THE REPRODUCTIVE BIOLOGY OF SELECTED TAXA
OF THE GENUS CERASUS DUHAM

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We used cytological and embryological methods to study reproductive cycle stages in Cerasus fruticosa Pall., Cerasus × eminens (Beck) Buia and Cerasus × mohacsyana (Jánca) Janchen from SW Slovakia, focusing on development of the male and female reproductive organs, fertilization processes and embryo formation. We found that reproductive potential was reduced by synergistic effects of negative biotic and abiotic factors. Despite the presence of degenerated, deformed pollen grains and their great variability of shape and size, a sufficient amount of normally developed viable pollen grains developed in anthers of C. fruticosa and C. × mohacsyana. Disturbed microsporogenesis in C. × eminens led to significantly lower production of viable pollen grains. We did not observe serious disturbances during megasporogenesis and megagametogenesis. Lower fruit set was caused by degeneration of ovules as a result of unsuccessful pollination, fertilization failure, or embryo degeneration during its initial development.

Key words: Cerasus taxa, female gametophyte, pollen grains, embryo.

INTRODUCTION

Cerasus fruticosa (European dwarf cherry, European ground cherry, Mongolian or steppe cherry) is a deciduous, xerophytic, winter-hardy, cherry-bearing shrub of the family Rosaceae native to Ciscaucasia, western Siberia, Kazakhstan, the area south of the Tian Shan Mts. (China), western Russia, Belarus, Poland, Ukraine, Moldova, Romania, Bulgaria, Hungary, Slovakia, Austria, the Czech Republic, Germany and Italy (Chrtke, 1992). It hybridizes naturally with Prunus cerasus to form P. × eminens and with P. avium to form P. × mohacsyana. P. × stacei is a spontaneous three-way hybrid of P. fruticosa, P. cerasus and P. avium (Wojcicki, 1991). The hybrid between Prunus fruticosa and Prunus mahaleb was described as the new taxon Prunus javorkae (Kárp.) Janchen (Hrotkó and Facsar, 1996). A recent study of stands in northern Poland found it to be disappearing there via genetic erosion or the disappearance of typical morphological characters (Boratynsky et al., 2003). Cerasus fruticosa and the hybrids Cerasus × eminens and Cerasus × mohacsyana are taxa of xerophytic vegetation which may play an important role in biocorridors. The fruits of C. fruticosa are used in cooking and for jams and jellies (as a sour cherry), and the species has medicinal uses as an astringent (Dzhangaliev et al., 2003). The flowers of all taxa support bee-keeping well, and the hardiness of the plants makes them desirable for grafting and for producing cultivars. The roots of the shrubs stabilize soil. C. fruticosa is planted in hedgerows as an ornamental windscreen. It is a potential gene source for drought- and cold-resistant stone fruits (Marhold and Wojcicki, 1992; Dzhangaliev et al., 2003).

Cerasus fruticosa is classed as vulnerable (VU) in Slovakia according to IUCN criteria (Feráková et al., 2001). It is also rare and endangered in Poland (Boratyński et al., 2003) due to genetic erosion by gene flow from plantations of C. vulgaris and C. avium and from reduction of its biotopes by human activity.

The reproductive biology of C. fruticosa and its hybrids is insufficiently known. The detailed research reported here is intended to help develop effective ways to preserve the remaining populations of C. fruticosa in their natural conditions. We used cytological and embryological methods to precisely describe the stages of the reproductive cycle of C. fruticosa and two of its hybrids (Cerasus × eminens, Cerasus × mohacsyana), and estimated their generative reproductive ratios.
MATERIALS AND METHODS

DESCRIPTION OF LOCALITIES
OF THE STUDIED TAXA

The material for study was sampled in 2003-2005 from the following locations: for C. × eminens from Stúrovo, Višok (SW Slovakia); for C. fruticosa from Salka, Sovie Vinohrady Nature Reserve (SW Slovakia) and from Pyramidá hill (Zoborské vrchy hills, Tríbeč Mts.); for C. × mohacSYana from Zobor hill (Zoborské vrchy hills, Tríbeč Mts.).

The Sovie Vinohrady Nature Reserve is on the southeast slopes of the Ipeľská Pahorkatina Region 1–1.5 km west of Salka village. In the lower part of this site are remnants of former orchards and vineyards; in the upper parts the slopes are covered mainly by grassland vegetation with sparse shrubs. These upper areas are gradually becoming forested. The basic phytocoenoses are formed particularly by xerophilous grasses of the genus Festuca, replaced by Stipa species after burning. Floristically it is an extremely rich natural ecotype where different types of xerophilous vegetation blend together; we can find typical xerophilous phytocoenoses, especially from the Festucion valesiaceae alliance (e.g., Festuco-Stipetum capitatae, Pulsatillo-Festucetum valesiaceae), and the rarest xerophilous species of the Slovak flora on the shallow soils around the ridges (e.g., Amygdalus nana, Crambe tataria, Himantoglossum caprinum, Iris graminea, Sternbergia colchiciflora, Stipa dasypylla). Višok I was made a nature reserve in view of the many Continental, Pannonian, Pontic and Mediterranean species found there (Svobodová and Ulrych, 2000; Bogová, unpubl. data).

The Višok site is the collective name for two hills – Višok I (Višok Nature Reserve) and Višok II (Dankov Vrch hill). Višok I is located in the southeastern and Višok II in the southwestern part of the hills of Belianske kope near the town of Stúrovo (Bogová, unpubl. data). The Višok site belongs to the phytogeographical district of the Pannonian flora, but there is a noticeable effect of the Kováčovské kope hills (Burdá hills) which belong to the phytogeographical district of the Matricum flora (Svobodová and Ulrych, 2000). The climate in this area is very warm and dry, with 10.3°C average annual temperature and 584 mm annual precipitation. The primary phytocoenosis of these areas is downy oak forest, preserved only on the north and east slopes. Forest glades with many xerophilous non-forest species can be found on shallow substrate. The basic phytocoenoses are formed particularly by xerophilous grasses of the genus Festuca, replaced by Stipa species after burning. Floristically it is an extremely rich natural ecotype where different types of xerophilous vegetation blend together; we can find typical xerophilous phytocoenoses, especially from the Festucion valesiaceae alliance (e.g., Festuco-Stipetum capitatae, Pulsatillo-Festucetum valesiaceae), and the rarest xerophilous species of the Slovak flora on the shallow soils around the ridges (e.g., Amygdalus nana, Crambe tataria, Himantoglossum caprinum, Iris graminea, Sternbergia colchiciflora, Stipa dasypylla). Višok I was made a nature reserve in view of the many Continental, Pannonian, Pontic and Mediterranean species found there (Svobodová and Ulrych, 2000; Bogová, unpubl. data).

PLANT MATERIAL AND METHODS

Collected flower buds, flowers and seeds were fixed in FAA or NAV fixatives. Dehydration of fixed samples, infiltration, paraffin embedding, cutting and staining were based on techniques described by Pazourková (1986) and Erdelksá (1986). Serial sections of ovaries and fruits were cut 5–12 μm thick and stained with Heidenheim's hematoxylin. The slides were examined and photographed with an Axioplan 2 bright field light microscope (Carl Zeiss, Jena) and a Sony DXC-S500 color digital camera. Pollen grains isolated from flowers were immediately germinated in vitro in culture medium (100 ml distilled water + 1 g agar + 5, 10 g saccharose). Pollen germination was evaluated at 20°C. The pollen grain germination rate was determined after 6–24 h. The number of germinated pollen grains was averaged from 100 pollen grains from three random microscope fields for each culture condition. The number of flowers and the number of normally developed fruits were used to calculate the percentage of fruit.
RESULTS

In all studied taxa the floral buds begin to form and differentiate in the summer of the year before the flowers reach full anthesis. Flower buds usually appear on shoots from mid-May to early June. During the summer the flower parts differentiate in the buds: the primordia of the flower perianth in the buds were visible in July, and primordia of the pistil and anthers were clearly seen in August. Flower buds collected at the end of October were in a state of dormancy. In dormant floral buds of the studied taxa, archesporia differentiated into microsporangia of the primordial anthers, but the gynoecia were less developed. Ovule primordia taken at this time showed small protuberances on the margins of the placentae. After winter dormancy the next differentiation of both generative organs continued in March or the beginning of April, depending on the weather.

MEGASPOROGENESIS, MEGAGAMETOGENESIS

Pistils of the Cerasus taxa were unicarpellate; usually only one pistil was found in a single flower, but there were also multi-pistil flowers (Tab. 1). Two pistils per flower regularly occurred in flowers of C. × eminens at the Víšok II site. All studied taxa produced a larger number of pistils, usually 3 to 4 per flower, each year during remontant flowering in summer.

The ovary contained two ovules, which were anatropous, bitegmic and crassinucellate. Development of the female gametophyte was slower than that of the male gametophyte. A multicellular archesporium comprised of 3–8 cells differentiated in the micropylar region of the nucellus at the beginning of April (Fig. 1). Only one of the archesporial cells enlarged to form a megasporocyte by mid-April. Two successive divisions of the megasporocyte (meiosis I and II with cytokinesis) gave rise to a linear tetrad of megaspores by the end of April. The three megaspores at the micropylar end degenerated, leaving a single chalazal functional megaspore (Fig. 2). Three mitotic divisions of the chalazal megaspore led to the formation of 2-, 4- and 8-nucleate female gametophytes (Figs. 3–6). Female gametophyte development was monosporic, following the Polygonum type. The mature female gametophyte of the studied taxa was oval and located in the micropylar region of ovules. It was formed by the egg apparatus and two nearby polar nuclei. These two polar nuclei fused into one central diploid nucleus shortly before fertilization. The egg apparatus included the egg cell and two pyriform synergids. There were three antipodes, which were ephemeral and very nearly degenerated; therefore they did not occur in the mature gametophyte. In late April and early May we observed only one female gametophyte in each ovule of the studied taxa. The developmental stages of the female gametophyte during anthesis differed between the studied taxa. We most frequently observed undifferentiated 8-nucleate or mature female gametophytes. The micropyle was formed by both integuments. The inner and outer integuments were 5- or 6-layered. Primary and secondary ovules did not develop at the same time; development of the female gametophyte was faster in the primary ovule. Typically, the secondary ovule aborted shortly after pollination, and the primary ovule generally was fertilized.

After nuclei of the female gametophyte degenerated, which they did particularly after unsuccessful fertilization, the ovules were observed to degenerate. The most common cause of failed fertilization was bad weather during the flowering period, sharply reducing the number of pollinators. The incidence of degenerated female gametophytes lowered fruit set. Megagametophyte development can be influenced by many factors: genotype, level of plant growth regulators, assimilates stored in the flowers (starch reserves), and environmental factors. We also observed pistils damaged by insect pests.

MICROSPOROGENESIS, MICROGAMETOGENESIS

The juvenile anthers of the studied taxa were tetrasporangiate (Fig. 7). The wall between two pollen chambers disintegrated at anther maturity. The anther wall consisted of the epidermis, an endothecium with well-developed fibrous thickenings, and one or two ephemeral middle layers. The tapetum was single-layered, with secretory cells usually containing 2–5 nuclei. In C. × eminens we observed degeneration of the tapetum layer in several anther microsporangia during differentiation of microsporocytes; we also found highly vacuolated tapetum cells during microsporogenesis. These observations indicate disturbed tapetum development as one of the reasons for undeveloped pollen grains. Sporogenous cells differentiated immediately after the end of winter dormancy, usually in the first half of March (Fig. 8). Microsporocytes differentiated in the second half of March (Fig. 9).
Figs. 1–6. Development of female gametophyte. Fig. 1. Multicellular archesporium of C. fruticosa (16.4.2003, Pyramida hill). Fig. 2. Functional megaspore in ovule of C. × mohacsyana (22.4.2004, Zobor hill). Fig. 3. Two-nucleate female gametophyte of C. fruticosa (16.4.2003, Pyramida hill). Fig. 4. Four-nucleate female gametophyte of C. fruticosa (16.4.2003, Pyramida hill). Fig. 5. Central region of mature female gametophyte of C. × mohacsyana (28.4.2004, Zobor hill). Fig. 6. Micropylar region of mature female gametophyte of C. × mohacsyana (28.4.2004, Zobor hill). Bar = 50 μm in Figs. 1–3, 5,6. Bar = 100 μm in Fig. 4. AC – archesporial cells; CN – central nucleus; EC – egg cell; FM – female gametophyte; FMe – functional megaspore; N – nucellus; Sy – synergids.
Development of male gametophyte. **Fig. 7.** Tetrasporangiate anthers with archesporial cells of *C. fruticosa* (11.3.2003, Salka). **Fig. 8.** Sporogenous cells in anther of *C. fruticosa* (14.3.2003, Salka). **Fig. 9.** Microsporocytes in anther of *C. fruticosa* (21.3.2003, Pyramída hill). **Fig. 10.** Early anaphase I of meiotic division of microsporocytes in anther of *C. fruticosa* (1.4.2004, Salka). **Fig. 11.** Tetraedric tetrads of haploid microspores of *C. fruticosa* (1.4.2004, Salka). **Fig. 12.** Haploid microspores separated from tetrads of *C. fruticosa* (1.4.2004, Salka). Bar = 50 μm in all figures. AC – archesporial cells; Co – connective; E – epidermis; End – endothecium; Mi – microspores; Mis – microsporangia; ML – middle layer; Msc – microsporocytes; SC – sporogenous cells; T – tapetum; Td – tetrads.
onset and course of microsporogesis occur synchronously, depending on the position of the flower primordia in buds and the position of stamens in the flower. We also recorded asynchronous development of pollen grains in the flower buds, in which both anthers with male archesporial cells and
microsporocytes occurred at the same time. Microsporogenesis was observed in early April at the study sites and took place simultaneously (Fig. 10). Meiotic divisions of the microsporocytes led to the formation of tetradic tetrads of haploid microspores (Figs. 11, 12). Mature pollen grains were 2-celled and tricolporate, and were released from the anthers around the second half of April. C. fruticosa pollen grains varied in shape and size. In the pollen of the hybrid taxa C. × mohacsyana and especially C. × eminens we found great variability of shape, large amounts of degenerated pollen, as well as degeneration of all anther microsporangia. We assume that the hybrid origin of the taxa was most responsible for the formation of degenerated pollen grains, but that fluctuations of temperature and precipitation during pollen development were also factors. These types of disturbed microsporogenesis significantly hampered the production of viable pollen, especially in C. × eminens. C. fruticosa and C. × mohacsyana produced sufficient quantities of viable pollen. The germination rates we found for C. fruticosa were 24.4% (Pyramid site, 5% saccharose), 36.5% (Pyramid site, 10% saccharose), 48.3% (Salka site, 5% saccharose) and 19.7% (Salka site, 10% saccharose). For the hybrid taxon C. × mohacsyana the germination rates were 21% (5% saccharose) and 16.2% (10% saccharose), and for C. × eminens 0.2% (5% saccharose) and 0% (10% saccharose).

We observed degenerated stamens, dry stamens and insect-damaged stamens in all three taxa. Their high numbers of degenerated pollen grains and damaged anthers decreased their reproductive potential.

POLLINATION, FERTILIZATION, EMBRYOGENESIS

The studied taxa bloomed from around mid-April, depending on the weather (especially temperature), and the flowering period continued for about two weeks. C. fruticosa and C. × eminens began to bloom first at the more southern locations (Víšok II, Sovie Vinohrady). C. fruticosa and C. × mohacsyana at Pyramid and Zobor began to bloom about a week later. Bees were the most important pollinators.

Unfavorable weather during the flowering period had a negative impact on the number of insect pollinators, overgrowth of pollen tubes and stigma secretion, resulting in unsuccessful pollination and fertilization.

All three taxa were porogamous; the pollen tube grew into a female gametophyte, the sperm cells were released, the first sperm cell fused with the egg cell, creating the zygote, and the second sperm cell fused with the central cell diploid nucleus of the female gametophyte (Fig. 13).

Embryogenesis conforms to the Asterad type. After successful fertilization the female gametophyte markedly elongated; development of the endosperm haustorium started in the chalazal region of the ovules shortly after fertilization. We recorded the first stage of embryogenesis in early May. The zygote was present in most of the analyzed ovules at this time. We observed globular embryos with a short suspensor in mid-May (Fig. 14), heart-shaped (Fig. 15) and torpedo-shaped embryos in the second half of May, and mature embryos in late June (Fig. 16). Endosperm formation was first nuclear but later the tissue became cellular. Cellularization started at the micropylar pole. Free endospermal nuclei were recorded in the chalazal region of the developing ovules even in heart-shaped and torpedo-shaped embryo stages. The endosperm was gradually consumed during embryogenesis and only remnants were found in a thin layer surrounding the mature embryo. No serious disturbances were noted during embryogenesis and endosperm development. Rarely, we observed degeneration of the zygote in the early stages following fertilization. Only one seed developed in each fruit.

In C. × eminens ovaries all the ovules degenerated completely, so no fruits developed. Our observations indicate that low pollen viability and the lack of other pollen donors for the studied population were responsible for that.

In the field we found damage to the reproductive organs of the examined taxa caused by insect pests and diseases, significantly reducing fruit set. Fruit set of C. × mohacsyana and C. fruticosa was low during the whole research period. C. × mohacsyana developed fruits only occasionally, and normal fruits of C. fruticosa developed from only 0.8% of the flowers in 2004. No C. × eminens fruits developed during the entire study.

The average morphometric measurements of C. fruticosa fruits were as follows: ripe fruits 14 mm long and 16 mm wide, kernel 9 mm long and 7 mm wide, and mature seeds 7.9 mm long and 6.2 mm wide. All mature fruits contained normally developed seeds.

DISCUSSION

Flower fertility and fruit set development are analyzed over time in order to establish any temporal relationships between megagametophyte and microgametophyte development. They are also studied to discover malfunctions limiting fruit set (Thompson and Liu, 1973). The Cerasus taxa we studied occur in localities of southern Slovakia characterized by very hot and dry climate. These climatic conditions, especially in warmer years, adversely affect the course of differentiation. In hot
dry summers we observed damage to and desiccation of flower buds during their formation and differentiation, recorded annually in all taxa studied, particularly in *C. fruticosa* plants at Pyramidá hill; this location, on a steep, rocky, dry south slope, has the most extreme ecological conditions. We also found that the late spring frosts were harmful to reproductive organ development. Members of the genus *Prunus* vary in their sensitivity to spring frost. Miranda et al. (2005) found the lowest resistance to spring frost in *P. avium* plants (syn. *Cerasus avium*); in other species resistance increased in the following order: *P. dulcis → P. persica → P. salicina → P. spinosa*.

We observed regular formation and differentiation of two to four pistils per flower in *C. × mohacsyana* and *C. fruticosa* taxa at Pyramidá hill. Fruits did not develop from these flowers: ovules were formed in the pistils but no female gametophytes developed in them.

Jia (2008) found that only 8.9% of the ovules in double pistils of *P. salicina* cultivars developed, while 24.3% of those in normal pistils developed. In *P. avium* cv. Satohminshiki, Beppu and Kataoka (1999) found that higher temperatures had a demonstrable impact on the presence of additional pistils in the flowers. A single pistil was found in all checked flower buds at 25°C, but the percentage of flowers with double pistils increased markedly at temperatures above 30°C, with two pistils in more than 80% of the flowers at 35°C. Engin and Ünal (2008) also reported flowers with two pistils in *C. avium* plants.

In the genus *Cerasus* there usually are two ovules in the ovary. This number is considered normal and has been documented by several authors, particularly for sour cherries. Burgos and Egea (1994) and Alburquerque et al. (2002) reported the frequent occurrence of ovules without female gametophytes and the occurrence of 2- or 4-nuclear female gametophytes in *P. armeniaca* cultivars at anthesis.

Furukawa and Bukovac (1989) reported up to 25–40% ovules that were 4-nuclear or less, or degenerated, during flowering of *P. cerasus* cv. Montmorency. Hedhly et al. (2003) found that increasing the temperature by only 5–7°C increased the degeneration rate as well as the number of degenerated ovules during flowering of *P. avium* cultivars.

The female gametophyte of *Cerasus* develops rapidly in the primary ovule, while the secondary ovule degenerates shortly after pollination: Arbeloa and Herrero (1991) mention an interval not longer than 2 weeks after anthesis. Survival of the primary ovule and its greater capacity for fertilization generally is found in representatives of the subfamily Prunoideae.

The structure of the anther wall of all studied taxa was typical for representatives of the family Rosaceae. The same anther wall structure has been described for *P. salicina* (Radic et al., 2008). The tapetum was secretory and its cells contained 2–5 nuclei. In *C. × eminens* we observed disturbed tapetum development during sporogenesis, resulting in degeneration of tapetum layers, and large vacuoles in the tapetum cells. Radic et al. (2008) observed large vacuoles in tapetum cells of *P. salicina* anthers with sterile pollen grains.

The male gametophyte develops more rapidly than the female gametophyte. Microsporogenesis is simultaneous, as is typical for most representatives of Rosaceae (Podrubnaja-Arnoldi, 1982). During microsporogenesis we recorded asynchronous development of pollen grains. In addition to anthers with microsporocytes we observed anthers with male archesporial cells in buds. Ostrolucká and Križo (1989) also observed this phenomenon in the genus *Quercus*, and Dušišová (unpubl. data) in some representatives of Ericaceae. Our observations of 2-celled tricolporate pollen grains are consistent with those of Cerović and Mićić (1999) in *C. avium* and Arbeloa and Herrero (1991) in *P. persica*, we found antipode degeneration before fertilization. These authors observed antipode degeneration 3 days after they developed.

In our samples the female gametophyte was mostly mature and well differentiated in *C. fruticosa* ovules during flowering, although 8-nuclei undifferentiated female gametophytes appeared sporadically. The female gametophyte in ovules of the hybrid taxa *C. × mohacsyana* and particularly *C. × eminens* did not develop or else degenerated. Degeneration of nuclei or whole female gametophytes during flowering was observed in ovules of cv. Čačansky Rubin sour cherry (Cerović and Mićić, 1999). Egea and Burgos (1994) and Alburquerque et al. (2002) reported the frequent occurrence of ovules without female gametophytes and the occurrence of 2- or 4-nuclear female gametophytes in *P. armeniaca* cultivars at anthesis.
with observations of the majority of representatives of Rosaceae. We did not find the 3-celled pollen grains described by Jakovlev (1985) or the tetracolporate pollen grains observed in anthers of *C. vulgaris* (Miaja et al., 2000). In the hybrid taxa, particularly in *C. × eminens*, we recorded degenerated pollen grains and great variability of pollen shape. Miaja et al. (2000) found low germination and abnormal pollen grains in *C. vulgaris*. Radice et al. (2008) noted differences in the development of sterile pollen grains in *P. salicina*, clearly evident from the microsporocyte stage; the atypical shape of those sterile pollen grains was characteristic. The percentage of viable pollen grains was significantly lower in *C. × eminens*; the number of normally developed and viable pollen grains was sufficient in *C. fruticosa* and *C. × mohacysyana*. Perez and Moore (1985) found a high rate of pollen germination in 18 *Prunus* species (82–97%) and much lower pollen germination in their hybrids (1–40%). Miaja et al. (2000) reported less than 25% germination of *C. vulgaris* pollen in vitro.

As in the case of macrosporogenesis and microsporogenesis, development of the embryo and endosperm in the three studied taxa generally followed the patterns known in the family Rosaceae. Embryogenesis in *C. × mohacysyana* and *C. fruticosa* followed the course as described by Keserović (1999) for *C. avium* and *C. vulgaris*. We did not observe defects in endosperm development of the type noted by Dittmann and Stösser (1999) for some *Prunus* species.

As we found in our study, *C. fruticosa* showed very low fruit set at the Sovie Vinohrady site in 2004, where only 0.8% of the flowers developed to normal fruits. Similarly, Ivanišová (unpubl. data) found marked variability of *C. fruticosa* fruit set depending on the location and year: fruit set of this species varied from 0.49% to 7.22% at the Sovie Vinohrady site and from 1.86% to 10.5% at the Pyramid hill. Baranec (1996) reported similar data from Pyramid hill, where only 2.1% to 9.1% of *C. fruticosa* flowers developed to mature fruits. Clearly, fruit set generally is low in *C. fruticosa*, and seems typical for other representatives of subfamily Prunoideae as well: fruit set lower than 30% has been found in *Crataegus monogyna*, *Cerasus mahaleb* and *Prunus spinosa* (Guitián et al. 1992). Low fruit set together with hybridization with related species of genus *Cerasus* appear to be a major reason for the decline of *C. fruticosa* populations from natural habitats not exposed to direct human impacts.

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REFERENCES


HROTKO K. and FACSAR G. 1996. Taxonomic classification of Hungarian populations of *Prunus fruticosa* (Pall.)


