culture-tube screening of 20 *Stenotaphrum* clones against all four southern chinch bug sources (PDP-1, PDP-2, PDP-Field, and STD). Clones were eight African crosses (FX-1, FX-2, FX-10, FX-14, FX-33, FX-40, FX-41, and FX-43), seven Floratam progeny (FX-3, FX-4, FX-5, FX-11, FX-20, FX-35, and FX-89), FX-332, FX-703, Floratam, Bitterblue, and PI-365031, which had been selected from a larger germplasm based on novelty or superior performance in previous field trials (Busey, unpublished). Only FX-332 was diploid ($2n$= 18). Plant introduction (PI) parents were not included, because of poor field performance. There were three replications (pairs of southern chinch bugs in culture tubes) of 80 southern chinch bug source × grass clone combinations. Southern chinch bug survival and number of eggs were recorded weekly for 4 wk. In this bioassay only, oviposition rate was calculated as the total egg production divided by the number of weeks, 4; thus, females that had died contributed zero, and were not considered missing. Excrement production (an indicator of the degree of feeding activity) was rated visually on a scale of 0 to 3, with 0 = no visible milky deposit on culture tube; 1 = one droplet present, <1 mm diam.; 2 = noticeable multiple-droplet excrement deposit; and 3 = extremely heavy excrement deposit.

The second resistance bioassay was a verification of six possibly resistant clones from the previous bioassay (FX-40, FX-5, FX-2, FX-10, FX-33, and PI 365031), compared with Floratam. There were 10 replications (pairs) involving PDP-1 southern chinch bugs and 8 replications with PDP-2 southern chinch bugs. Excrement production was again rated.

The third resistance bioassay was a comparison of the effect of prior food sources, Floratam and Florida Common, in a factorial design involving present food sources Floratam and FX-33. FX-33 was evaluated in this and the subsequent bioassay, because it had previously provided the lowest oviposition rate of any *S. secundatum*. PDP-Field southern chinch bugs had been maintained in bins for 105 d on prior food sources. Surviving males and females were paired and randomly assigned to present food sources in culture tubes. The design was completely randomized. Replications for different treatment combinations varied from 8 to 11.

The fourth resistance bioassay was a reconfirmation of FX-33 resistance, involving southern chinch bug congregations in bins. The experimental design was completely randomized. Sources were PDP-1 and PDP-2 southern chinch bugs combined and, in another set of bins, PDP-Field southern chinch bugs. At the time this bioassay was conducted, PDP-Field southern chinch bugs had had 92 d to acclimate to laboratory conditions. In addition to FX-33 and Floratam, PDP colony southern chinch bugs were also tested on Bitterblue as an additional, unfamiliar control, but in that comparison the number of females was too small and variable for consideration of oviposition.

Inflorescences of the most resistant *S. secundatum* clones (FX-10, FX-33) and their parents and *S. dimidiatum* PI 365031 were fixed in 3:1 absolute ethanol/glacial acetic acid for 24 h, stored in 70% ethanol v/v, and pollen mother cells were squashed and stained by the iron-acetocarmine technique (Belling, 1921). Diakinesis and metaphase I chromosome configurations were observed with phase-contrast microscopy.

RESULTS AND DISCUSSION

In the preliminary screening bioassay, STD southern chinch bugs had low oviposition rates (Fig. 1A) on all polyploid hosts, except for Bitterblue (13.4 eggs female^{-1} wk^{-1}), which is known to be susceptible. Oviposition rates of STD southern chinch bugs on other polyploids were 0 to 4.7 eggs female^{-1} wk^{-1}, and the excrement production rating was generally low, <1.5. This was consistent with a hypothesis of plant resistance to STD southern chinch bugs. PDP-Field southern chinch bugs had negligible oviposition (mean 1.3 eggs female^{-1} wk^{-1}; Fig. 1B), and there was no difference among hosts in insect oviposition rate. The excrement rating of PDP-Field southern chinch bugs was generally high, ≥ 1.5. The inconsistency of these results was attributed to the PDP-Field southern chinch bugs having recently been removed from the field, and having been in a phase of acclimation to laboratory conditions. The PDP colonies (Fig. 1C) were adapted (>5 eggs female^{-1} wk^{-1}) on about one-half of the polyploid St. Augustinegrasses, including

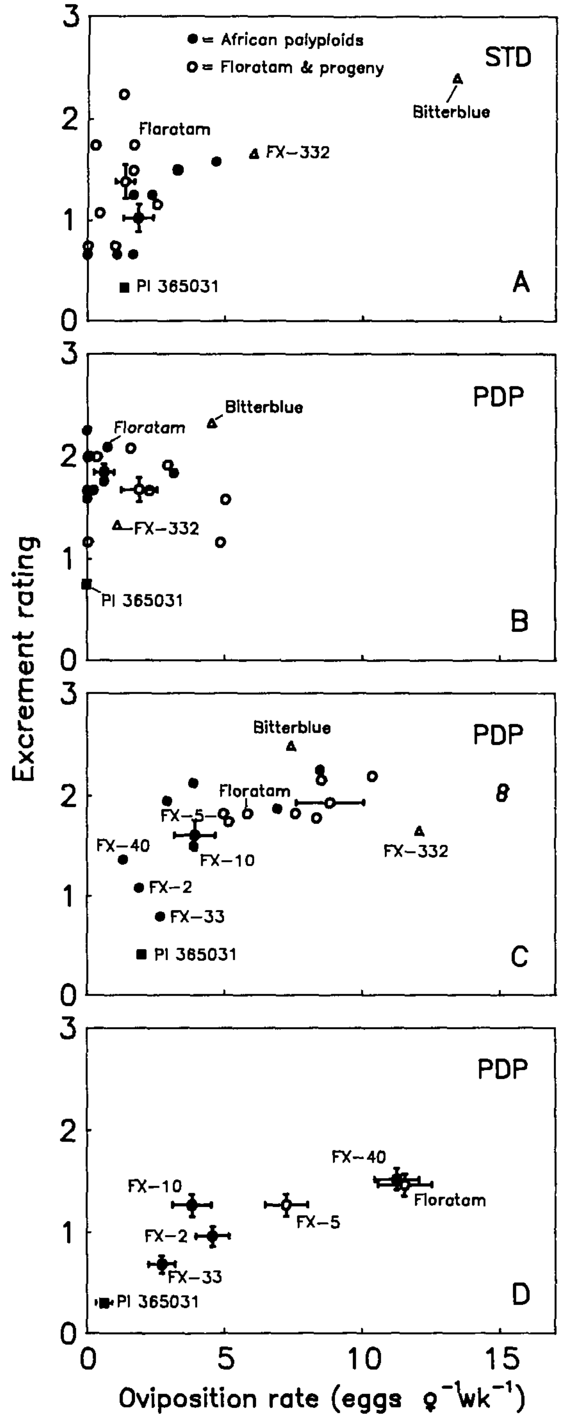

Fig. 1. Oviposition rate and excrement rating (0 = no visible milky deposit to 3 = extremely heavy deposit) of southern chinch bug colonies on *Stenotaphrum* clones. A, B, and C are 4-wk screening bioassays. (A) Standard southern chinch bug colony, means of three pairs; (B) PDP (polyploid damaging population) southern chinch bug field population; (C) PDP southern chinch bug colonies, means of six pairs; and (D) confirmation bioassay, lifetime data, PDP colonies, means of 18 pairs. Bars represent standard errors of clone group means (African polyploids and Floratam & progeny; A–C), or individual clones (D).

Floratam and all its progeny, and those clones were deemed susceptible to PDP southern chinch bugs. All clones with an excrement rating <1.5 had low oviposition rates. African germplasm provided for low oviposition rates (Fig. 1C), and mean PDP colony performance (oviposition rate and excrement deposit) was used to select six clones for confirmation of resistance. Only one selected clone, FX-5, was a Floratam offspring.

In the second resistance bioassay, FX-2, FX-10, FX-33, and PI 365031 supported shorter adult longevity and fewer cumulative lifetime eggs per PDP southern chinch bug than Floratam (Table 1). This confirmed the resistance of those clones. FX-5 supported lower cumulative egg production than Floratam, but no different longevity. FX-40 did not differ in any measure of resistance from Floratam. Among the resistant clones, *S. dimidiatum* PI 365031 was highly resistant (≤2 eggs female^{-1} wk^{-1} and ≤5 eggs lifetime^{-1}), whereas *S. secundatum* FX-2, FX-10, and FX-33 were moderately resistant (≤5 eggs female^{-1} wk^{-1} and ≤25 eggs lifetime^{-1}). The most resistant clones, FX-33 and PI 365031, supported low excrement ratings (Fig. 1D). Whereas the preliminary screening had been based on only three replications, and this subsequent verification was based on 18 replications, the latter means estimates would be expected to be more precise. One clone, FX-33, was selected for more intensive testing.

In the third resistance bioassay, prior food source had no effect on cumulative egg production or longevity, and there was no prior × present interaction. This was important, because it showed that the resistance differences observed were not a short-term

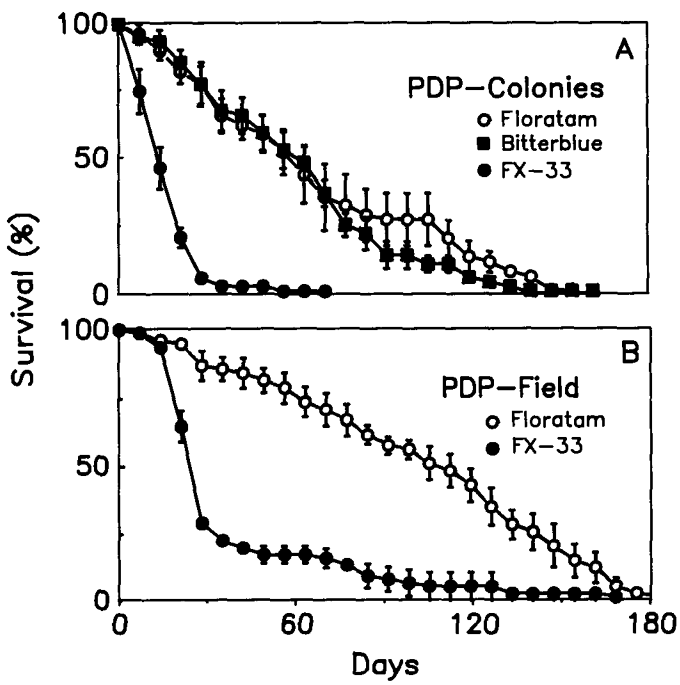

Fig. 2. Survival of PDP (polyploid damaging population) southern chinch bugs on three *S. secundatum* clones. Each line represents mean survivals of four replications (congregations in bins) involving a total of at least 60 southern chinch bugs: (A) colonies; (B) field recollections. Bars represent standard errors for bins of each grass clone and bug source.

phenotypic plasticity, which would be erasable by 105-d exposure to a different host. For purposes of illustration, means from both prior food sources are presented (Table 1). Present food sources differed greatly in cumulative lifetime egg production (12 eggs for FX-33 and 176 for Floratam). These values showed that high oviposition rates of field bugs on Floratam developed with laboratory acclimation over time, but that resistance of FX-33 was still apparent.

In the fourth resistance bioassay, PDP field and colony southern chinch bugs had rapid mortality when confined on FX-33 congregations in bins (Fig. 2). By 2 to 3 wk, higher mortality ($P < 0.05$) was recorded from PDP southern chinch bugs confined on FX-33 than from those confined on Floratam or Bitterblue. There was no difference in longevity between insects on Floratam and the unfamiliar (but susceptible) Bitterblue (Table 1). Longevities on FX-33 were shorter ($P < 0.05$), for both field and colony southern chinch bugs, than on Floratam.

It was difficult to get good chromosome squashes of *S. dimidiatum* PI 365031, because the metaphase chromosomes clumped tightly. This clone had about $2n=54$ at diakinesis, with virtually complete bivalent pairing, in the few cells scored. The parent *S. secundatum* accessions from Africa (PI 290888, PI 293666, PI 300127, and PI 300130) and their PDP-southern-chinch-bug-resistant intercross progeny FX-10 and FX-33 had $2n=30$ chromosomes, with primarily bivalent pairing (Table 2) and regular dissociation at anaphase I (Fig. 3). The African germplasm had high crossed seed set (43% and 70% for original clones and crossed progeny, respectively). African introductions PI 300127 and PI 300130 and progeny FX-10 and FX-33 had laminar hairs, a trait not previously recognized in *S. secundatum*. Several open-pollinated progeny of Floratam had laminar hairs, which indicated that the African introductions were the probable pollen parents of these Floratam progeny. This was confirmed by the reduced lifetime oviposition of PDP southern chinch bugs on FX-5, a Floratam progeny.

The African $2n=30$ St. Augustinegrass germplasm is distinctive and includes clones with acceptable turf characteristics, while PI 365031 *S. dimidiatum* is extremely coarse and of poor density. The abundant laminar hairs of some polyploid *S. secundatum* clones

Table 1. Mean adult longevity, oviposition rate, and cumulative lifetime oviposition of ployploid damaging population southern chinch bugs bioassayed on eight *Stenotaphrum* clones.

Bioassay†	Confinement unit	Source of bugs	*Stenotaphrum* clone	Longevity‡		Oviposition: Weekly rate		Oviposition: Cumulative	
				d		eggs female^{-1} wk^{-1}		eggs lifetime^{-1}	
2	Mated pairs	Colony	Floratam	45.4ab§	(36)¶	11.5a§	(90)#	65a§	(17)††
			FX-40	49.7a	(36)	11.2a	(105)	70a	(18)
			FX-5	37.8bc	(36)	7.2b	(91)	38b	(18)
			FX-2	25.9d	(36)	4.5c	(76)	20bc	(18)
			FX-10	29.9cd	(36)	3.8c	(58)	14bc	(18)
			FX-33	21.2d	(36)	2.7cd	(43)	7c	(18)
			PI 365031	9.5e	(36)	0.6d	(30)	1c	(18)
3	Mated pairs	Field	Floratam	58.8b	(20)	18.2a	(82)	149a	(10)
			FX-33	16.5c	(22)	5.2b	(32)	15b	(11)
			Floratam‡‡	86.3a	(16)	18.8a	(89)	209a	(8)
			FX-33‡‡	21.6c	(18)	3.0b	(22)	7c	(9)
4	Congregations	Colony	Floratam	61.3a	(4)				
			Bitterblue	56.4a	(4)				
			FX-33	11.4b	(4)				
4	Congregations	Field	Floratam	97.0a	(4)	15.6a	(94)	199a	(4)
			FX-33	32.7b	(4)	4.5b	(46)	19b	(3)
Weighted mean			Floratam	72.7		16.0		125	
			FX-33	21.8		3.9		14	

† Bioassay 2 = verification of preliminary culture-tube screening; 3 = comparison of effect of prior food source; 4 = reconfirmation of FX-33 resistance.
‡ Longevity includes only time on bioassay clones and does not include time on prior food sources.
§ Clone means within columns and within confinement-unit/source-of-bugs combinations followed by the same letter are not significantly different ($k = 100$; $P \approx 0.05$; Waller-Duncan k-ratio t-test).
¶ Numbers in parentheses indicate numbers of southern chinch bugs (mated pairs), or number of bins (congregations).
# Numbers in parentheses indicate number of weekly egg observations.
†† Numbers in parentheses indicate number of females (mated pairs) or number of bins (congregations).
‡‡ Prior food source was Florida Common; elsewhere, it was Floratam.

Table 2. Origin, chromosome number, and mean chromosome association of metaphase I and diakinesis pollen mother cells of African clones of *Stenotaphrum secundatum*, and two progeny.

	Clone	Origin	(2*n*)	Cells examined	Chromosome association: I	II	III	IV
			no.					
(A)	PI 290888	Pretoria, Rep. of So. Africa	30	36	0.03	14.94	0.03	0.00
(B)	PI 293666	Kigimbani, Tanzania	30	28	0.00	14.00	0.00	0.39
(C)	PI 300127	Rep. of So. Africa	30	29	0.41	14.21	0.00	0.00
(D)	PI 300130	Uitenhage, Rep. of So. Africa	30	65	0.17	14.95	0.02	0.00
	FX-33	(C × D) × (B × A)	30	42	0.21	14.62	0.02	0.10
	FX-10	(A × B) × (C × D)	30	25	0.48	14.52	0.00	0.00

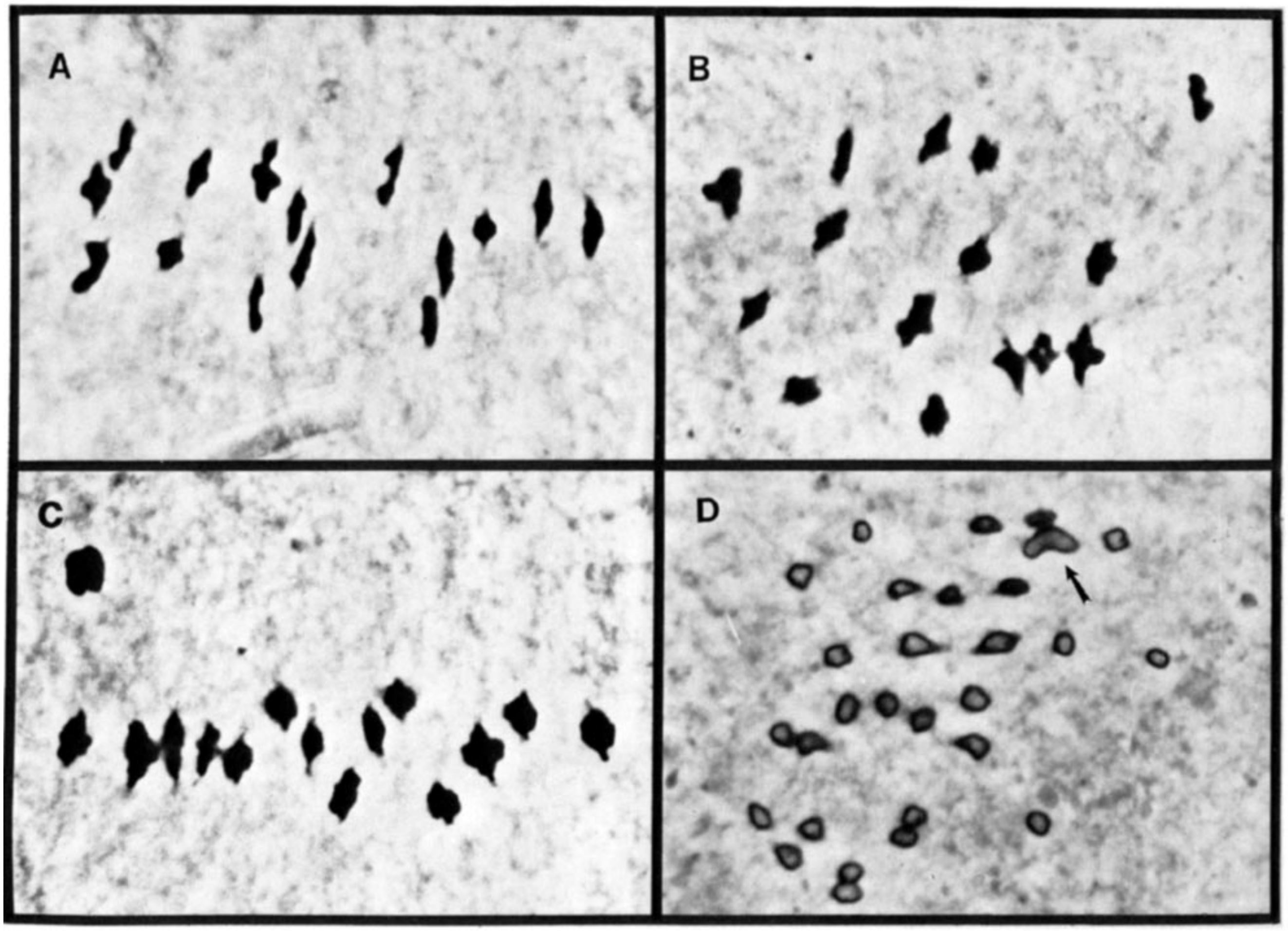

Fig. 3. Photomicrographs of chromosomes of $2n=30$ *S. secundatum* clones: (A) Metaphase I of PI 300130 showing 15 bivalents, × 1850; (B) Metaphase I of FX-10 showing 15 bivalents, × 1850; (C) Metaphase I of FX-33 showing 15 bivalents, × 1850; (D) Anaphase I of FX-33 showing 28 univalents and one late-dividing bivalent (arrow), × 2450.

help further distinguish the African germplasm. In *Stenotaphrum*, only a form of *S. micranthum* (Desv.) C.E. Hubb. from the North Tongan Island of Tafahi has laminar hairs (Sykes, 1970). Although classified morphologically in the same group as Bitterblue (Busey et al., 1982), the African polyploid germplasm has generated clones resistant to the PDP southern chinch bug. There is variation in PDP southern chinch bug resistance among the African germplasm; e.g., FX-40 is susceptible. The $2n=30$ chromosomes of the African germplasm pair regularly and intercrosses are easily made, as well as putative crosses onto Floratam. Previous breeding use of Floratam's southern chinch bug resistance was limited to mutation breeding, because of low seed set. With the present African polyploid germplasm, the high level of resistance to southern chinch bugs, even against the Floratam-killing PDP southern chinch bug, can be manipulated in crossing and selection for this and other traits.

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