A New Generation of Clonal Seed Orchards of Wild Cherry. Selection of Clones and Spatial Design

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Abstract

Forest policy in Flanders (Belgium) strongly promotes the use of indigenous hardwoods, among which wild cherry (*Prunus avium* L.), for re- and afforestation and for stand conversion,. This line of policy generates a strong demand for high quality forest reproductive material, which cannot be met by the currently available basic material. The selection and breeding programme attempts to remedy the discrepancy between supply and demand by creation of a new generation of clonal seed orchards characterised by (i) a high yield and (ii) a high genetic quality and diversity of the offspring. This goal was achieved by selection of 52 genotypes based on half-sib progeny trials. Yield and genetic quality of the offspring was enhanced to a further extend by adjusting the spatial design of the seed orchard to the phenologically and gametophyticaly cross-compatibility of the selected genotypes. Background pollination is reduced to a minimum by establishment of the seed orchard at a minimum distance of 400 m from other wild cherry populations and sweet cherry plantations

Introduction

The use of wild cherry (*Prunus avium* L.) for re- and afforestation is motivated by the acknowledgement of its high silvicultural, ecological and economical importance. Wild cherry is a tolerant tree species allowing the establishment of mixed forest stands and is a relatively fast growing hardwood producing very high quality timber. Furthermore, the species is often mentioned as a potential alternative to poplar for the afforestation of abandoned and set-aside farmland. Through the implementation of the EU Council Directive 1999/105/EC in the Flemish Forest Decree, the certification of forest reproductive material of wild cherry became compulsory and, thus, the genetic quality of the reproductive material had to be warranted. The present annual demand for reproductive material, amounting to 30 kg of

pure seed on average, cannot be met by the available indigenous basic material. Firstly, reproductive material of the category "source-identified" is not allowed for forestry purposes, i.e. wood production. On the other hand, only two Flemish seed stands and one seed orchard are presently recorded in the Belgian Catalogue of Forest Basic Material.

The area of the seed stands is very restricted, *viz* "Vrebos" (0.28 ha) and "Rattenberg" (0.37 ha). Consequently, the yield is very low, being about 10 kg of pure seeds in good crop years. Moreover, the harvest is not cost-effective as the cost price is about four times the selling price. Observation of the fruit morphology of the cherries harvested in "Vrebos" strongly indicates an introgression from sweet cherry cultivars. Moreover, this seed stand is characterised by a predominant regeneration through root suckering resulting in a marked clonal structure: the 402 individuals only represent 64 different genotypes with a fairly low genetic distance (~ 0.23). Therefore, the seed stand "Vrebos" is likely to be removed from the above-mentioned catalogue. Finally, the potential for the selection of additional seed stands is limited, due to the occurrence of wild cherry as individual trees or small clusters scattered throughout mixed forest stands.

The sole seed orchard "Mommedeel", established in 1988 and covering 0.82 ha, suffers from recent severe dieback of the constituents due to the unsuitable site conditions and to the abortion of the grafts.

The selection and breeding programme attempts to remedy the discrepancy between supply and demand by creation of a new generation of clonal seed orchards characterised by (i) a high yield and (ii) a high genetic quality and diversity of the offspring. Additionally, seed orchards offer the possibility for intensive management and the advantage of less labour-intensive harvest.

The adopted research strategy pursues a fourfold aim:

i. Assessment of the genetic diversity of the basic collection with a view to detecting identical or closely related accessions.
ii. Identification of the very best clones within the basic collection by assessment of adaptive traits in half-sib progeny trials
iii. Designing the layout of the seed orchard, i.e. determination of the optimal spatial arrangement of the clones within the orchard.
iv. Establishment of minimum isolation standards with regard to surrounding natural populations of wild cherry and cultivated sweet cherries.

Material and methods

From the early 80's till the late 90's, 168 phenotypically superior plus trees of wild cherry were selected in 27 wild cherry populations covering its entire distribution area in Belgium. Vegetative copies of these plus trees, obtained by grafting or budding, were planted in seven experimental comparative plots.

The genetic diversity of this basic collection was assessed by six AFLP markers and 11 highly informative microsatellite markers with DP values ranging from 0.76 to 0.95 (Clark *et al*. 2003, Downey *et al*. 2000, Vaughan *et al*. 2004). Estimation of the genetic distance D between individuals was based on the proportion of shared alleles: $D = 1 - P$ with $P = \Sigma_u S/2u$ where the number of shared alleles S is summed over all loci u.

In 1995, seeds were harvested in seven multiclonal plantations established with vegetative replica of the selected plus trees. This resulted in the establishment of 13 half-sib progeny trials covering a total surface of 15.3 ha. Adaptive traits such as vigour (height growth), morphology (stem straightness, branching habit, apical dominance), phenology (flushing, bud set, St. Johns sprouts) and disease resistance were assessed. Wild cherry is subject to two major diseases, viz anthracnosis, a fungal leaf disease caused by *Blumeriella jaapii* and bacterial canker caused by *Pseudomonas syringae* with three distinct pathovars, namely pv *syringae*, pv *morsprunorum* and pv *avii*. For each trait, the narrow sense heritability h^2_A as well as the general combining ability GCA was determined, allowing the construction of a selection index: $I = \Sigma_i (h^2_A.GCA)_i$ with i = 1 to n and n = number of traits.

Wild cherry is entomophilous, with bumble bees (*Bombus* spp.) acting as main pollen vectors. Bumble bees display a particular foraging behaviour, which leads one to suspect a small-scaled patch-like pollination pattern within wild cherry populations (Goulson *et al*. 1998, Heinrich 1976).

As a test-case, one of the above-mentioned multiclonal plantations was selected, consisting of 65 accessions and surrounded by eight 'natural' wild cherry populations situated at various distances. In order to unravel the pollen flow, i.e. assessment of internal mating patterns as well as pollen input from outside the plantation, a parenthood analysis was carried out using

11 microsatellite markers and the self-incompatibility genotype (DP = 0.98). The objective was to trace the father tree of 60 half-sibs randomly chosen within the offspring of 16 selected accessions, using the exclusion method. When determining the mating pattern, the allogamous nature of wild cherry was taken into account. This trait is governed by a single multi-allelic *S*-locus with gametophytic action which controls self-incompatibility (SI) and cross-incompatibility between individuals with the same SI-genotype. The SI-genotype is determined by concensus and allele-specific PCR (Sonneveld et al. 2001, Sonneveld *et al*. 2003).

Results and conclusions

Analysis of AFLP and microsatellite markers revealed four groups of identical genotypes. As wild cherry often regenerates through root suckering, plus trees selected within the same population are likely to be identical. Consequently, the basic collection was reduced to 152 genetically different accessions with a genetic distance ranging from 0.46 to 0.92.

The selection index I was determined for all accessions. No significant differences of the I-values were found between the 13 progeny trials. A total of 52 genotypes was finally selected, showing an I-value higher than the median value and a genetic distance varying between 0.72 and 0.89.

Parenthood analysis of the 960 half-sibs showed that 21 % of the sires was located outside the plantation. Tracing of external pollen donors revealed that 80 % of the pollen input came from sires situated within a wild cherry population located at a distance of 400 m from the plantation studied.

Assessment of the internal pollen flux revealed that 75 % of the sires was located within a distance of 10 m from the mother tree, i.e. twice the planting distance (Fig. 1). Paternal contribution was influenced by flowering period and *S*-(in)compatibility but not by flowering abundancy. Finally, the parent exclusion probability exceeded 0.99.

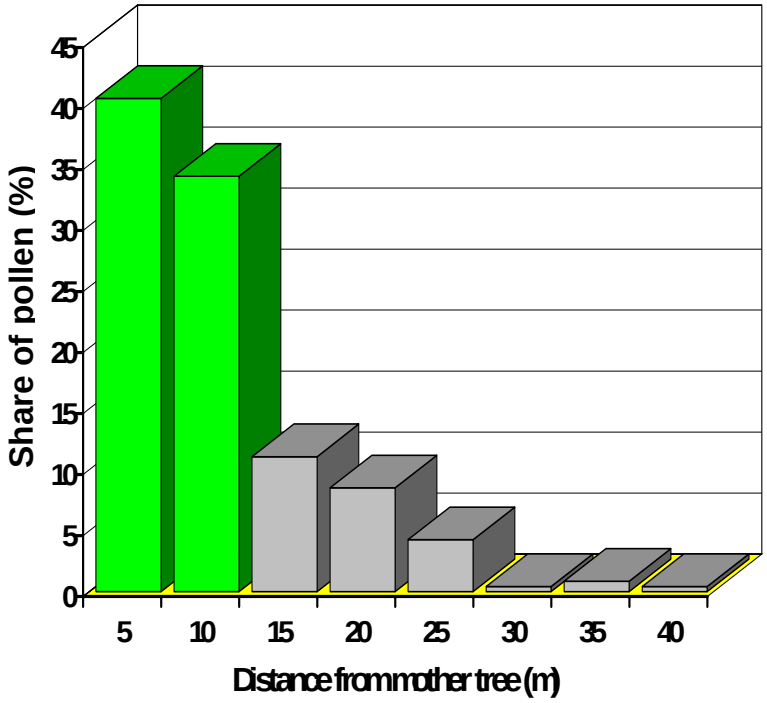

Figure 1. Relation between pollen contribution of sires and their distance from the mother tree in a multiclonal plantation of wild cherry.

As the paternity analysis revealed a small-scaled patch-like pollination pattern, neighbouring trees in the new generation seed orchards should be phenologically compatible, i.e. display an overlapping flowering period as well as gametophytically compatible, i.e. have a different SI-genotype.

Flowering phenology of the 52 selected genotypes was observed in four plantations in 2002 and 2004 by recording the share of fully opened flowers every 48 hours during the whole flowering period. When assessing the overlap between the flowering period of two accessions, it has to be taken into account (i) that fully opened flowers are only receptive for 38 hours and (ii) that the pollen in the pollen bags of bumble bees are viable during 12 hours. The flowering phenology of an accession was not influenced by its location within the populations. No significant differences were found between sites and the observations of 2002 and 2004 appeared to be concordant.

Observation of the flowering phenology resulted in a cross-tabulation of the overlap in flowering period for all possible combinations between the 52 selected accessions. When

adopting a tresshold value of 25 % for the overlap in flowering period, 66 % of all combinations appeared to be phenotypically cross-compatible.

The self-incompatibility genotype of the 52 accessions was determined and confirmed by carrying out controlled crosses. In addition, six 'new' *S*-alleles, previously unknown in sweet cherry cultivars, were detected and numbered S_{17} to S_{22} (De Cuyper *et al.* 2005). Few of the genotypes (11 %) appeared to be cross-incompatible.

In view of the establishment of the seed orchard, selected genotypes were propagated vegetatively by grafting on the dwarfing rootstock “Gisela 5”. Furthermore, traditional pomological techniques are applied to increase fruit production, such as planting in espalier, use of bird nets and placing of several bumble bee nests. Early and abundant flowering is induced by adequate pruning, tearing and bending of branches, weed control and root pruning.

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