*Floriculture and Ornamental Biotechnology* ©2013 Global Science Books

# Gender Inheritance and Identification of Male Sterility Gene *RSMS1* in Intra- and Inter-specific Crosses of Dioecious *Rosa setigera* Michaux

**David C. Zlesak[1*] • Kathy Zuzek[2] • Stan C. Hokanson[3]**

[1] University of Wisconsin-River Falls, Department of Plant and Earth Science, 410 S. 3rd Street, River Falls, WI 54022, USA
[2] University of Minnesota Extension, 4100 220th St W STE 100, Farmington, MN 55024, USA
[3] University of Minnesota, Department of Horticultural Science, 1970 Folwell Avenue, St. Paul, MN 55108, USA

*Corresponding author*: * **david.zlesak@uwrf.edu**

## ABSTRACT

*Rosa setigera* Michaux (prairie or blackberry rose; $2n=2x=14$) is the only dioecious rose species and only member of the Synstylae section (=Systylae) native to North America. Although flowers have male and female structures to attract pollinators, only one gender is typically functional per genotype. Intra- and interspecific crosses were made to document gender segregation in progeny. Seventeen of the 19 intraspecific crosses did not deviate from a 1:1 female:male ratio. The remaining two families shared a parent, with one family having significantly more and the other less of each gender. Interspecific crosses were only successful with female *R. setigera* genotypes, indicating the existence of unilateral interspecific cross incompatibility. All $F_1$ hybrids were male-sterile and female-fertile. Segregation for male fertility was observed in subsequent generations. Segregation data support the conclusion that a single gene (*RSMS1*) controls male sterility with a dominant allele needed for the male-sterile phenotype. This gene has high penetrance, but in some interspecific populations has weakened expressivity as seen by very low rates of *in vitro* pollen germination (<1%) and abnormal pollen tube growth. Potential applications and new research opportunities related to these advancements in understanding interspecific cross compatibility and gender inheritance in *R. setigera* are discussed.

**Keywords:** Cryptic dioecy, rose, Systylae, Synstylae, unilateral interspecific incompatibility
**Abbreviations:** ***RSMS1***, *Rosa setigera* male sterility 1; $\chi^2$, chi-square

## INTRODUCTION

*Rosa setigera* Michaux ($2n=2x=14$; blackberry or prairie rose) is native to the Eastern half of the United States (New York to Florida and West to Nebraska and Texas) and Southwestern Ontario (Lewis 1958; Kemp *et al*. 1993). The species typically grows in open, sunny areas in or on the edge of prairies. As species succession leads to greater canopy coverage and shading, stands of *R. setigera* decline and eventually die out.

*Rosa setigera* has a strong resemblance to blackberries (*Rubus* sp.), another genera in subgenus Rosoidae of the family Rosaceae. Similarities can be seen in leaf morphology, lax growth habit, frequent tip layering, and flowering season. *Rosa setigera* flowers in late June and early July when most sympatric rose species have finished flowering. Two key features distinguish *R. setigera* from the other 130+ rose species: 1) it is the only dioecious rose species (plants are functionally either male or female) and 2) it is the only member of the section Synstylae (synonym Systylae; Walter Lewis, pers. comm.) of *Rosa* native to North America (most Synstylae species are native to Asia) (Lewis 1958; Krüssmann 1981; Kemp *et al*. 1993). Section Synstylae members are characterized by elevated, loosely or tightly adhered styles; a climbing or rambling growth habit; and blooms borne in clusters at ends of stems (Lewis 1958; Krüssmann 1981; Kemp *et al*. 1993). Interestingly, *R. setigera* has much stronger similarity with North American Cinnamomeae species for flavonoid and enzymatic profiles than other Synstylae members (Grossi *et al*. 1998).

The form of dioecy found in *R. setigera* is cryptic dioecy; male and female plants produce flowers that have both male and female structures in order to attract pollinators, but only one gender is typically functional per individual (Kevan *et al.* 1990). Rare hermaphroditic and neuter *R. setigera* individuals have been reported, but have not been preserved for future research. The relative occurrence in natural populations of such individuals has not been extensively surveyed (Erlanson 1934; Lewis 1958). Kevan *et al*. (1990) report finding only functionally male or female *R. setigera* individuals in southwestern Ontario. Plants characterized for gender consistently displayed the same gender over years (Lewis 1958; Kevan *et al.* 1990).

Outcrossing enhances genetic variability within species. In roses, outcrossing is common especially in diploid species and is primarily facilitated by gametophytic self-incompatibility (Cole and Melton 1986; Ueda and Akimoto 2001; MacPhail and Kevan 2009). Dioecy is a very effective means to ensure outcrossing. Dioecy has arisen multiple times within the plant kingdom and can be found across different taxonomic ranks: whole plant families (e.g. Salicaceae), genera (e.g. *Humulus*), or species (e.g. *R. setigera*) (Westergaard 1958; Kevan *et al.* 1990). Cryptic dioecy in *R. setigera* has been associated with gender-associated differences in floral development and changes in overall plant architecture (Kevan *et al.* 1990). Kevan *et al*. (1990) report that male *R. setigera* plants typically have more flowers per inflorescence than females and that on the day after flowers open, full petal size is reached more quickly in females than males.

Because roses are a valuable crop, much work is being done to develop new rose cultivars that will have increased value. The trend is for continued specialization and divergence between germplasm groups for the different market

Received: 16 November, 2012. Accepted: 2 January, 2013.

Original Research Paper

classes (Zlesak 2006). Sterility genes, gender-associated floral traits, and other valuable traits from *R. setigera* can aid rose breeders in cultivar development across the different market classes. *Rosa setigera* was used in the development of a limited number of mainly climbing rose cultivars for landscape use in the late 19th to mid 20th centuries (e.g. 'American Pillar', 'Doubloons', and 'Jean Lafitte'; Young and Schorr 2007) well before cryptic dioecy was recognized in this species and before pedigrees were routinely recorded and reported.

Inheritance of gender in *R. setigera* is understudied. The key report by Lewis and Basye (1961) describes an $F_1$ population (n=70) resulting from crossing one genotype of *R. setigera* (female-fertile and male-sterile) x *R. brunonii* Lindl., another member of section Synstylae. The seedling population possessed an overall rate of 96% defective pollen (based on staining with 1% acetic-orcein), and plants produced numerous fruit implying female fertility and male sterility. Lewis and Basye (1961) suggested that *R. setigera* might confer a dominant factor governing male sterility, but they did not generate additional, segregating populations to develop a genetic model of inheritance with gene and allele number (Walter Lewis, pers. comm.). The current authors' preliminary findings regarding interspecific hybridization with *R. setigera* include: 1) no confirmed hybrids were generated despite numerous pollinations using male *R. setigera*; and 2) successful hybrids have been readily generated using female *R. setigera* only when Synstylae-derived diploid males (roses derived from the polyantha and hybrid musk commercial classes; unpublished data) were used.

Because *R. setigera* is the only dioecious species within *Rosa*, it provides a rare and valuable opportunity to study the origin and inheritance of dioecy. Understanding the reproductive biology of this species would also be valuable to strategically introgress sterility genes and other valuable traits (i.e. floral morphology related to gender and disease resistance) into modern rose cultivars. The objectives of this study are 1) to generate intra- and interspecific crosses with *R. setigera* genotypes and document the genders of the resulting progeny; and 2) to begin to understand the inheritance of gender through segregation patterns over successive generations.

## MATERIALS AND METHODS

### Plant materials

Parental genotypes with their female and male fertility are listed in **Table 1**. Gender of *R. setigera* and other parents was determined using hanging drop pollen germination assays (as described by Zlesak 2004) and confirmed by the presence or absence of fruit in the fall (male-sterile genotypes produced fruit and male-fertile genotypes did not) (**Fig. 1**). Anthers from at least two blooms per genotype were bulked a day before dehiscence and allowed to air dry and dehisce in an open 35 mm film canister under ambient laboratory conditions. The germination medium consisted of 1.5% sucrose and 40 ppm boric acid in distilled water. A volume of 20 µl of medium was used per assay and assays were allowed to incubate at room temperature for 2 hr before being observed. Two pollen germination assays were performed per parental genotype.

Four of the *R. setigera* clones were located at the University of Minnesota Landscape Arboretum. The female genotype (19720391) was obtained as a plant in 1972 from the former Joseph J. Kern Rose Nursery in Mentor, Ohio. The other three genotypes 19670317-1, 19670317-2, and 3MD were males. Genotypes 19670317-1 and 19670317-2 were grown from seed obtained from Boerner Botanical Gardens in Hales Corners, Wisconsin in 1967. Information on the source of the plant(s) from Boerner Botanical Gardens or Joseph J. Kerm Rose Nursery was no longer available. The origin of the male clone 3MD is not known. The ten *R. setigera* plants designated as FS1 through FS10 were part of a naturalized landscape in front of a visitor center at Fort Snelling in Minneapolis, MN. Their origin could not be ascertained.

Although 19670317-2 is classified as a male, it occasionally does have very limited female fertility. In two of the years between 1999 and 2012 less than 1% of the flowers developed into small hips containing one to a few viable achenes each (**Fig. 2**). In 2007, achenes were collected and successfully germinated (unpublished data). In comparison, female-fertile 19720391 typically produces hips containing more than 10 achenes each from nearly 100% of the flowers each year.

In contrast to *R. setigera* which blooms only early in the growing season, the diploid parents derived from other Synstylae section species all have repeat or continuous flowering throughout the growing season and possess both male and female fertility. The diploid polyantha, hybrid musk, and shrub roses descended from Synstylae section species (primarily *R. multiflora*) that were used in interspecific crosses with *R. setigera* included: 95-1, 95-2, 'ZLEMartinCipar' (trade name: Oso Happy™ Candy Oh!), 'Mevrouw Nathalie Nypels', Poly A, and 'Robin Hood' (Zlesak *et*

**Table 1** Female and male fertility of rose genotypes used as parents to study the inheritance of gender from *Rosa setigera*.

| Genotype | Female fertile | Male fertile |
|---|---|---|
| ***R. setigera* genotypes** | | |
| 19670317-1 | no | yes |
| 19670317-2 | no[a] | yes |
| 19720391 | yes | no |
| 3MD | no | yes |
| FS1 | no | yes |
| FS2 | yes | no |
| FS3 | no | yes |
| FS4 | no | yes |
| FS5 | yes | no |
| FS6 | yes | no |
| FS7 | no | yes |
| FS8 | yes | no |
| FS9 | no | yes |
| FS10 | no | yes |
| **Non-*R. setigera* based genotypes** | | |
| 95-1 | yes | yes |
| 95-2 | yes | yes |
| 'Mevrouw Nathalie Nypels' | yes | yes |
| Poly A | yes | yes |
| 'Robin Hood' | yes | yes |
| 'ZleMartinCipar' | yes | yes |
| **Interspecific *R. setigera* hybrids ((19720391 x 95-1) open pollinated)** | | |
| 1Setmon | yes | no |
| 20000067-22 | yes | no |

[a] During some growing seasons 19670317-2 has had very limited female fertility.

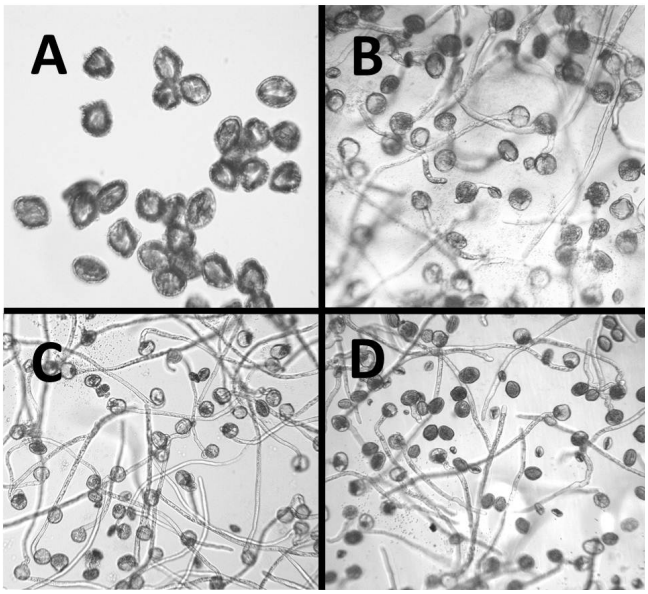


**Fig. 1 Pollen germination assays of a representative subset of parental genotypes; *Rosa setigera* parents: (A) 19720391 and (B) FS1, and non-*R. setigera* Synstylae-derived parents: (C) 95-1 and (D) 'Robin Hood'.**

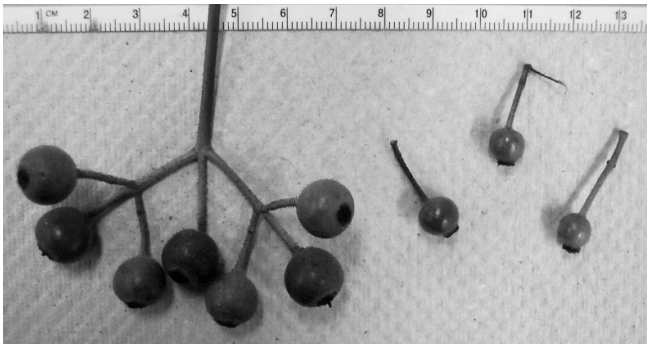

**Fig. 2 Representative hips of female *Rosa setigera* genotype 19720391 (left) and primarily male, but weakly hermaphroditic, *R. setigera* genotype 19670317-2 (right).**

**Table 2** Intra-specific *Rosa setigera* populations with gender segregation and chi-square analysis using an expected 1:1 segregation ratio for gender.

| Cross | | Number of progeny | | | $\chi^2$ value | *P* value |
|---|---|---|---|---|---|---|
| **Female** | **Male** | **Females** | **Males** | **Total** | | |
| 19720391 | FS3 | 0 | 1 | 1 | 1.00 | 0.317 |
| 19720391 | FS4 | 0 | 1 | 1 | 1.00 | 0.317 |
| 19720391 | FS7 | 2 | 0 | 2 | 2.00 | 0.157 |
| 19720391 | FS9 | 0 | 2 | 2 | 2.00 | 0.157 |
| 19720391 | FS10 | 8 | 5 | 13 | 0.69 | 0.405 |
| 19720391 | 19670317-2 | 23 | 26 | 49 | 0.18 | 0.668 |
| 19720391 | 19670317-1 | 19 | 38 | 57 | 6.33 | 0.012 |
| 19720391 | 3MD | 24 | 26 | 50 | 0.08 | 0.777 |
| FS2 | 19670317-1 | 12 | 4 | 16 | 4.00 | 0.046 |
| FS2 | FS1 | 11 | 5 | 16 | 2.25 | 0.134 |
| FS2 | FS7 | 7 | 8 | 15 | 0.07 | 0.796 |
| FS2 | 19670317-2 | 4 | 3 | 7 | 0.14 | 0.705 |
| FS5 | FS1 | 2 | 8 | 10 | 3.60 | 0.058 |
| FS5 | FS3 | 5 | 3 | 8 | 0.50 | 0.480 |
| FS5 | FS7 | 4 | 7 | 11 | 0.82 | 0.366 |
| FS6 | 19670317-1 | 50 | 48 | 98 | 0.04 | 0.840 |
| FS6 | FS3 | 6 | 2 | 8 | 2.00 | 0.157 |
| FS7 | FS3 | 7 | 3 | 10 | 1.60 | 0.206 |
| FS8 | 19670317-1 | 5 | 4 | 9 | 0.11 | 0.739 |
| Total | | 189 | 194 | 383 | 0.07 | 0.791 |

*al.* 2005; Zlesak, 2009). The roses 95-1 and 95-2 are selections derived from a commercial seed source (*Rosa* 'Polyantha Angel Rose Mixed', Thompson & Morgan Seedsmen, Inc., Jackson, NJ). Poly A is a selection derived from two rounds of open pollination of 95-1.

## Intra- and interspecific crosses

Standard emasculation and pollination techniques common in rose breeding programs were used to generate hybrid rose populations. Populations reported in this study were generated between 1999 and 2007. Anthers were collected a day before anthesis and allowed to dehisce in open 35 mm film canisters in the laboratory. If pollen was not used within two days of anther collection, canisters of dried pollen were sealed and stored at -20°C until the day of use. Flowers used as females were emasculated before they reached anthesis. Petals were removed as part of the process, but sepals were retained. Crosses were labeled and upon hip ripening, achenes were removed and cold stratified at 4-10°C for several weeks in moist peat moss to overcome physiological dormancy and promote germination. Progeny that displayed non-recurrent flowering (flowering in late spring to early summer on branches from vernalized buds) took two to three years to first flower. In advanced generations, recurrent or continuous flowering was recovered and such progeny typically began flowering 6-10 wks after germination.

Intraspecific *R. setigera* populations were generated from crosses within and between genotypes growing at the University of Minnesota Landscape Arboretum and Fort Snelling and are listed in **Table 2**. Successful inter-specific populations are listed in **Table 3**.

Two open-pollinated populations were generated from separately collecting and bulking seed of individuals from two crosses (19720391 x 95-1) and (19720391 x 'ZleMartinCipar'). Due to male sterility in the $F_1$ interspecific hybrids, the expected male parent(s) of these open-pollinated populations were repeat blooming diploid polyantha populations growing in an adjacent bed. Selections 20000067-22 and 1Setmon are male-sterile diploid repeat-flowering genotypes derived from open pollinated seedlings of the population (19720391 x 95-1). 20000067-22 was used as a female parent with the males Poly A and 'Robin Hood'. The roses 20000067-22, 1Setmon, 95-1 and 95-2 were used as females in crosses with the male-fertile *R. setigera* clones 19670317-1, 19670317-2, and 3MD. Ten or more pollinations using each male *R. setigera* genotype were made onto each female parent.

## Gender determination of hybrid populations

Genders of individuals from $F_1$ interspecific populations were determined using hanging drop pollen germination assays as previously described. Due to the large numbers of assays required, especially among intraspecific *R. setigera* genotypes, a modified pollen germination assay was used after a sample of ~30 *R. setigera* genotypes displayed comparable pollen germination results with both techniques. Instead of a hanging drop assay, cover slips were incubated with the 20 µl drop of pollen germination medium and pollen on top of the cover slip. Cover slips were placed on a layer of distilled-water-soaked paper towels on the bottom of a sealable plastic container (~3,000 cc in volume). After cover slips were set on paper towels, the container was sealed to allow the relative humidity to rise to $\geq$98% as determined by a hygrometer. After incubation, cover slips were inverted over standard glass slides and observed. Incubation time for the modified assays was also 2 hr.

## RESULTS

Interspecific $F_1$ crosses were obtained only when *R. setigera* served as the female parent. All of the $F_1$ interspecific hybrids were classified as male-sterile and possessed female fertility as evidenced by fruit set and viable offspring (**Table 3**). The $F_1$ interspecific hybrids also expressed the expected dominant trait of non-recurrent bloom like *R. setigera*. Non-recurrent flowering seedlings would have been expected from crosses between females 95-1, 95-2, 20000067-22, and 1Setmon and male *R. setigera* genotypes, but these crosses were largely unsuccessful. There were two seeds that resulted in two seedlings from 20000067-22 x 19670317-2. Both seedlings were without prickles and were repeat flowering like 20000067-22; repeat flowering and prickles are recessive traits (Semeniuk 1971; Debener 1999) and were unexpected phenotypes for this cross. One of the two seedlings was male-fertile and the other was male-sterile.

Although interspecific $F_1$ crosses were classified as male-sterile, there was a very low rate of abnormal pollen germination in 19720391 x 'ZleMartinCipar' $F_1$ genotypes and the open-pollinated population of this family (**Fig. 3**). Less than 1% of pollen germinated and after the 2 hr incubation, pollen tubes were <20% in length and generally more twisted and wider than pollen tubes from the typical male-fertile genotypes.

The open-pollinated progeny of 19720391 x 95-1 segregated for male fertility and fit a 1:1 male-sterile:fertile ratio, consistent for outcrossing with the neighboring male-fertile diploid polyantha populations (**Table 3**). However, the open-pollinated progeny of 19720391 x 'ZleMartinCipar' more closely fit a 3:1 male-sterile:fertile ratio (chi-square value=0.44 ; *P*=0.501) than a 1:1 male-sterile:fertile ratio, consistent with the possibility of the seedlings' being primarily the result of self-fertilization. The two populations generated by crossing 20000067-22 as a female with Poly A and 'Robin Hood' as males fit a 1:1 male-sterile:fertile ratio.

Gender segregation of intraspecific crosses is provided in **Table 2**. Of the 19 families, all but two (19720391 x

**Table 3** Inter-specific *Rosa setigera* populations with segregation for male fertility and chi-square analysis using an expected 1:1 male sterile:fertile ratio in advanced, segregating populations.

| Cross | | Number of progeny | | | $\chi^2$ value | *P* value |
|---|---|---|---|---|---|---|
| **Female** | **Male** | **Male sterile** | **Male fertile** | **Total** | | |
| 19720391 | 95-1 | 19 | 0 | 19 | | |
| 19720391 | 'Mevrouw Nathalie Nypels' | 5 | 0 | 5 | | |
| 19720391 | 'ZleMartinCipar' | 5 | 0 | 5 | | |
| FS8 | 'ZleMartinCipar' | 3 | 0 | 3 | | |
| 20000067-22 | Poly A | 41 | 41 | 82 | 0.00 | 1.000 |
| 20000067-22 | 'Robin Hood' | 2 | 1 | 3 | 0.33 | 0.564 |
| Open-pollinated populations (seed bulked within family) | | | | | | |
| (19720391 x 95-1) | | 9 | 9 | 18 | 0.00 | 1.000 |
| (19720391 x 'ZleMartinCipar') | | 10 | 2 | 12 | 5.33 | 0.021 |

19670317-1 and FS2 x 19670317-1) fit a 1:1 female:male ratio ($P \leq 0.05$). Both aberrant families have 19670317-1 as the male parent, but differed in which gender was significantly more abundant.

## DISCUSSION

Interspecific unilateral incompatibility may be present in *R. setigera*. Crosses with other diploid Synstylae-derived genotypes were successful only when *R. setigera* served as the female parent. Unilateral interspecific incompatibility has been documented in many plant families including Rosaceae, in particular *Rubus*, a closely allied genus of *Rosa* (Keep 1968; Liedl and Anderson 1993). Interestingly, crosses with 20000067-22 and 1Setmon as females with male *R. setigera* clones (i.e., 19670317-1, 19670317-2, and 3MD) did not result in successful hybrids. Both 20000067-22 and 1Setmon are maternal grandchildren of female *R. setigera* genotype 19720391, inheriting its male sterility factor, and should share the same cytoplasm. The same group of *R. setigera* male genotypes produced viable progeny directly with 19720391.

Seventeen rose cultivars are recorded as direct descendants of *R. setigera*; 13 have *R. setigera* listed as the maternal parent and four have *R. setigera* listed as the paternal parent (Young and Schorr 2007). Most cultivars are from the late 19$^{th}$ or early 20$^{th}$ century, when many breeders routinely collected and raised open-pollinated seed and later used their best judgment to infer pedigrees. Another reported *R. setigera* hybrid is selection 90-300 from the Texas A&M rose breeding program. It has been listed as *R. wichurana* x *R. setigera* in one publication (Ma *et al.* 1997) and as the reciprocal cross in another publication (Kim and Byrne 1996). Records in the Texas A&M breeding program confirm that *R. setigera* served as the maternal parent for this intrasectional hybrid (David Byrne, pers. comm.). A concerted effort to cross a diverse group of Synstylae section species with male and female *R. setigera* genotypes would be of value to better characterize the presence and extent of unilateral interspecific incompatibility.

Consistent with Lewis and Basye (1961), interspecific $F_1$ hybrids with female *R. setigera* were 100% male-sterile. The male sterility factor in interspecific $F_1$ populations has been attributed to a dominant factor by Lewis and Basye (1961). Lewis and Basye did not raise additional generations from these $F_1$ hybrids to ascertain how many genes control male sterility, and their action (Walter Lewis, pers. comm.). The current study is the first report where additional generations were generated and segregation of male sterility documented. The two seedlings that resulted from crossing 20000067-22 x 19670317-2 are likely not true hybrids: they lack two dominant characteristics (non-repeat bloom, and prickles) expected to be inherited from 19670317-2 (Semeniuk 1971; Debener 1999). Both seedlings possessed the recessive traits found in 20000067-22: repeat flowering and lack of prickles. The seedlings may have been the result of pollen contamination in the greenhouse, or apomixis. Apomixis via diplospory would be consistent with the phenotypes of these seedlings (i.e. 2*n* eggs with some meiotic recombination). Apomixis has been documented in roses, including data that suggest diplospory (Crespel *et al.* 2001; Zlesak *et al.* 2007).

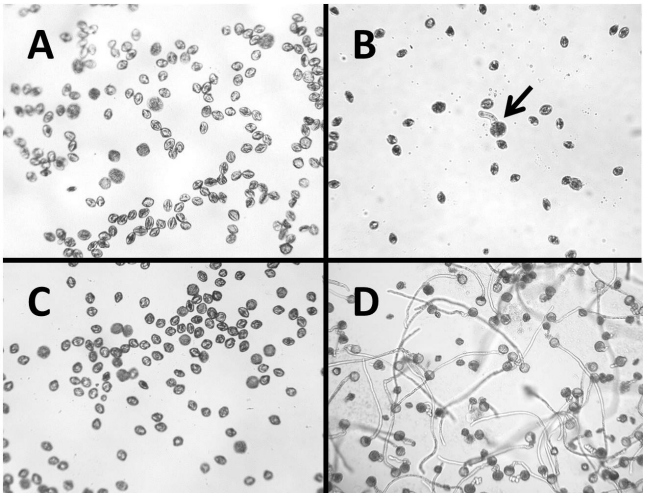


**Fig. 3 Pollen germination assays of representative interspecific hybrids within the populations: (A) 19720391 x 95-1, (B) 19720391 x 'ZleMartinCipar' (arrow points to a germinated pollen grain growing at a slower rate than typical), (C) 20000067-22 x Poly A (male-sterile seedling), and (D) 20000067-22 x Poly A (male-fertile seedling).**

Segregation patterns fit a 1:1 male-sterile:fertile ratio for progeny for the 19720391 x 95-1 open-pollinated population and the controlled crosses 20000067-22 x Poly A and 20000067-22 x 'Robin Hood'. This supports a single gene model with a dominant allele controlling male sterility. The name *Rosa setigera* male sterility 1 (*RSMS*1) is proposed for this gene. The data support the conclusion that female *R. setigera* genotypes are homozygous and the allele (*Rsms*1) they possess is dominant and results in male sterility in interspecific crosses.

*Rsms*1 appears to have high penetrance and generally high expressivity. Interestingly, the population 19720391 x 'ZleMartinCipar' displayed weakened expressivity with occasional pollen grains exhibiting atypical germination and growth (**Fig. 3**). There may be a unique interaction between the genetic backgrounds of 19720391 and 'ZleMartinCipar' influencing the pathway of *Rsms*1 and leading to weakened expressivity. The open-pollinated progeny from bulked seeds of 19720391 x 'ZleMartinCipar' fit a 3:1 rather than a 1:1 male-sterile:fertile ratio, consistent with the possibility of self-fertilization due to very limited male fertility in $F_1$ hybrids. Surveying more interspecific populations (a wider group of female *R. setigera* parents and diploid Synstylae-derived cultivars) to determine how common weakened expressivity of *Rsms*1 is, and whether there is a dosage effect, would be of value.

Seventeen of the 19 intraspecific *R. setigera* populations fit a 1:1 female:male ratio (**Table 2**). More work is needed to elucidate the genetic model controlling gender inheritance in *R. setigera*. Unilateral interspecific incompatibility so far has hindered the generation of interspecific populations using male *R. setigera* genotypes and obtaining

populations that may segregate for female sterility. Perhaps inheritance of gender is as simple as a one-gene three-allele model for *RSMS1*, wherein females are homozygous for *Rsms1*, males are heterozygous with a unique allele conferring the male gender, and there is a third allele that is homozygous and recessive to *Rsms*1 in non-*R. setigera* derived germplasm. Populations derived from aberrant genotypes, like 19670317-2 (typically male, but in some years displaying very limited female fertility), can be helpful in better understanding gender segregation in intra- and interspecific populations and can aid in the development of a more comprehensive model of gender inheritance. Obtaining other aberrant individuals may be possible: Erlanson (1934) and Lewis (1958) report hermaphroditic and neuter individuals.

In *Rubus*, a close genera to *Rosa*, dioecy is present in some members, and a two gene model controlling dioecy has been proposed (Jennings 1988). When the dominant allele is present at each locus a hermaphroditic individual results; when an individual is homozygous recessive at both loci individuals are neuter; and when an individual has a dominant allele at only one locus it is either male or female, depending on which locus has the dominant allele. Shared phenotypic characteristics between *R. setigera* and some *Rubus* species (e.g. similar flowering time which is different than most other rose species, lax growth habit, and similarities in foliar characteristics) along with dioecy suggest *R. setigera* may be an introgressive hybrid with *Rubus*. More effort is warranted to explore potential genetic relationships between *R. setigera* and dioecious *Rubus* members.

Furthering the understanding of gender inheritance in intra- and interspecific crosses with *R. setigera* opens up new research opportunities. Identifying the male sterility gene *RSMS1* sets the stage for this gene to be cloned, better characterized (origin and pathway), and introgressed into additional cultivated germplasm. Sterility can have practical use in commercial breeding programs to aid in generating controlled crosses and also to serve as a tool to limit gene flow (e.g. transgenics containing genes of concern). Additionally, following morphological differences in relation to gender in interspecific crosses (i.e. inflorescence architecture and petal expansion rate) can aid commercial cultivar development. More foundational ecological and evolutionary questions can be pursued by developing a more thorough characterization of unilateral interspecific self-incompatibility and the genetic relationship between *R. setigera* and *Rubus* sp.

## ACKNOWLEDGEMENTS

The authors thank Virge Klatt, Brandi Miatke, Steven Lott, Michelle Grabowski, and Adam Larson for their field and laboratory assistance and Peter Harris and Dr. Walter Lewis for their helpful comments in the preparation of this paper.

## REFERENCES

**Cole P, Melton B** (1986) Self- and cross-compatibility relationships among genotypes and between ploidy of the rose. *Journal of the American Society for Horticultural Science* **111**, 122-125

**Crespel L, Meynet J, Jacob Y, Zhang D, Gudin S** (2001) Identification of apomictic plants in *Rosa hybrida* L. by AFLPs. *Acta Horticulturae* **547**, 51-55

**Debener T** (1999) Genetic analysis of horticulturally important morphological and physiological characters in diploid roses. *Gartenbauwissenschaft* **64**, 14-20

**Erlanson EW** (1934) Pollen analysis for rose breeders. *American Rose Annual* **19**, 63-68

**Grossi C, Raymond O, Jay M** (1998) Flavonoid and enzyme polymorphisms and taxonomic organisation of *Rosa* sections: Carolinae, Cinnamomeae, Pimpinellifoliae and Synstylae. *Biochemical Systematics and Ecology* **26**, 857-871

**Jennings DL** (1988) *Raspberries and Blackberries - Their Breeding, Diseases and Growth*, Academic Press, London, 230 pp

**Keep W** (1968) Incompatibility in *Rubus* with special reference to *R. idaeus* L. *Canadian Journal of Genetics and Cytology* **10 (2)**, 253-262

**Kemp JR, Kevan PG, Posluszny U** (1993) Morphological differences and changes of the gynoecium in short-lived flowers of *Rosa setigera* Michaux and their relationship to dioecy. *International Journal of Plant Sciences* **154**, 550-556

**Kevan PG, Eisikowitch D, Ambrose JD, Kemp JR** (1990) Cryptic dioecy and insect pollination in *Rosa setigera* Michx. (Rosaceae), a rare plant of Carolinian Canada. *Biological Journal of the Linnean Society* **40**, 229-243

**Kim Y, Byrne DH** (1996) Interspecific hybrid verification of *Rosa* with isozymes, *HortScience* **31**, 1207-1209

**Krüssmann G** (1981) *The Complete Book of Roses*, Timber Press, Portland, 436 pp

**Liedl BE, Anderson NO** (1993) Reproductive barriers: Identification, uses and circumvention. In: Janick J (Ed) *Plant Breeding Reviews*, New York, John Wiley and Sons Inc., pp 11-154

**Lewis WH** (1958) A Monograph of the Genus *Rosa* in North America. III. *R. setigera*. *The Southwestern Naturalist* **3**, 154-174

**Lewis WH, Basye RE** (1961) Analysis of nine crosses between diploid *Rosa* species, *Proceedings of the American Society for Horticultural Science* **78**, 572-579

**MacPhail VJ, Kevan PG** (2009) Review of the breeding systems of wild roses (*Rosa* spp.). In: Zlesak DC (Ed) Roses. *Floriculture and Ornamental Biotechnology* **3 (Special Issue 1)**, 1-13

**Ma Y, Byrne DH, Chen J** (1997) Amphidiploid induction from diploid rose interspecific hybrids. *HortScience* **32**, 292-295

**Semeniuk P** (1971) Inheritance of recurrent blooming in *Rosa wichuraiana*. *The Journal of Heredity* **62**, 203-204

**Ueda Y, Akimoto S** (2001) Cross- and self-incompatibility in various species of the genus *Rosa*. *The Journal of Horticultural Science and Biotechnology* **76**, 392-395

**Westergaard M** (1958) The mechanism of sex determination in dioecious flowering plants. *Advances in Genetics* **9**, 217-281

**Young MA, Schorr P** (2007) *Modern Roses 12, The Comprehensive List of Roses in Cultivation or of Historical or Botanical Importance*, Pediment Publishing, Battle Ground, WA, 576 pp

**Zlesak DC** (2004) The hanging drop pollen germination assay- A tool to assess fertility. *Rose Hybridizers Association Newsletter* **35 (3)**, 10-12

**Zlesak DC** (2005) Trifluralin-mediated polyploidization of *Rosa chinensis minima* (Sims) Voss seedlings. *Euphytica* **141**, 281-290

**Zlesak DC** (2006) *Rosa hybrida* L. In: Anderson NO (Ed) *Flower Breeding and Genetics: Issues, Challenges, and Opportunities for the 21st Century*, Springer, Dordrecht, pp 695-738

**Zlesak DC** (2009) Pollen diameter and guard cell length as predictors of ploidy in diverse rose cultivars, species, and breeding lines. In: Zlesak DC (Ed) *Roses*. *Floriculture and Ornamental Biotechnology* **3 (Special Issue 1)**, 53-70

**Zlesak DC, Bradeen JM, Anderson NO** (2007) The use of AFLP markers to resolve clonal origin and integrity in rose, hydrangea, and lily. *Floriculture and Ornamental Biotechnology* **1 (1)**, 51-60

**Zlesak DC, Thill CA, Anderson NO** (2005) Trifluralin-mediated polyploidization of *Rosa chinensis minima* (Sims) Voss seedlings. *Euphytica* **141**, 281-290