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Selection of Early and Late Flowering *Robinia pseudoacacia* from Domesticated and Introduced Cultivars in Korea and Prediction of Flowering Period by Accumulated Temperature

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Abstract : The objectives of this study were to select early, late, and abundant flowering trees of black locust from domesticated and introduced cultivars, and prediction of flowering period by calculation of accumulated temperature in spring. Four cultivars (Debreceni-2, Pusztavacs, Jaszkiseri, and Rozsaszin AC) from Hungary and a cultivar from Beijing, China, were introduced, propagated by seed and planted in a seed orchard. For domesticated black locust, 63 cultivars from 10 locations throughout the country were selected and propagated by root cutting. Criteria for selection of domesticated cultivars were abundant flowering, long flowering period, or abundant nectar production with, if possible, straight stems. Accumulated temperature was calculated from data of a nearby weather station by accumulating daily maximum temperature minus 5 degree Celsius from January 1 up to the date reaching 880 degrees. Daily mean temperature was also used to calculate accumulated temperature up to the date reaching 450 degrees. The percentages of two-year and three-year-old flowering trees propagated by root cutting were higher than that of trees propagated by seeds, while four-year-old trees all flowered regardless of propagation methods. Among the domesticated cultivars, all the cultivars from Ganghwa showed abundant flowering with highest nectar production of 6.5 ul per flower, which was 100% more than other domesticated cultivars and 50% more than Debreceni-2 cultivar with highest nectar production among the introduced cultivars from Hungary. At the end of the eight years of observations, two trees of Debreceni-2 cultivars and a tree from Beijing, China were selected for early flowering trees which flowered 2 to 3 days earlier than average trees, while a tree of Debeceni-2 and three trees from Bejing were selected for late flowering trees which flowered 2 to 3 days later than average trees. It is possible to extend the flowering period of black locust by 4 to 6 days by planting early and late flowering cultivars together. Abundant flowering trees were unable to be selected due to severe damages by leaf gall midges which killed many trees and reduced the crown size of the remaining trees in the seed orchard, and which were first found in Korea in 2001 and now damaging most of the black locust forests in Korea. The prediction of flowering period by accumulated temperature indicated that black locust flowered to a peak when accumulated daily maximum temperature reached 880 degrees Celsius, and when daily mean temperature reached 450 degrees.

Key words **:** *sum of temperature, black locust, Debreceni-2, Pusztavacs, Jaszkiseri*

Introduction

Black locust (*Robinia pseudoacacia* L.) belongs to a genus of *Robinia* that has about 20 species in tree and shrub forms worldwide. It has a wide range of distribution in the native North America (Hanover *et al.*, 1992), relatively high degree of genetic diversity (Bongarten, 1992; Chang *et al.*, 1998), and is a timber tree of some importance (Harlow *et al.*, 1979).

Black locust has been introduced to many parts of the world due to its many useful characteristics. It was introduced to Europe including Germany (Molnar, 1998) and Hungary (Keresztesi, 1988). It was first introduced to Korea in late 19th century and has relatively small genetic variation (Hong *et al.*, 2002) and peculiar flowering physiology (Lee and Hong, 2005). It has been planted in many parts of Korea to produce woods, fiber,

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honey, and forage (Park, 1996). Black locust has adapted well to various sites, such as open fields after clear cutting, abandoned mines, cut-slopes for new roads, land fills for garbage disposal, and eroded lands after forest fires (Korean Society of Black Locust Research, 1992). Due to its capability of fixing atmospheric nitrogen into ammonia, black locust has been extensively planted as a pioneer species in erosion control sites with poor and dry soil (Korean Society of Black Locust Research, 1997). Forest statistics indicated that during the 32 years of reforestation projects by Forest Service from 1960 to 1992 a total of 324 thousands ha of lands were planted by black locust (Park, 1996).

In addition, black locust produces unique wood, which has been proved to be very useful by farmers. They planted and managed the black locust forests for fuel, forage for cattle, woods for props, wagon wheels, and flooring. In addition to above uses, black locust has been extensively used for honey production. Among the 198 honey-producing woody plants in Korea (Lee, 1998), black locust contributes to 70% of total honey production in Korea (Park, 1996).

Hungary which has been known to improve genetic resources of black locust during the last 200 years has developed more than 30 varieties of black locust for various purposes including extension of honey harvesting period and more nectar production (Keresztesi, 1988). In Korea black locust in a single location usually flowers for only 7 to 9 days (Ministry of Agriculture and Forestry, 1999), which is considered very short compared with other honey-producing plants. Therefore, the Korean apiculturists collect honey from black locust by continuous moving along the flowering trees from south to north throughout the country. It is desirable to have black locust trees or cultivars that flower early or late to extend the flowering period and that produce more nectar than average trees. In addition prediction of flowering period in advance will help apiculturists to plan their travels throughout the country.

The objectives of this study were to select early and late flowering black locust trees which have been domesticated and introduced from Hungary and China and to predict flowering period by calculation of accumulated temperature.

Materials and Methods

1. Introduction of cultivars

Black locust growing in Hungary was introduced from Hungary Forest Research Institute in 1988. As shown in Table 1, a total of five cultivars were introduced by seeds. Among them Debreceni-2 is one of the most commonly planted cultivars in Hungary for honey production due to its abundant flowering, late flowering, and high nectar production (Keresztesi, 1988). Pusztavacs is not a name of a cultivar, but is a name of a seed production area known for its straight stem for timber production (Redei, 1998). Jaszkiseri is known for its straight stem with high resistance to frost in autumn (Redei, 1997). A Chinese cultivar was introduced for its resistance to drought with a long flowering period and abundant flowering.

Korean cultivars were selected from 10 locations throughout the country from Hapcheon at the southern most location to Cheolwon at the northern most location. A total of 63 cultivars were selected for this study. Criteria for selection of domesticated black locust cultivars were abundant flowering, long flowering period, and abundant nectar production (Ministry of Agriculture and Forestry, 1999).

2. Raising planting stocks

Seeds introduced from Hungary and China were pretreated to break seed coat dormancy. Black locust seeds have waxy seed coats to prevent water infiltration into the seeds. Seeds were placed into a container made of

Table 1. Characteristics of black locust cultivars selected from Korea and introduced from Hungary and China.

Country of origin	Names of cultivars	Characteristics
Hungary	Debreceni-2	Abundant flowering, late flowering, with pink flowers and nectar production of more than 5 ul per flower.
	Pusztavacs	Straight stem; Not a name of a cultivar but a name of seed production area.
	Jaszkiseri	Straight stem with high frost resistance
	Rozsaszin AC	fast growth, abundant and late flowering with pink flowers
	Mixed seeds	not known
China	Beijing	Long flowering period and resistance to drought
Korea	Hapcheon, Daegu, Gumi, Cheonan, Suwon, Yeoju, Wonju, Ganghwa, Paju, Cheolwon	Hapcheon and Daegu for straight stem, Cheonan and Wonju for late flowering, Ganghwa for abundant flowering and high nectar production.

woven net and submerged into boiling water for three seconds to make cracks on the surface of the seed coats. Then the seeds were immediately put into cold water to cool them down. With this kind of treatment seeds germinated within a week and percents of germination were more than 95%.

The above treated seeds were sown into a nursery bed with mulching using rice straws. Seeds were sown to produce 50 seedlings per m^2. One out of two seedlings were rouged to obtain seedlings with straight stems and height at least 1.6m at the end of the first growing season.

Selected black locust mother trees growing in Korea were propagated by root cutting. Cut rootings were taken from the mother trees in mid March. The sizes of the rootings varied slightly among the locations, but were at least 1.0 cm in diameter and 15 cm in length.

3. Establishment of seed orchard, planting, and maintenance

Seed orchards were established in a flat land with sandy loam soil, which had been maintained as a pitch pine forest for about 30 years. One-year-old black locust seedlings or root cuttings were planted with a spacing of 1.5m by 1.0m to have a planting density of 6,000 plants per ha. The seed orchard had a total area of 1.3 ha. At the beginning of the second growing season after planting in the seed orchard, stems of all the trees were cut at the base to produce straight stems.

4. Survey for flowering

Starting from the second year after planting in the seed orchard, a few trees started to flower with small number of flowers. Height growth was recorded for the first three years and straightness of stems was also recorded. Flowering characteristics were recorded every year to select individuals with early flowering, late flowering, abundant flowering, or pink flowers. Seasonal dynamics of flowering was followed as directed by Koltowski and Mah (1998).

5. Calculation of accumulated temperature for prediction of flowering period

To understand a relationship between floral development and local weather and to predict flowering period based on daily temperature, accumulated temperature was calculated from weather data collected from a weather station located 3km east from the seed orchard. Accumulated temperature is defined as accumulated daily mean temperature or daily maximum temperature minus five degrees Celsius starting from January 1 to the peak day of flowering in May (Lee and Kim, 1987; Murray *et al.*, 1989). The peak day of flowering was estimated to be the fourth day from the beginning of flowering (Koltowski and Mah, 1998). Usually black locust flowers for about 7 to 9 days in a single location. The flowering and weather data were compared for seven years from 2000 to 2006.

In case of the year of 1999, accumulated daily maximum temperature was compared with the date of the peak flowering from 15 locations throughout the country.

Results and Discussion

Table 2 shows flowering from 1999 to 2003 of black locust trees propagated by either root cutting or seeds. One-year-old trees were transplanted to the seed orchard and 5% of trees propagated by root cutting started to flower in the same year of the transplanting, while less than 0.5% of seedlings flowered. It suggested that vegetative propagation of mature black locust trees promoted early flowering. Two years after the transplanting about 34% of the three-year-old trees from seeds flowered, and the percent of flowering trees increased to 54% in the four-year-old trees, while 100% of trees in the seed orchard flowered when they were five years old. First flowering showed no difference between cultivars or between countries of origin.

Table 3 shows results of early selection for pink flowers and abundant flowering individuals from three-year-

Table 2. Percent of flowering black locust trees from 1999 to 2002 propagated by seed and root cutting.

Propagation method	Cultivars	Year of planting	No. of trees planted	Percents of flowering trees (%)				
				1999	2000	2001	2002	2003
Root cutting	Korea	1999	199	5.0	stem removed	38.4	70.3	100
		2000	180	-	14.4	57.2	56.5	100
Seeds	Debreceni-2	1999	565	0.5	stem removed	38.4	61.2	100
	Pusztavacs		834	0.1		27.7	47.5	100
	Mixed seeds from Hungary		572	0.3		30.3	38.7	100
	Jaszkiseri	2000	278	-	0.3	20.5	42.8	100
	Beijing, China		444	-	0.8	25.7	53.8	100

One-year-old trees were transplanted to a seed orchard.

Table 3. Early selection of pink flowers and abundant flowering individuals of three-year-old black locust trees from Korea and introduced cultivars from Hungary and China.

Names of cultivars	No. of trees observed	Abundant flowering		Percents of trees from Hungary with pink flowers or percent of abundant flowering in trees from Korea.
		No. of trees	Percents (%)	
Hungary Debreceni-2	565	23	4.1	Percents of trees with pink flowers: 2.1%
Hungary Pusztavacs	834	24	2.9	Percents of trees with pink flowers: 0%
Hungary Mixed seeds	373	8	2.2	Percents of trees with pink flowers: 0%
Korean cultivars (1999 planting)	199	25	12.6	Hapcheon 2.6%, Cheonan 18.3%, Wonju 17.1%, Ganghwa 46.1%
Korean cultivars (2000 planting)	180	30	16.7	Yeoju 5.4%, Gumi 2.2%, Paju 94.7%, Cheolwon 39.1%

Table 4. Percents of abundant flowering and early or late flowering of black locust trees two (Jaszkiseri and Beijing cultivars) or three (remaining cultivars) years after planting.

Names of cultivars	No. of trees observed	Extent of abundant flowering (%)					Flowering period (%)				Pink flowers (%)
		No flowers	Very little flowers	Medium flowers	Abundant flowers	Very abundant flowers	Very early flowering	Early flowering	Late flowering	Very late flowering	
Korean cultivars	319	35.7	21.9	31.7	8.2	2.5	0.3	1.3	2.8	5.3	0
Debreceni-2	565	38.8	26.2	20.0	10.4	4.6	1.2	6.9	4.6	1.6	2.1
Pusztavacs	834	52.5	21.8	14.8	8.8	2.2	0.7	5.0	3.0	0.5	0
Jaszkiseri	278	57.2	21.6	15.8	4.3	1.1	0	0.7	3.2	2.2	4.3
Mixed seeds	372	61.3	18.6	12.4	5.9	1.9	0	2.4	3.7	0.5	0
Beijing	264	46.2	22.7	24.6	5.3	1.1	0	2.7	3.0	4.2	0

old trees. Debreceni-2 cultivar from Hungary is known to have pink flowers (Keresztesi, 1988). However, only 2% of the trees in Debreceni-2 cultivar showed pink flowers and even they had pale pink only in the lower parts of their petals. As shown in Table 1, the Debreceni-2 cultivar was introduced to Korea, because it had abundant and late flowering with pink flowers. Unfortunately Debreceni-2 may not be used in Korea for an ornamental purpose due to pale pink flowers in only 2% of the individuals. We may have to try to introduce this cultivar again by vegetative propagation to maintain deep pink color in the petals as shown in some parts of Hungary.

Table 3 also shows abundant flowering of some Korean cultivars. Ganghwa and Paju cultivars were selected based on the abundant flowering. Particularly Ganghwa cultivar should receive a special attention by bee keepers, because all the selected individuals from Ganghwa had abundant flowering and high nectar production. In the earlier study before the selection in 1997 on the amount of nectar production in Ganghwa, we found that the average nectar production of this cultivar showed 6.5 ul per flower at the peak of flowering period, which was 100% more than national average in Korea and 50% more than Debreceni-2 from Hungary. The Ganghwa cultivars were selected from several stands in Ganghwa-gun, Yangdo-myeon, Geonpyong-ri in Incheon city.

Table 4 shows the extent of abundant flowering and early or late flowering period of black locust trees two or three years after planting. Debreceni-2 was introduced for its abundant flowering in Hungary. In this table, 15% of Debreceni-2 individuals showed abundant or very abundant flowering, while other cultivars including Korean cultivars showed less percentage than Debreceni-2. Debreceni-2 also showed highest percentage of late flowering among the introduced cultivars. It may agree well with previous research on this cultivar (Redei *et al.*, 2002). Pink flowers were observed in few trees of Debreceni-2, Pusztavacs, and Jaszkiseri. However, the percentage was still lower than 4.3%, which was considered meaningless in using Hungarian cultivars for pink flowers as a horticultural purpose.

Table 5 shows a process and a final result of selection of early and late flowering individuals from a seed orchard. During the five years of observations to select early and late flowering individuals, no single tree had been selected five consecutive years as early or late flowering individuals. However, some trees have been selected as early flowering trees during the last four consecutive years, and other trees as late flowering ones for three years. Finally two trees of Debreceni-2 cultivar and a tree from Beijing, China were selected as early flowering trees. In addition, a tree of Debreceni-2 and three trees from Beijing were selected for late flowering.

The early flowering trees flowered about two or three days earlier than remaining trees, while late flowering trees flowered about two or three days later than remaining trees. It means that flowering period of black locust

Table 5. Selection of early and late flowering individuals of black locust trees from a young seed orchard in Suwon, Korea.

Names of cultivars	2002		2003		2004		2005		2006		Final selection	
	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
Debreceni-2	1.8	0.2	1.4	0.2	0.5	0.2	0.2	0.2	0.9	0.2	2 trees	1 tree
Pusztavacs	6.4	0	0.8	0	0.2	0	0.1	0	0.7	0	0	0
Mixed seeds from Hungary	1.9	0	0.5	0	0	0	0	0	0.3	0	0	0
Beijing	1.1	0	1.1	0	0.4	1.1	0.4	0.8	1.1	1.1	1 tree	3 trees
Korean cultivars (1999 planting)	2.5	0	1.0	0	0.5	0	0	0	0	0	0	0
Korean cultivars (2000 planting)	5.0	0	3.3	0	1.7	2.8	0.6	0.6	0	0	0	0
Remarks			10 trees were same as previous ones. Leaf gall midge damages observed.		5 trees were same as previous ones. Leaf gall midge damages again		Some selected trees killed by gall midges		More selected trees killed by gall midges		Previous 4 years data coincided.	Previous 3 years data coincided.

Unit: percents of total trees in each cultivar

in a single location might be extended about four to six days from average flowering period of about eight to ten days. Even though a flowering period in a location has been reported to be about 4 to 7 days in 20-year-old black locust stands (Kim *et al.*, 1986) and in bigger isolated trees (Koltowski and Mah, 1998), we may extend flowering period up to 15 days in a single location, if early and late flowering cultivars are planted at the same time with average flowering trees. The maximum flowering period may not coincide with possible honey-collecting period for honey bee keepers, because they may not wait until to the last day of fading honey production at the end of the flowering season.

As shown in Table 5, it is unfortunate that the black locust trees in the seed orchard experienced severe damages by insects, known as an yellow locust midge, *Obolodiplosis robiniae*, (Woo *et al.*, 2003) starting in 2001. The five consecutive years of insect attacks eventually killed many trees and selection of early and late flowering trees might have been influenced by the heavy mortality of most of the cultivars. Particularly selection of abundant flowering trees was almost impossible due to reduction of crown size after the heavy damages by the insects.

Table 6 shows development of early aborted, decayed, and filled seeds of black locust trees at the end of October in the seed orchard. Filled seeds were found to be 54.2% in the late October, while 29.4% were aborted during the process of maturation and 16.3% were decayed by the time of seed collection for winter storage. The 54% of filled seeds at the time of seed harvest in autumn is considered high enough to collect seeds for winter storage.

Table 6. Development of early aborted, filled, and decayed seeds after full growth collected in late October from an young black locust seed orchard.

Names of cultivars	Filled seeds (%)	Decayed seeds after full growth (%)	Early aborted seeds (%)
Korean cultivars	45.1	20.2	34.3
Debreceni-2	56.9	15.2	27.9
Pusztavacs	46.2	8.1	45.7
Jaszkiseri	66.2	14.7	19.1
Mixed seeds	77.2	9.4	13.4
Beijing	33.6	30.4	36.0
Average	54.2	16.3	29.4

Table 7 shows prediction of flowering period of black locust based on accumulated daily maximum temperature from January 1st to a date in May until accumulated daily maximum temperature minus 5 degree Celsius reached 880 degrees. The accumulated daily maximum temperature of 880 degrees seemed not to match with actual flowering date in all the years except 2003. When the predicted and actual dates were converted to Julian date for a t-test, the predicted date was not significantly different from the actual date at 5% level. The accumulated temperature of most of the years was less than necessary to approach the date of actual peak flowering date. However, it may be explained by the fact that the weather station in Suwon was located in the center of the city, while the seed orchard was located about 3km west near a mountain area, which made the daily temperature of the seed orchard about 2 degrees lower than at the weather station. Considering the difference between the two locations, the accumulated daily maximum temperature of 880 degree Celsius seemed to be useful to predict

Table 7. Prediction of date of the peak flowering of black locust in the seed orchard based on accumulated daily maximum and daliy mean temperature minus five degree Celsius from January 1 up to the 880 degrees or 450 degrees, respectively. The reason of actual later flowering tendency compared with predicted date is cooler temperature in the seed orchard than in Suwon weather station.

Year	Actual date of peak flowering	Predicted date of peak flowering based on daily maximum temperature	Number of days off the predicted date by maximum temperature	Predicted date of peak flowering based on daily mean temperature	Number of days off the predicted date by mean temperature
2000	May 20	May 16	+4	May 18	+2
2001	May 15	May 16	- 1	May 18	-3
2002	May 12	May 10	+2	May 15	-3
2003	May 16	May 16	0	May 15	+1
2004	May 16	May 8	+8	May 14	+2
2005	May 20	May 17	+3	May 18	+2
2006	May 20	May 15	+5	May 17	+3

Table 8. Prediction of date of the peak flowering of black locust in the year of 1999 in 15 locations throughout South Korea based on accumulated daily maximum temperature minus five degree Celsius from January 1 up to the 880 degrees.

Locations	Actual date of peak flowering	Predicted date of peak flowering based on daily maximum temperature	Number of days off the predicted date by daily maximum temperature
Daegu	May 6	May 6	0
Ulsan	May 7	May 4	+3
Gwangju	May 8	May 8	0
Jinju	May 10	May 13	-3
Jeonju	May 12	May 12	0
Gangreung	May 12	May 9	+3
Uljin	May 12	May 13	-1
Seoul	May 15	May 14	+1
Cheongju	May 15	May 15	0
Andong	May 17	May 17	0
Gunsan	May 18	May 21	-3
Wonju	May 21	May 22	-1
Suwon	May 24	May 20	+4
Sokcho	May 25	May 17	+8
Cheolwon	May 30	May 30	0

flowering date in black locust.

The accumulated daily mean temperature in Table 7 also matched well with the peak flowering date in black locust. The data indicated that black locust needed to have 450 degrees of accumulated daily mean temperature minus 5 degrees for flowering. In contrast Lee and Kim (1987) reported that black locust needed to have accumulated daily mean temperature of 280 degree. However, their calculation was based on the single year of 1986 and it is possible that it was not calculated properly.

Accumulated temperature may be applied to phenology of other flowering trees. For example, Jung *et al.* (2005) used accumulated temperature to study heating requirement for flowering in Japanese cherry. They found that Japanese cherry required 124 degrees when daily mean temperature minus 7 degrees Celsius was used for calculation of accumulated temperature.

Temperate trees have chilling requirement for breaking dormancy before spring flowering (Taira, 2003; Cesaraccio *et al.*, 2004). Calculation of accumulated temperature would be influenced by the chilling requirement. However, present study with black locust did not calculate the duration of winter chilling. We assumed that the normal chilling period of two months in November and December in Korea would be enough to break dormancy in black locust. January may be considered to contribute to the both chilling and heating requirements depending on the lowest and highest daily temperature in January.

Table 8 shows prediction of flowering dates in a single year of 1999 in 15 locations throughout the country with different latitude. This table also indicated that the accumulated daily maximum temperature reached 880 degrees for black locust to flower. The actual flowering date in the six locations out of 15 total locations in this study matched precisely with daily maximum temperature, while actual flowering date of eight locations showed one to four days of differences from predicted date based on accumulated daily maximum temperature. When the predicted and actual dates were converted to Julian date for a t-test, the predicted date was not significantly different from the actual date at 5% level. In case of Sokcho city the difference was eight days and the accumulated temperature seemed not to match with actual flowering date. It is assumed that Sokcho is located in the east side of the Taebaek Mountain Range which has a different weather pattern from rest of the cities and that the accumulated temperature of different number may be used for this location.

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