Variation for 2n Pollen Production and Male Fertility in Wild *Solanum* Germplasm Resistant to *Phytophthora infestans* (Mont.) de Bary (US-8)

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ABSTRACT

Wild potato species possess genetic variability for valuable traits including resistance to *Phytophthora infestans*, the causal agent for potato late blight disease. Breeding schemes using 2n gametes are a powerful tool for introgressing these traits. Forty-five plant introductions (PIs) across 12 *Solanum* species representing various Endosperm Balance Numbers (EBN) and having resistance to *P. infestans* (US-8, isolate ND 95-2) were screened for 2n pollen production and male fertility. Species evaluated were 2x(1EBN) *S. bulbocastanum*, *S. cardiophyllum*, *S. pinnatisectum*, and *S. trifidum*; 2x(2EBN) *S. berthaultii*, *S. megistacrolobum*, *S. microdontum*, and *S. verrucosum*; 4x(2EBN) *S. fendleri* and *S. stoloniferum*; 6x(4EBN) *S. guerreroense*; and 2x(unknown EBN) *S. polyadenium*. Acetocarmine staining and cytological analyses were used to determine 2n pollen production and pollen stainability from genotypes grown across three locations. Based on frequency, genotypes were assigned to 2n pollen production categories (0≤1%, Low=1%-10%, Medium=11%-60%, and High≥60%) and pollen stainability categories (0≤1%, 1=1%-5%, 2=6%-10%, 3=11%-20%, 4=21%-50%, and 5≥50%). Eleven of the 12 species and 37 of the 45 PIs examined contained genotypes producing ≥1% 2n pollen with ranges of 0%-63.6% and 0%-81.8%, respectively. Dependence was found between location and pollen stainability, EBN and pollen stainability, but not for location and frequency of genotypes with 2n pollen. Stability for 2n pollen production was observed across environments. Among the 1EBN germplasm, dependence was found between 2n pollen and pollen stainability. Resistance to *P. infestans* was found to be independent of 2n pollen production; therefore, identifying genotypes combining 2n pollen production, fertility, and resistance to *P. infestans* was possible.

INTRODUCTION

Wild *Solanum* species possess a wealth of genetic and allelic diversity for economically important traits that can be introgressed into cultivated potato. These traits include resistance to both abiotic and biotic stresses as well as quality-related traits like high specific gravity and low reducing sugars (Bamberg et al. 1994; Hanneman and Bamberg 1986; Lauer and Shaw 1970; Yerk and Peloquin 1989). Late blight, caused by the pathogen *Phytophthora infestans*, is a widespread and aggressive disease that costs the potato industry substantial income due to chemical control through fungicides, reduced yield through foliar and tuber destruction, and loss of infected tubers

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Abbreviations:
ANOVA, analysis of variance; CIP, International Potato Center; EBN, Endosperm Balance Number; FDR, first division restitution; df, degrees of freedom; (M)AUDPC, (mean) area under the disease progress curve; NRSP-6, Inter-Regional Potato Introduction Project; PI, plant introduction; *ps*, parallel spindles; SDR, second division restitution.

during storage. Since all major potato cultivars are susceptible to this disease (Inglis et al. 1996; Jenkins 2000), producers rely heavily on the use of fungicides for its control. However, recent migration of A2, metalaxyl-insensitive races of the pathogen from Mexico (Goodwin et al. 1995) has made management of this disease increasingly difficult (Kato et al. 1997; Platt 1994). Breeding for resistance to *P. infestans* using the relatives of cultivated 4x potato is of great interest to breeders because there is genetic variability among the wild potato species for resistance to new and old races of the pathogen (Bamberg et al. 1994; Douches et al. 2001; Zlesak and Thill 1999). This resistance can be exploited using 2n gametes and EBN manipulation for the purpose of introgression of durable resistance into cultivated potato (Hanneman 1999).

Gametes with the sporophytic chromosome number (2n) are useful tools for introgression and are found throughout *Solanum* species (Quinn et al. 1974; Watanabe and Peloquin 1991). These 2n gametes provide major benefits for introgression, including successful endosperm development due to a 2:1 maternal to paternal ratio of genetic factors in the endosperm (Johnston and Hanneman 1980, 1982), increased pollen germination and viability (Simon and Peloquin 1976), and transmission of large amounts of heterozygosity and epistasis to progeny (Hermsen 1984; Peloquin et al. 1989). Peloquin (1983) estimated that transmission of heterozygosity was about 80% for first division restitution (FDR) 2n gametes and about 40% for second division restitution (SDR) 2n gametes. Therefore, for traits where heterozygosity and epistatic interactions are important, 2n gametes provide substantial benefit over introgression methods requiring somatic chromosome doubling (Mendiburu and Peloquin 1977; Tai and De Jong 1997). Identifying wild species genotypes having both resistance to *P. infestans* and 2n gametes can especially be useful for introgression of resistance into cultivated potato. The objectives of this research were to determine if *Solanum* species, PIs within species, and genotypes with PIs identified as having resistance to *P. infestans* also have variability for 2n pollen production and male fertility.

MATERIALS AND METHODS

Forty-five PIs representing 12 *Solanum* species that Zlesak and Thill (1999) previously identified as having resistance to *P. infestans* were screened for 2n pollen production and pollen stainability (Table 1). Resistance screens were conducted using a *P. infestans* isolate provided by Dr. Gary Secor at North Dakota State University (US-8, ND 95-2). *Solanum* germplasm was obtained as botanical seed from the Inter-Regional Potato Introduction Project (NRSP-6) at Sturgeon Bay, Wisconsin. Genotypes were grown at one of three Minnesota locations and genotypes were individually sampled by bulking pollen from two to five flowers. The three locations were field environments at Rosemount, MN, (Waukegan Silt Loam, summer 1999), and Morris, MN, (Doland Silt Loam, summer 2000), and the greenhouse environment at the University of Minnesota, St. Paul, MN, (summer 1999 and winter 1999/2000).

TABLE 1—Solanum *species and plant introductions evaluated for 2n pollen production and male fertility.*

Solanum species (ploidy)	Plant introductions (PIs)[1]
1EBN	
S. bulbocastanum (2x)	243345, 243504, 243505, 243506, 243509, 243512, 275192
S. cardiophyllum (2x)	283062, 283063
S. pinnatisectum (2x)	184764, 184774, 190115, 230489, 253214, 275231, 275232, 275233, 275234, 275236, 347766
S. trifidum (2x)	283064, 283065, 283104
2EBN	
S. berthaultii (2x)	265857, 265858, 473331
S. fendleri (4x)	225661
S. megistacrolobum (2x)	195210
S. microdontum (2x)	195185, 218224, 473166, 473171
S. stoloniferum (4x)	161158, 161178, 195166, 205510, 230490, 239410
S. verrucosum (2x)	161173
4EBN	
S. guerreroense (6x)	161727
Unknown EBN	
S. polyadenium (2x)	230480, 275237, 275238, 310963, 320342

[1]PIs obtained from NRSP-6, Potato Introduction Station, Sturgeon Bay, WI 54235.

Pollen staining was conducted by placing pollen in aceto-carmine on a glass microscope slide for 30 sec and then stirring in a drop of glycerin to ensure uniform distribution of pollen grains. Large-sized pollen, approximately 1.25-1.4x the diameter of typical n pollen, was considered 2n. Based on the frequency of 2n pollen, genotypes were assigned to 2n pollen production categories: 0≤1%, Low=1%-10%, Medium=11%-60%, and High≥60%. Additionally, genotypes were assigned to pollen stainability categories based on the frequency of plump, well-stained pollen grains: 0≤1%, 1=1%-5%, 2=6%-10%, 3=11%-20%, 4=21%-50% and 5≥50% (Yerk 1987). Genotypes with ≥1% 2n pollen were considered 2n pollen-producing and those with ≥6% pollen stainability were considered male-fertile (Hermundstad and Peloquin 1985).

A group of 25 clones that were identified as having ≥11% 2n pollen at Rosemount in 1999 were planted in the field at Morris in 2000. They were planted in one block with one replication of two plants per clone to determine stability of 2n pollen production and pollen stainability over clonal generations and environments. One sample was collected per genotype with pollen bulked from two to five flowers.

Evaluation of resistance to *P. infestans* (US-8, ND 95-2) of PIs was performed in the field at the University of Minnesota, Late Blight Nursery at Rosemount, MN, (Zlesak and Thill 1999). Six-week-old seedlings of up to 48 genotypes per PI were brought to the field on August 23, 1998 and placed between inoculated border rows of the cultivar Norchip. Twelve days after field placement they were directly inoculated with *P. infestans* to guard against escapes. Foliar late blight disease assessments were taken twice a week for seven readings using the CIP (International Potato Center) rating scale of 1 to 9, where 1=no visible lesions, 5=35%-65% necrotic tissue, and 9=100% necrotic tissue (Henfling 1987). CIP evaluation scores were converted to mean percent defoliation for the corresponding range and used to calculate the area under the disease progress curve (AUDPC) and relative AUDPC for each genotype (Campbell and Madden 1990). AUDPC for resistance to *P. infestans* was available for 514 of the 733 genotypes screened for 2n pollen and pollen stainability.

Chi-squared analysis was used to determine dependence between germplasm groups and frequency of genotypes possessing ≥1% 2n pollen, germplasm groups and pollen stainability, and 2n pollen and pollen stainability. Dependence was determined by comparing the calculated chi-square value to the critical chi-squared value at $\alpha = 0.05$ (Moore and McCabe 1999) and an odds statistic was calculated for comparisons where dependence was found (Agresti 1996). For comparing locations and frequency of genotypes producing 2n gametes, only PIs having genotypes sampled from multiple environments were considered. An analysis of variance (ANOVA) was used to determine dependence between 2n pollen producing genotypes and relative AUDPC.

RESULTS

Dependence was not found between location and frequency of 2n pollen-producing genotypes (χ^2, 2 *df* = 1.39, P = 0.499), allowing the data to be pooled over locations. Dependence was found between species and 2n pollen-producing genotypes (χ^2, 11 *df* = 178.77, $P < 0.0001$) and pollen stainability (χ^2, 55 *df* = 290.18, $P < 0.0001$) (Table 2). Since dependence was

TABLE 2—*Frequency of 2n pollen and male fertility among* Solanum *species resistant to* Phytophthora infestans *(US-8, ND 95-2) as represented by area under the disease progress curve (AUDPC).*

		Plant introductions			Genotypes					
Solanum species (ploidy)	Mean AUDPC[1]	n	No. with 2n pollen	% 2n pollen	n	No. with 2n pollen[2]	% 2n pollen	Odds stat.[3] % 2n pollen	No. male fertile[4]	% male fertile
1EBN										
S. bulbocastanum (2x)	649b	7	5	71.4	74	16	21.6	0.29	68	91.9
S. cardiophyllum (2x)	555a	2	2	100	35	6	17.1	0.21	35	100
S. pinnatisectum (2x)	1178d	11	11	100	239	152	63.6	1.75	233	97.5
S. trifidum (2x)	1542g	3	3	100	29	4	13.8	0.16	20	69.0
2EBN										
S. berthaultii (2x)	1458f	3	3	100	69	23	33.3	0.48	69	100
S. fendleri (4x)	1265e	1	1	100	21	1	4.8	0.05	21	100
S. megistacrolobum (2x)	1932h	1	1	100	4	1	25.0	0.33	4	100
S. microdontum (2x)	1536g	4	3	75.0	57	6	10.5	0.12	55	96.5
S. stoloniferum (4x)	1435f	6	5	83.3	150	15	10.0	0.11	149	99.3
S. verrucosum (2x)	1404ef	1	1	100	19	6	31.6	0.46	19	100
4EBN										
S. guerreroense (6x)	1888h	1	0	0	12	0	0	<0.01	4	33.3
Unknown EBN										
S. polyadenium (2x)	1075c	5	2	40.0	24	7	30.0	2.43	22	91.7

[1]AUDPC values (Zlesak and Thill 1999) followed by the same letter do not differ significantly using an LSD at $\alpha = 0.05$.
[2]Genotypes having ≥1% 2n pollen are considered 2n pollen producing.
[3]The odds statistic can be used to compare two species at a time using an odds ratio (Agresti 1996).
[4]Genotypes with ≥6% stainable pollen are considered male fertile (Hermundstad and Peloquin, 1985).

TABLE 3—*Frequency of 2n pollen and male fertility among* Solanum *plant introductions resistant to* Phytophthora infestans *as represented by area under the disease progress curve (AUDPC).*

Species (ploidy)	PI Code	PI	AUDPC[1]	n[2]	Male fertile[3] No.	Male fertile[3] %	2n pollen genotypes[4] 0	Low	Med	High	No.	%	Odds stat.[5]
1EBN													
S. bulbocastanum (2x)	1	243345	840 c	3	3	100	3	0	0	0	0	0.0	
$\chi 2$, 6 *df* = 11.31,[6]	2	243504	746 c	9	9	100	9	0	0	0	0	0.0	
P = 0.079	3	243505	548 b	12	9	75.0	10	2	0	0	2	16.7	
	4	243506	574 b	12	12	100	11	1	0	0	1	8.3	
	5	243509	727 c	12	11	91.7	9	3	0	0	3	25.0	
	6	243512	267 a	9	9	100	7	2	0	0	2	22.2	
	7	275192	1007 d	17	15	88.2	9	8	0	0	8	47.1	
S. cardiophyllum (2x)	8	283062	460 b	21	21	100	17	4	0	0	4	19.0	
$\chi 2$, 1 *df* = 0.83,	9	283063	1113 a	14	14	100	12	1	0	1	2	14.3	
P = 0.362													
S. pinnatisectum (2x)	10	184764	1268 ef	20	20	100	12	6	1	1	8	40.0	0.66
$\chi 2$, 10 *df* = 28.22,	11	184774	1184 bcd	25	24	96.0	6	13	6	0	19	76.0	3.17
P = 0.001	12	190115	1225 de	23	20	87.0	6	8	9	0	17	73.9	2.83
	13	230489	1196 bcd	20	20	100	4	13	1	2	16	80.0	4.0
	14	253214	1207 cd	23	23	100	5	15	3	0	18	78.3	3.6
	15	275231	1146 b	21	19	90.5	14	7	0	0	7	33.3	0.5
	16	275232	1172 bc	22	22	100	4	16	2	0	18	81.8	4.5
	17	275233	1074 a	22	22	100	8	13	1	0	14	63.6	1.75
	18	275234	1325 f	19	19	100	11	7	1	0	8	42.1	0.73
	19	275236	1020 a	22	22	100	7	13	2	0	15	68.2	2.14
	20	347766	1183 bcd	22	22	100	10	9	3	0	12	54.5	1.2
S. trifidum (2x)	21	283064	1272 a	5	3	60.0	4	1	0	0	1	20.0	
$\chi 2$, 2 *df* = 0.22,	22	283065	1418 b	7	5	71.4	6	1	0	0	1	14.3	
P = 0.895	23	283104	1866 c	17	12	70.6	15	2	0	0	2	11.8	
2EBN													
S. berthaultii (2x)	24	265857	1571 b	28	28	100	18	7	2	1	10	35.7	
$\chi 2$, 2 *df* = 4.96,	25	265858	1538 b	20	20	100	15	3	2	0	5	25.0	
P = 0.083	26	473331	1287 a	21	21	100	13	7	1	0	8	38.1	
S. fendleri (4x)	27	225661	1265	21	21	100	20	1	0	0	1	4.8	
S. megistacrolobum (2x)	28	195210	1932	4	4	100	3	1	0	0	1	25.0	
S. microdontum (2x)	29	195185	1543 b	11	11	100	11	0	0	0	0	0.0	
$\chi 2$, 3 *df* = 1.97,	30	218224	1313 a	20	20	100	17	3	0	0	3	15.0	
P = 0.578	31	473166	1701 bc	6	5	83.3	5	0	1	0	1	16.7	
	32	473171	1748 c	20	19	95.0	18	1	1	0	2	10.0	
S. stoloniferum (4x)	33	161158	1332 b	24	24	100	23	1	0	0	1	4.2	0.04
$\chi 2$, 5 *df* = 20.74	34	161178	1458 c	38	38	100	37	1	0	0	1	2.6	0.03
P = 0.0009	35	195166	1711 d	24	24	100	20	3	1	0	4	16.7	0.2
	36	205510	1748 d	20	20	100	13	6	1	0	7	35.0	0.54
	37	230490	1296 b	22	21	95.5	20	1	0	1	2	9.1	0.1
	38	239410	1157 a	22	22	100	22	0	0	0	0	0.0	<0.01
S. verrucosum (2x)	39	161173	1404	19	19	100	13	3	0	3	6	31.6	
4EBN													
S. guerreroense (6x)	40	161727	1888	12	4	30.8	12	0	0	0	0	0.0	
Unknown EBN													
S. polyadenium (2x)	41	230480	1019 a	6	5	50.0	5	1	0	0	1	16.7	
$\chi 2$, 4 *df* = 6.77	42	275237	1049 a	1	1	100	1	0	0	0	0	0.0	
P = 0.148	43	275238	1035 a	3	3	100	3	0	0	0	0	0.0	
	44	310963	1175 b	11	10	90.9	5	0	0	6	6	54.5	
	45	320342	1179 b	3	3	100	3	0	0	0	0	0.0	

[1]Within a species AUDPC values (Zlesak and Thill 1999) followed by the same letter do not differ significantly, LSD at $\alpha = 0.05$.
[2]Number of genotypes examined.
[3]Genotypes with ≥ 6% stainable pollen are considered male-fertile (Hermundstad and Peloquin 1985).
[4]Categories of 2n pollen production, 0≤1%, Low=1%-10%, Medium=11%-60%, and High≥60%.
[5]An odds statistic is computed within species having a significant $\chi 2$ at P=0.05 to compare PIs (Agresti 1996).
[6]Chi-square analysis was used to test for dependence between PI and frequency of genotypes with 2n pollen within species.

found, an odds statistic was computed for each species and species can be compared two at a time as a ratio. For instance, it is six times (1.75/0.29) more likely to find genotypes producing 2n pollen in *S. pinnatisectum* than *S. bulbocastanum*. The percentage of 2n pollen-producing genotypes among species ranged from 0% (*S. guerreroense*) to 63.6% (*S. pinnatisectum*) with 2n pollen being identified in 11 of the 12 species examined. Diploid and tetraploid germplasm differed in their frequency of 2n pollen-producing genotypes with 40.2% and 9.4%, respectively (χ^2, 1 df = 32.68, $P < 0.0001$). Dependence was found between EBN categories (1, 2, 4, and unknown) and frequency of 2n pollen-producing genotypes (χ^2, 3 df = 81.84, $P < 0.0001$). However, when *S. pinnatisectum* is removed from the analysis, there is no longer dependence (χ^2, 3 df = 5.37, P = 0.146). The percentage of male-fertile genotypes (≥6% stainable pollen) ranged from 33.3% for *S. guerreroense* to 100% for *S. cardiophyllum*, *S. berthaultii*, *S. fendleri*, *S. megistacrolobum*, and *S. verrucosum* (Table 3).

Thirty-seven of the 45 PIs examined had 2n pollen-producing individuals (Table 2) and dependence between PIs within a species and frequency of genotypes with 2n pollen was found for only *S. pinnatisectum* (χ^2, 10 df = 28.22, P = 0.001) and *S. stoloniferum* (χ^2, 5 df = 20.74, P = 0.0009) (Table 3). The odds statistic is used to compare PIs within these two species, for example, within *S. pinnatisectum* it is 4.8 times (3.17/0.66) more likely to find 2n pollen-producing genotypes in PI 184774 than PI 184764. Within PIs differences in expression of 2n pollen, low to high, were shown by the number of observed individuals across 2n pollen categories (Table 3). For instance, *S. pinnatisectum* PI 190115 and PI 275232 were similar for frequency of 2n pollen-producing genotypes (73.9% and 81.8%, respectively); however, PI 190115 had 53% of its genotypes in the medium or high 2n pollen category, while PI 275232 had 11% in these upper categories. In addition, 2n pollen-producing genotypes in the medium or high categories were found in six of 12 species examined (range of 2%-*S. stoloniferum* to 25%-*S. polyadenium*) and among species with genotypes in the medium or high 2n pollen categories, the greatest number of individuals were found within *S. pinnatisectum* (32) and the least within *S. microdontum* (2).

All genotypes with the exception of one, identified as having ≥11% 2n pollen at Rosemount in 1999 and re-sampled at Morris in 2000, produced 2n pollen. Sixteen of the 25 clones remained in the same 2n pollen category, and eight clones moved up or down one category. However, clones varied for pollen stainability across environments with 11 genotypes remaining in the same pollen stainability category, 7 genotypes moving up (+) or down (-) one pollen stainability category, 6 genotypes moving +/- two categories, and 1 genotype moving +/- three categories. Nevertheless, all clones had ≥6% pollen stainability in both environments and should be male-fertile (Hermundstad and Peloquin 1985).

Dependence was found between location and pollen stainability (χ^2, 10 df = 56.88, $P < 0.0001$), data not shown, as well as EBN group and pollen stainability category (χ^2, 15 df = 135.37, $P < 0.0001$) (Table 4). In addition, when pollen stainability categories were grouped as male-sterile (≤5%) or male-fertile (≥6%), dependence was found with location (χ^2, 2 df = 13.19, P = 0.001), data not shown, and EBN (χ^2, 3 df = 115.76, $P < 0.0001$) (Table 4). Dependence between 2n pollen production and pollen stainability was found only among the 1EBN germplasm (χ^2, 15 df = 53.2, $P < 0.0001$) and not within the other EBN groups (Table 5).

An ANOVA indicated no significant differences between genotypes with or without 2n pollen and relative AUDPC across species (F, 513 df = 2.682, P = 0.102); excluding *S. pinnatisectum* (F, 283 df = 0.244, P = 0.622) and within *S. pinnatisectum* (F, 229 df = 1.436, P = 0.232) similar results were observed. A scatterplot plotting PIs according to mean AUDPC and percentage 2n pollen-producing individuals revealed 12 PIs being above average for both traits (Figure 1, + / + quadrant). The species represented by the 12 PIs include three 2x(1EBN) species: *S. bulbocastanum* (3 PIs), *S. cardiophyllum* (1 PI), and *S. pinnatisectum* (8 PIs).

DISCUSSION

Wild species genotypes having gametes with the sporophytic chromosome number (2n) have been found across many

TABLE 4—*Dependence between Endosperm Balance Number (EBN) and pollen stainability / male fertility among* Solanum *genotypes.*

	Pollen stainability						
	Male-sterile[1]		Male-fertile				
EBN	<1%	1-5%	6-10%	11-20%	21-50%	>50%	Total
1	9	12	7	13	31	305	377
2	1	2	5	9	32	271	320
4	5	3	2	1	0	1	12
Unknown	2	0	1	0	1	20	24
Total	17	17	15	23	64	597	733

χ^2, 15 df = 135.37, $P < 0.0001$, EBN and pollen stainability.
χ^2, 3 df = 115.76, $P < 0.0001$, EBN and male fertility.

[1]Based on Hermundstad and Peloquin (1985).

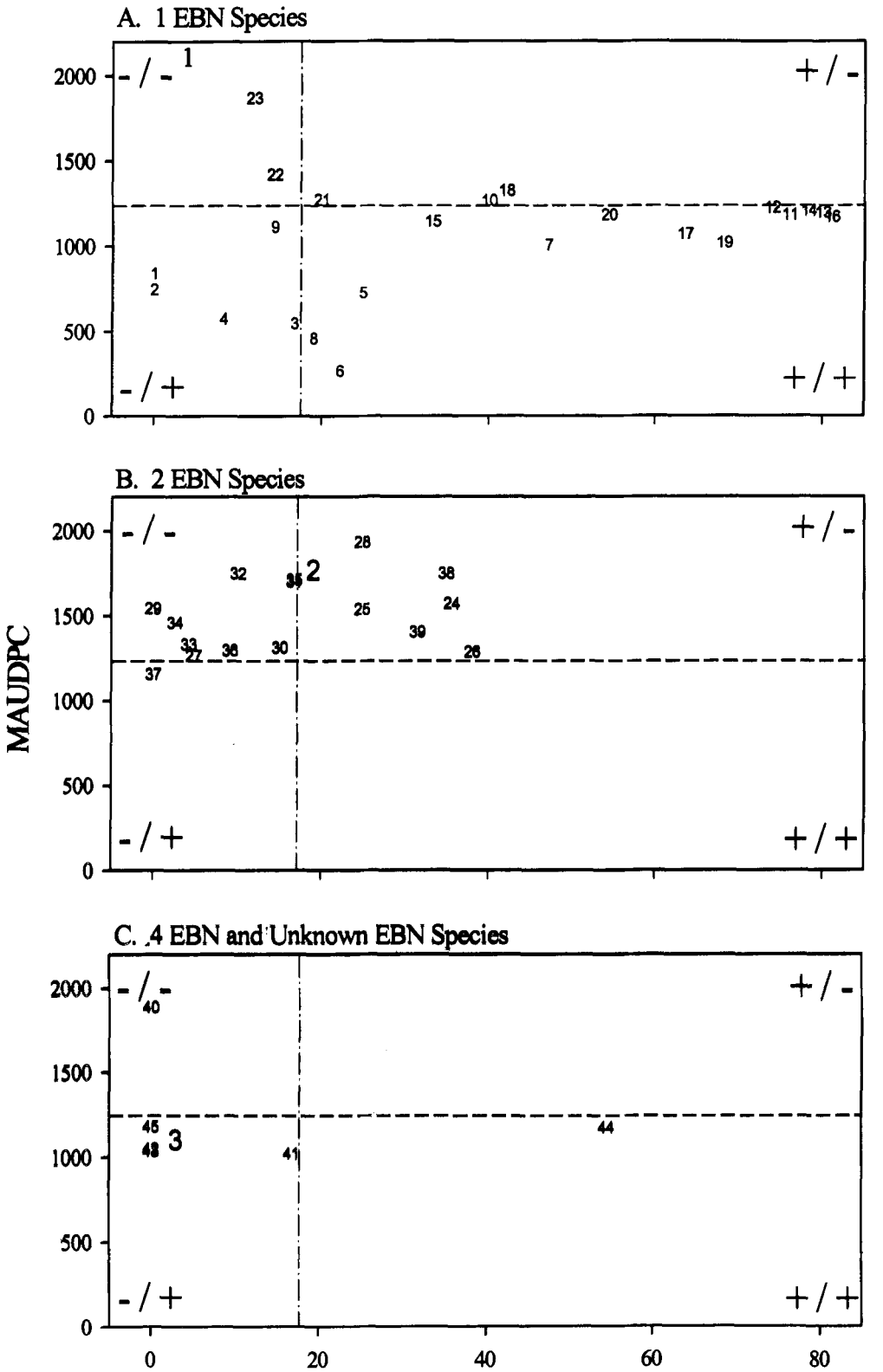

FIGURE 1.
Scatter plot comparing *Solanum* plant introductions for mean area under the disease progress curve (MAUDPC) and percent 2n pollen production.

LEGEND FIGURE 1.
The figure is partitioned into sections A, 1 EBN; B, 2 EBN; and C, 4 EBN and unknown EBN *Solanum* species and plant introductions as found in Table 3. The x-axis (% 2n pollen production) is partitioned by a vertical line into below (-) and above (+) average 2n pollen production. The y-axis (MAUDPC) is partitioned by a horizontal line into below average MAUDPC (-, high MAUDPC values) and above average MAUDPC (+, low AUDPC values); lower MAUDPC scores are desirable (+).

[1]Performance of plant introductions for % 2n pollen vs MAUDPC is indicated (x, y) with coded identification numbers of plant introductions (PIs) as found in Table 3.

[2]In panel B, 2EBN species, *S. microdontum* PI 473166 (Code 31) and *S. stoloniferum* PI 195166 (Code 35) are overlapping.

In panel C, unknown EBN species, *S. polyadenium* PI 275237 (Code 42) and *S. polyadenium* PI 275238 (Code 43) are overlapping.

TABLE 5—*Dependence between pollen stainability and 2n pollen among 1, 2 and other Endosperm Balance Number (EBN)* Solanum *species genotypes.*

Pollen stainability[2]	2n pollen[1] 0	Low	Medium	High	Total
	1EBN species				
<1%	9	0	0	0	9
1-5%	6	4	2	0	12
6-10%	2	3	1	1	7
11-20%	4	6	3	0	13
21-50%	10	12	9	0	31
>50%	168	120	14	3	305
Total	199	145	29	4	377
χ^2, 15 *df* = 53.2, *P* < 0.0001					
	2EBN species				
<1%	1	0	0	0	1
1-5%	2	0	0	0	2
6-10%	4	0	1	0	5
11-20%	8	1	0	0	9
21-50%	27	2	3	0	32
>50%	226	35	5	5	271
Total	268	38	9	5	320
χ^2, 15 *df* = 14.5, *P* = 0.487					
	4EBN and unknown EBN species				
<1%	7	0	0	0	7
1-5%	3	0	0	0	3
6-10%	2	1	0	0	3
11-20%	1	0	0	0	1
21-50%	1	0	0	0	1
>50%	15	0	0	6	21
Total	29	1	0	6	36
χ^2, 15 *df* = 16.1, *P* = 0.375					
	Total				
<1%	17	0	0	0	17
1-5%	11	4	2	0	17
6-10%	8	4	2	1	15
11-20%	13	7	3	0	23
21-50%	38	14	12	0	64
>50%	409	155	19	14	597
Total	496	184	38	15	733
χ^2, 15 *df* = 47.8, *P* < 0.0001					

[1]Categories of 2n pollen production, 0≤1%, Low=1%-10%, Medium=11%-60%, and High≥60%.
[2]Genotypes with ≥6% are considered male-fertile (Hermunstad and Peloquin 1985).

Solanum series and species (Quinn et al. 1974; Watanabe and Peloquin 1991). These 2n gametes are important to potato improvement by allowing germplasm transfer from species to cultivated potato following sexual crosses between parents differing in EBN (Hanneman 1999). For example, 2n = 3x(2EBN) plants, from seed having successful endosperm development, can be obtained from crosses between 2x(2EBN) germplasm and 2x(1EBN) clones via 2n gametes (Ehlenfeldt and Hanneman 1984). Finding 2n pollen-producing genotypes among 11 of the 12 species (Table 2) and 37 of the 45 PIs (Table 3) having significant variability for resistance to *P. infestans* (Zlesak and Thill 1999) is encouraging in this regard. The literature also supports many findings that demonstrate the usefulness of 2n gametes in breeding, especially for traits where heterozygosity and epistatic genetic interactions are important (Tai and De Jong 1991). This is due to the mechanisms of 2n gamete formation and the unique genetic consequences resulting from these mechanisms, as discussed by Hermsen (1984) and Peloquin et al. (1989).

The International Potato Center (CIP) places the integration of technologies toward developing durable resistance to late blight among their highest research priorities, as have many potato-breeding programs worldwide (International Potato Center 2001). This is especially evident when looking at the rapidly advancing field of genetic mapping of late blight resistance genes in cultivated *S. tuberosum* (Oberhagemann et al. 1999) and species, *S. pinnatisectum* (Kuhl et al. 2001), *S. bulbocastanum* (Naess et al. 2000) backgrounds. Little is known, however, about the genetic relationship between genes involved in horizontal resistance to *P. infestans*. Since, horizontal resistance is assumed to be controlled by multiple genes (Agrios 1997), late blight-resistant species producing 2n pollen by parallel spindles, a first division restitution (FDR) mechanism, would be useful because characteristics of the parents can be transferred nearly intact to their progeny. This implies that large epistatic interactions are transferred nearly intact and that favorable intra- and interlocus interactions are maintained from parent to offspring (Mok and Peloquin 1975).

Resistance to *P. infestans* and 2n pollen production are not significantly associated. The relatively low P value (0.102) between 2n pollen-producing genotypes and AUDPC can be misleading, suggesting that germplasm resistant to *P. infestans* may have slightly more 2n pollen-producing genotypes than more susceptible germplasm. *S. pinnatisectum* was the most represented species and had a relatively high frequency of 2n pollen-producing genotypes and was relatively resistant to *P. infestans*. When *S. pinnatisectum* is considered by itself ($P = 0.232$) or is excluded from analysis ($P = 0.622$), the P values rise dramatically, indicating that *S. pinnatisectum* had a strong influence on the analysis.

A scatter-plot plotting PIs using mean AUDPC and percentage 2n pollen-producing genotypes (Figure 1) reveals 12 PIs that fall into the most favorable quadrant (+ / +, having an above average frequency of 2n pollen-producing genotypes and below

average mean AUDPC). Such PIs are especially valuable to breeders for introgression of resistance, and it is interesting to note that all 12 PIs are Mexican 2x(1EBN) species. Since the center of origin of *P. infestans* is suspected to be Mexico (Neiderhauser 1991), finding greater resistance among PIs from Mexico was not unexpected. Although the EBN of *S. polyadenium* is not conclusive, this species tends to group closely with the Mexican 1EBN species according to chloroplast DNA restriction site variation (Spooner and Sytsma 1992) and preliminary crossing data of Louwes and Hekstra (1989). Variation across PIs within a species for 2n pollen and other traits of interest should be taken into account when making decisions about which PIs to use for introgression. In many cases superior PIs within a species can be identified that possess relatively high levels of 2n pollen as well as other traits of interest. For example, a breeder may choose *S. berthaultii* PI 473331 over PIs 265857 and 265858 because it has significantly more *P. infestans* resistance and similar 2n pollen production (Table 3). However, if a PI within a species lacks 2n pollen, yet was a superior candidate for resistance to *P. infestans* such as 4x(2EBN) *S. stoloniferum* PI 239410, one may choose to pursue alternative methods of introgression. It is noteworthy that diverse methods are available, and Hanneman (1999) has extensively reviewed this area.

When using 2n pollen frequency data to estimate the gene frequency of parallel spindles (*ps*) an over-estimation of *ps* can be made, but Iwanaga and Peloquin (1982) suggest this to be small. Parallel spindles formation is suggested as being the primary mechanism of 2n pollen production in potato (Mok and Peloquin 1975). The lack of dependence between 2n pollen production and AUDPC suggests that *ps* may be far apart on the same chromosome or on different chromosomes than most genes conferring resistance to *P. infestans*. In practice, as either 2n pollen or resistance to *P. infestans* is selected first in a relatively large population, there should still be sufficient variability among the selected population to make gain selecting for the other trait. Screening more PIs and genotypes within a PI for 2n pollen production may lead to the detection of 2n pollen in species and PIs where none were previously found. For PIs such as *S. stoloniferum* PI 239410 that have been identified as possessing relatively high levels of resistance to *P. infestans* and a low frequency of 2n pollen (Table 3), it may still be possible to select genotypes having both traits.

Once genotypes are identified as being 2n pollen producers, one can be confident that most will continue to produce 2n pollen in other environments, suggesting a high penetrance of *ps*. Only one of the 25 genotypes that was rated in the medium or high 2n pollen category from Rosemount lacked detectable 2n pollen in the next clonal generation at Morris. In addition, sampling across locations yielded no significant differences between environments for detecting 2n pollen-producing genotypes, suggesting flexibility in the choice of environments used to screen germplasm for 2n pollen. Since recessive meiotic mutants genetically control the development of 2n pollen (Mok and Peloquin 1975), the genetic potential for 2n gametes is inherently present in clonally propagated genotypes, although the expressivity of 2n gametes may change over environments (Haynes et al. 1987; McHale 1983; Owen et al. 1988). For pollen stainability, however, dependence was found among the three environments and also among the EBN groups (Table 4). Although significant differences were found, most genotypes were above a 6% minimum that Hermundstad and Peloquin (1985) found effective in determining usable levels of male fertility. If male fertility becomes a barrier to introgression, maximizing cultural conditions to avoid stresses such as high temperatures would be beneficial because heat stress is known to depress flowering and pollen viability (Bamberg 1995).

Among the 1EBN germplasm it was interesting to find dependence between 2n pollen production and pollen stainability, while such dependence was not found between these traits among the other EBN groups (Table 5). Perhaps the dependence is due to modifier gene(s) that regulates *ps* expression. The existence of modifier genes associated with the expression of 2n pollen is supported by the possibility to select for genotypes with increased frequency of 2n pollen using recurrent selection in red clover (Parrot and Smith 1986) and diploid potato (McHale 1983; Ortiz and Peloquin 1992). Peloquin and Ortiz (1992) highlight the advantages of selecting for high levels of 2n gametes, particularly for production systems using true potato seed. However, Watanabe and Peloquin (1989) report that the percentages of 2n pollen among 2n pollen-producing plants rarely exceeded 50% and were generally between 5% and 30% in their study. They went on to suggest benefits associated with fecundity for diploids able to keep 2n gamete production at relatively low levels, thereby permitting efficient crossing with other diploids, yet still allowing for sexual polyploidization.

Diploid species in this study produced 2n pollen at a greater frequency than tetraploid species (40.2% and 9.4%, respectively), and 2n gametes have been suggested to be the primary mode of polyploidization in the evolution of polyploid potatoes (den Nijs and Peloquin 1977; Iwanaga and Peloquin 1982; Quinn et al. 1974). If the gene frequency for *ps* were the same between diploid and tetraploid species, there would be fewer 2n pollen-

producing genotypes among tetraploids since the gene needs to be nulliplex (*pspspsps*) for expression. The two tetraploid species examined in this study are disomic tetraploids (*S. fendleri* and *S. stoloniferum*). Watanabe and Peloquin (1991) suggest that disomic tetraploid species often have a *ps* frequency similar to that of diploids they were derived from and that tetrasomic polyploids often have a higher *ps* frequency than their diploid ancestors.

The presence of 2n gametes is commonly found across a diverse range of wild potato species (Quinn et al. 1974; Watanabe and Peloquin 1991). Therefore, breeders attempting to introgress traits of interest from the wild *Solanums* can select species, PIs, and genotypes within a PI that possess relatively high levels of both 2n gametes and economic trait(s) of interest. Germplasm that has an EBN value identical to ploidy, i.e., 2x(2EBN) and 4x(4EBN), is relatively easy for breeders to access (Hanneman 1999) and requires a straightforward breeding approach (Table 6-b and d). Diploid (2EBN) germplasm can be crossed with 2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids to capture wild germplasm in a form that will tuberize under long-day conditions (Hermundstad and Peloquin 1986). Then, F_1 progeny can be crossed with cultivars via 2n gametes to produce 4x(4EBN) progeny that can again be crossed with cultivars. In addition, 2x(2EBN) species can be crossed directly with cultivars via 2n gametes (Table 6-b). Germplasm differing in ploidy and EBN, i.e., 2x(1EBN) and 4x(2EBN), offers breeding challenges due to progeny having odd ploidy or aneuploidy (Table 6-a and c). Diploid (1EBN) or 4x(2EBN) germplasm requires a modification of the analytical breeding scheme (Mendiburu and Peloquin 1977) as originally proposed by Chase (1963). Diploid (1EBN) germplasm can be crossed with 2x(2EBN) haploids of *S. tuberosum* or haploid-species hybrids by 2n gametes or crossed to the same 2x(2EBN) after somatic chromosome doubling of the 2x(1EBN), which results in 3x(2EBN) progeny (Table 6-a and c).

TABLE 6—*Breeding schemes*[1] *for sexual introgression of wild* Solanum *species into* S. tuberosum *and steps using 2n gametes.*

Germplasm	Requiring 2n gametes	Cross to:	Progeny	Requiring 2n gametes	Cross to:	Progeny
(a.)						
2x(1EBN)	yes	2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids	3x(2EBN) hybrid	yes	4x(4EBN) cultivar	5x(4EBN)
				no	2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids	potential aneuploid hybrid (~2EBN)
				yes	2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids with 2n gametes	5x(4EBN) hybrid
(b.)						
2x(2EBN)	no	2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids	2x(2EBN) hybrid	yes	2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids with 2n gametes	4x(4EBN) hybrid
				yes	4x(4EBN) cultivars	4x(4EBN) hybrid
	yes	4x(4EBN) cultivar	4x(4EBN) hybrid	no	4x(4EBN) cultivar	4x(4EBN) hybrid
(c.)						
4x(2EBN)	no	2x(2EBN) *S. tuberosum* haploids or haploid-species hybrids	3x(2EBN) hybrid	yes	4x(4EBN) cultivar	5x(4EBN) hybrid
	yes	4x(4EBN) cultivar	6x(4EBN) hybrid	no	4x(4EBN) cultivar	5x(4EBN) hybrid
(d.)						
4x(4EBN)	no	4x(4EBN) cultivar	4x(4EBN)	no	4x(4EBN) cultivar	4x(4EBN)

[1]Breeding schemes were derived from Carputo et al. (1997), Chase (1963), Ehlenfeldt and Hanneman (1984, 1988), Hanneman (1999), and Mendiburu and Peloquin (1977).

Zlesak and Thill (2001) obtained a 3x(2EBN) hybrid after somatic chromosome doubling a *S. pinnatisectum* genotype and crossing it as female to a 2x(2EBN) haploid-species hybrid. Triploids generally have reduced fertility and the potential to produce aneuploid progeny. However, they may be crossed to cultivars via 2n gametes resulting in 5x(4EBN) hybrids that can be crossed to 4x(4EBN) cultivars producing tetraploids and aneuploids. This breeding scheme has been successfully used by Carputo et al. (1997) and Ehlenfeldt and Hanneman (1988) using the South American species *S. commersonii* 2x(1EBN). Additionally, Adiwilaga and Brown (1991) were successful using the Mexican species *S. stoloniferum* 4x(2EBN) and the Mexican and USA species *S. fendleri* 4x(2EBN). In these instances, obtaining 3x hybrids was essential for success, and the limited success using the Mexican 2x(1EBN) species may be due to reproductive barriers in addition to EBN (Novy and Hanneman 1991). Sexual hybrids have been obtained employing "bridging-species" tactics using *S. verrucosum* (Hamernik et al. 2001; Hermsen et al. 1976) and *S. acaule* and *S. phureja* (Hermsen et al. 1973). Functional 2n gametes in these hybrids would allow crossability to 4x(4EBN) cultivars or breeding lines.

Although breeding schemes involving 2x(1EBN), 2x(2EBN), 4x(2EBN), and 6x(4EBN) germplasm provide breeding challenges, the rewards are great in terms of access to genes for trait(s) of economic importance. This is the case with the Mexican 2x(1EBN) germplasm having exceptionally strong resistance to *P. infestans* (Zlesak and Thill 1999).

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