第31卷 第10期 中南林业科技大学学报 Vol. 31 No. 10
2011年10月 Journal of Central South University of Forestry & Technology Oct. 2011

Cross compatibility of *Ilex glabra* (L.) A. Gray with *Ilex* × *meserveae* S. Y. Hu and *Ilex verticillata* (L.) A. Gray

ZHANG Dong-lin[1], SUN You-ping[2], JIN Xiao-ling[3], HAN Li-juan[4]

(1. Dept. of Plant, Soil and Environmental Sciences, University of Maine, 5722 Deering Hall, Orono, ME 04469, USA;
2. Central South University of Forestry and Technology, Changsha 410004, Hunan, China;
3. School of Landscape Architecture, Central South University of Forestry and Technology, Changsha 410004, Hunan, China;
4. Department of Biology, Changchun Normal University, Changchun, Jilin 130032, China)

Abstract: *Ilex glabra* (L.) A. Gray (inkberry) is a native evergreen shrub with dark green foliage, which is of interest to nursery growers in the northern landscapes in US. Inkberry was hybridized with cold-hardy male *Ilex verticillata* (L.) A. Gray (common winterberry) and *Ilex* × *meserveae* S. Y. Hu (meserve holly) to breed cold-hardy cultivars. Cross pollination of inkberry wild species and its five cultivars ('Chamzin', 'Compacta', 'Densa', 'Nigra', and 'Shamrock') with both male plants was carried out in the greenhouses to test their compatibility. Cross compatibility of common winterberry and meserve holly with inkberry significantly varied among inkberry and its cultivars. Inkberry 'Chamzin' and 'Densa' were more compatible with either common winterberry or meserve holly, while the wild species and 'Compacta' were less compatible; 'Nigra' and 'Shamrock' were almost incompatible with them. Pollen germination observed in situ with a fluorescence microscope supported the above results. Pollen germination of common winterberry and meserve holly on the stigma of wild species 'Chamzin', 'Compacta', and 'Densa' was greater than that on inkberry 'Nigra' and 'Shamrock' stigma in both 2008 and 2009. Following the pollen tube growth, a dramatic reduction of developed pollen tubes was observed as they grew along the style and into the ovary. The percentage of pollen tubes reaching the ovary of the wild species and 'Chamzin', 'Compacta', or 'Densa' was much higher than that of 'Nigra' or 'Shamrock', which increased the probability of their tubes entering into the ovule and setting fruit. Most of their seeds were aborted. Less than 2 seeds per fruit were developed when common winterberry and meserve holly was the pollinator, while over 3 seeds per fruit were produced when they pollinated with compatible pollen, *Ilex glabra* 'Pretty Boy'. Reproduction barriers, including the inhibition of pollen germination, pollen tube growth to the style and the ovary, and the lack of fertilization, resulted in the cross incompatibility of inkberry with both cold hardy species. Future cross-pollination studies should consider the incompatibility of cultivar variations.

Key words: common winterberry; fruit set; inkberry; meserve holly; pollen germination; seed set

CLC number: S722.3; Q949.754.6 **Document code:** A **Article ID:** 1673－923X(2011)10－0001－09

收稿日期: 2011-05-16

基金项目: 国家林业局948项目(2008-4-14)

作者简介: 张冬林(1962—),男,湖北浠水人,美国乔治亚大学博士,缅因大学终身教授,中南林业科学大学特聘教授,从事森林培育、植物学及生态学教学和科研工作;E-mail:donglin@maine.edu

通讯作者: 孙友平(1979—),男,湖南长沙人,美国缅因大学博士,现在克莱姆森大学从事药用植物生物技术及生态生理博士后研究;E-mail:youpins@clemson.edu

1 Introduction

Ilex L. (holly) comprises at least 500 deciduous and evergreen shrubs or trees with economic importance as crops and ornamental plants (Galle, 1997; Loizwau and Spichiger, 2004). *Ilex* × *meserveae* S. Y. Hu (meserve holly), a hybrid between I. rugosa F. Schmidt (prostrate holly) and *I. aquifolium* L. (english holly), is an evergreen tree or shrub that attains a maximum height of 1.8～3.6 m and a maximum width of 2.4～3.6 m. The leaves are glossy and bluish to dark green with prominent spiny margins. Fruits are showy red or yellow berries (Dirr, 1998). Meserve holly is a cold-hardy broadleaf evergreen shrub withstanding temperatures as low as －29 to －34 ℃ (Dirr, 1998; Rhodus, 2007). On the other hand, *Ilex verticillata* (L.) A. Gray (common winterberry) is a deciduous multi-stemmed shrub that grows to a height of 1.8～3.0 m. It bears bright red fruits which ripen in the fall and persist through winter (Dirr, 1998). The fruiting branches are often used considerably as a Christmas decoration. Common winterberry is a very cold-hardy plant, tolerating temperatures down to about －35 ℃ (Iowa State University Extension, 2007). Cappiello and Littlefield (1994) reported that little or no damage was found on these two species when they were planted in the Lyle E. Littlefield Ornamentals Trial Garden at University of Maine in Orono. *Ilex glabra* (L.) A. Gray (Inkberry) is a native evergreen shrub with glossy, evergreen foliage and black berries. It grows to a mature height of 1.8 to 2.4 m and a width of 2.4 to 3.0 m (Dirr, 1998). It is a desirable ornamental plant for northern landscapes. However, inkberry is less cold hardy than the above two species (Cappiello and Littlefield, 1994). It is of practical importance to breed a cold hardy hybrid with shiny and high quality evergreen foliage for northern nursery growers and landscape specialists.

Inter-specific hybridization and introgression could be used to tap genes of ornamental importance for horticulture improvement programs. However, reproduction barriers that make introgression difficult are common between divergent relatives and ornamental species. These barriers, such as genetic incompatibility, lack of fertilization, endosperm failure, embryo abortion and seedling lethality, play an important role in any part of the plant's reproductive cycle (Hodnett *et al.*, 2005). Although many *Ilex* hybrids recently have been bred, patented, and marketed (US Patent and Trademark Office, 2008; USDA National Agricultural Library, 2007), the biological nature of the incompatibility system(s) that prevent hybridization and/or seed development of *Ilex* species is yet to be understood. The authors observed that fruit set of inkberry artificially cross-pollinated with meserve holly and common winterberry varied among cultivars, which suggested that some degree of incompatibility existed between them (unpublished data). Eisenbeiss (1990) reported that a wide range of compatibility between *Ilex* species resulted in full fruit set and fertile seed, to no fruit or seed at all. He also summarized the compatible *Ilex* species under garden conditions. However, there are no reports in the literature characterizing reproductive barriers between inkberry, meserve holly and common winterberry.

The objectives of this research were to: 1) investigate the cross-hybrid compatibility of inkberry cultivars with both cold hardy meserve holly and common winterberry; and 2) observe meserve holly and common winterberry pollen germination and tube growth in inkberry pistils to determine if pistil-pollen interactions are reproduction barriers to production of inter-specific hybrids.

2 Materials and Methods

2.1 Artificial Pollination

Female *Ilex glabra* (L.) A. Gray (inkberry) wild species and its cultivars 'Chamzin', 'Compacta', 'Densa', 'Nigra', and 'Shamrock' were used for pollination with male *Ilex verticillata* (L.) A. Gray (common winterberry) and *Ilex* × *meserveae* S. Y. Hu (meserve holly) in 2008 and 2009. Pollination was also conducted among wild species and cultivars of inkberry in 2009 with 'Pretty Boy' as the pollinator (control: inkberry × inkberry cultivars). All selected plants were grown in 3.3 L plastic pots with Metro-mix 560 (Scotts-sierra Horticultural Products Company, Marysville, OH) and fertilized with control released fertilizer (Peters Professional 15 N-4.4 P-24.9 K, Scotts-sierra Horticultural Products Company, Marysville, OH). By the end of November, they were moved into cold storage at 3.9 ℃ for over-wintering about 5 months. One group of plants was then moved into a glass greenhouse at University of Maine (Orono, ME) on 14 May 2008 and 8 May 2009 to induce flowering for pollination with meserve holly. Other plants stayed outside until they were moved into the greenhouse on 24 June 2008 and 22 June 2009 for pollination with common winterberry or inkberry 'Pretty Boy'. During blooming (4 ~ 14 June and 25 June-4 July 2008; 26 May-6 June and 28 June-8 July 2009), hand pollination was carried out as the protocol described by Dafni (1992). In brief, the recently opened male flowers of potted plants of common winterberry and meserve holly were collected and stored in clear vials in a cooler at about 4 ℃ until use. To avoid dosage interference, pollen was shaken onto clear micro-slides and then brushed onto the stigma surfaces of inkberry cultivars. To ensure active stigma receptivity, all pollinations were done between 0 ~ 8 hours. Four or less flowers were pollinated on each branch to eliminate the influence of nutrient competition on fruit development. After pollination, all late opening flowers were removed using tweezers. Plants were arranged in a randomized complete block design in each of 3 glass greenhouses. Three blocks (three greenhouses) were used for hand pollination. In each block (greenhouse), each treatment had 1 to 12 plants as sub-samples based on the available plants. Plants were randomly arranged in each greenhouse. Two weeks after pollination, all pollinated plants were moved outside the greenhouses.

2.2 Pollen-Pistil Interaction

To evaluate pollen-pistil interaction for each case, ten flowers were collected 24 hr after pollination in 2008, and thirty flowers were collected 48 hr after pollination in 2009. Pistils were processed using a modified protocol described by Kho and Baer (1968). They were fixed in formaldehyde-acetic acid-alcohol (FAA, 46.3% ethyl alcohol, 5.5% formaldehyde, 3.5% methanol, 2.5% glacial acetic acid and 42.2% H_2O) (Fisher Scientific, Chicago, IL) and stored at −4 ℃ until examined. Pistils were cleared and softened in 0.8 Mol NaOH overnight, stained with 0.04% (w/v) aniline blue in 0.1 Mol KH_2PO_4 for approximately 30 min, and mounted on microscope slides in 50% 0.1 Mol KH_2PO_4 and 50% glycerol. The slides were kept in the dark until observation under a Leitz fluorescence microscope (K. A. Dawson Co., Belmont, MA). Fluorescence was induced using 390 to 420 nm light filtered from a mercury lamp with a 450 nm emission filter (Martin, 1959; Dumas and Knox, 1983). Pollen germination on inkberry pistils and pollen tube growth into the style and ovary were noted. Pollen germination percentage and percentages of pollen tube growth to the style and the ovary were then calculated.

3 Fruit and Seed Set

On 1 Oct. 2008 and 2009, the number of developed fruits was counted. The percentage of fruit set was then calculated using the following equation: (number of fruit on plants ÷ number of flowers pollinated) × 100%. On 15 Oct. 2009, all fruits in each treatment were harvested and pooled for seed collection. The number of developed seeds was recorded for each fruit. Developed seeds were plump and solid, while aborted seeds were shriveled and papery.

4 Data Analysis

All data including the percentage of pollen germination on stigma, pollen tube growth to style and ovary, fruit set and the number seed per fruit were included in the analysis of variance (ANOVA). A two way ANOVA was performed using Statistical Analysis Systems (SAS Version 9.1, SAS Institute, Inc., Cary, NC). Student-Newman-Keuls Test at $P \leqslant 0.05$ was applied for means separation.

5 Results and Discussion

5.1 Pollen-Pistil Interaction

A significant difference of pollen germination percentage ($P < 0.0001$ for 2008 and 2009) and the percentage of pollen tube growth into the style ($P < 0.0001$ and $P = 0.0039$ for 2008 and 2009, respectively) was observed between common winterberry and meserve holly as pollinators in both years. The percentage of their pollen tube growth into the ovary was also significant between both pollinators in 2008 ($P = 0.0094$), but not in 2009 ($P = 0.67$). Pollen germination percentage ($P = 0.13$) and percentage of pollen tube growth into the style ($P = 0.86$) among inkberry cultivars were not significant in 2008; however, they differed among inkberry cultivars ($P < 0.0001$) in 2009. The percentage of pollen tubes that grew into the ovary differed among inkberry cultivars ($P < 0.0001$) in 2008, but not in 2009 ($P = 0.12$). A significant interaction between pollinators and cultivars for pollen germination percentage ($P < 0.0001$) and percentage of its pollen tube growth into the style ($P < 0.0001$) and ovary ($P = 0.0006$) was found in 2009, but not in 2008 ($P > 0.11$).

Compared with 2008 pollination, pollen germination of both common winterberry and meserve holly were much lower in 2009 (Table 1 and 3). This might have resulted from higher humidity during the 2009 bloom season since higher relative humidity tends to decrease pollen viability (Dubay and Murdy, 1983; Perveen *et al.*, 2007). In both years, pollen germination of common winterberry and the percentage of its pollen tube growth into the style were higher than that of meserve holly regardless of the cultivars (See Table 1 and 3). Pollen germination on inkberry stigma surface was 25.5%～39.2% for common winterberry and 9.8%～23.9% for meserve holly in 2008 (See Table 1), and 14.6%～21.1% for common winterberry and 6.7%～18.0% for meserve holly in 2009 (Table 2). It is not surprising since an interspecific hybrid such as meserve holly could have a high degree of pollen sterility (Eisenbeiss, 1990). Compared with pollen of inkberry 'Pretty Boy' on inkberry stigma surfaces (control), pollen germination of common winterberry and meserve holly were 3.2%～33.0% (common winterberry) and 17.4%～69.3% (meserve holly) less (See Table 2). This reduced the opportunity of their tubes growing into the ovule and then fruit and seed setting.

Table 1　Pollen germination and tube growth in *Ilex glabra* pistils following pollination with *Ilex* × *meserveae* and *Ilex verticillata* in 2008

Female	Male	Pollen Germination z /%	% Pollen Tube Growth to the: z	
			Style	Ovary
Ilex glabra	*Ilex* × *meserveae*	21.5 ±3.0 bcdy	11.3 ± 2.1 bc	1.8 ± 0.4 bc
Ilex glabra 'Compacta'		23.9 ±1.2 abcd	12.8 ± 1.5 bc	0.5 ± 0.2 c
Ilex glabra 'Densa'		21.3 ± 6.3 bcd	11.2 ± 4.9 bc	0.6 ± 0.4 c
Ilex glabra 'Nigra'		9.8 ± 2.7 d	5.9 ± 2.0 c	0.0 ± 0.0 c
Ilex glabra 'Shamrock'		17.7 ± 2.5 cd	12.0 ± 1.8 bc	0.0 ± 0.0 c
Ilex glabra	*Ilex verticillata*	39.2 ± 5.5 a	21.7 ± 3.4 ab	3.3 ± 0.9 a
Ilex glabra 'Compacta'		28.2 ± 2.5 abc	17.4 ± 1.9 ab	0.9 ± 0.4 bc
Ilex glabra 'Densa'		25.5 ± 2.4 abc	13.8 ± 1.8 bc	2.2 ± 0.7 ab
Ilex glabra 'Nigra'		35.5 ± 3.5 ab	23.7 ± 3.2 a	0.2 ± 0.2 c
Ilex glabra 'Shamrock'		33.1 ± 4.5 abc	20.1 ± 3.2 ab	0.0 ± 0.0 c

z Values are means ± standard error of 10 pistils.

y Different letters in columns indicate that they are significantly different ($P \leqslant 0.05$) according to Student-Newman-Keuls mean separation.

Table 2　Pollen germination and tube growth in *Ilex glabra* pistils following pollination with *Ilex* × *meserveae* and *Ilex verticillata* in 2009

Female	Male	Pollen Germination z /%	% Pollen Tube Growth to the: z	
			Style	Ovary
Control y		21.8 ±1.2 ax	5.5 ±0.5 a	1.3 ±0.2 a
Ilex glabra	*Ilex* × *meserveae*	14.8 ±1.2 b	0.9±0.2 d	0.1±0.0 b
Ilex glabra 'Chamzin'		8.8 ±0.8 c	1.5±0.5 cd	0.4±0.1 ab
Ilex glabra 'Compacta'		15.1 ±1.5 b	3.0±0.5 bc	0.7±0.4 ab
Ilex glabra 'Densa'		18.0 ±1.0 ab	5.1±0.9 a	0.9±0.3 a
Ilex glabra 'Nigra'		6.7 ± 0.7 c	0.8±0.2 d	0.3±0.1 ab
Ilex glabra 'Shamrock'		14.7 ±0.7 b	0.8±0.2 d	0.3±0.1 ab
Ilex glabra	*Ilex verticillata*	18.6 ±0.7 ab	2.7±0.4 cd	0.6±0.2 ab
Ilex glabra 'Chamzin'		17.9 ±1.0 ab	4.5±0.6 ab	1.0±0.2 a
Ilex glabra 'Compacta'		15.8 ±1.1 b	3.0±0.4 bc	0.5±0.1 ab
Ilex glabra 'Densa'		21.1 ±1.3 a	2.9±0.5 bc	0.1±0.1 b
Ilex glabra 'Nigra'		14.6 ±0.8 b	2.4±0.3 cd	0.3±0.1ab
Ilex glabra 'Shamrock'		14.9 ±1.0 b	1.2±0.4 cd	0.3±0.2 ab

z Values are means ± standard error of 30 pistils. y *Ilex glabra* pollinated with compatible pollen of 'Pretty Boy' was used as control. x Different letters in each column indicate that they are significantly different ($P \leqslant 0.05$) according to Student-Newman-Keuls mean separation.

Table 3　Fruit set of *Ilex glabra* accessions following pollination with *Ilex* × *meserveae* and *Ilex verticillata* on 1 Oct. 2008 and 2009

Taxa (Female)	Fruit Set /% z				
	Ilex glabra 'Pretty Boy'	*Ilex* × *meserveae* (Male)		*Ilex verticillata* (Male)	
	2009	2008	2009	2008	2009
Ilex glabra	96.3 ± 1.3 ay	47.8 ± 3.0a	20.3 ± 5.3 ab	26.0 ± 2.5b	11.7 ± 3.5b
Ilex glabra 'Chamzin'	97.2 ± 1.0 a	39.0 ± 7.8 ab	17.2 ± 6.6 ab	65.9 ± 3.2a	72.0 ± 3.8a
Ilex glabra 'Compacta'	83.8 ± 5.2 a	21.2 ± 4.0bc	24.1 ± 7.8 ab	29.5 ± 1.6b	14.4 ± 2.2b
Ilex glabra 'Densa'	96.3 ± 0.7 a	56.6 ± 6.7a	57.8 ± 3.3 a	72.3 ± 1.9a	72.4 ± 2.6a
Ilex glabra 'Nigra'	97.8 ± 1.1 a	4.7 ± 0.8c	12.3 ± 4.4 ab	13.1 ± 1.1x	2.7 ± 0.7b
Ilex glabra 'Shamrock'	98.8 ± 1.0 a	2.0 ± 0.6c	5.6 ± 1.0 b	1.7 ± 0.2c	0.2 ± 0.1b

z Values are means ± standard error of three replications. y Different letters in each column indicate that they are significantly different ($P \leqslant 0.05$) according to Student-Newman-Keuls mean separation. x Data of preliminary experiment in 2007 are presented as a reference but were excluded from the analysis.

Following pollen tube growth, the number of pollen tubes observed in the style was dramatically reduced by 33.2%~45.9% (2008) and 74.9%~91.9% (2009) for common winterberry, and 32.2% ~ 47.4% (2008) and 71.7%~94.6% (2009) for meserve holly (See Table 1 and 3). This trend was observed in *Sorghum bicolor* pistils following pollination with other Sorghum species (Hodnett *et al.*, 2005). At this stage, the number of pollen tubes was 18.2% ~ 78.2% (common winterberry) and 7.3%~85.5% (meserve holly) less than that of inkberry 'Pretty Boy' (control). This stage further decreased the possibility of their tubes entering into ovule and thus reduced fruit setting.

In 2008, the percentage of pollen tube growing to the ovaries of inkberry wild species and its cultivars 'Compacta', 'Densa' and 'Nigra' was less than 3.3% and 1.8% when common winterberry and meserve holly were used as a pollinator, respectively (See Table 1). However, pollen tubes were never observed in the ovaries of the inkberry 'Shamrock', which might be due to a small sample size or short period after pollination. In 2009, less than 1.0% and 0.9% of pollen tubes grew to the ovaries of inkberry when pollinated with common winterberry and meserve holly, respectively (See Table 2). These numbers only accounted for 76.9% and 69.2% of that when inkberry 'Pretty Boy' was used as a pollinator. The probability of fruit setting was further decreased at this stage.

5.2 Fruit Set

A significant difference in fruit set occurred between pollen sources (2008: $P = 0.005$, 2009: $P = 0.0047$), among inkberry cultivars ($P < 0.0001$ for both years) and interaction of both factors (2008: $P = 0.044$, 2009: $P < 0.0001$).

A large variation in fruit set occurred among inkberry cultivars pollinated with common winterberry in both years (See Table 3). In 2008, fruit set for inkberry 'Densa' and 'Chamzin' was 72.3% and 65.9%, respectively, while in 2009 fruit set was about 72%. Less fruit were observed on the inkberry wild species and its cultivars 'Compacta' in both years. 'Nigra' and 'Shamrock' had 13.1% and 1.7% fruits set, respectively, in 2008, while only 2.7% and 0.2% fruits was observed in 2009. Similarly, fruit set varied to some degree among inkberry cultivars when pollinated with meserve holly (See Table 3). Inkberry 'Densa' had the highest fruit set in both years. The fruit set of inkberry wild species, 'Chamzin', and 'Compacta' ranged from 21.2% to 47.8%, but fruit set on inkberry 'Nigra' and 'Shamrock' in 2008 was only 4.7% and 2.0%, respectively. In 2009, fruit set for inkberry wild species, 'Chamzin', 'Compacta', and 'Nigra' ranged from 12.3% to 24.1%, while fruit set for inkberry 'Shamrock' was only 5.6%. In addition, when inkberry wild species, 'Chamzin', 'Densa', 'Nigra', and 'Shamrock' pollinated with inkberry 'Pretty Boy', the fruit sets were 96.3% or more. Even on inkberry 'Compacta', there still had 83.8% of fruit setting (See Table 3). These were much higher than that pollinated with common winterberry and meserve holly.

Based on hand pollination for two years, the fruit set of inkberry differed from one cultivar to another when pollinated with common winterberry or meserve holly. This was in agreement with previous reports by Vargs *et al.* (2002). They found that fruit set of Prunus dulcis (Mill.) D. A. Webb (almond) differed considerably among cultivars. Davies and Buchanan (1979) reported the fruit set of Vaccinium ashei Reade (rabbiteye blueberry) varied with its cultivars. Our results also indicated that most inkberry cultivars produced more fruits when pollinated with common winterberry than with meserve holly. This was reasonable since pollen germination of common winterberry on inkberry stigma was much higher and a higher percentage of its pollen tubes grew into inkberry ovaries. Similar results were found in Prunus cerasus L. (sour cherry); cultivars with higher pollen germination produced higher fruit set (Milutinovic *et al.*, 1998). In addition, when highly-compatible intraspecific pollen was used for pollination of inkberry cultivars, there was over 83.8% fruit set of inkberry cultivars with no significant difference among cultivars ($P = 0.06$) (See Table 3). This suggested no self sterility existed among cultivars. Thus, inter-specific compatibility barriers among these two *Ilex* species and one interspecific hybrid might be responsible for the lower fruit set and large variation among cultivars.

5.3 Seed Set

Seed sets were significant among pollen sources ($P < 0.0001$). Inkberry and its cultivars produced more seeds per fruit when pollinated with compatible pollen, inkberry 'Pretty boy'. The number of seeds per fruit was over 3 when inkberry 'Pretty boy' was the pollinator, while less than 2 when common winterberry and meserve holly (See Table 4). The number of seeds per fruit significantly varied with inkberry cultivars ($P < 0.0001$). When inkberry 'Pretty boy' was the pollen source, about 5 seeds per fruit were observed for inkberry 'Chamzin', 'Densa', and 'Nigra', 4 seeds per fruit for inkberry 'Shamrock', while 3 for inkberry and inkberry 'Compacta' (See Table 4). However, when pollinated with common winterberry, inkberry, inkberry 'Chamzin', 'Densa', and 'Nigra' produced only one seed per fruit (See Table 4). Some plants didn't produce any seed. Similarly, inkberry 'Chamzin', 'Densa', and 'Shamrock' with meserve holly as pollen source had about one seed per fruit, while 2 seeds per fruit was recorded for inkberry 'Nigra' (See Table 4). In addition, an interaction of both pollen sources and inkberry cultivars was also observed ($P < 0.0001$). These results implied that common winterberry and meserve holly had lower probability of fertilization.

Table 4 Seed sets of inkberry accessions following pollination with *Ilex* × *meserveae* and *Ilex verticillata* in 2009

Accessions(Female)	Number of Seeds per Fruit		
	Ilex glabra 'Pretty Boy'	*Ilex* × *meserveae*	*Ilex verticillata*
Ilex glabra	3.0 ± 0.1z ey (100)x	0.2 ± 0.1 c (36)	1.1 ± 0.2 a (29)
Ilex glabra 'Chamzin'	5.2 ± 0.1 a (71)	0.9 ± 0.2 b (26)	1.3 ± 0.2 a (86)
Ilex glabra 'Compacta'	3.4 ± 0.2 d (51)	0.3 ± 0.1c (67)	0.4 ± 0.1c (62)
Ilex glabra 'Densa'	4.7 ± 0.2 b (52)	1.0 ± 0.2 b (50)	1.1 ± 0.1a (125)
Ilex glabra 'Nigra'	4.6 ± 0.2 b (55)	2.4 ± 0.3 a (40)	1.3 ± 0.5 ab (7)
Ilex glabra 'Shamrock'	3.9 ± 0.2 c (51)	0.8 ± 0.4 bc (10)	0.4 ± 0.1 bc (52)

z Values are means ± standard error. y Different letters in each column indicate that they are significantly different ($P \leqslant 0.05$) according to Student-Newman-Keuls mean separation. x Data in parentheses are the number of seeds observed.

Other factors, such as ovule penetration, endosperm failure and/or embryo abortion may account for lower fruit and seed set. Therefore, a histological study should be conducted to determine what results in low seed development after pollination. Seed germination would provide evidence as to whether or not fertilization is occurring and if the developing embryo aborts or the endosperm deteriorates. Both histological study and germination study are still under way.

In summary, cross compatibility of meserve holly or common winterberry with inkberry varied among inkberry wild species and its cultivars. The inkberry wild species, its cultivars 'Chamzin' and 'Densa' had greater compatibility with either meserve holly or common winterberry, while 'Compacta' and 'Nigra' were less compatibile and 'Shamrock' was almost incompatible with either meserve holly or common winterberry. Reproduction barriers, including the inhibition of pollen germination, pollen tube growth to the style and the ovary, and lack of fertilization, resulted in varying degrees of the cross incompatibility of inkberry with both cold hardy species. Further cross pollination should consider should consider the incompatibility of cultivar variations.

Acknowledgments: Maine Agriculture, Forestry, and Experiment Station Publication #3144. We thank Dr. Stephanie Burnett, Dr. Michael E. Day, Dr. Mike Greenwood, Dr. Renae Moran, and Dr. John Smagula for reviewing the manuscript. We gratefully Brad Libby for his help in plant production and nursery management; Kelly Edwards for his technical assistance in using the fluorescence microscope.

Literature Cited:

[1] Cappiello P E, L E Littlefield. Woody landscape plant cold-hardiness ratings[P]. Maine Agricultural and Forest Experiment Station Technical Bulletin 156, 1994.

[2] Dafni A. Pollination ecology: a practical approach[M]. Oxford USA: Oxford University Press Inc., NY., 1992.

[3] Davies F S, D W Buchanan. Fruit set and bee activity in four Rabbiteye blueberry cultivars[C]// Proceeding of Florida State Horticultural Society, 1979. 92: 246-247.

[4] Dirr M A. Manual of Woody Landscape Plants[C]// 5th ed. Stipes Publishing. Co. Champaign, Ill., 1998.

[5] Dumas C, R B Knox. Callose and determination of pistil viability and incompatability[J]. TAG Theoretical and Applied Genetics, 1983, 67: 1-10.

[6] Eisenbeiss G K. Sexual compatibilities among holly species [J]. Holly Society Journal, 1990, 8 (1): 7-10.

[7] Galle F C. Hollies: the genus *Ilex*[M]. Portland, Oregon: Timber Press,.

[8] Hodnett G L, B L Burson, W L Rooney, S L Dillon, H J Price. Pollen-pistil interaction result in reproductive isolation between *Sorghum bicolor* and divergent Sorghum species [J]. Crop Science, 1997. 45: 1403-1409.

[9] Iowa State University Extension. ISU Extension News Release: Winterberry adds color to the winter landscape, 2009. November 24. <http://www.extension.iastate.edu/newsrel/2002/dec02/dec0221.html>.

[10] Kho Y O, J Baer. Observing pollen tubes by means of fluorescence[J]. Euphytica, 1968, 17: 298-302.

[11] Martin F W. Staining and observing pollen tubes in the style by means of fluorescence[J]. Stain Technology, 1959. 34(3): 125-128.

[12] Milutinovic M, V Rakonjac, Nikolic D. Functionality of pollen and fruit set in sour cherry cultivars[J]. Acta Horticulturae, 1998, 468: 591-594.

[13] Rhodus T. OSU Pocket Gardener, Shrubs, *Ilex* × meservea [DB/OL]. 2009-11-24. http://hcs.osu.edu/pocketgardener/source/index.html.

[14] US Patent and Trademark Office. Patent full-text and full-page image databases[DB/OL]. 2009-11-30. http://patft.uspto.gov/.

[15] USDA National Agricultural Library. Article Citation Database, *Ilex* hybrids. 2009-11-24. http://agricola.nal.usda.gov/cgi-bin/Pwebrecon.cgi.

[Edited by QIU De-yong]

光滑冬青与密苏威冬青和美国冬青杂交亲和性的研究

张冬林[1]，孙友平[2]，金晓玲[3]，韩丽娟[4]
（1. 美国缅因大学植物、土壤和环境科学系，奥罗诺，缅因州 04469，美国；
2. 中南林业科技大学，湖南 长沙 410004；3. 中南林业科技大学 风景园林学院，湖南 长沙 410004；
4. 长春师范大学 生物系，吉林 长春 130032）

摘　要： 光滑冬青是一种美国原产的常绿灌木，其叶质地光滑、深绿色；深得美国北部园林和苗圃种植者的喜爱。为培育其抗寒品种，在温室条件下对六个优良光滑冬青栽培变种与两种缅因当地抗寒性极强的冬青栽培变种（密苏威冬青和美国冬青）进行了人工控制授粉，并对其杂交亲和性进行了测定。结果表明：光滑冬青与密苏威冬青和美国冬青杂交亲和性差异显著。光滑冬青 Chamzin 和 Densa 栽培变种与密苏威冬青和美国冬青杂交亲和性较高，光滑冬青自然种和 Compacta 栽培变种与密苏威冬青和美国冬青杂交亲和性较低，而光滑冬青 Nigra 和 Shamrock 栽培变种基本与密苏威冬青和美国冬青不相容。荧光显微镜对 2008 年及 2009 年花粉原位萌发观测的情况也支持这一结果。密苏威冬青和美国冬青花粉在光滑冬青自然种，Chamzin，Compacta 和 Densa 栽培变种柱头上的萌发率明显高于 Nigra 和 Shamrock 栽培变种柱头上的萌发率。花粉管在花丝中的生长受到不同程度的抑制，只有极小部分的花粉管进入到子房中。密苏威冬青和美国冬青花粉管到达光滑冬青自然种、Chamzin，Compacta 和 Densa 栽培变种子房的比例明显高于到达 Nigra 和 Shamrock 栽培变种子房的比例，大大增加了花粉管进入胚珠的几率，并最终受精结实。对果实中种子统计后发现，大部分种子败育。当密苏威冬青和美国冬青花粉与光滑冬青授粉后，每个果实中只有不到 2 个种子发育完全，而当光滑冬青花粉与光滑冬青授粉后，每个果实中有超过 3 个种子完全发育，部分达到 6 个。因此，抑制花粉萌发及花粉管在花丝中及子房中生长、受精失败等因素导致光滑冬青与密苏威冬青和美国冬青杂交不亲和，在今后的冬青杂交育种中，应当考虑栽培品种间杂交亲和性差异。

关键词： 美国冬青；结果率；光滑冬青；密苏威冬青；花粉萌发；结子率