Comparison of mechanical, biological and chemical methods for controlling Black cherry (*Prunus serotina*) in Flanders (Belgium)

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Abstract

Large scale application of Black cherry (*Prunus serotina* Ehrh.) in forestry and the typical ecological characteristics of the species resulted in its dominance of the shrublayer in homogeneous pine forests on poor sandy soils in Flanders. For reasons of nature conservation Flemish forest policy and management tries to transform these forests into more natural and structural deciduous forests. Controlling Black cherry is seen as a major condition for succes in this matter.
In this study the lethal effect of mechanical, biological and chemical control methods was tested using an experimental block design. The methods consisted of girdling and felling with periodical removal of new sprouts, stump and stem treatment with mycoherbicide based on the Silverleaf disease fungus (*Chondrostereum purpureum* Pouzar), leaf and stump treatment with the herbicide glyphosate and stem treatments with the same herbicide using different techniques like the Hack & Squirt method, the EZject Capsule Injection System and the Silvaxe Tree Injector. The results indicated that a proper comparison can only be made after evaluation in the second autumn after treatment at the earliest. All chemical control methods, except one (Silvaxe Tree Injector System), showed a significant higher proportional mortality than mechanical and biological control methods. No statistical proof was found of a seasonal influence on mortality for the chemical methods. Application of the Hack & Squirt method and the EZject Capsule Injection System in summertime resulted in a significantly higher proportional mortality than leaf treatment and stump treatment in autumn. Lethal doses of glyphosate for different dbh-classes were calculated resulting in easy to use application procedures for stem treatment. The high lethal effect of the Hack & Squirt method confirmed their practical value. No significant seasonal influence on mortality nor any significant impact of mycoherbicide concentration was found for the biological stump treatments. Stem treatment with mycoherbicide proved inappropriate. The best season for girdling appears to be springtime while additional test indicate that wintertime could be even more appropriate. The lowest significant proportional mortality for girdling and felling with one additional removal of sprouts was found in summertime. A high variability in proportional mortality was detected for the mechanical methods and in a lesser degree for the biological methods, sometimes approximating or even equaling the mortality rate of chemical methods. If environmental considerations have to be taken into account or if chemical control is legally forbidden (e.g. forest reserves in Flanders) biological stump treatment and girdling offer a valuable alternative. Their variable mortality can lead to unpredictable results though.

Keywords: *Prunus serotina, block design, girdling, felling, mycoherbicide, Chondrostereum purpureum P., glyphosate*

1. Introduction

At the beginning of this century Black Cherry (*Prunus serotina* Ehrh.) was predominantly used in Belgian forestry in coppice, for fixation of continental dunes, as shrublayer in pine stands and together with other broadleaved tree species for fire prevention in shelterbelts around new pine stands (Rouffignon 1899, C.J.Q. 1921, De Neunheuser 1922, Goblet d'Alviella 1922, Masson 1920). It wasn't until after 1930 that the species was planted on a large scale underneath pine stands on poor sandy soils for reasons of soil improvement (Ab. 1951, Misson 1930, Muys et al 1992). This fact, together with the species specific ecological characteristics of large reproductive capacity and tolerance (Starfinger 1990), formed an ideal starting point for further dissemination resulting in massive dominance of the shrublayer in an estimated 50,000 ha of pine forests, especially in the Campine Region of Flanders (Muys & Maddelein 1993, Van Den Meersschaut & Lust 1996). This current situation is attended by important negative consequences for nature conservation and forestry. The dominant occurrence of Black cherry hampers all transformation of homogeneous coniferous plantations into more natural and structural deciduous forests. Controlling Black cherry is generally recognized as a major condition for achieving this goal. This study makes a contribution to the control of this species by comparing the lethal effect of several mechanical, biological and chemical methods. The main aim was to find environmentaly friendly alternative control methods for the chemical methods with the same or comparable lethal effectiveness. Seasonal influence on mortality of some methods was investigated along with the impact of site effects and of different concentrations of mycoherbicide for the biological methods. Chemical non-felling techniques were adopted and examined for these purposes after determination of the required lethal dose of herbicide in function of different dbh-classes. Finally the importance of the evaluation period (required time-lag between treatment and evaluation) was investigated.

2. Material and methods

2.1. Study site and experimental design

The tests were executed in the State Forests 'Staatsbos Ravels' (province of Antwerp - town of Ravels) and 'Pijnven' (province of Limburg - towns of Hechtel/Eksel) in the period 1994-1995. Both forests belong to the Campine Region of Flanders and are located on predominantly poor sandy soils (Table 1). They were planted at the beginning of this century on former heathlands and mainly consist of coniferous tree species (mostly Pine) in the upperstorey and Black cherry in de understorey.

In order to incorporate different site conditions a block design was elaborated consisting of 4 blocks, 2 in each forest. Within each block the same methods for controlling Black cherry were carried out and every method or treatment was applied on a hundred trees, totalling 400 trees per treatment. The minimum stemdiameter at breast height (dbh) of the trees was put to 3 cm.

Table 1. Overview of the block design with a description of dominant tree species and soil type

Block	Forest	Dominant tree species	Soil type
A	Staatsbos Ravels	Scots pine	moderately wet to wet loamy sandy soil with a conspicuous humus and/or iron B horizont (Sdg - Seg)
B	Staatsbos Ravels	Corsican and Scots pine	moderately wet to wet sandy soil (gradually becoming fine-grained in depth) with a conspicuous humus and/or iron B horizont (Zdgy - Zegy)
C	Pijnven	Corsican pine	dry to moderately dry sandy soil with a conspicuous humus and/or iron B horizont (Zbg - Zcg)
D	Pijnven	Corsican pine	very dry to moderately wet sandy soil without profile development (Zap) + dry sandy soil with a conspicuous humus and/or iron B horizont and with a gravel substrate occurring on a low to medium depth (20-125 cm) and with a thin humus upper soil layer (< 20 cm) ((t)Zbgl)

2.2. Control methods

A total of nine different methods for controlling Black cherry were tested using the experimental block design. They are grouped into three major categories namely mechanical, biological and chemical methods.

2.2.1. Mechanical methods

The mechanical control methods tested in this study are based on the exhaustion of trees by interrupting or affecting their nutrient and water supply, eventually resulting in their death.
The most commonly used and widespread method is girdling (Londo, 1991). In this study trees were girdled at a height of approximately 1,5 m using a draw shave (or plane-iron) with a convex blade. The bark (phloem and cambium layer) was removed over a variable lenght of 10 to 20 cm. The trees were girdled on three different moments of the year (spring (end of May 1995), summer (end of July and end of August 1995) and autumn (end of September and beginning of October 1994)) in order to

examine seasonal influence on mortality. Each individual tree received at least one additional treatment, which included the removal of new-grown leafy sprouts under the wound using a chopper and cutting through the newly overgrown bark tissue on the wound using a tree scribe. This operation was carried out approximately 6 weeks after girdling, except for the trees that were girdled in autumn. The additional treatment of the latter was carried out in spring of the following year. Some were treated in this manner twice.
The second tested mechanical control method consisted in felling the tree at a height between 0.5 to 1 m. As with girdling the trees were felled on the same three different moments of the year (spring, summer and autumn) in order to examine seasonal influence on mortality. Each individual tree received at least one additional treatment, which included the removal of new-grown leafy sprouts using a chopper. The period in which these operations were carried out is the same as those for girdling. Some were treated in this manner twice.

2.2.2. Biological methods

The biological control methods tested in this study are based on the application of the fungus Silverleaf disease (*Chondrostereum purpureum* Pouzar) as a biological control agent (myco-herbicide). Besides being a saprophyte on dead wood Silverleaf disease also has parasitic properties, especially on plants of the family Rosaceae (de Jong 1988). In natural circumstances basidiospores can germinate on fresh tree wounds. Once germinated the mycelium is able to colonize the wood after which the disease symptoms become visible, a dull gleaming silvery discoloration of the leaves. This symptom is caused by one or several still unidentified toxines excreted by the mycelium into the sapflow and upto the leaves where they are able to tear the epidermis from the palissade-parenchyme (Bishop 1979, Scheepens & Hoogerbrugge 1988, Deacon 1984). This proces hampers photosynthesis and transpiration. The real cause of death though is the interruption of the sapflow because the mycelium blocks the wood vessels (Butler & Jones 1949). The myco-herbicide used in this study was produced by the Dutch firm Koppert Biological Systems (P.O. Box 155, 2650 AD Berkel en Rodenrijs, the Netherlands) as a ready to use product based on a watery solution of mycelium of the fungus. It is recently commercialized under the trade name 'Biochon' (de Jong et al 1998). In general it is applied on fresh wounds in a 10% concentration with water.
The first tested biological method was stump treatment with myco-herbicide. The trees were felled at ground level and the fresh wound was immediately inoculated with mycelium of the fungus on its entire surface by means of a brush. The first stump treatment was executed at the beginning of December 1994 using a 10% concentration of the product in water. In the following spring (middle of June 1995) similar stump treatments were made with 10%, 5% and 2% concentrations. The production firm advises application only during the months of April, May, June, September, October and November. The mycelium can survive during winter and summer months though its growth is rather limited due to respectively low temperatures and (probably) a low concentration of soluble carbonhydrates in the wood (Butler & Jones 1949, Scheepens & Hoogerbrugge 1988, Bishop 1979).
The second method was entirely based on the growth characteristics of mycelium in the wood and its blocking effect. With a chainsaw two opposed cuts were made on both sides of the stems each covering half of the cross-section of the stem. An average distance of 0.5 m was kept between both cuts to assure the strenght of the stem and prevent it from breaking. The upper- and underside of both cuts were inoculated with a 10% mycelium concentration using a portable pump sprayer. The treatment was carried out in springtime (middle of June 1995). The rather rapid growth of 4 mm/day of

the mycelium in vertical direction (de Jong & Scheepens 1985) should eventually lead to the blocking of the entire cross-section. Inoculation of both halves of the cross-section is important because lateral growth of the mycelium is very slow (Butler & Jones 1949).

2.2.3. Chemical methods

The five chemical control methods in this study are based on the application of herbicides containing the active component glyphosate. All treatments were made with the herbicide Viking which contains 270 gram glyphosate per liter product. It's a systematic, non-selective contact herbicide with a broad application spectrum ranging from annual and perennial grasses to woody plants (Grossbard & Atkinson 1985, Lanz 1977, Roediger 1979, Sacher 1978). After uptake through leaves or wounds in the stem or branches the herbicide is translocated to the rootsystem where it interferes with the 'Shikimate-acid cycle', a metabolic proceschain characteristic to plants (Freedman 1991, Van Oorschot & Van Rensen 1990).

The first tested chemical method was leaf treatment. The trees were felled at ground level at the end of Januari 1995. Almost all of them grew new leafy sprouts which were sprinkled in the second half of July 1995 with a recommended 1.4% Viking solution in water using a portable pump sprayer (Jager 1977, Jager & Oosterbaan 1979, Oosterbaan 1981, van den Tweel 1984, Miller & Mitchell 1990, Eijsackers 1991).

The second tested chemical method was stump treatment. The trees were felled at ground level and the fresh wound was immediately treated with a recommended 7% Viking solution in water using a brush (Jager & Oosterbaan 1979, van den Tweel 1984). The stump treatment was applied in autumn (beginning of November 1994) and summer (middle of July 1995).

The following three chemical methods can be grouped as 'stem treatments' because the trees aren't felled but killed while standing. The techniques and instruments involved are adopted from North America where they are used for chemical thinnings in forestry practice. Stem treatment with herbicides was illustrated for other tree species like North American *Populus sp., Prunus sp., Tsuga sp., Salix sp., Betula sp.* and *Acer sp, Alnus rubra, Pinus contorta, Pseudotsuga menziesii, Betula pubescens* and *Quercus robur (Scholz & Wulf 1998, Taylor 1992, Wagner et al 1994).* The first stem treatment tested in this study is the EZject Capsule Injection System, a product of Monsanto Canada Inc (55 Murray Park Road, Winnipeg, Manitoba, Canada). The instrument consists of a 1.5 m long metal cilinder containing 4 seperate loaders for a total of 400 capsules and a springsystem for shooting or ejecting the capsules into the stem. Each capsule is made out of a coppery shell (like an empty gun shell) filled with pure glyphosate (0.15 gram) mixed with a sucrose solution. The instrument is placed at a 30 to 45° angle against the stem as close to the ground as possible. Metal teeth prevent it from slipping away. A capsule is partially shot into the stem by a short but powerful stroke. Trees with a dbh < 20 cm require one capsule every 5 cm stem circumference. Bigger trees require one every 3 cm stem circumference. The EZject Capsule Injection System was applied in winter (beginning of February 1995) and summertime (middle of July 1995).

The second stem treatment was the Hack and Squirt method. With a hatchet notches are made around the stem under an angle of 45° and at an average stem height of 1 m. Each notch is sprinkled with a certain amount of herbicide using an ordinary spray bottle. In order to know the exact number of notches and the exact amount of herbicide per tree the lethal dose of herbicide needed to be calculated in function of the tree diameter. These calculations were based on the prescriptions for the EZject Capsule Injection System and are presented in table 2.

Table 2. Calculated lethal doses of glyphosate and Viking, number of notches and amount of Viking used in this study in function of different dbh-classes

dbh-class (cm)	Stem circumference (cm)	Lethal dose of glyphosate (gram)	Lethal dose of Viking (ml)	Number of notches	Amount of Viking used (ml)
< 5	< 15	0.75	1.7	1	2
5 - 10	15 - 30	0.90	3.3	2	4
10 - 15	30 - 45	1.35	5.0	3	6
15 - 20	45 - 60	1.80	6.7	4	8
20 - 25	60 - 80	4.05	15.0	8	16
> 25	> 80	> 4.05	> 15.0	> 8	> 16

These calculations resulted in an easy to use procedure. For trees with dbh < 20 cm every 15 cm stem circumference a notch is made. For trees with dbh > 20 cm the number of notches increases to every 10 cm. Every single notch is sprinkled with 2 ml of pure viking herbicide. The Hack & Squirt method was applied in summertime (middle of July 1995).
The third stem treatment was based on the use of the Silvaxe Tree Injector (Silvicultural Implement Company Inc, P.O. Box 36542, Birmingham, Al 35236[1]) which combines the actions of the Hack & Squirt method in one single action. The instrument looks like an ordinary axe but is attached to a reservoir, worn as a backpack, by a plastic tube. The axe-handle is hollow and the blade is internaly provided with a springsystem that allows an automatical injection of an average amount of 1 ml of herbicide into the stem with every stroke. Based on the calculations in table 2 an easy to use procedure could be derived. For trees with dbh < 20 cm every 7 to 8 cm stem circumference a notch is made. For trees with dbh > 20 cm the number of notches increases to every 10 cm. This method was applied in springtime (beginning of April 1995).

2.3. Additional tests

In the forest 'Staatsbos Ravels' three additional girdling tests were executed on a total surface of 1.25 ha. The site was characterized by moderately wet to moderately dry sandy soils with a conspicuous humus and/or iron B horizont and with dominance of Scots pine in the upper treelayer. All Black cherry trees with a dbh > 5 to 7 cm were taken into account. The first test was executed at the end of November 1994 on a surface of 0.25 ha. After girdling the trees didn't receive any additional treatment. The two other tests took place at the end of February 1995, both on a surface of 0.5 ha. Only one of them received an additional treatment six weeks after girdling.
Additional biological tests were performed at the same locations as the experimental block design in both forests. At the beginning of December 1994 a hundred trees in block B received two machete cuts on opposite sides of the stem. Each cut was treated with a 10% concentration of the mycoherbicide previously mentioned, using a portable pump sprayer. Application of a pump sprayer requires a low spray pressure and a large diameter of the spray head (Ø ≥ 1.5 mm) because the mycelium is very vulnerable and sensible to fragmentation (Wagner et al 1994). Another test, a stump

[1] The Silvaxe Tree Injector was supplied by the Ben Meadows Company (3589 Broad Street, Atlanta, Georgia 30341, USA)

treatment with mycoherbicide, was situated in block A and executed in the middle of June 1995. A hundred trees were felled and treated with a 10% concentration of mycoherbicide using a portable pump sprayer. In the same period another stump treatment with mycoherbicide was executed in block C and D using a brushcutter for felling instead of a chainsaw. Again a 10% concentration and a portable pump sprayer were used. In order to assess natural infection by Silverleaf disease and natural mortality trees were felled at ground level at the same location. The stumps didn't receive any additional treatment (= blanc test).
At the same locations as the experimental block design in the forest 'Staatsbos Ravels' additional chemical tests were made using the Silvaxe Tree Injector. The tests were executed in both blocks (A and B) in the middle of July 1995. In each block a hundred trees were treated with pure Roundup (356 gram glyphosate per liter product) instead of Viking.

2.4. Overview

For convenience the different tests were encoded by means of a letter and cipher combination. Table 3 gives a global overview of the different codes used. The first letter in the code normally refers to the block of the experimental block design, except for the additional girdling tests which were executed elsewhere.

Table 3. Overview of the tested control methods and their corresponding codes

Code	Description of the test
A,B,C,D1a1	girdling in autumn '94 + one additional treatment (= removal of new-grown sprouts and overgrown bark tissue)
A,B,C,D1a2	girdling in autumn '94 + two additional treatments
A,B,C,D1b1	girdling in spring '95 + one additional treatment
C,D1b2	girdling in spring '95 + two additional treatments
A,B,C,D1c	girdling in summer '95 + one additional treatment
A,B,C,D2a1	tree felling in autumn '94 + one additional treatment (= removal of new-grown
A,B,C,D2a2	sprouts)
A,B,C,D2b	tree felling in autumn '94 + two additional treatments
A,B,C,D2c	tree felling in spring '95 + one additional treatment
	tree felling in summer '95 + one additional treatment
A,B,C,D3a	stump treatment with a 10% concentration of mycoherbicide in autumn '94
A,B,C,D3b	stump treatment with a 10% concentration of mycoherbicide in spring '95
A,B,C,D3c	stump treatment with a 5% concentration of mycoherbicide in spring '95
A,B,C,D3d	stump treatment with a 2% concentration of mycoherbicide in spring '95
A,B,C,D4	stem treatment (chainsaw cuts) with a 10% concentration of mycoherbicide in spring '95

A,B,C,D6	leaf treatment with herbicide
A,B,C,D7a	stump treatment with herbicide in autumn '94
A,B,C,D7b	stump treatment with herbicide in summer '95
A,B,C,D8a	Silvaxe Tree Injector application in spring '95
A,B8b	Silvaxe Tree Injector application in summer '95
A,B,C,D9a	EZject Capsule Injection System application in winter '94-'95
A,B,C,D9b	EZject Capsule Injection System application in summer '95
A,B,C,D10	Hack & Squirt method in summer '95
C,D3e	tree felling at ground level in spring '95 with no additional treatment (blanc test)
B11	stem treatment (machete cuts) with a 10% concentration of mycoherbicide in autumn '94
A12	stump treatment with a 10% concentration of mycoherbicide in spring '95 using a portable pump sprayer
C,D13	stump treatment with a 10% concentration of mycoherbicide in spring '95 using a brushcutter for felling
G1	girdling in autumn '94 without additional treatment
G2	girdling in winter '95 + one additional treatment
G3	girdling in winter '95 without additional treatment

3. Results

Most tests were evaluated in the autumn of 1995 and the autumn of 1996. The results of the girdling tests are presented in table 4. A distinction is made between dead trees, trees with a dead canopy but with leafy sprouts underneath the girdling wound and trees with a green canopy. A,B,C,D1c were not evaluated in '95 because the time-lag between application and evaluation was too short to see any meaningful results yet. D1a1, D1a2, D1b1 and D1b2 were undeliberately destroyed during the test period.

Table 4. Proportional mortality for the girdling tests as evaluated in the autumn of 1995 and 1996

Test	Evaluation in '95				Evaluation in '96			
	# trees	dead (%)	dead canopy (%)	green canopy (%)	# trees	dead (%)	dead canopy (%)	green canopy (%)
A1a1	48	23	73	4	43	63	35	2
B1a1	40	13	73	15	34	38	62	0
C1a1	47	4	89	7	44	7	93	0
D1a1	51	18	63	19	-	-	-	-
Total	186	15	74	11	121	35	64	1
A1a2	49	14	74	12	51	59	37	4
B1a2	55	29	66	5	56	77	23	0
C1a2	49	33	61	6	42	43	57	0
D1a2	47	23	49	28	-	-	-	-
Total	200	25	63	12	149	61	38	1
A1b1	85	39	36	25	88	58	36	6
B1b1	100	75	24	1	104	95	4	1
C1b1	46	28	72	0	39	56	44	0
D1b1	44	11	75	14	-	-	-	-
Total	275	46	44	10	231	74	23	3
C1b2	49	49	47	4	41	54	46	0
D1b2	55	18	76	6	-	-	-	-
Total	104	33	63	5	41	54	46	0
A1c	-	-	-	-	100	6	85	9
B1c	-	-	-	-	77	7	84	9
C1c	-	-	-	-	75	11	89	0
D1c	-	-	-	-	52	25	75	0
Total	-	-	-	-	304	11	84	5
G1	198	24	34	42	200	44	16	40
G2	300	67	27	6	300	90	9	1
G3	287	55	32	13	268	83	14	3

The results of the biological tests are presented in table 5. A distinction is made between dead trees, sick trees showing Silverleaf disease symptoms and healthy trees with vital fresh green sprouts.

Table 5. Proportional mortality for the biological tests as evaluated in the autumn of 1995 and 1996

Test	Evaluation in '95				Evaluation in '96			
	# trees	dead (%)	sick (%)	healthy (%)	# trees	dead (%)	sick (%)	healthy (%)
A3a	83	22	55	23	91	46	4	50
B3a	100	10	79	11	97	64	5	31
C3a	97	28	41	31	92	57	5	38
D3a	100	35	50	15	86	84	0	16
Total	381	24	56	20	366	62	4	34
A3b	89	15	47	38	92	28	15	57
B3b	104	22	36	42	101	75	6	19
C3b	102	4	74	24	109	53	21	26
D3b	96	38	40	18	77	70	9	21
Total	391	20	49	31	379	57	13	30
A3c	92	22	58	20	93	59	5	36
B3c	103	5	49	46	105	39	15	46
C3c	98	3	69	26	108	53	24	23
D3c	100	58	28	14	90	87	3	10
Total	393	22	51	27	396	58	13	29
A3d	95	26	50	24	97	66	8	26
B3d	100	18	25	57	104	60	8	32
C3d	100	17	37	46	95	44	21	35
D3d	94	60	17	23	84	85	8	7
Total	389	30	32	38	380	63	11	26
A4	-	-	-	-	78	1	-	-
B4	-	-	-	-	59	3	-	-
C4	-	-	-	-	35	3	-	-
D4	-	-	-	-	17	0	-	-
Total	-	-	-	-	189	2	-	-
A12	88	17	70	13	87	60	9	31
B11	97	10	36	54	63	16	-	-
C13	96	6	78	16	96	78	10	12
D13	102	75	14	11	83	91	5	4
Total	198	42	45	13	179	84	8	8

The results of all the other tests are presented in table 6. Here only the percentage of dead trees is given.

Table 6. Proportional mortality for all other tests as evaluated in the autumn of 1995 and 1996

Test	Evaluation in '95		Evaluation in '96		Test	Evaluation in '95		Evaluation in '96	
	# trees	dead (%)	# trees	dead (%)		# trees	dead (%)	# trees	dead (%)
A2a1	39	23	41	63	A2a2	49	14	47	30
B2a1	48	27	46	52	B2a2	55	29	48	48
C2a1	43	7	38	8	C2a2	48	29	57	53
D2a1	51	29	48	63	D2a2	59	52	42	95
<u>Total</u>	<u>181</u>	<u>22</u>	<u>173</u>	<u>48</u>	<u>Total</u>	<u>211</u>	<u>31</u>	<u>194</u>	<u>55</u>
A2b	-	-	88	26	A2c	-	-	75	0
B2b	-	-	115	43	B2c	-	-	81	11
C2b	-	-	104	48	C2c	-	-	94	7
D2b	-	-	60	92	D2c	-	-	62	23
<u>Total</u>	-	-	<u>367</u>	<u>49</u>	<u>Total</u>	-	-	<u>312</u>	<u>10</u>
A6	86	94	90	93	A10	93	97	71	97
B6	94	84	99	95	B10	94	98	95	100
C6	89	75	94	91	C10	98	100	105	100
D6	97	99	78	99	D10	97	98	66	100
<u>Total</u>	<u>366</u>	<u>88</u>	<u>361</u>	<u>94</u>	<u>Total</u>	<u>382</u>	<u>98</u>	<u>337</u>	<u>99</u>
A7a	92	97	96	96	A7b	77	99	83	100
B7a	85	79	89	89	B7b	73	95	76	95
C7a	93	84	92	87	C7b	85	99	88	97
D7a	93	91	79	97	D7b	68	93	-	-
<u>Total</u>	<u>363</u>	<u>88</u>	<u>356</u>	<u>92</u>	<u>Total</u>	<u>303</u>	<u>96</u>	<u>247</u>	<u>97</u>
A9a	94	75	89	94	A9b	88	92	81	99
B9a	102	81	96	92	B9b	100	93	97	99
C9a	103	97	100	99	C9b	101	98	98	100
D9a	93	92	63	100	D9b	91	96	37	100
<u>Total</u>	<u>392</u>	<u>86</u>	<u>348</u>	<u>96</u>	<u>Total</u>	<u>380</u>	<u>95</u>	<u>313</u>	<u>99</u>
A8a	101	14	99	41	A8b	84	39	76	59
B8a	100	15	83	40	B8b	100	41	89	69

C8a	91	23	92	28
D8a	87	30	63	67
Total	379	20	337	42
C3e	90	4	92	25
D3e	85	28	73	68
Total	175	16	165	44

Total	184	40	165	64

For further statistical analysis proportional mortality values are used. Otherwise applying absolute numbers of evaluated trees could affect comparability between different control methods because the differences are often quite big.

In order to evaluate the influence of the evaluation time (1995 - 1996) 16 different control methods were grouped into one dataset. Only methods which were evaluated in both periods and with data for at least 3 blocks were taken into account (*1a1, *1a2, *1b1, *2a1, *2a2, *3a, *3b, *3c, *3d, *6, *7a, *7b, *8a, *9a, *9b and *10, with '*'=A,B,C and D). Four methods (*1a1, *1a2, *1b1 and *7b) had one missing value for block D for the evaluation period '96. These missing values were calculated using the Yates hand calculation procedure resulting in respectively the following values: 50, 74, 84 and 112 (Neter et al 1990). The dataset (including calculated data for the missing values) proved not to follow a normal distribution (χ^2 *p<0.001*) so that a normal multifactor analysis of variance (ANOVA) for a block design could not be performed. Instead a nonparametric ANOVA for a block design (Friedman Two-Way Analysis by Ranks procedure) was applied. Proportional mortality seemed to be significantly higher during the evaluation period '96 than during the period '95 (*Friedman p<0.001*). Also a significant difference between the blocks (*Friedman p<0.001*) and between the control methods (*Friedman p<0.001*) was detected.

For reasons of confirmation the dataset without missing values was treated and tested as a one-way design instead of a block design. This dataset also proved not to be normally distributed (χ^2 *p<0.001*) so that a nonparametric ANOVA (Kruskal-Wallis One-Way Analysis by Ranks) was applied. There was significant proof that data from both evaluation periods seemed to come from different populations (*Kruskal-Wallis p<0.001*), which confirms the previous result. On the contrary though no significant difference between the blocks was found (*Kruskal-Wallis p>0.05*), probably due to the fact that the variance between the methods masks the variance between the blocks. The previous Friedman-test is therefore more meaningful because it compares data pairwise.

The data from the evaluation period '96 were considered into more detail. Following the same previously specified criteria 20 different control methods were grouped into one dataset (*1a1, *1a2, *1b1, *1c, *2a1, *2a2, *2b, *2c, *3a, *3b, *3c, *3d, *4, *6, *7a, *7b, *8a, *9a, *9b and *10, with '*'=A,B,C and D). Their average proportional mortality rate is presented in figure 1.

Again the missing values for the same methods were calculated using the Yates hand calculation procedure resulting in respectively the following values: 56, 80, 90 and 117 (Neter et al 1990). The dataset (including calculated data for the missing values) proved not to follow a normal distribution (χ^2 *p<0.001*) so that a normal multifactor analysis of variance for a block design could not be performed. Again the Friedman Two-Way Analysis by Ranks procedure was applied. A significant influence of the blocks (*Friedman p<0.001*) and a significant difference between the control methods (*Friedman* *p<0.001*) was found. In order to examine which block differed from another the data per block were considered separately resulting in 4 datasets. Each of these datasets proved to have a normal distribution (*Kolmogorov-Smirnov p>0.05*). Only block C proved to be significantly different from block D (*t-test p<0.05*) while the difference between block A and block D seemed to be just not significant (*t-test p=0.0521907*).

In order to examine the difference between mechanical, biological and chemical control methods the original dataset without missing values was considered. This dataset also proved not to be normally distributed (χ^2 *p<0.001*), even after several attempts to normalize, so that a nonparametric ANOVA (Kruskal-Wallis One-Way Analysis by Ranks) was applied. There seemed to exist a significant difference between the three mentioned groups of methods (*Kruskal-Wallis p<0.001*). On the other hand no significant influence of the blocks was found (*Kruskal-Wallis p>0.05*). For the latter preference is given to the Friedman-test because of reasons mentioned before. In order to examine which group of methods differed from another the data were considered separately per group. Only the data of the chemical methods proved not to follow a normal distribution (*K-S p<0.05*). No significant difference was found between the mechanical and biological methods (*t-test p>0.05*). On the other hand both groups of methods proved to be significantly different from the chemical methods (*Mann-Whitney U p<0.001*).

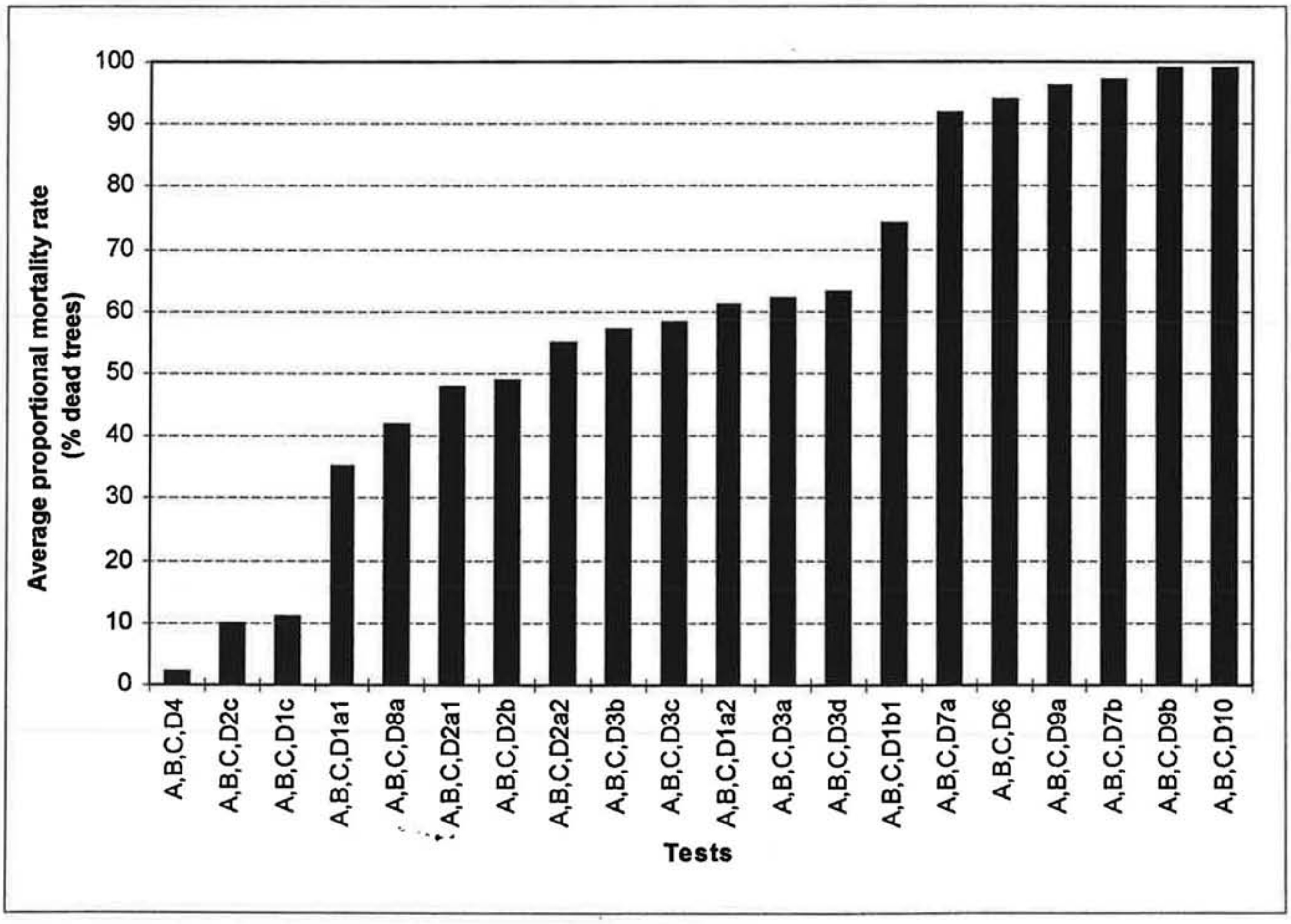

Figure 1. The average proportional mortality rates of the different tested control methods

Next each group of methods was investigated separately. The data of the chemical methods was normalized by excluding method A,B,C,D8a (*K-S* $p>0.05$). Considering its average proportional mortality rate this method can be treated as an outlier. On each group of methods a normal multifactor ANOVA for a block design could be performed.

The mechanical methods proved to be significantly different ($p<0.001$) while at the same time there was a significant influence of the blocks ($p<0.01$). Figure 2 presents the result of a multiple range test for the different methods.

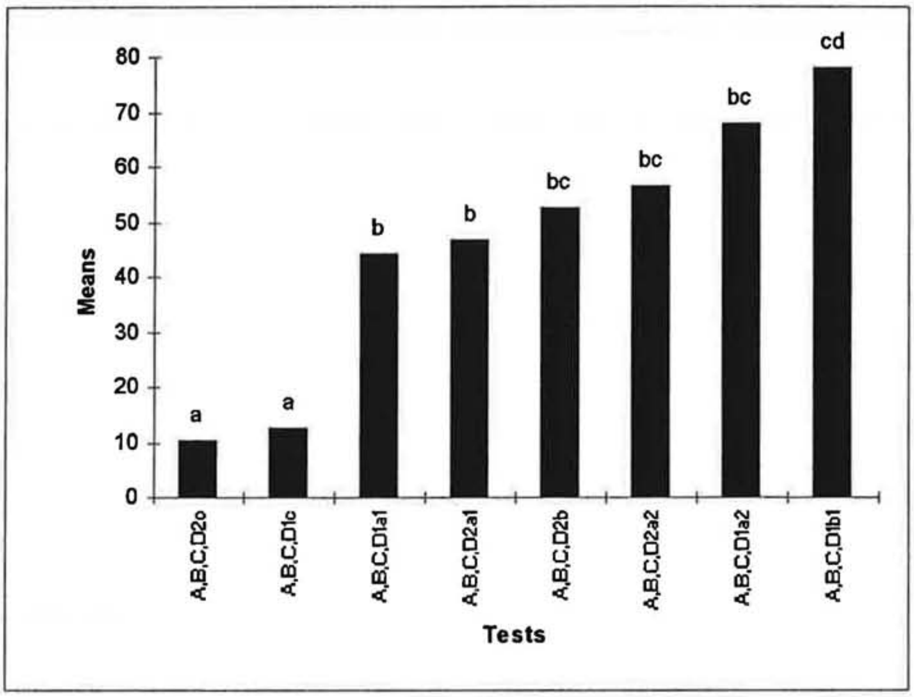

Figure 2. Result of a multiple range test for the mechanical methods (Different letters denote statistically significant differences (p<0.05). If at least one letter is shared no significant differences exist)

Girdling (*1c) and felling (*2c) in summertime with one additional treatment seem to result in a significantly lower proportional mortality. Girdling and felling in autumn with one (*1a1 resp. *2a1) or two (*1a2 resp. *2a2) additional treatments don't lead to a significant different result. There seems to exist a significant seasonal influence for girdling with one additional treatment. The best season appears to be springtime. Concerning the blocks proportional mortality lies significantly higher in Block D than in the others. Because of the three missing values in block D for the girdling method this conclusion is mainly based on the felling method (Table 4 and 6).

A multiple range test was carried out on the girdling and the felling methods separately. For the felling methods the previous results were confirmed. For the girdling methods no significant difference was found between method *1a1 and *1b1 ($p>0.05$). Also the blocks proved to have no significant influence any more ($p>0.05$).

The biological methods proved to be significantly different ($p<0.001$) while at the same time there was a slight significant influence of the blocks ($p=0.0492$). A multiple range test shows that the

proportional mortality of a stem treatment with mycoherbicide (*4) is significantly lower in comparison with the other methods (*$p<0.05$*) for which no mutual significant differences were detected (*$p>0.05$*). Concerning the blocks proportional mortality in block D lies significantly higher than in block A and C.
In order to examine seasonal influence on stump treatment with mycoherbicide a separate multiple range test was performed on the methods *3a and *3b. Treatment in autumn didn't result in a significantly different proportional mortality than treatment in springtime (*$p>0.05$*), which confirms the result from the previous multiple range test. On the other hand there was no significant difference between the blocks any more (*$p>0.05$*).
In order to examine the influence of the concentration of mycoherbicide another separate multiple range test was performed on the methods *3b, *3c and *3d. The applied concentrations had no significant effect on the proportional mortality (*$p>0.05$*), which also confirms the result from the previous multiple range test. Again no significant diffirence between the blocks was found (*$p>0.05$*).

The chemical methods proved to be significantly different (*$p<0.05$*) while at the same time there was no significant influence of the blocks (*$p>0.05$*). Figure 3 presents the result of a multiple range test for the different methods.

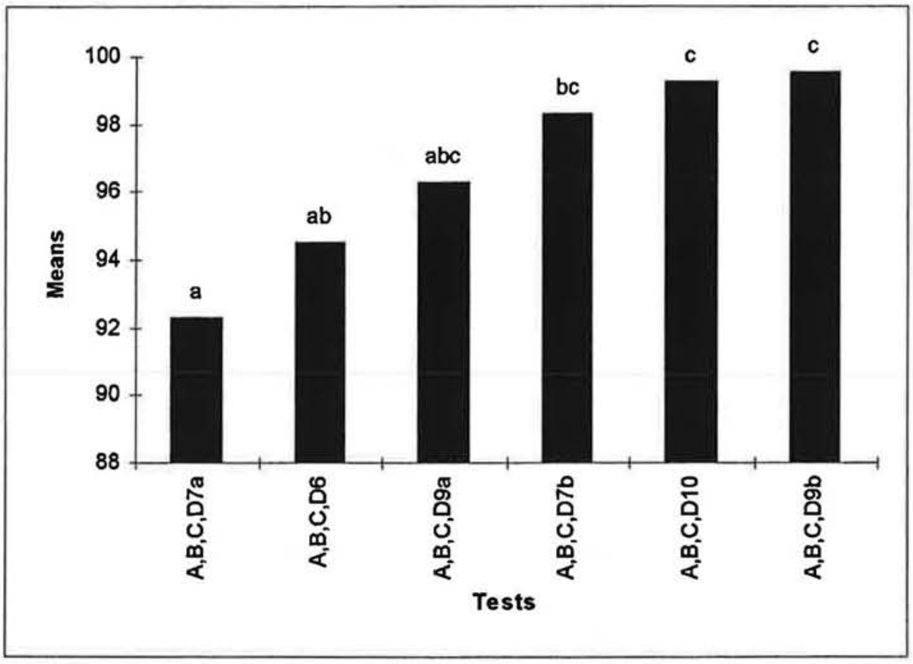

Figure 3. Result of a multiple range test for the chemical methods (Different letters denote statistically significant differences (p<0.05). If at least one letter is shared no significant differences exist)

Application of the Hack & Squirt method and the EZject Capsule Injection System in summertime result in a significantly higher proportional mortality than leaf treatment and stump treatment with herbicide in autumn. The latter proves to lead to a significantly better result when applied in summertime, while no significant effect of application period could be detected for the EZject Capsule Injection System.

In order to examine seasonal influence of the last two methods and to confirm the previous conclusions a multiple range test was performed on both methods separately. This time no significant seasonal influence was found for stump treatment with herbicide (*$p>0.05$*), while the previous conclusion was confirmed for the EZject Capsule Injection System (*$p>0.05$*). Again no significant influence of the blocks was found (*$p>0.05$*).

Next the stump treatments with mycoherbicide and herbicide were examined separately. A new dataset was formed with the following methods : *3a, *3b, *3c, *3d, *7a and *7b. This dataset proved to have a normal distribution (*K-S $p>0.05$*) so that a normal multifactor ANOVA for a block design could be performed. Both methods (*$p<0.001$*) and blocks (*$p<0.05$*) proved to be significantly different. A multiple range test shows that proportional mortality lies significantly higher in Block D than in the others (*$p<0.05$*). Stump treatment with herbicide leads to significantly better results than with mycoherbicide (*$p<0.05$*). Separate multiple range tests for both biological and chemical stump treatments confirm previous conclusions.

4. Discussion

Evaluation during the second autumn after treatment generally results into significantly higher proportional mortality rates. This is especially important for the mechanical and biological control methods because their effect is based on gradual exhaustion and development of the Silverleaf disease (de Jong 1988, Scheepens & Hoogerbrugge 1988). The effect of herbicides on the other hand manifests itself rather quick so that evaluation during the first autumn already gives proper and reliable results. Comparison of mechanical, biological and chemical control methods is therefore only justified during the second autumn after treatment at the earliest.

Except for the Sylvaxe Tree Injector technique, average proportional mortality of the chemical control methods is significantly higher than of the mechanical and biological methods. Considering each mechanical and biological method a very high variability can be detected between the blocks (Table 4, 5 and 6), often equaling the average proportional mortality of the chemical methods. On the other hand for almost all of these methods mortality is less than 50% in one or more blocks, sometimes even decreasing underneath 10%. Application of these methods can therefore lead to unpredictable results in contrast with chemical methods for which variability in proportional mortality is quite small (Table 6). Explanatory factors for these big differences in mortality for each method on different sites were not considered. Possible factors could be site condition, weather condition on the moment of application, dbh-class distribution, dominance structure,... These factors could certainly be subjects for further detailed research.

The results from and the analysis of the experimental block design indicate a significant seasonal influence on mortality for girdling with one additional treatment. Springtime seems to be the best season for girdling, while girdling in summertime leads to very poor results. Girdling in wintertime was not included in the experimental block design but results from table 4 show that for G2 (girdling in wintertime with one additional treatment) a proportional mortality of 90% is reached. Wintertime could therefore be the most appropriate period for girdling instead of springtime. Because this test was performed on only one location generalization is not completely justified though. On the other hand it gives a valuable indication because 300 trees were included. The result from G3 (girdling in wintertime without additional treatment), with a proportional mortality of 83% and executed on 268 trees, even strenghtens this presumption. Further tests ought to be carried out which could statistically confirm this. The result from G1 (girdling in autumn without additional treatment) illustrates that the girdling itself was badly performed. 40% of the trees are still alive and show a green canopy. This indicates that the trees were not girdled deep enough so that locally the cambium layer remained untouched resulting in a new connection between the canopy and the root system, thus restoring the downward sapflow. No significant differences were found between girdling in autumn with one or two additional treatments although the average proportional mortality of the latter was almost twice as big (Table 4). Clear differences between girdling and felling methods could not be assessed explicitly. A higher mortality can be expected when the number of additional treatments is drastically increased. Van der Kruis (1990) found that a single removal on a yearly basis of all new leafy sprouts on stumps of felled trees had little or no effect. Increasing the removal to 2 to 3 times during the vegetation period for 2 years finaly minimalized the number of vital stumps.

The results from and the analysis of the experimental block design indicate no significant seasonal influence nor any influence of the concentration of mycoherbicide on mortality for stump treatment with mycoherbicide. Dutch investigations with the same or similar myceliumsuspensions confirm these conclusions (Malais personal communication 1995, Scheepens & Hoogerbrugge 1988). Considering all results of the stump treatments with mycoherbicide, on average only 25% of the stumps was already dead during the first autumn after treatment. Proportional mortality increased to an average level of 63% during the second autumn. Remarkable is the same percentage of dead and sich trees for both periods namely 73%. 80% of all sick trees in the first autumn was dead during the second observations a year later, which stresses again the importance of the evaluation period. An average of 10% of the trees was found sick during the second evaluation period. Changes are real that part of these trees will eventually die too, increasing proportional mortality even more. Although a significant difference in mortality between chemical and biological stump treatment was detected, results are sometimes quite alike. In 4 of the 19 test-cases of biological stump treatment proportional mortality exceeded 80%. On the other hand stem treatment with mycoherbicide results in extreme low mortality rates eliminating its potential for practical application. Natural mortality after felling (*3e) can be interpreted as relative high (44%). Differences with actual stump treatment could not be statistically detected because the test was not performed in all 4 blocks. Nevertheless average mortality after biological stump treatment is still ± 20% higher which indicates an actual effect of the biological agent.

The Silvaxe Tree Injector System gave the poorest results of all chemical methods. Applying Roundup instead of Viking could increase average proportional mortality with 22% up to 64%. Practical application on a greater scale in other State forests in Flanders led to similar results. Van Den Meersschaut & Lust (1996) found that only one fourth of the supposed amount of herbicide (1 ml/stroke) or calculated lethal dose is actually injected. Considering the extreme high proportional mortality rate of the other stem treatments with herbicide in this study and the positive results for the same or similar stem treatments tested by other researchers (Jager & Oosterbaan 1979, Scholz & Wulf 1998, Spaeth et al 1994, Taylor 1992, Wagner et al 1994), the rather bad results with the Silvaxe Tree Injector System can be ascribed to malfunctioning of the instrument. The high lethal effect of the Hack & Squirt method proves the value of the calculated lethal doses in table 2 and the proposed easy to use application procedure. They are approximately in line with those applied by other autors. Roediger (1974) found that 1ml of herbicide every 5 cm of dbh was sufficient to kill species like beech, oak and birch. Woolfenden (1988) achieved positive results with 2 ml of Roundup for spruces with dbh less than 10 cm. Ogilvie (1984) concludes that 3 ml of Roundup is enough to kill four different conifer species (Japanese larch, Sitka spruce, Scots pine and Douglas fir) of the same diameter class. Also Wolf (1984) found that 2 to 3 ml was sufficient to kill species like Common alder, beech, oak, birch, Hornbeam, spruce and Scots pine with an average dbh of 5 to 7 cm. Besides their high lethal effect stem treatments offer other advantages : the accessibility of the stands is guarateed, temporary increase of the amount of dead wood resulting in a higher nature conservation value through creation of niches for xylobiontic invertebrates, hole-breeding birds, epifytes and fungi, slight alteration of the forest micro-climate, less disturbance of breeding birds and thus applicable during the breeding season and independent on weather conditions. Moreover Van Den Meersschaut & Lust (1996) proved these methods were more than twice as labour extensive as leaf and stump treatment with herbicides. One major drawback of these methods is their herbicide expenditure though. Van Den Meersschaut & Lust (1996) calculated the consumption of herbicide for a model stand with an average stem number of 641 trees/ha and an average dbh of 11 cm (minimum dbh = 5 cm). The herbicide consumption of the Hack & Squirt method and the EZject Capsule Injection System amounted to respectively 3.46 and 2.47 liter/ha while that of leaf and stump treatment amounted to respectively 1.22 and 0.58 liter/ha. These negative consequences are partly compensated by the fact that both methods and especially the EZject Capsule Injection System deal with a direct local injection of the herbicide into the tree. This in contrast with for example leaf treatment where a solution of herbicide is sprayed into the atmosphere where it can be distributed by the wind affecting other non-target plants (Scholz & Wulf 1998, Van Den Meersschaut & Lust 1996). Statistical analysis of seasonal influence on the lethal effect of stump treatment with herbicide showed contradictory results so that no general conclusion can be formulated. Jager & Oosterbaan (1979) indicate a high global mortality of 88% after stump treatment in January, March, April, June, September and November but suggest that application in springtime can result in lower mortality rates because of an optimal upward sapflow. Leaf treatment with herbicide gave good results although Oosterbaan (1981) and van den Tweel (1984) advise spraying in August and September.

5. Conclusion

For comparison of mechanical, biological and chemical methods for controlling Black cherry evaluation of mortality has to be posponed till the second autumn after treatment at the earliest. All chemical control methods, except one (Silvaxe Tree Injector System), result in a significant higher proportional mortality than mechanical and biological control methods. If environmental considerations have to be taken into account or if chemical control is legally forbidden (e.g. forest reserves in Flanders) biological control methods and girdling offer a valuable alternative. When applying biological and mechanical methods it is necessary to realize that proportional mortality can be very variable and thus quite unpredictable resulting in an increased labour intensity. On the other hand if chemical control methods are chosen stem treatments like the Hack & Squirt method deserve priority. Besides their effectiveness they are attended by important advantages like easy to apply, labour extensive, low disturbance and higher nature conservation values. Black cherry infested stands can consist of a big variety of diameter and height classes (from seedlings to mature trees) so that one control method is often inadequate and a combination of different methods is necessary. Different possible strategies for controlling Black cherry are formulated by Van Den Meersschaut & Lust (1996).

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