

CONSERVATION MEASURES FOR AUTOCHTHONOUS OAKS IN FLANDERS

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ABSTRACT

Oaks have always been economically esteemed. For hundreds of years seeds of high quality provenances have been transported throughout Europe. Especially in Flanders, since the Middle Ages the most densely populated region of Europe north of the Alps, forests have suffered from an exceptional high human impact, including import of allochthonous seeds. Consequently, autochthonous oaks are extremely rare and threatened nowadays. An inventory aiming at the localisation of autochthonous growth sites of woody plants in Flanders revealed a number of places where old coppice stools of both native oak species *Quercus robur* and *Q. petraea* survive. Because these relics hold valuable historical and autochthonous genetic information, conservation strategies are indispensable. Preservation acts include the delineation of autochthonous provenance areas as conservation units for both species based on cpDNA haplotypes. The production of autochthonous forest reproductive material is an instrument to spread the genetic information. Therefore, old coppice stands are approved as official seed sources, which allows appropriate certification and commercialisation. In addition, the coppice practice is being reinstalled on a small scale as a response to historical ecological concerns. Finally, *ex situ* gene conservation is attained by vegetative propagation of the largest stools and thereby creating living gene banks that can serve as future seed orchards.

Key words: *Quercus*, coppice, gene bank, conservation, seed source, molecular markers, grafting.

INTRODUCTION

Conservation genetics of autochthonous woody plants

The higher the impact of man on the world's ecosystems, the stronger the belief that we have a moral responsibility to take care of the world's biodiversity heritage (e.g. FRANKEL *et al.* 1995). This reflection is incorporated in the Convention on Biological Diversity (CDB 1992) where it is stated that biodiversity should not only consider the diversity of species, communities and ecosystems. It directs to descend the ladder of biological hierarchy down to the genetic level, as genetic diversity is a necessary means of survival in dynamic ecological equilibria. Therefore, conservation of the patterns of genetic diversity within species is an objective, for the same reasons and with the same importance as conservation of species diversity. Being sessile and long-living organisms, woody plants maintain the highest levels of intraspecific genetic variation of all life forms (HAMRICK *et al.* 1992) as an indispensable prerequisite for securing evolutionary flexibility (e.g. GREGORIUS & KLEINSCHMIT 1999; NEWTON *et al.* 1999). Next to the CDB convention, that covers all

living organisms, the Ministerial Conferences on the Protection of Forests in Europe (ANONYMOUS 1998) explicitly emphasised the urgency of the preservation of local autochthonous genetic resources, in order not to lose the genetic heritage. Since spontaneous colonisation after the last glaciation, autochthonous trees and shrubs have only rejuvenated naturally or have been artificially propagated with material from strict local origin (HEYBROEK 1992). Autochthonous populations can thus be seen as the direct descendants of the plants that colonised a particular region during the post-glacial migration. Because they grew and reproduced during at least thousands of years in that region, they are assumed to be well adapted to local biotic and abiotic conditions. Distribution areas of native species are often defined on a large scale and their borders tend to coincide with a country or administrative zone. In contrast, autochthonous provenance areas are delineated primarily by ecological criteria. Information on genetic diversity of autochthonous populations further adjusts this demarcation. Here, provenance is the original geographic area where seeds were obtained, whereas seed source only refers to the area where the seeds were harvested.

Strategies for the preservation of autochthonous oaks: state of the art

Since 1997 actions are undertaken towards the protection of the autochthonous woody plants in Flanders in general and towards oaks specifically. These are undertaken jointly by the Forest and Green Area Division of the Flemish Community and the Institute of Forestry and Game Management, (COART *et al.* 1998). As stated by BOWEN (1999), conservation efforts should preserve the processes of life. This includes not only the fixation of autochthonous genomes in living gene banks, but also the maintenance of an evolutionary potential for the genetic diversity to evolve in a natural way so that adaptation to changing growth conditions is possible. In practice, there are several goals for this conservation of autochthonous populations in Flanders. Firstly, as Flanders is a highly populated region, human pressure on remaining semi-natural habitats is large. Therefore, remaining populations should be protected in the first place from further demolition, both *in situ* through habitat protection as *ex situ*, in living gene banks. Secondly, these remaining populations can be the source for plantations in existing forests and in new semi-natural woodlands. It is indeed important to preserve and use all oak diversity present and not only focus on stands with economic superior characteristics. Also, as Flanders' landscapes are shaped largely by human activities, cultural historical concerns play an additional role in the conception of the conservation measures.

As a first step, a systematic inventory of growth sites harbouring autochthonous trees and shrubs was started and is up to date going on (MAES & RÖVEKAMP 1998; RÖVEKAMP & MAES 1999; MAES & RÖVEKAMP 2000; RÖVEKAMP & MAES 2000; RÖVEKAMP *et al.* 2000; OPSTAELE 2001). For oak, only coppice wood is assigned an autochthonous status because this historical silvicultural practice creates the possibility to attain much older ages than non-coppice trees. Coppicing exploits the capacity of certain forest species, including oak, to generate sprouts (suckers) at the base of the stem (stump) once it is cut. The coppice is cut at regular intervals *e.g.* 15-30 years. When the coppice system is abandoned, old coppice stools can be recognised as the individual sprouts have grown out as stems and these remain in a circular form. The larger the diameter or circumference of such a group of stems, the older the original tree.

The age of single oak trees in hedgerow or farmland in the Netherlands is assessed up to 450 to 720 years (MAES 1998). In comparison, oak coppice stools with circumference of 30 m at soil level are roughly estimated to reach ages up to 1500 years (MAES 1998).

Because of their old age, coppice stools may date from before the period of intensive translocation of acorns and planting material that probably started in the Middle Ages in Flanders and became important during the reconversion of coppice to high forest (TACK *et al.* 1993). As years with abundant acorn production are dispersed in time and natural regeneration in Flanders is rare, oaks were regularly planted (BARY-LENGER & NEBOUT 1993). Nowadays, coppice oak stands are relics within today's silvicultural practice of high forest that sometimes survived demolition for fixation of land dunes, or just by chance.

The preservation of the stools both *in situ* and *ex situ* are the first necessary conservation acts. As public organisations or institutions that own most coppice stands are aware of the precious value, protection against destruction is not an immediate issue. Reinstalling the coppice practice of neglected stools is not only important from a cultural historical point of view, it can also extend the life-span of the stools. The collection of the largest stools in *ex situ* living gene banks holds a twofold purpose. The long-term preservation of the remaining genotypes is achieved and future seed harvest will produce autochthonous plant material. As autochthony implies adaptation to local growth conditions (HEYBROEK 1992) and their old ages prove vitality, the old coppice stands can be a valuable basis for future (re)forestation that aim at the conservation of biodiversity. Spontaneous natural regeneration cannot be relied upon for the spread of the autochthonous genetic information of oaks in Flanders as there are too little old coppice oak stands left as starting populations. The production of autochthonous forest reproductive material is a better means to achieve this aim. The delineation of provenance areas for autochthonous oaks is an obligatory step towards the commercialisation of plant material. An autochthonous provenance area is the region where the individuals of a species are autochthonous and is therefore the unit of conservation management. Seeds harvested in different seed sources within the provenance area can be mixed and the derived plant material should only be planted within this area.

Objectives

General conservation strategies for forest trees in Europe, including oak, have been put forward within the scope of EUFORGEN (BORELLI *et al.* 2001). Also, a combination of field data and molecular marker analyses gives the most relevant insights for the development of practical management and conservation plans (*e.g.* BOWEN 1999; CRUZAN 1998; KING & BURKE

1999). Here we develop a range of practical conservation measures for autochthonous oaks in Flanders, starting from a field study and, where applicable, refining the information with molecular marker data. Included are the delineation of autochthonous provenance areas as conservation units, official approval of seed sources for the production of plant material, the preservation of autochthonous genomes in living gene banks and the reinstallation of the coppice practice. The presented approach can be considered as a case study for several economically important native forest tree species in Flanders. For all, autochthonous populations are rare and sometimes regionally extinct (MAES & RÖVEKAMP 1998, RÖVEKAMP & MAES 1999, MAES & RÖVEKAMP 2000, RÖVEKAMP & MAES 2000, RÖVEKAMP *et al.* 2000, OPSTAELE 2001).

MATERIAL AND METHODS

Inventory of autochthonous woody plants

MAES (1991) and MAES *et al.* (1993) developed criteria to evaluate the autochthonous character of a tree or shrub population. Important criteria regarding the location are: (i) the location was afforested on the topographic map of 'de Ferraris' (18th century) or on other historical topographic maps (DE KEERSMAEKER *et al.* 2001); (ii) the location gives an undisturbed impression; (iii) the ecological conditions of the location are similar to the conditions in the natural area of prevalence of the species; (iv) plant species, indicative for old growth forest and ancient undisturbed woodlands (TACK *et al.* 1993) are present in herbaceous, shrub or tree layer and (v) the location lies within the natural distribution range of the species. Important criteria regarding the tree or shrub are: (i) the tree or shrub is a wild individual and no cultivated variety and (ii) trees are old or old coppice stools are present. In addition, historical sources and oral information on flora, soil condition and geography can be used to further establish the autochthonous character of the location. The set of criteria shall be only partially applicable in most situations and the autochthonous character of a population can only be established with an absolute certainty in a very limited number of cases.

Grafting oaks

Rootstocks were three-year-old *Quercus robur* plants originating from an official seed stand in Belgium and bought in a commercial nursery by the end of February 2002. In the same month scions were cut from 23 old

coppice stools within the 2 stands Speelbos 97–83 and Gemeentebos 98–133 (Table 1). For each mother tree 10 scions were divided in two parts and two different grafting methods were applied. For the first one, the above ground part of the rootstock was cut and the scion was cleft grafted on the central root. After grafting the plants were potted. For the second method scions of the same mother plants were cleft grafted on the stem of a rootstock which is a standard procedure (KRÜSSMANN 1981), cutting the rootstock about 15 cm above ground level. Rootstocks were in this case potted before grafting.

Measuring tree rings

A representative stool was chosen and a stem disc at the base of the cut stem was air dried and polished with sandy paper (1200). Ring widths were measured with a linetab (Heidelberg, Germany).

Molecular analyses

Chloroplast diversity studies were carried out following the protocols of DUMOLIN-LAPÈGUE *et al.* (1997) and PETIT *et al.* (2002a).

Microsatellite analysis to confirm identity of oak stools was carried out as described in COART (2003).

RESULTS

Survey of autochthonous oaks in Flanders

The inventory of autochthonous trees and shrubs in Flanders (Materials and methods) revealed the last relics of old coppice stands of both native species *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. (Table 1). The largest stools of both species are mainly found in the Northern parts of Flanders, on sandy soils in the Campine area. CpDNA analysis showed that two main European migration lineages, from the Iberian and from the Italian peninsula refugia of the last glaciation, have reached Flanders (PETIT *et al.* 2002b; KÖNIG *et al.* 2002; COART 2003). This analysis validated the autochthonous evaluation of coppice oak stands in the field study (COART 2003). Most of these stands are monotypic and all are descendants of the Iberian and the Italian peninsula migration routes. This is in contrast with the analysis of several official seed stands and different old line plantations on farmland borders in Flanders that were shown to be more polytypic (COART 2003). *Q. petraea* and *Q. robur* of the Italian

Table 1. Summary of most important autochthonous oak sites in Flanders.

Nr. ^a	Species ^b	Location name	City	Autochthony quotation ^c	Chloroplast type ^d	Nr. of stools with circumference > 8 m ^e	Ownership	Surface (ha)	Acknowledgement as seed source; if no: reason
1	<i>Q. petraea</i> *	QPwindelsteen00-MA3	Maasmechelen	A	1	0*	City	5	Yes
2	<i>Q. petraea</i> *	QPklaverberg98-86,87	As	A	1	26	City	10	Yes
3	<i>Q. petraea</i> *	QPopgrimbic00-MA13	Opgrimbie	A	1	5*	City	4	Yes
4	<i>Q. petraea</i> *	QPgemeentebos98-117	Hechtel-Eksel	A/B	1	5	Military domain	2	No, mixture with <i>Q. robur</i>
5	<i>Q. petraea</i> *	QPkabouterkensberg97-101	Kasterlee	B	10+12	0	City	1	No, small number of relative small coppice stools and mixture of haplotypes
6	<i>Q. petraea</i>	QPremsart97-170	Sint-Joris-Weert	A/B	10	0*	State	2	No, singled coppice and planted oaks in neighbourhood
7	<i>Q. robur</i>	QRrobertii98-79	Gruitrode	A	1	3	State	0.5	No, small number of trees and mixture with <i>Q. petraea</i>
8	<i>Q. robur</i>	QRgemeentebos98-133	Hechtel-Eksel	A	10	6	Military domain	4	Yes
9	<i>Q. robur</i>	QRkabouterkensberg97-101	Kasterlee	B	10+12	12	City	1	No, mixture of haplotypes
10	<i>Q. robur</i>	QRspeelbos97-83	Wetteren	B	10	7	State	4	Yes
11	<i>Q. robur</i>	QRmeerskant99-224	Laarne	A	10	0*	Private	0.25	Yes
12	<i>Q. robur</i>	QRmeerdaalwoud97-166	Sint Joris Weert	B	12	0*	State	1	No, planted oaks adjacent
13	<i>Q. robur</i>	QRbeerzel01	Beerzel	A	-	5	City	1	Not yet, unknown haplotype
14	<i>Q. robur</i>	QRkonijnenberg01	Vosselaar	A	-	10	City	3	No, planted oaks adjacent and unknown haplotype

^{a)} Corresponds with numbering on Fig. 1a and 1b.

^{b)} Mixture with *Q. × rosacea* is indicated with *.

^{c)} Evaluation of autochthony in field survey (MAES & RÓVEKAMP 1998): A = certainly autochthonous, B = possibly autochthonous.

^{d)} Haplotype according to PETT *et al.* (2002b). 1 originates from Italian migration lineage, 10 and 12 are from Iberian migration lineage.

^{e)} Singled coppice is indicated with*.

migration lineage (haplotype 1) grow in the High Campine (Fig. 1b and 1c), that is considered as the east border of the distribution area of this haplotype (KÖNIG *et al.* 2002). Descendants of the Spanish lineage (haplotypes 10 and 12) are more dispersed over Flanders (Fig. 1b and 1c). To be sure that coppice stools measuring circumferences at soil level of 20 m and more are not the result of oaks planted in circles some 100 years ago, microsatellite analysis (Materials and methods) was performed on three stools, two from Speelbos97–83 and one from QPkloverberg98–86,87 (Table 1). In all cases up to 15 different stems in the circle were analysed and proven to be clones. This is in agreement with the results of BAKKER (2001) who analysed oak stools in ancient woodlands in a similar way.

Autochthonous provenance areas in Flanders

Autochthonous provenance areas are defined in the first place by ecological criteria that comprise mainly climatic and soil conditions. Roughly, Flanders consists of sandy soils in the north and loamy soils in the south, and climatic influence extends from atlantic to subatlantic from west to east. In combination with the coastal area this division reflects the phytogeographic regions as defined by LAMBINON *et al.* (1998) and leads to the major autochthonous provenance areas in Flanders (Fig. 1a). The largest coppice stools for both *Q. robur* and *Q. petraea* grow in the northern part of Belgium, within sandy Flanders (atlantic climate, sandy to loamy sandy soils) and the Campine (subatlantic climate, sandy soils) (Fig. 1b and 1c). On two locations in Meerdaal forest in Brabant District East (subatlantic climate, loamy soils) only singled coppices are present (Table 1). A further division of the Campine results in low and High Campine, according to the elevation above sea level that is lower or higher than 50 m

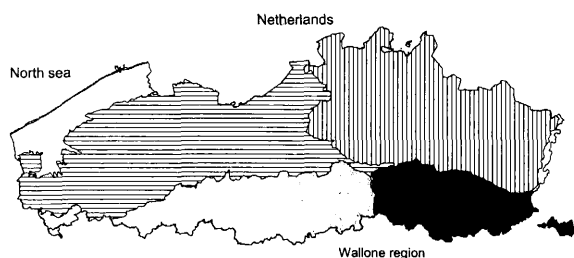


Fig. 1a. General provenance areas for autochthonous trees and shrubs in Flanders. White area: Coastal area (dunes and polders), horizontal barred area: Sandy Flanders (soil type: sand to sandy loam), vertical barred area: the Campine (soil type: sand), light grey area: Brabant District East (loamy soils) and dark grey area: Brabant District West (loamy soils).

respectively. In the High Campine soils are poorer and dryer in comparison to the low Campine.

Next to ecological conditions, knowledge on the spatial distribution of the genetic diversity of autochthonous populations further refines the delineation of provenance areas. As the current pattern of cpDNA diversity reflects the evolutionary history of oaks since the last glaciation, it is applicable in the delineation of conservation units (NEWTON *et al.* 1999; PETIT *et al.* 2002a). Autochthonous *Q. petraea* and *Q. robur* of the Italian migration lineage (haplotype 1) grow in the eastern part of Flanders, which points out the eastern border of the distribution area of this lineage. Descendants of the Spanish lineage (haplotype 10 and 12) can be found dispersed in Flanders (COART 2003; KÖNIG *et al.* 2002). All coppice and singled coppice stands analysed show a uniform haplotype indicating undisturbed populations, except Qpkabouterkensberg 97–101 and Qrkabouterkensberg 97–101 (Table 1). In this case mixing of haplotypes 10 and 12, both originating from the Iberian peninsula lineage, can be a natural phenomenon as both haplotypes migrated together (PETIT *et al.* 2002b), although human influence cannot be excluded.

Conservation units for *Q. petraea*

For *Q. petraea* a provenance area is drawn that consists of the High Campine (n° 1 to 4 in Table 1 and Fig. 1b). A distinction between Low and High Campine is relevant because of the geographic grouping of stands 1 to 4, all monotypic for haplotype 1, in the latter (Fig. 1b). In the Low Campine only one stand is present, QPkabouterkensberg97–101, that shows Iberian peninsula descendants (haplotypes 10 and 12 in Fig. 1b and Table 1). The smaller circumferences of the stools (5 to 6 m at soil level), that indicate a younger age, the low

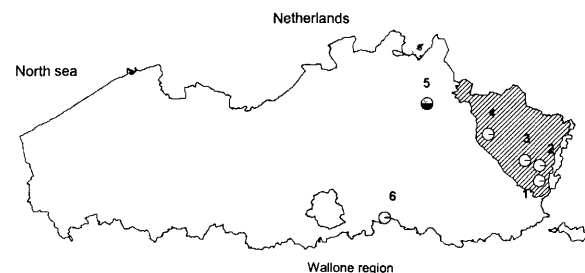


Fig. 1b. Location of autochthonous *Quercus petraea* sites and provenance area. Tilted bars: provenance area of the High Campine. Shading within circles indicates the haplotypes: white: haplotype 1, light grey haplotype 10, dark grey: haplotype 12. Circles with one type of shade indicate stands with monotypic haplotype, different shadings indicate polytypic stands.

number of stools, the mixed haplotypes and the absence of other similar stands in the neighbourhood are all reasons not to define a provenance area for the Spanish lineage descendants. Haplotype 10 is present in the singled coppice stand QPrenisart97-170 (Table 1) in Meerdaalforest located in Brabants District East. Meerdaalforest is a large forest of 1300 ha. About 40 % of the surface holds oak stands. For QPrenisart97-170, the unfeasibility to assess the age of singled coppice as the circumference of the original stools cannot be traced, is a disadvantage. Singling may have been performed on extended old stools or on narrow and younger stools. Younger stools are possibly planted with allochthonous provenances. Or, younger stools can be offspring of original autochthonous material, but one to several generations may have already passed since the Middle Ages when imported oaks planted in the neighbourhood may have influenced the genetic information through pollination. Because cpDNA is maternally inherited, genetic pollution of the autochthonous genomes through pollination with allochthonous fathers is impossible to trace. CpDNA analysis of oak stands in the neighbourhood, that were in origin maintained in a coppice with standards system, showed the presence of Balkan lineage descendants (haplotype 7, PETIT *et al.* 2002b) intermixed with Spanish lineage haplotypes (unpublished results E. COART). As the Balkan lineage does not naturally occur in Flanders (PETIT *et al.* 2002b; KÖNIG *et al.* 2002), the presence of this haplotype 7 definitely proves import of allochthonous seeds. Therefore, improvement of autochthonous seed sources in Meerdaalforest (see lower) is less advisable as allochthonous influence in the offspring would be evident through both near and distant pollen flow (STREIFF *et al.* 1999; BUIEVELD *et al.* 2001). These are the main reasons not to define a provenance area in Brabants District East. As there are no autochthonous stands for *Q. petraea* in the sandy Flanders area (Fig. 1b) there is again no ground for defining a provenance area.

Conservation units for *Q. robur*

In QRrobertii98-79 only a few stools of *Q. robur* are present that show the Italian haplotype 1 (no. 7 in Table 1 and Fig. 1c) and they are intermixed with *Q. petraea* and *Q.x rosacea* stools. Also, no similar Italian peninsula descendant stands in the neighbourhood are present. These are the reasons not to define a provenance area for this haplotype. In contrast, QRgemeentebos 98-133 is a stand with many coppice stools, monotypic for haplotype 10 and a good candidate for improvement as official seed source (see lower). As

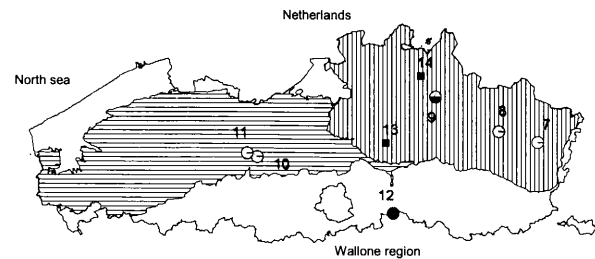


Fig. 1c. Location of autochthonous *Quercus robur* sites and provenance areas. Horizontal bars: provenance area of Sandy Flanders, vertical bars: provenance area of the Campine. Shading within circles indicates the haplotype: white: haplotype 1, light grey haplotype 10, dark grey: haplotype 12. Circles with one type of shade are monotypic stands, different shadings indicate polytypic stands. Squares indicate stands that are not yet tested for haplotype presence.

haplotype 10 is also present in QRkabouterkensberg 97-101 (n° 9 in Table 1 and Fig. 1c), an autochthonous provenance area for the whole Campine is defined, taking into account only the Spanish lineage descendants (haplotype 10). The haplotype of QRbeerzel01, QRkonijnenberg01 and QRgerheide01 still have to be determined (n° 13, 14 and 15 in Table 1 and Fig. 1c). The sandy Flanders region (Fig. 1c) is defined as another provenance area for *Q. robur*. Only Spanish descendants are known (n° 10 and 11 both monotypic for haplotype 10, Table 1 and Fig. 1c). For Brabants district east no provenance area is defined. The same negative aspects that hamper the improvement as seed source as mentioned above for QPrenisart97-170 are present for QRmeerdaalwoud97-166 with singled coppice stools in Meerdaal forest (no. 12 in Table 1 and Fig. 1c).

Improvement of autochthonous seed sources and accompanying legislation

As provenance areas are defined as conservation units, *in situ* seed sources can be officially approved to allow appropriate certification and commercialisation of harvested acorns and the marketing of forest reproductive material. A first criterion for the validation of *in situ* seed sources is an appropriate isolation of allochthonous pollen sources in the neighbourhood. Diverse studies demonstrated fertilisation of oaks by pollen originating from trees outside the stand (*e.g.* STREIFF *et al.* 1999; BAKKER 2001). Therefore, the criteria for buffer zones where no or little contaminating pollen sources should be present are difficult to draw up. Within the presented potential seed sources (Table 1), QRkonijnenberg01 and the stands in Meer-

daal forest (QPrenisart97–170 and Qrmeerdaalwoud 97–166) are discarded because of an oak plantation right next to the stand or vast amounts of possible allochthonous stands in the vicinity, respectively.

As economic quality of autochthonous offspring from the seed sources is unknown, the next criterion for commercial seed production is an appropriate certification. European legislation (EU directive 1999/105/EG) necessitates the adaptation of national legislation on the marketing of forest reproductive material by the beginning of 2003. In Flanders' legislation, the possibility is included to certify reproductive material of autochthonous oaks within the category 'source identified', so there is no need for economic assessments. Also, the Flemish legislation allows the addition of autochthonous seed sources on a list of recommended provenances. The usage of these provenances, both by private owners and by public organisations, is extra subsidised by the Flemish Community. The seed sources as indicated in Table 2 will be approved in the course of 2003.

Table 2. Number of autochthonous seed sources to be approved as category 'source identified' in 2003. CpDNA haplotype is indicated between brackets.

Provenance area	High Campine	Campine	Sandy Flanders
Species			
<i>Q. petraea</i>	3 (1)	–	–
<i>Q. robur</i>	–	1 (10)	2 (10)

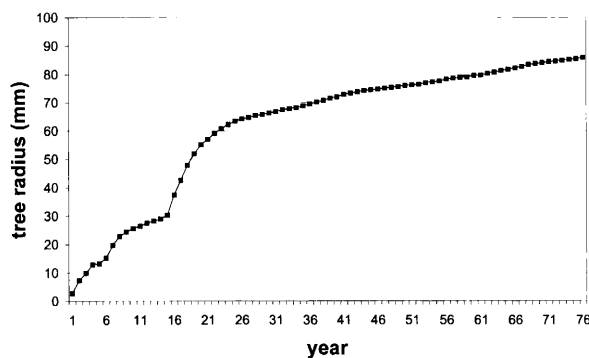


Fig. 2. Cumulative tree ring widths of coppice oak.

Living gene banks

The oldest stools that show circumference at soil level of 8 m and more are collected for *ex situ* preservation. To exclude some of the possible pollination from allochthonous fathers, the mother plants are vegeta-

tively propagated. Therefore, two grafting methods were assessed in the winter of 2001–2002 (Material and Methods). To our surprise, grafting on the central root of rootstocks gave a success rate many times higher than standard grafting on the stem of rootstock (Table 3 and Fig. 3). This grafting technique allows easy propagation for the living gene banks. These will be planted separately per species, per provenance area and per haplotype. This partitioning is a necessary cautiousness for long-term projects as views may change in the future when new insights may grow from innovating scientific analyses or from novel silvicultural methods. The plantations will be laid out according to standard practices for seed orchards.

A cultural reflection: reinstalling the coppice practice

Coppice wood as a silvicultural practice was abandoned at the end of the 19th century and the beginning of the 20th century. From a historical cultural viewpoint the coppice practice was reinstalled on a small scale in QR Robertii98–79 (Table 1). The aim is to apply the coppice system on a surface of 5 ha consisting of 10 compartments of each 0.5 ha and a rotation cut every 20 years. To this purpose, an area of 625 m² was cut in the winter of 2001–2002 and stools were protected with fences against wild life damage. 23 stools and 14 individual trees sprouted the next growing season. 9 stools and 5 individual trees died. Thus, stools can be lost and decisions on reinstalling coppice practice of old and neglected stools should be considered carefully. To date the last coppice cut in QR Robertii98–79, growth rings were counted on a representative pool of a coppice stool and showed that it was performed in 1927 or 1928 (Fig. 2). The chronology of tree ring widths show that during the first 25 years the stems grew fast, indicating the absence of competition for light. The next year growth was strongly hampered probably by crown closure, as the stems of the coppice wood grow locally in high densities.

DISCUSSION

Molecular contributions to the conservation efforts

Application of intraspecific variation, measured with molecular tools, for the conception of conservation strategies of autochthonous woody plants is relevant as this information can be supplementary to field data and, as NEWTON *et al.* (1999) declared, should be the focus of conservation genetics. BAKKER (2001) stated that for practical conservation measures for the autochthonous

Table 3. Success rate of two grafting techniques. The source stand of the scions (column 'source material'), the number of grafts and the number of mother trees from which scions were cut (between brackets) are indicated. The succeeded plants were counted after the first growth season.

Source material	Grafts on central root stock		Grafts on above ground stem of stock (normal graft)	
	grafts	succeeded	grafts	succeeded
QRspeelbos97-83	65 (13)	29 (12)	52 (13)	1 (1)
QRgemeentebos98-133	25 (5)	11 (4)	23 (5)	1 (1)
QRrobertii98-79	20 (4)	14 (4)	20 (4)	1 (1)



Fig. 3a. Scions grafted on stem of potted root stocks. Picture taken during first growth season.



Fig. 3b. Scions grafted on central root of root stocks. Picture taken during first growth season.

oaks in the Netherlands molecular markers are of aid in species identification, clone identification, description of genetic variation and structure of populations and paternity analysis. To our perception, mainly the two last topics are most interesting. Although the separation of the closely related oak species is possible with molecular techniques (MUIR *et al.* 2000; COART *et al.* 2002), leaf morphology also clearly differentiates both species (^{Kremer} *et al.* 2003). Furthermore, the hybrid *Q. × rosacea*, that shows intermediate traits between the parent species, occurs when *Q. petraea* is present. Therefore hybrids and *Q. petraea* are considered together in conservation actions (Table 1).

As stated before, the conservation of autochthonous oaks in Flanders focuses on the protection of the remaining populations and on the usage of the autochthonous genetic diversity for future plantations. Especially the second goal requires the delineation of conservation units as a first necessary conservation step (KING & BURKE 1999). Heritable genetic information offers an objective means for the demarcation of these working units. Here, the local geographic distribution of cpDNA diversity of old coppice stands in Flanders (COART 2003; KÖNIG *et al.* 2002) contributed to the delineation of provenance areas, the conservation units

for autochthonous oak populations. They reflect evolutionary lineages because cpDNA is inherited maternally and provides information on the maternal history, returning to the time of the last glaciation (PETIT *et al.* 2002a; PETIT *et al.* 2002b). But, it does not trace influences of pollination and more important, it was shown that any correlation between phenotypic traits and haplotype has disappeared (KREMER *et al.* 2002). It can therefore be postulated that the lack of proven correlation with adaptive traits makes cpDNA diversity less relevant in conservation. However, the old autochthonous coppice stands represent both a biological (imprint of colonisation routes) and cultural heritage (of coppice practice). For these reasons, we consider the cpDNA data relevant in delineating conservation units, as long as the current levels of nuclear diversity and patterns of differentiation among populations are not disregarded. Altogether, keeping haplotype lineages separate is a conservation strategy that operates from a cautious point of view. Why mixing haplotypes as natural forces kept them separate?

It could be reasoned that gathering acorns from one or a few superior individuals and sowing or planting the seedlings would create monotypic stands, the ones that we consider autochthonous. However, two arguments

contradict this hypothesis. First of all, the evaluation of the autochthonous character based on cpdiversity was in almost all cases in agreement with the field evaluation (see results). Secondly, a comparison between allochthonous and autochthonous stands in Flanders clearly showed that allochthonous stands are more polytypic in comparison to the autochthonous ones (COART 2003).

Natural distribution of autochthonous *Q. petraea*

Q. petraea has no coppice remains in the western part of Flanders. One possible explanation is that *Q. petraea* does not naturally occur in this region. The ecological amplitude of *Q. petraea* goes beyond the borders of *Q. robur* on extremely dry and acid sandy soils (e.g. BARY-LENGER & NEBOUT 1993). GALOUX (1953) already noticed that pedunculate oak takes the place of sessile oak in the High Campine when the water table became less deep. Possibly *Q. robur* showed more affinity for lowland and river valleys in sandy Flanders, whereas sessile oak survived better on dry and extremely poor sandy soils, that are more typical for the High Campine. In analogy, natural stands of sessile oak are more abundant on lighter, drier and more acid soils in comparison to *Q. robur* in England (e.g. TANSLEY 1949). In the natural distribution area of *Q. petraea* drawn by BARY-LENGER & NEBOUT (1993) sandy Flanders is not included. But, the latter data are based on recent surveys and do not rule out the possibility of extinction of *Q. petraea* as a second explanation. *Q. petraea* does follow the coastal line in France roughly up to the Belgian-French border and is present in England, so extinction due to human impact in sandy Flanders is reasonable. The fixation of poor and acid sand dunes occurring more regularly in the Campine may have augmented the chances of survival of sessile oak in this region, whereas in the western part of Flanders they may have suffered more from deforestations and intensive forest use in the past.

Expansion of the autochthonous genetic information

The commercialisation of autochthonous plant material is a means to expand the autochthonous genetic information and will be accompanied with proper communication with the end user, a condition clearly stated by KING & BURKE (1999). Several considerations should be taken into account. Firstly, autochthonous plant material holds no guarantee for economic quality. Therefore, these provenances are only advised in plant actions where nature conservation is a more important

issue in comparison to wood production. This will involve rather a minority of oak plantations in Flanders. In the view of future climatic changes and taking in consideration the widely dispersed oak decline, vitality and local adaptation may become important criteria for plantations of oak next to economic motives.

Furthermore, recent research pointed out that both for *Q. petraea* and *Q. robur*, recommended provenances exhibit slightly lower within-population diversities and have higher heterozygote deficiencies than autochthonous stands, although these differences were not significant (COART 2003). It is thus clear that in order to safeguard the genetic variability in future oak forests, there is no reason to choose recommended provenances over autochthonous populations as seed sources. For plantations of oak with a productive function recently a list of recommended provenances for Flanders is elaborated

(<http://www.ibw.vlaanderen.be/ned/bossen/genet/text/aanbev%20herk%20folder2.pdf>) that includes 11 official seed stands for *Q. robur* and 2 stands (in the same forest) for *Q. petraea* in Flanders. In comparison, 4 stands for *Q. robur* and 2 for *Q. petraea* will be approved as autochthonous seed stands (Table 2) that will be added to the mentioned list of recommended provenances. Each autochthonous stand in Table 1 consists of a minimum of 30 individuals, but most of them count many more trees (column 'surface' in Table 1). Also, the survey of Flanders for autochthonous trees and shrubs is not yet finished, so the detection of additional autochthonous oak stands can be expected.

In the High Campine area all autochthonous seed sources are located for *Q. petraea*. In this area it is recommended to plant autochthonous *Q. petraea* also in regular forests. Because many conifers have been planted during the past centennial for the coal mining industry only 6.7 % of the forests in the Campine are oak stands with basal area above 50%. This type of stands count up to 13.2 % and 15.2 % of the forests in the loam region (Brabant District East together with Brabant District West) and sandy Flanders respectively (Forest and Green Area Division 2002). Thus, the amount of possible allochthonous oak plantation is smaller in the Campine in comparison to the other regions. In addition, on the High Campine plateau oak is the only indigenous deciduous tree species that survives properly on the poor and dry sandy soils. As the usage of indigenous species is promoted in general, planting of allochthonous provenances of oak should be held back. The preservation of the remaining autochthonous stands from being mixed in large amounts with allochthonous genomes through pollen flow is more valuable. A selection of *Q. petraea* coppice stools in the High Campine for which offspring shows high

economic value meets the aims of both conservationists demanding autochthony and foresters working towards wood quality. This program will be started in 2003.

Secondly, as autochthony is regarded to be related to local growth conditions, plant material originating from a provenance area should be planted within this area. A first major drawback is the provenance areas that do not cover the whole of Flanders territory. When autochthonous plant material of oak is highly recommended outside these areas, it is advised either to abandon oak and look for other autochthonous species, or to use material that originates from the official seed sources. A second major drawback lies within the current Flemish legislation. Autochthonous provenances can be appropriately certified and, even more, the usage will be subsidised. These subsidies are dependent on the species and the provenance used, but does not restrict the planting area. The only way to tackle this problem is an appropriate communication with the end-user. Finally, as mentioned before, the living gene banks

A sustainable silvicultural practice

Autochthonous woody plants are the last indicators of the natural woody vegetation on a particular location. Still, anthropogenic influences on the autochthonous growth sites are impossible to think away. Even more, in the case of coppice oak, the silvicultural practice enlarged the life-span of the stools. During the last centennial memories of historical human activities have faded quickly. This includes the historical coppice practice that was reconverted to high forest by the end of the 19th and the beginning of the 20th century. Because the coppice system supplies a self-renewing and thus durable energy production, coppice practice on larger areas nowadays may not only be of interest from a cultural-historical point of view. Since the increasing consciousness of consumers for the green issues, marketing of coppice products may become realistic, such as charcoal, oak bark for tanning, rustic poles, furniture,... Our small-scale experiment showed that reinstalling the coppice practice on neglected stools is not without danger of losing autochthonous genotypes, but is a feasible act when the largest stools are protected in *ex situ* collections.

Conclusion and future perspectives

In summary, a field survey together with molecular marker data gave us the necessary tools to work out conservation units for autochthonous oaks and to define strategies for the extension of autochthonous gene

sources. Future cpDNA analysis is needed for some recently surveyed coppice stands. In addition, appropriate molecular markers may contribute to proper control of seed lots during the marketing process.

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