Domestication of wild roses for fruit production

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Appendix

Papers I-IX

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Uggla, M. & Nybom, H. 1999. Domestication of a new crop in Sweden – dogroses (*Rosa* sect. *Caninae*) for commercial rose hip production. *Acta Horticulturae* ISHS 484, 147-151.

II. Werlemark, G., Carlson-Nilsson, U., Uggla, M. & Nybom, H. 1995. Effects of temperature treatments on seedling emergence in dogroses, *Rosa* Sect. *Caninae* (L). *Acta Agriculturae Scandinavica, Section B, Soil and Plant Science* 45, 278-282.

III. Nybom, H., Carlson-Nilsson, U., Werlemark, G. & Uggla, M. 1997. Different levels of morphometric variation in three heterogamous dogrose species (*Rosa* sect. *Caninae*, Rosaceae). *Plant Systematics and Evolution* 204, 207-224.

IV. Werlemark, G., Uggla, M. & Nybom, H. 1999. Morphological and RAPD markers show a highly skewed distribution in a pair of reciprocal crosses between hemisexual dogrose species, *Rosa* sect. *Caninae*. *Theoretical and Applied Genetics* 98, 557-563.

V. Uggla, M. & Carlson-Nilsson, B.U. 2004. Screening of fungal diseases in offspring from crosses between *Rosa* sections *Caninae* and *Cinnamomeae*. *Scientia Horticulturae*. In press.

VI. Uggla, M., Gao, X. & Werlemark, G. 2003. Variation among and within dogrose taxa (*Rosa* sect. *Caninae*) in fruit weight, percentages of fruit flesh and dry matter, and vitamin C content. *Acta Agriculturae Scandinavica, Section B, Soil and Plant Science* 53, 147-155.

VII. Uggla, M., Gustavsson, K.-E., Olsson, M.E. & Nybom, H. 2004. Changes in colour and sugar contents in rose hips (*Rosa dumalis* L. and *R. rubiginosa* L.*)* during ripening. *Journal of Horticultural Science & Biotechnology* . In press.

VIII. Uggla, M. 2004. Changes in fruit quality and fruit detachment force in rose hips (*Rosa spinosissima* L.) during ripening. Manuscript.

IX. Gao, X., Björk, L., Trajkovski, V. & Uggla, M. 2000. Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *Journal of the Science of Food and Agriculture* 80, 2021-2027.

Contents

Abstract

Uggla, M. 2004. Domestication of wild roses for fruit production. Doctoral thesis.
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The utilization of rose hips has a long tradition in Sweden, where they are used for the manufacturing of a popular dessert soup rich in vitamin C. In the mid 1980s a project was initiated at Balsgård, situated in the south of Sweden, with the aim to develop rose cultivars as a field crop for rose hip production. In the present thesis, some aspects of the domestication process in wild roses for fruit production are reported and discussed. Most of the papers concern species within section *Caninae*. All of these species are polyploid and characterized by their unique meiosis with unequal distribution of chromosomes from the parents to the offspring.

The plant breeding program was divided into three steps. In the first step, plant material was acquired from different sources. The second step involved intraspecific and interspecific crosses in section *Caninae* and the third step concerned intersectional crosses between sections *Caninae* and *Cinnamomeae*. Seed germination was studied with different temperature treatments, and differences in amount of germination among the species were noted. Morphological diversity within and between three species, belonging to section *Caninae*, *R. dumalis*, *R. rubiginosa* and *R. villosa* was investigated. *Rosa dumalis* demonstrated the most pronounced intraspecific variation, whereas *R. rubiginosa* was very homogenous. Similar results were obtained when offspring plants were screened for fruit traits, such as fruit weight, % fruit flesh, % dry matter and vitamin C. Matroclinal inheritance was demonstrated with molecular markers (RAPD) in a pair of reciprocal crosses between *R. dumalis* and *R. rubiginosa*. The development of colour and some other fruit characteristics during ripening was studied for 6 weeks in *R. dumalis*, *R. rubiginosa* and *R. spinosissima*. For *R. dumalis* and *R. rubiginosa* it is possible to use colour as an indicator of optimum harvesting time. In *R. spinosissima* the fruits should be harvested in the middle of September, when most of the fruit traits have reached an optimum. Seedlings from 11 intersectional crosses (*Caninae* and *Cinnamomeae*) were screened for blackspot, leaf spot, powdery mildew and rust. Blackspot was the most severe disease followed by leaf spot, whereas symptoms of powdery mildew and rust were rare.

Key words: *Rosa* sect. *Caninae*, genetic diversity, matroclinal inheritance, fruit ripening, fungal diseases, antioxidants.

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Introduction

Utilization of rose hips has a long tradition in Sweden, where they are used for the manufacturing of a popular dessert soup rich in vitamin C. This dessert soup has been manufactured industrially for more than 50 years, mainly from dehydrated fruit flesh. The raw material is imported from different countries, e.g. from Chile. Chile is one of the largest producers of rose hips in the world with an annual export of 3600-4500 tonnes of dehydrated rose hips to Europe, handpicked from wildgrowing plants (Joublan *et al*., 1996). The special aroma desired in the rose hip dessert soup has been found mainly in species belonging to section *Caninae*.
In 1985, a plant domestication project was initiated in Sweden for the development of varieties suitable for mechanical harvesting and growing techniques for establishment of commercial plantations.

Utilization and plant breeding of rose hips

Roses belong to one of the most popular groups of ornamental plants and have a long history. During 4700 years, the rose has been a companion to humankind, mainly for the rose fragrance, but also for rose oil and rose water, and for cosmetic and medical purposes (Gustavsson, 1998). The genus *Rosa* includes more than 100 species in the temperate and subtropical zones of the Northern hemisphere. Roses are deciduous, rarely evergreen, and upright or climbing shrubs, with more or less prickly branches (Krüssman, 1981). The fruit, the rose hip, is a pseudocarp or false fruit, consisting of fleshy walls surrounding a cavity containing the single seed (Graham & Primavesi, 1993).

The genus *Rosa* L. belongs to the family Rosaceae. According to the system of Rehder 1940, it is divided into four subgenera; *Hulthemia*, *Platyrhodon*, *Hesperhodos* and *Eurosa* (Wissemann, 2003*)*. The first three subgenera include only few species. The subgenus *Eurosa*, (or with more modern nomenclature: *Rosa)*, comprises 10 sections. The sections *Caninae* and *Cinnamomeae* are the largest and comprise about 50 and 80 species, respectively. Rose hips, especially from wild species within section *Caninae* and to a lower extent also section *Cinnamomeae*, have a long tradition as raw material for tea, jam and juice but they are seldom consumed fresh. Already in 1551, *R. rubiginosa* appears to have been in culture, followed by *R. canina* in 1737 and *R. dumalis* in 1872 (Gustavsson 1998). The burnet rose or Scotch rose, *R. spinosissima* L. (syn. *R. pimpinellifolia* L.) belongs to section *Pimpinellifoliae* and has been used as an ornamental plant since the 17th century (Gustavsson, 1998).

The section *Caninae* comprises species with upright or arching stems, mostly with straight or curved, hooked prickles. They are found mostly in Europe, but occur also in North Africa and Southwest Asia (Zielinski, 1985). Species in section *Cinnamomeae* are upright shrubs and have stems with mostly straight prickles, paired below the nodes, sometimes bristly (Krüssman, 1981). *Rosa spinosissima* is a low shrub, native to southern Europe, mainly in the coastal areas, but also introduced and cultivated and sometimes naturalized (Nilsson, 1967). This species lacks the particular rose hip aroma, but the high contents of anthocyanins makes it interesting for production of potent antioxidants.

Rose hips contain high amounts (130-6694 mg/100 g) of vitamin C (Joublan *et al.*, 1996; Kovacs *et al*., 2000; Demir & Özcan, 2001;), carotenoids (Razungles *et al.*, 1989; Hodisan *et al.*, 1997; Hornero-Mendez & Minquez-Mosquera, 2000), phenolic compounds (Hvattum, 2002), and folates (Strålsjö *et al.*, 2003). The seeds contain unsaturated and polyunsaturated fatty acids (Szentmihályi *et al.*, 2002). Recently, anti-inflammatory properties (Winther *et al*., 1999; Larsen *et al.*, 2003; Warholm *et al.*, 2003) and antioxidant capacity (Daels-Rakotoarison *et al.*, 2002; Halvorsen *et al*., 2002) has been demonstrated, as well as antimutagenic activities (Karakaya & Kayas 1999) Table 1.

Table 1. *Nutritional and phytochemical compounds in rose hips reported in the last ten years*

Compounds	Year	Authors
Anti-inflammatory activity	1999	Winther *et al.*,
	2003	Larsen *et al.*
	2003	Warholm *et al.*
	2001	Rossnagel & Willich
Antioxidant activity	1999	Paper IX
	2001	Moure *et al.*
	2002	Daels-Rakotoarison *et al.*
	2002	Halvorsen *et al.*
	2002	VanderJagt *et al.*
Anti-mutagenic activities	1999	Karakaya & Kavas
Anti-ulcerogenic activity	2003	Gürbüz *et al.*
Anthocyanins	2001	Lachman *et al.*
	2001	Oszmianski *et al.*
Carotenoids	1997	Hodisan *et al.*
	2000	Hornero-Mendez & Minguez-Mosquera
Folates	2003	Strålsjö *et al.*
Flavonoids	1998	Karakaya & Nehir
Unsaturated, polyunsaturated fatty acid	1999	Zlatanov
	2002	Szentmiháli *et al.*
Phenolic compounds	2002	Hvattum
Secondary metabolites	1995	Hashidoko
Vitamin C	1996	Joublan *et al.*
	2000	Demir & Özcan
	2000	Kovács *et al*.
	2003	Paper VI
	2004	Ercisli & Esitken

Plant breeding programmes for rose hip production have been initiated in some countries, e.g. former Czechoslovakia (Jicinská, 1976; Simanek, 1982; Halasova, 1988; Halasova & Jicinska, 1988;), Germany (Stritzke, 1962), and Russia (Friedrich & Schuricht, 1985). Some cultivars are described in the literature, Table 2. Recently, native roses have been evaluated for quality traits in Chile (Joublan *et al.*, 1996), Hungary (Kovács *et al.*, 2000), the Nordic countries (Paper VI) and Turkey (Demir & Özcan, 2001; Ercisli & Esitken, 2004) (Table 1).

The Swedish plant breeding programme was performed in three steps (Paper I). In the first step, plant material was aquired from different sources. The second step involved intra- and interspecific crosses in section *Caninae* and the third step comprised interspecific crosses between sections *Caninae* and *Cinnamomeae*.

Table. 2. *Cultivars of rose hips from different countries*

Varieties	Country	Authors
Karpatia	former Czechoslovakia	Simanek, 1982
Vitaminj	Russia	Friedrich &
Pozdnedpelyij	"	Schuricht, 1985
Besshipnij	"	"
Vorontsovskij	"	"
Pillnitzer Vitamin-Rosen PiRo 3	Germany	"
Sylwia, Sylwana	Poland	Milewski, 1974

Domestication programmes in Sweden

In the middle of the 1980's, domestication and plant breeding of several new small fruits were initiated at the former division of horticultural plant breeding - Balsgård, the Swedish University of Agricultural Sciences in the south of Sweden. A project was started to domesticate lingonberry (*Vaccinium vitis-idaea* L.) (Gustavsson, 1999) followed by rosehips (Paper I), sea buckthorn (*Hippophae rhamnoides* L.), black chokeberry (*Aronia melanocarpa* (Michx). Ell.) (Jeppsson, 1999) and Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl.) (Rumpunen, 2001). Apart from the plant breeding work, it was necessary to develop suitable agronomic practices, methods for assessing fruit quality, and estimate the need for pest and disease resistance (Paper I). A rose group was initiated at Balsgård, in order to cover several aspects of the domestication process. Inheritance of the genomes in *Rosa* section *Caninae* was studied using microsatellite DNA markers (Nybom *et al.,* 2004). Morphometric and molecular variation in the Nordic dogroses was evaluated (Olsson, 1999) as well as genetic variation and reproductive strategies (Werlemark, 2000b). Variation in *Rosa* with the emphasis on improvement of winter hardiness and resistance to *Marssonina rosae* (blackspot) was determined especially in ornamental roses but also including some analyses of dogrose genotypes (Carlson-Nilsson, 2002).

Cytology

The dogrose taxa (section *Caninae*) are polyploid, 2n=28, 35 (the most common) or 42. In Sweden, only *R. villosa* subsp. *mollis* is tetraploid, whereas the rest of the species are pentaploid. The section *Cinnamomeae* comprises diploid (e.g. *R. cinnamomea*), tetraploid (e.g. *R. pendulina*), hexaploid (e.g. *R. nutkana*) and octoploid (*R. acicularis*) species (Krüssman, 1981; Wissemann, 2003). The chromosome numbers in section *Pimpinellifoliae* are diploid or tetraploid, thus e.g. *R. spinosissima* is tetraploid.

Traits within the section Caninae

The species in section *Caninae* are characterized by their unique meiosis, which results in an unequal number of chromosomes being transmitted to the daughter cells from the seed parents (Täckholm, 1920; Gustavsson, 1944). The dogroses have only 7 chromosomes in the male gametes and 21, 28 or 35 chromosomes (depending on ploidy level) in the female gametes. The dogroses are self-compatible (Jicinska 1976) but their large flowers are designed for pollination by insects and outcrossing is probably common**.** Matroclinal inheritance for molecular markers (Paper IV; Werlemark, 2000b; Werlemark & Nybom, 2001) as well as some morphological and fruit quality traits (Gustavsson, 1944) has been reported in crosses using dogrose species as pistillate parents. Several authors have reported the occurrence of apomixis, i.e. seed production without prior fertilization (Täckholm; 1920, Kroon & Zeilinga, 1974; Wissemann & Hellwig, 1997; Werlemark, 2000a). Previous studies of morphometric and molecular variation have demonstrated large differences within and between taxa in amount and distribution of genetic variability (Nybom *et al*., 1996; Paper III; Olsson, 1999). *Rosa rubiginosa* appears to be the most homogenous taxon both within and between populations. *Rosa dumalis* is reported to be rather heterogenous and has two subspecies, *R. dumalis* subsp. *coriifolia* and subsp. *dumalis* which are sometimes quite difficult to distinguish from one another. *Rosa villosa* shows considerable variation among populations but high within-population homogeneity.

Plant breeding aspects

Seed germination

Seed propagation is used for rootstock production of e.g. *R. canina* and *R. rubiginosa* (Leemans, 1964) since dogrose seedling offspring is fairly homogenous (Kroon & Zeilinga; 1974, Suszka & Bujarska-Borkowska, 1987). For a commercial rose hip plantation in Sweden, a large number of plants (4000 plants/ha) is necessary. Therefore seed propagation could be a suitable propagation method than the labour intense softwood cuttings. The seed is in both exogenous and endogenous dormancy when the hip is ripe (Gudin *et al.,* 1990) and contains growth inhibitors, and the embryo itself needs after-ripening (Gordon & Rowe, 1982). To ensure germination, it is necessary to weaken the pericarp by some means, e.g. acid or by supplying high temperature. How fast the seed germinates is determined by both genetic and environmental factors, e.g. mother plant environment and temperature influences the rate of embryo development (Gudin *et al.,* 1990). Therefore it is important to study how the seeds from different dogrose species respond to some simple temperature treatments.

Other fruit quality aspects

In several plant breeding programmes, fruit size, percentage of fruit flesh, soluble solids content, percentage of dry matter and vitamin C content are important quality traits. Large differences in these traits have been reported in fruits from ornamental roses, species and hybrids (Kaack & Falk Kühn, 1991). Determination of optimal harvesting time is one of the major issues within a domestication

programme. In many species, abscission of fruit can be used as an indicator of maturity since the ease of fruit abscission increases during ripening in e.g. raspberries, blackberries and boysenberries (Given, 1985). In contrast, rose hips do not abscise from the branches when ripe and, therefore, unpicked fruits usually remain on the branches until the following season. However, the ability to harvest rose hips mechanically is necessary for a commercial rose hip production, and more power is needed to separate fruits from branches compared to e.g. black currants (Olsson, pers. comm.). Fruit detachment force (FDF) is therefore a trait that should be screened during ripening.

In most fleshy fruits, ripening is associated with changes in colour, titrateable acidity, fruit firmness, soluble solids, degradation of starch and, in climacteric fruits, ethylene production. Therefore, assessment of optimal harvesting date is important in order to obtain rose hips suitable for the desired processed product. In rose hips, reports dealing with compositional changes during ripening are rare, except for vitamin C content (Ernst & Stritzke, 1958; Rouhani *et al*., 1976). In many fruit crops, colour is an important parameter, both for the appearance and for determination of fruit maturity. Colour measurement with the CIELAB (L*, a*, b*) coordinates and relationships between different quality traits has been reported from e.g. raspberry (Moore, 1997) and peach (Byrne *et al*., 1991).

In *Rosa spinosissima*, contents of anthocyanins (Acy content) in the rose hip is an important trait. Anthocyanins belong to the flavonoids, which is a group of polyphenolic compounds. The flavonoids are reported to have a wide range of biological effects, e.g. anti-bacterial and anti-inflammatory activity (Cook & Samman, 1996).

Fungal diseases

The same fungal diseases affect the wild roses as the ornamental roses. However, some species appear to be less susceptible against different fungal diseases, e.g. *R. rubiginosa* (Carlson-Nilsson, 2002), *R. rugosa* (Svedja & Bolton, 1980), *R. acicularis* Lindley, *R. bella* Rehder & Wilson, *R. marginata* Wallroth, *R. moschata* Herrmann, and *R. nutkana* Presl (De Vries & Dubois, 2001). One of the most severe diseases on fieldgrown roses is blackspot caused by the fungus *Diplocarpon rosae* Wolf (asexual stage *Marssonina rosae* (Lib.) Died.) characterized by black spots on the leaves. Infected leaves produce large quantities of ethylene and the plants defoliate (Horst, 1983). New growth is produced as a result of the defoliation and new infections may take place. This process weakens the plant, reduces growth and may even cause death of the plant (Carlson-Nilsson, 2002).

Powdery mildew (*Sphaerotheca pannosa* (Wallr. ex Fr. (Lév.)) is sometimes claimed to be the most widespread disease in roses (Horst, 1983). The disease reduces the flower production and cause weakening of the plants (Agrios, 1978). This disease also attacks the fruits. Leaf spot (*Sphaceloma rosarum* Pass) was observed at Balsgård for the first time in 1996 (Carlson–Nilsson, 2000) and presently appears to increase in ornamental roses at Balsgård. It causes serious problems with spotting on the leaves, which turn yellow and fall off (Horst, 1983). Fungi belonging to the genus *Phragmidium* Link cause rust. Orange aecidospores attack leaves and other green parts of the plant (Horst, 1983).

Material and methods

Plant material

Paper I

The material was obtained from interspecific crosses in section *Caninae* and between sections *Caninae* and *Cinnamomeae*.

Table 3. *Progeny groups from which seedlings were obtained for seed germination treatments (a= Paper II), measuring of genetic variation (b=Paper III), quantitative traits analysis (c=Paper VI). Dk (Denmark), N (Norway), S (Sweden) and SF (Finland). Numbering of localities refers to a previously published map (Nybom et al. 1996).*

Locality		*R. dumalis* subsp. *coriifolia*	*R. dumalis* subsp. *dumalis*	*R. rubiginosa*	*R. villosa* subsp. *mollis*
1	Tjörnedala, S		bc		ab
2	Tjurkö, S				ab
3	Falkenberg, S	abc	abc		ab
4	Kungsbacka, S		ab		ab
5	Fjärås, S	ab	ab		ab
6	Skepparslöv, S				abc
10	Kurrebo, S	ab	ab		ab
11	Tosteberga, S			abc	
12	Måryd, S			bc	
13	Kjugekull, S			abc	
14	Lemmeströ, S			ac	
20	Hornbæk, Dk		c	c	
23	Fynshoved, Dk				c
25	Rosenvold, Dk		c		
28	Næsby, Dk	c			
29	Fjellerup, Dk	c		c	
31	Klintehamn, S	c			
34	Halls fiskeläge, S			c	
35	Lummelunda, S			c	
38	Ytterrör, S			c	
39	Borgholm, S			c	
40	Egby, S				c
42	Gårdby, S	c			
50	Ullensvang, N		c		
52	Kløvi, N			c	
54	Njøsdalen, N				c
70	Piikkis, SF		c		

Paper II

Dogrose seeds were collected in the south of Sweden in 1987 from a total of 73 plants belonging to *R. dumalis* subsp. *coriifolia*, *R. dumalis* subsp. *dumalis*, *R. rubiginosa*, *R. sherardii* var. *venusta* and *R. villosa* subsp. *mollis*. A putative hybrid between *R. canina* and *R. dumalis* was also included (Table 3).

Paper III

Seeds were collected in the south of Sweden in 1988 (Paper I). Offspring plants (a maximum of 40 seedlings from each original seed source plant) were planted in a randomised design in a field trial with a total of more than 1000 offspring plants from *R. dumalis* subsp. *coriifolia*, *R. dumalis* subsp. *dumalis*, *R. rubiginosa*, *R. sherardii* var. *venusta* and *R. villosa* subsp. *mollis* (Table 3).

Paper IV

A pair of reciprocal crosses was performed between *R. dumalis* and *R. rubiginosa*. The parental material was selected from different locations in the south of Sweden. We analysed 37 plants of *R. dumalis* X *R. rubiginosa* and 45 plants of *R. rubiginosa* X *R. dumalis* for RAPD markers. Morphological measurements were conducted on 30 and 40 plants, respectively.

Paper V

649 seedlings were obtained from crosses between sections *Caninae* and *Cinnamomeae*. The pistillate parents were selections developed at Balsgård. The staminate parents were selections obtained from open pollination of the Russian variety ‘Uralskij Champion‘.

Paper VI

The study included *R. dumalis* subsp. *coriifolia*, *R.. dumalis* subsp. *dumalis*, *R rubiginosa* and *R. villosa* subsp. *mollis*, collected from Danmark, Norway, Sweden and Finland (Table 3). 117 offspring plants from 39 mother plants were used in this study.

Paper VII

Fruits were harvested from a local orchard. Two selections of *R. rubiginosa* and *R. dumalis* from the Balsgård breeding program were used.

Paper VIII

Eight seedlings of *R. spinosissima* from a local nursery in the north of Sweden were analysed.

Paper IX

Eight samples of *R. canina* and *R. moschata* were obtained from Fundacion Santiago, Chile and ten samples of *R. dumalis* subsp. *dumalis*, *R. dumalis* subsp.

coriifolia, *R. rubiginosa* and *R. villosa* subsp. *mollis* from an experimental field at Balsgård.

Seed germination

Fruits were harvested from several individual plants per species, with each species being sampled at 3-5 localities (Paper II). The achenes were extracted from the hips using a mechanical juice mixer. In December 1987, 400 achenes from each harvested plant were sown in two different pots. The pots were then covered with a thin layer of fine sand. Altogether, a total of ca. 50 000 achenes were sown. The pots were divided into two groups, which were subjected to different treatments. Pots in Treatment 1 were kept moist a 20°C for 12 weeks and after that moved to 5°C and kept moist for another 12 weeks. Pots in Treatment 2 were instead exposed to 5°C for the entire 24 weeks. After the 24 weeks, all pots were taken outdoors for germination. The emerged seedlings were removed and counted in June 1988. The pots with the remaining non-germinated seeds from both treatments were kept outdoors until October, when they were taken indoors and kept at 5°C under moist conditions. At the end of March 1989 the pots were taken outdoors again. This time numerous seedlings emerged in the spring. These were now registered as resulting from Treatment 3 (former Treatment 1) and Treatment 4 (former Treatment 2), respectively.

Measurements of genetic variation

Morphology

For the study in paper III, the fifth fully developed leaf (counting from the shoot apex) was collected in August-September from a shoot of the current year's growth. These leaves were Xeroxed and measured, 1) length (mm), 2) width (mm) and 3) distance from leaflet base to broadest part (mm). On the apical leaflet, 4) number of leaf teeth were counted along one cm of margin (=leaf serration). Two ratios were also employed in the subsequent analyses, leaflet shape (1/2) and leaflet base (3/1). One apical flower was collected from each plant when in full bloom, i.e. when at least 50% of the flower buds had opened. In the field, the following characters were scored, 5) pedicel length (mm), 6) ovary length (mm) and 7) width (mm), as well as 8) number of glandular hairs along one side of the ovary and pedicel. Ovary shape (7/6) was also included as a character. The petals were removed and measurements were made on the Xerox copies, 9) length (mm) and 10) width (mm) of one sepal (avoiding the largest as well as the smallest sepal, and 11) mean number of sepal lobules on the lobulated sepals (usually 3 out of the 5 sepals are lobulated).

In Paper IV, three flowers were collected from each plant. In the field we measured; length and width of the ovary. The petals were removed and, the remainder brought into the laboratory and photocopied. In August the largest leaf was collected from three shoots on each plant. The leaf was pressed and the quotient length/width was measured on a subapical leaflet.

Molecular markers

One of the most widely used techniques for DNA studies is the PCR (polymerase chain reaction) procedure and a simple way of employing the PCR technique without prior knowledge of DNA sequences, is the RAPD (random amplified polymorphic DNA) (Williams et al., 1990). The aim in Paper IV, was to study the inheritance/transmittance of molecular markers from the parental plants to the progeny plants in a reciprocal cross between *R. dumalis* and *R. rubiginosa*. A total of 38 plants of *R. dumalis* X *R. rubiginosa* and 40 plants of *R. rubiginosa* X *R. dumalis* were analysed together with the parental plants. DNA was extracted according to Holm (1995). One hundred primers were tested for amplification of bands that could differentiate between the two parental plants. DNA from the parental plants and a molecular-weight markers were present on each gel and photographed under UV light.

Measurements of fruit quality

Antioxidants

For Paper VIII, anthocyanins were determined with the pH differential method (Fuleki & Francis, 1968; Wrolstad, 1976) and expressed as cyanidin-3-glucoside using the molar absorbance of e=26 900 and molecular weight as 445.2. Absorbance was recorded at 515 nm with a Beckmann spectrophotometer DB.

Vitamin C was measured by iodide-titration (Paper VIII), HPLC (high performance liquid chromatography) (Paper VI) and using ferric-TPTZ reagent (Paper IX).

Total antioxidant power was measured with two different test systems, 1) FRAP (the ferric-reducing antioxidant power) (Benzie & Strain, 1999) and 2) TEAC (Trolex-equivalent antioxidant capacity) (Paper IX). Folin-Coicalteu reagent was used to determine the total phenolics, and total carotenoids were measured using $CHCl_3$ extract and hexane and detected at 460 nm.

Other fruit quality parameters

Fruit weight was determined in papers I, VI, VII and VIII. Mean fruit weight was calculated as the mean of 10 fruits in all papers, except in paper VIII, where 50 fruits were used. Percentage of fruit flesh (w/w) was determined after separating the seeds from the flesh by hand. Dry matter was determined in fruit flesh, weighed and dried at 160°C to a constant weight (<0.1% g in 20 s) measured by an automatic dryer (Paper VIII). Also, dry matter was determined by weighing the fruit flesh before and after drying at 110°C for 24 hours (Papers I, VI and VIII).

Content of soluble solids was measured in one drop of homogenized juice (fruit flesh and water) with a refractometer and expressed as °Brix taking dilution into account, (Papers VII and VIII).

Sugar content was determined by HPLC. Fruit flesh was extracted in absolute ethanol and kept frozen (-20°C) until analysis.

Total acidity was measured in fruit flesh homogenized in distilled water and titrated with 0.1 M NaOH to an end-point of pH 8.1. The result was expressed as malic acid, g/100 g.

External colour was determined with a Minolta Chroma Meter CE-200. The CIELAB coordinates (L*, a*, b*) were measured to calculate hue angle (H°)=tan-1(b*/a*) and chroma ($(a^{*2}+b^{*2})0.5$) where +a* represents increasing redness, –a* increasing greenness, +b* increasing yellowness and –b* increasing blueness (Smedley, 1995). Hue describes the visible colour and chroma describes the brightness or intensity of the hue (Perkins-Veazie, 1992).

Fungal diseases

In two consecutive years, disease symptoms of blackspot, powdery mildew, leaf spot and rust were assessed by visual inspection of plants in a field trial (Paper V). All four diseases were screened simultaneously using the same scale. The plants were divided into two levels (lower and upper). Each level was rated on a four-point scale: F=free of disease; L=light infection [20% of the plant area (leaves, stems, thorns) infected]; M=moderate infection (21-50% of the plant area infected); S=severe infection (51-100% of the plant area infected). The ratings for the levels were combined and transformed to numeric values (0-9) according to a formula previously applied for evaluation of blackspot (Carlson-Nilsson & Davidson, manuscript submitted).

Statistical analysis

The statistical analyses in this thesis were performed with SPSS (Norusis, 1990), SuperANOVA (Abacus Concepts, 1989) and SYSTAT (SYSTAT Statistics, 1992) statistical program packages. Variation between species and seed germination treatments were analysed by one-way analyses of variance (Anova) in Paper II. Differences between species were compared with the Scheffé a posteriori test, which is exact also for groups with a variable number of cases.

Nested analyses of variance were used in Papers III and VI. Also, nested analyses of variance between the three species as compared to the variation among populations within species were estimated in Paper III. Differences between individual offspring families for the eleven characters were carried out with univariate analyses of variance. Pairwise comparisons were carried out with Scheffé and LSD (least significant difference). Canonical variates analyses (CVA=discriminant analyses of variance) to estimate intraspecific variation were used in papers IV and VII. CVA aims to obtain maximum separation among predeterminated groups. The distinctiveness of the populations was estimated with reclassification tests. The percentage of correctly classified plants can be regarded as an indication of the ability of the chosen characters to separate the investigated populations.

In paper VI, mean values for the disease scores were calculated separately for each offspring family. One-way analyses of variance with a non-parametric Kruskal-Wallis test were conducted on offspring families derived from the same pistillate parent and on three families that had the same genotype as staminate parent. Spearman rank correlations calculated on all eleven crosses separately were used to find possible interferences between the four different diseases.

In Paper VIII, the aim was to study variation between fruit traits and harvest dates. For each species, linear and quadratic regression analyses were used to

investigate relationships between (1) hue value and (2) total sugar (fructose and glucose) and °Brix, and between (1) chroma and (2) total sugar and soluble solids, and between (1) total sugar and (2) soluble solids. We used one-way analysis of variance, based on three replicates each harvest week. The Scheffé test was used for pairwise comparisons. Differences between sampling dates were analysed with ANOVA for each trait using seedlings as error term, in paper VIII. Linear and quadratic regression analyses were used for all seedlings taken together.

Mean and standard deviation for antioxidant activity and the content of phytonutrients were calculated in Paper IX.

Results and discussion

Seed germination

Ornamental roses are mainly propagated by cuttings or grafting whereas rootstocks are propagated by seeds (Leemans, 1964). For rose hip production, dogrose plants are mainly propagated by cuttings. Due to the *Canina*-meiosis, the offspring is usually homogenous and therefore seed propagation could be an alternative to propagation by cuttings. Endogenous and exogenous dormancy is found in the seeds (Gudin *et al*., 1990) and the seeds can be treated with different methods e.g. by immersion in sulphuric acid prior to stratification (Roberts, 1979). A more simple temperature treatment method (warm and cold period) has been reported by Suszka & Bujarska-Borkowska (1987) to produce good results when germinating *R. canina* rootstock selections.

Our results in Paper II with different temperature treatments showed that the warm and cold treatment (12 weeks at 20°C followed by 12 weeks at 5°C) produced a higher seed germination compare to only a cold period (24 weeks at 5°C). For all species except *R. rubiginosa*, most of the germination occurred during the second year. The largest increase in germination between the first and second year was found in *R. dumalis* subsp. *dumalis*, 7.1% and 23.2% respectively, and in *R. dumalis* subsp. *coriifolia*, 14.7% and 32.8%, respectively. By contrast, in *R. rubiginosa* germination decreased from 18.8% to 6.4%.

In Paper I, germination ranged between 4 and 65% for seeds obtained from intraspecific and interspecific crosses in section *Caninae*. *Rosa rubiginosa* as seed parent showed high seed germination (47-65%) compared to e.g. *R. dumalis* (11-39%). However, in Paper I, results are reported only from the first year and there is a possibility that the germination would have been higher the second year. Intersectional crosses were conducted between *Caninae* and *Cinnamomeae*, with *R. rubiginosa* as seed parent. The germination ranged from 10% to 71% (with *R. moyesii* and *R. rugosa* as pollen parent, respectively). The crosses in Paper I were performed in a greenhouse with controlled climate, which could explain the higher percentages of seed germination compared to in Paper II, where the seeds were collected in nature. Seed germination is affected by both genetic and environmental factors. The temperature influences the rate of embryo development and the thickness of the endocarp (Gudin *et al*., 1990). A high temperature and much light during the preceding harvest in hybrid tea rose, resulted in

considerably higher germination next year compared to years when harvest was conducted in low temperature and less light (Von Abrams & Hand, 1956).

Genetic variation

Within and between species

Genetic variation was measured using both vegetative and reproductive characters in Paper III. To minimize the effects of phenotypic plasticity, we only compared plants raised from seed and grown in a randomised design. The characters selected had proven useful in previous taxonomic treatises and were relatively easy to score.

First we investigated the general pattern for the distribution of genetic variability at different levels like offspring family, population and species. Means were calculated for each offspring family separately on a number of morphological characters. These means were plotted in pairs to illustrate the existing variation at different levels. Pedicel length and sepal lobation showed that *Rosa dumalis*, *R. rubiginosa* and *R. villosa* were very well differentiated. However, the two subspecies of *R. dumalis* overlapped completely. All offspring plants belonging to *R. rubiginosa* formed a close-knit group, with no differentiation among populations (Figure 1).

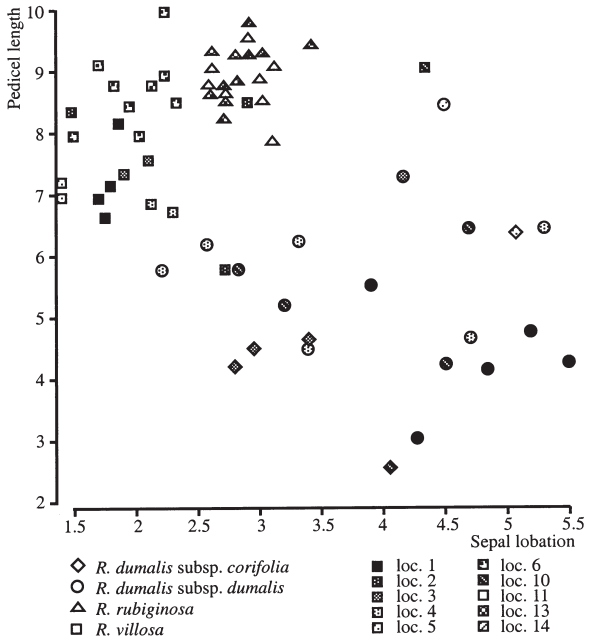


Figure 1. Character means plotted for pedicel length and sepal lobation, calculated for each offspring family separately (Fig. 2, Paper III).

In Paper VI, fruit weight, percentage of fruit flesh and percentage of dry matter was screened in four taxa. In that study we found that *R. rubiginosa* was well separated from the other taxa, whereas *R. villosa* and *R. dumalis* clustered together (Figure 2). In a RAPD-based study of variation in seven taxa in section *Caninae*, three groups were found, the *canina/dumalis* group, the *rubiginosa* group and the *sherardii/villosa* group (Olsson *et al.,* 2000). These results indicate that the three major subsections *Caninae* (*R. canina*, *R. dumalis* etc.), *Rubigineae* (e.g. *R. rubiginosa*) and *Vestitae* (e.g. *R. villosa*) are well separated, whereas differentiation within the subsections is more obscure.

To estimate the level of homogeneity within the various offspring families, coefficients of variation (CV) were calculated for seven characters. The mean CV for each population and character as plotted in a graph and showed that ovary length and ovary width had very low coefficients of variation, whereas pedicel length exhibited much higher and also more variable values. The CV of *R. rubiginosa* were overall quite low, indicating that offspring families were comparatively homogenous. The values for *R. villosa* and *R. dumalis* subsp. *coriifolia* were higher and not quite homogenous as in *R. rubiginosa*.

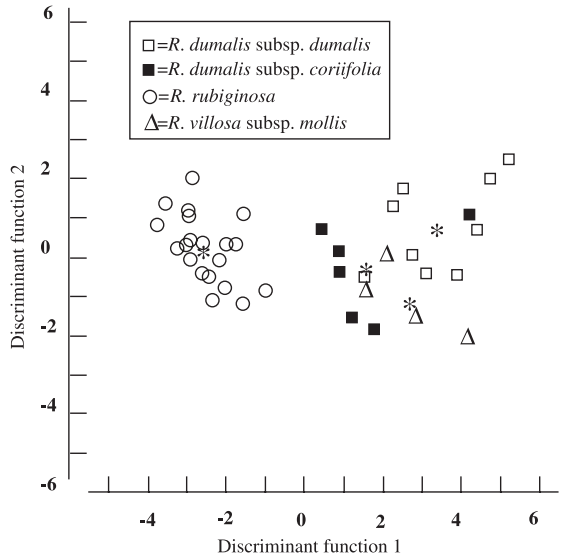


Figure 2. Canonical variates analysis (CVA) performed for percentage of dry matter, fruit weight and percentage of fruit flesh to separate four taxa. The stars denote taxon centroids (Fig. 1, Paper VI).

In Paper VI, analysis of intraspecific variation (three offspring plants from the same mother plant) showed that *R. dumalis* subsp. *coriifolia* was the most variable taxon, followed by *R. dumalis* subsp *dumalis* and *R. villosa*, whereas *R. rubiginosa* showed the most restricted variability. *R.osa dumalis* subsp. *dumalis* showed significant differences between offspring groups (in two years) in all three

traits, fruit weight, percentage of fruit flesh and dry matter. *R.osa dumalis* subsp. *coriifolia* differed in vitamin C and in 1994 for fruit weight and percentage of fruit flesh. *R. villosa* showed barley significant differences in fruit weight 1994 and 1995. Since the number of offspring groups available for harvesting was comparatively low, there is a possibility that we would have found more variation in this taxon with a larger sample of plant material. Although a large number of plants (60) were analysed in *R. rubiginosa*, still no intraspecific variation could be found. Our results are in accordance with previous studies of morphological characteristics in this taxon, where *R. rubiginosa* is very homogenous compared to all other investigated species in section *Caninae*, e.g. *R. dumalis* and *R. villosa* subsp. *mollis* (Paper III; Olsson, 1999). In *R.villosa* subsp. *mollis*, fruit weight and percentage of fruit flesh varied significantly among offspring groups, in one and two years respectively. Nybom *et al*. (1997) reported that *R. villosa* subsp. *mollis* showed considerable heterogeneity among populations but homogeneity within populations. *Rosa dumalis* is very heterogenous and the two subspecies are difficult to separate from one another (Paper III). Olsson *et al.* (2000) reported that neither leaflet-shape analysis nor molecular data could distinguish the two subspecies.

This means that for plant breeding purposes, only a few seedlings of *R. rubiginosa* are sufficient to obtain most of the genetic variation. However, in *R. dumalis and R. villosa*, several seedlings from different populations are necessary in order to obtain representative samples of the large variability.

Experimental crosses

Two offspring plant groups obtained from a reciprocal cross between *R. dumalis* subsp. *dumalis* and *R. rubiginosa* were measured both with morphological characters and with RAPD markers, in Paper IV. Offspring plants could be separated with four of seven morphological characters (sepal length, sepal serration, peak flowering and leaflet lenght/width quotient). Coefficient of variation for each character showed that the progeny group *R. dumalis* X *R. rubiginosa* was more variable compared to plants in the *R. rubiginosa* X *R. dumalis* progeny group.

With the RAPD method, one hundred primers were tested for amplification of bands that could separate the two parents. Eleven of these did not amplify any DNA fragments at all. Of the 89 remaining primers, 9 primers produced a total of 21 polymorphic bands, 11 specific for *R. dumalis* and 10 for *R. rubiginosa*. Matroclinal inheritance was indicated since all but one of the seed parent-specific markers were transmitted to all offspring plants (Figure 3). In contrast, only half of the staminate parent-specific markers were transmitted to the offspring plants, and none of them reached all the offspring plants. Jicinska (1976) reported matroclinal inheritance (leaf characters) in interspecific progeny plants from sect. *Caninae* as seed parent and *R. rugosa* as pollen parent, but she also reported that some characters were patroclinally inherited (prickles).

In Paper IV, 9 of the offspring plants did not inherite any of the pollen donor markers. These plants were assumed to be of apomictic origin. Werlemark (2000) reported that these offspring plants were studied also for male and female fertility in the form of pollen viability and fruit characters. The pure species had a pollen

viability of 20-30%, whereas most of the interspecific hybrid offspring plants had a pollen viability of <10%. These 9 plants with no pollen parent-specific RAPD markers, had the same pollen viability as the pure species. The possible occurrence of apomixis in dogroses has been mentioned in several reports by e.g, Gustavsson (1937), Kroon & Zeilinga (1974) and Werlemark (2000a). However, the reduced recombination within the sect. *Caninae* can make selfpollinated offspring plants difficult to separate from the apomictically derived offspring plants. Using RAPD, Werlemark & Nybom (2001) found apomictically derived offspring plants also in the cross combinations *R. rubiginosa* X *R. sherardii* and *R. villosa* X *R. sherardii*. Microsatellite DNA analyses have subsequently demonstrated that these plants are, in all likelihood of apomictic origin (Nybom *et al*., 2004).

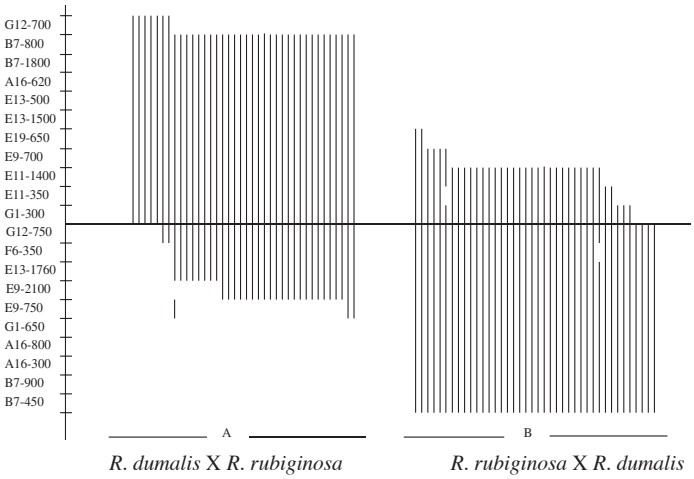


Figure 3. Distribution of RAPD bands in the progenies. The bands above the horizontal line are specific for *R. dumalis* and the bands below the line are specific for *R. rubiginosa*. Each vertical line represents a progeny plant (Fig. 3, Paper IV).

Fungal diseases

In a field trial with plants derived from crosses between sections *Caninae* (pistillate parent) and *Cinnamomeae* (staminate parent), we found that blackspot was the most serious disease, followed by leaf spot, whereas powdery mildew and rust were rare (Paper V). Only 3% of all assessed seedlings were free from blackspot, whereas about 25% were free from leaf spot. No less than 78% and 97% of the seedlings with *R. dumalis* and *R. rubiginosa* as pistillate parents, respectively, were free from powdery mildew, and a high percentage (97%) of all seedlings were free from rust. These results are in agreement with results from a

trial where 63 rose varieties were evaluated for infection against blackspot, powdery mildew and rust (De Vries & Dubois, 2001).

Blackspot showed the highest mean scores (3.58 to 6.52 in 1997 and 3.76 to 6.04 in 1998) of the four assessed diseases. Analyses of skewness and kurtosis indicated a normal distribution for blackspot disease scores when each family was studied separately. This may suggest polygenic resistance. In a study of crosses in tetraploid rose cultivars, the obtained segregation ratio instead provided evidence for the presence of a single dominant resistance gene against blackspot (von Malek & Debener, 1998). Xue and Davidsson (1998) studied 5 partial resistance components in 11 different rose cultivars. One of them was completely resistant to all isolates, whereas the other cultivars showed a partial resistance, probably controlled by multiple genes.

For the other three diseases, distribution of disease scores was heavily skewed towards low infection scores. For e.g. powdery mildew, scores were overall very low (0.02 to 1.09 in 1997 and 0.00 to 2.08 in 1998) in all families except in one of the *R. dumalis* crosses, where mean values were above 1.0. Carlson-Nilsson (2002) also found a low level of powdery mildew in different accessions of roses.

In our study, leaf spot infection was found in 75% of the plants. Mean scores varied between 0.92 and 2.46 in 1997, and between 0.98 and 2.96 in 1998. Two of the families, which have *R. rubiginosa* as pistillate parent and the same father (Uralski Champion o.p. 30258) contained a large number of disease resistant plants compared to other families. It is possible that the comparatively low susceptibility to leaf spot in these two families is caused by a major gene.

Family means for leaf spot varied more between families with *R. rubiginosa* as pistillate parent than between families with *R. dumalis* as pistillate parent. This suggests that the influence of the paternal genotype, Uralskij Champion o.p. is more evident in the *R. rubiginosa* seedlings than in the *R. dumalis* seedlings.

For powdery mildew we found more variation between family means when *R. dumalis* had been used as pistillate parent compared to when *R. rubiginosa* had been used. Overall, the resistance against powdery mildew was rather strong when we used *R. rubiginosa* as pistillate parent. In contrast, we found a higher number of susceptible seedlings in some crosses when *R. dumalis* was used as pistillate parent, suggesting that resistance is more variable in this species.

We found no differences in blackspot ratings among families in either of the two years. The scores can vary quite substantially from year to year and are affected by factors like weather conditions, physiological status of the plant as well as by the amount of the inoculum (Carlson-Nilsson, 2000).

Fruit quality

Ripening

Colour measurements

In this thesis, two ripening studies (Papers VII and VIII) are included. In Paper VII the two dogrose species *R. dumalis* and *R. rubiginosa* showed a similar colour development measured with a chroma meter, with significant changes in external colour between harvest dates. The rose hips darkened during ripening as evidenced by the decreasing L* values. The a* values showed a dramatic increase from a

greenish red to more intensely red colour until the fourth (September 26) harvest date, after which no significant changes were found. The b* values showed a slight decrease during ripening, indicating that rose hips became less yellow. Hue values decreased strongly in both species during the first three weeks but later on levelled out almost completely. Chroma increased during beginning of the ripening period, indicating a more pronounced colour saturation, but no significant increase was found in the two species after September 19 or 26, respectively. At the end of September and therafter, night temperatures in the South of Sweden often fall below zero which may have arrested the ripening process. In tomato, biosynthesis of the carotenoid lycopene was inhibited at temperatures below a certain limit (Dumas *et al*., 2003), and a similar inhibition of carotenoid synthesis of the yellow to red coloured pigments may have occurred in the rose hips and thereby affecting the colour values.

Anthocyanin content in *R. spinosissima* was measured with a spectrophotometer during ripening in Paper VIII. Contents varied considerably between seedlings (Figure 4). For most of them, a pronounced increase was noted until September 8. After this date, the values levelled out or even started to decrease again. This can be compared with i.e. sour cherry, where Acy content and soluble solids increased to a maximum content in the middle of the harvest period (Poll *et al*., 2003). Jeppsson & Johansson (2000) found that in black chokeberry all cultivars (except one), increased significantly in Acy content during maturation.

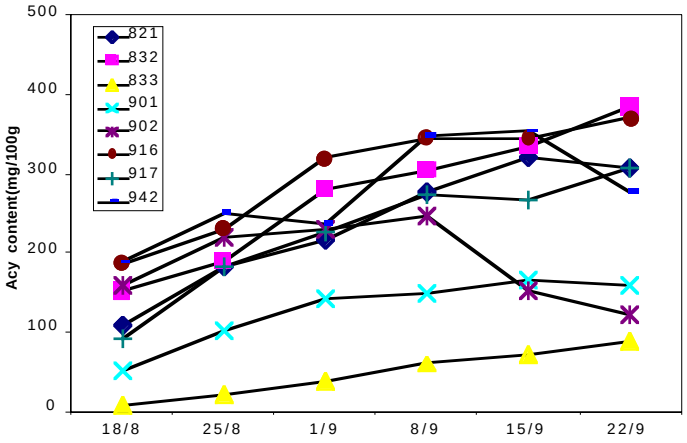


Fig. 4. Anthocyanin content (mg/100 g) in eight seedlings of *R. spinosissima* during ripening (Fig. 1, Paper VIII).

Fruit weight and percentage of fruit flesh and dry matter

In Paper VII, mean fruit weight and percentage of fruit flesh showed some significant variation between harvest dates in *R. dumalis* and *R. rubiginosa* but without any obvious trends that could be connected with ripening time. These findings are in accordance with a study on black chokeberry; in three out of six cultivars, fruit weight increased during the first two weeks and thereafter fluctuated up and down between sampling dates (Jeppsson & Johansson, 2000). Probably, the fruits had already terminated their growth at the onset of the harvesting.

However, both rose hips and black chokeberry were grown on light soils without irrigation, and the measured fluctuations could be due to differences in water uptake during ripening. That is in contrast to the ripening study of *R. spinosissima* (Paper VIII) where linear regression (R^2=0.14 and p=0.01) showed a relationship between fruit weight and harvest dates; mean weight calculated across all seedlings increased from 2.9 g the first week to 3.6 g the last week. Using linear and quadratic regression, we found a relationship also between percentage of fruit flesh and harvest dates.

Percentage of dry matter increased during ripening in all three studied species. In 1996, percentage of dry matter increased significantly during the whole period in *R. dumalis*, while in *R. dumalis* in 1997 and for *R. rubiginosa* in both years, percentage of dry matter increased significantly only until September 19 and thereafter decreased slightly (values not shown). In *R. spinosissima*, percentage of dry matter increased with time calculated across all seedlings from 18.8 % in August 18 to 23.4% in September 22. We also found significant differences in percentage of dry matter between seedlings.

Vitamin C and total acidity

In Paper VIII, I found a linear relationship between vitamin C and harvest dates (Figure 5). In fruits of *R. rugosa*, harvested at three ripening stages (unripe, half ripe and ripe), vitamin C increased with time (Ernst & Stritzke, 1958). Grading the rose hips with colorimetric data indicated that maximum content of ascorbic acid did not coincide with maximum color-agent concentration (Bakos *et al.*, 1981).

Both linear and quadratic regressions showed a relationship between harvest dates and total acidity. Mean of all seedlings together was 0.6 g/100 g the first harvest date, followed by 0.8 g/100 g between September 1 to 15. After this date, total acidity decreased again to 0.7 g / 100 g.

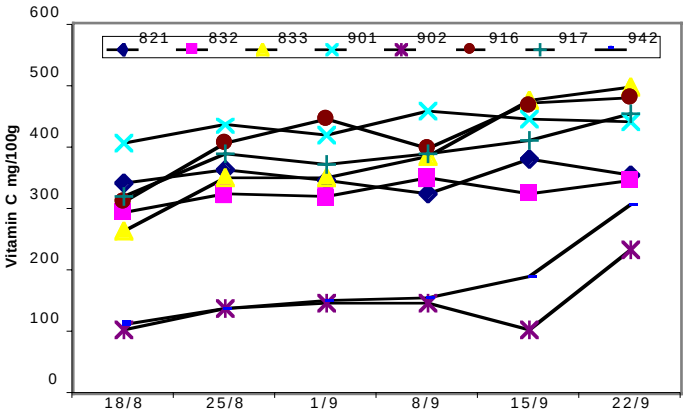


Fig. 5. Vitamin C content (mg/100 g) in eight seedlings of *R. spinosissima* during ripening. (Fig. 2, Paper VIII).

Fruit detachment force

Linear and quadratic regressions showed no significant changes in FDF during the harvest dates calculated across all eight seedlings (Paper VIII). According to Vakarelski (1978), who estimated removal force in different parts of rose plants, fruits from *R. rugosa* and *R. canina* needed a removal force of 10-20N (9.81 N=1.0 kg) to separate from the plant. In our study, removal force needed was only 0.60 kg to 0.83 kg. However, two of our seedlings, 902 and 942, showed a comparatively high mean in FDF, 0.95 kg and 1.00 kg respectively.

Changes in soluble solids

Content of soluble solids in the fruit flesh measured as °Brix values, increased significantly with time in all three species. In 1996, the °Brix value increased from 15.2% to 24.4% in *R. dumalis* and from 13.1% to 21.5% in *R. rubiginosa* during harvest period. In 1997 it ranged from 16.0% to 23.1% and from 17.3% to 25.7% (Paper VII). In *R.spinosissima,* soluble solids increased during the harvest period from 10.2% to 16.3% (Paper VIII).

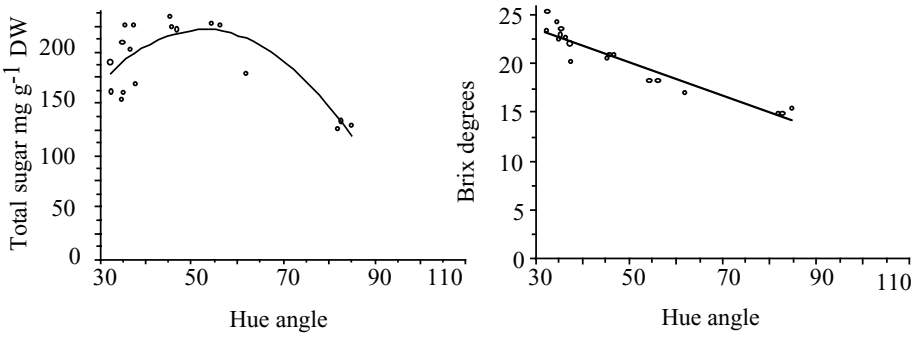
In paper VII, content of soluble solids was also measured by HPLC. In *R. dumalis*, the total sugar content was 131 mg/g DW at the first harvest date in 1996, compared to only 29 mg/g DW in *R. rubiginosa*. The total sugar content was much more similar in the two species during the last three weeks, ranging from 170 to 201 mg/g for *R. dumalis* and from 124 to 144 mg/g for *R. rubiginosa*. Results obtained in 1997, showed the same overall pattern with stable levels in sugars at the last harvest dates. Glucose was the major soluble sugar in both species followed by fructose, while no sucrose could be detected. The glucose and fructose contents varied significantly during the harvest period. *Rosa dumalis* reached a maximum of total sugar at September 19, with glucose accounting for 63-72% of the total sugar content. In *R. rubiginosa*, total sugar content increased until October 3, at which time 60-62% was glucose. Significant changes in glucose content could not be detected in either of the two species between the last three harvest dates.

Concordance among traits

The relationships between on the one hand fruit colour expressed as hue angle and, on the other hand, total sugar and °Brix values, were estimated in Paper VII, using linear and quadratic regression analyses with hue angle as the independent variable. When hue angle decreased, total sugar content increased. The linear plots (Figure 5), showed more variation in sugar content when hue values decreased below 45, indicating that sugar contents became more variable towards the end of the ripening period, especially in *R. dumalis*. This is probably due to the inhibition of carotenoid synthesis whereas the transport of sugars from leaves to fruits still continues. In both species, °Brix values increased when hue angle decreased (Figure 6).

In Paper VIII, a linear relationship was demonstrated between FDF and fruit weight. Obviously smaller fruits need lower FDF to separate from the branches. A relationship between FDF and fruit weight has been found also in other fruits, e.g. in orange (Kender & Hartmond, 1999).

R. dumalis 1996



R. rubiginosa 1996

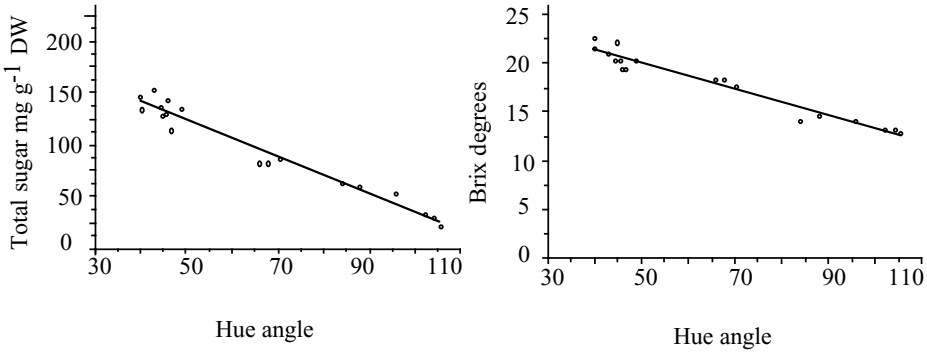


Figure.6. Quadratic regression between hue angle and total sugar (mg g-1 DW) and linear regression between hue angle and soluble solids (°Brix) measured in fruits of *R. dumalis*. Linear regression between hue angle and total sugar (mg g-1 DW), and between the hue angle and soluble solids (°Brix) measured in fruits of *R. rubiginosa*. Decreasing hue angle indicates increasing redness (Fig. 2, Paper VII).

Variation in fruit traits

In Paper VI, important fruit traits like fruit weight, percentage of fruit flesh, and percentage of dry matter were screened in *R. dumalis* subsp. *coriifolia*, *R. dumalis* subsp. *dumalis*, *R. rubiginosa* and *R. villosa* subsp. *mollis* during three years. In 1993, vitamin C content was also investigated. Pairwise comparisons among taxa based on progeny groups showed that *R. dumalis* subsp. *dumalis* had significantly larger fruits than the other taxa with a mean weight of 2.6 g. Fruit size is of minor importance if mechanical harvesting is used, but for home gardens and in smallholdings e.g. pick-your-own enterprises, a larger fruit size would be desirable. *Rosa rubiginosa* had significantly lower percentage of fruit flesh in all three years. However, this species has some other desirable traits such as good flavour, high productivity and high levels of field resistance to powdery mildew (Paper I). No significant differences could be observed between the four taxa in percentage of dry matter or vitamin C.

Although we found significant variation among taxa for the traits in Paper VI, the differences were rather small. Larger variations in the studied traits have been reported in other studies of various *Rosa* species, e.g. fruit size (Kaack & Falk Kühn, 1991). However, we studied only four closely related taxa, whereas Kaack & Falk Kühn (1991) included not only species from different sections, but also hybrids and ornamental roses. Also in vitamin C content larger variation between species has been reported for e.g. British and Chilean species (Melville & Pyke, 1947; Joublan *et al.,* 1996) but the samples were collected from wild growing plants over a large geographical area.

We found a positive correlation (0.70, $p<0.001$) between fruit weight and percentage of fruit flesh. Simultaneous selection for these traits is important in order to avoid time-consuming work with the separation of fruit flesh and seeds by hand. Negative correlations (-0.59 and –0.58, ($p<0.001$) respectively) were found between percentage of dry matter on one hand, and fruit weight and percentage of fruit flesh on the other hand. This means that larger fruits with a higher percentage of fruit flesh contain a lower percentage of dry matter. However, it is favourable to have large fruits and high percentage of fruit flesh since these traits affect the yield more than does the percentage of dry matter. We found no correlations between vitamin C content and the other three traits. However, we only measured vitamin C one cold and rainy year (1993) and there is a possibility that we could have had a different result in the following years when the weather was warmer and precipitation lower.

Antioxidants

In Paper IX, extracts of 18 samples of rosehips, representing six taxa, were evaluated for antioxidant capacity. Seven samples were obtained from Chile and ten samples were picked in our collections at Balsgård. Two different test systems were used, the FRAP (ferric-reducing antioxidant power) and the TEAC (Trolox-equivalent antioxidant capacity) assay. The FRAP assay measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of antioxidants. The TEAC assay is based on the ability of the antioxidant to react with or neutralize free radicals generated in the assay systems.

The FRAP assay measures antioxidants as reductants directly, whereas e.g. TEAC is more indirect and measures the inhibition of reactive species (free radicals) generated in the mixture (Halvorsen *et al*., 2002). The high FRAP value of the crude extracts ranged from 983.4 $\mu molg^{-1}$ (1.0 mmol/g) in *R. moschata* to 2187.1 $\mu molg^{-1}$ (2.2 mmol/g) in a *R. villosa* hybrid, equivalent to an antioxidant capacity of 86.5-192.5 mg ascorbate g^{-1} dried rosehip. The TEAC values varied from 457.2 $\mu molg^{-1}$ (0.5 mmol/g) in *R. canina* to 626.2 $\mu molg^{-1}$ (0.6 mmol/g) in *R. villosa*, equivalent to 114.3-156.5 mg Trolox g^{-1} dried rosehip. Halvorsen *et al*. (2002) screened total antioxidants in dietary plants. In the FRAP assay of the berry group (various small fruits), dogrose was exceptionally high and contained in average 39.46 mmol /100 g (0.4 mmol/g) compared to e.g. crowberry and blueberry (9.17 and 8.23 mmol /100 g, respectively). In another study, Oszmianski *et al*. (2001), reported antioxidant capacity in different berries. The highest antioxidant capacity was found in fruits from *R. spinosissima* (151.35 mmol Trolox eq./ml juice) compared to e.g. chokeberry (24.44 mmol Trolox eq./ml juice and black currant juice (21.82 mmol Trolox eq./ml juice).

In Paper IX, the FRAP and TEAC assays were used to evaluate three fractions of rose hip extracts, a phenolic, an ascorbic and a lipophilic extract. In both assays the sums of antioxidant capacity in the three fractions were slightly lower than in the crude extract. Hovewer, the phenolic extract made the major contribution to the antioxidant activity in both assays (overall mean was 90.5% and 75.7%, respectively). The ascorbic acid made a minor contribution to the total activity (8.6% and 16.9%) and the lipophilic component made an even smaller one (0.9% and 8.6%). However, the lipophilic antioxidant was the most efficient when comparison of activity was based on the relation between the total antioxidant capacity and the content of antioxidants.

Concluding remarks

When the rose hip programme was initiated at Balgård, the aim was to develop cultivars suitable for manufacturing of rose hip soup. However, in the last years, several reports have been published concerning nutritional and bioactive compounds in rose hips. In the future, there will probably be a demand for "healthy hips" and a market for completely new products. The need for different quality characters will of course change depending on the product. In order to make field cultivation of rose hips economically feasible, plant breeding efforts must be directed towards development of cultivars with characters required for the different and perhaps highly specialized end products. In addition, there will always be a need for basic research in genetics, resistance, field production etc.

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