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Report Title: Development of an Effective Trap and Lure for Detection of the Brown Spruce Longhorn Beetle, *Tetropium Fuscum* (Coleoptera: Cerambycidae)

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**Development of an Effective Trap and Lure for Detection of
the Brown Spruce Longhorn Beetle, *Tetropium fuscum*
(Coleoptera: Cerambycidae)**

Year-end Report to the Fundy Model Forest, March 2003

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March 2003

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ABSTRACT

Synthetic lures, including a blend of monoterpenes similar to that emitted from the cortex of spruce trees ("spruce blend"), were tested for attraction of the brown spruce longhorn beetle, *Tetropium fuscum* in field trapping experiments conducted in Halifax, NS, Switzerland, and Poland in 2002. Traps baited with spruce blend captured significantly more *T. fuscum* (in Nova Scotia) and *T. castaneum* (in Switzerland) than unbaited traps. The addition of an ethanol lure to spruce blend-baited traps significantly increased capture of *T. fuscum* (in N.S. and Poland) and *T. castaneum* (in Poland). The Colossus-dry traps caught the most *T. fuscum* but mean catch did not differ significantly among the four trap designs tested. Cortical volatiles were sampled *in-situ* at regular intervals from May to September 2002 from girdled and ungirdled red and Norway spruce at Acadia, NB, to determine the effects of wounding and moisture stress on the relative concentration of terpenes. Although results were not consistent among all sampling dates, girdled trees of both species emitted relatively greater percentages of (-) β -pinene, (+) 3-carene, and bornyl acetate and lower percentages of sesquiterpenes than did ungirdled trees. Our results indicate that the addition of an ethanol lure synergizes attraction of the spruce blend to both *T. fuscum* and *T. castaneum*, another wood borer on the Canadian Food Inspection Agency's list of unwanted quarantine pests. The probability of detecting *T. fuscum* and *T. castaneum* in areas where either is present will be increased by the addition of an ethanol lure to cross-vane traps baited with spruce blend.

INTRODUCTION

The brown spruce longhorn beetle, *Tetropium fuscum* Fabr., (Coleoptera: Cerambycidae), is a quarantine pest recently found established near the port of Halifax, Nova Scotia. In its native Europe *T. fuscum* mainly attacks weakened Norway spruce but in Nova Scotia it is infesting and killing apparently healthy red spruce. An attractant-baited trap capable of detecting the presence of *T. fuscum* at low densities would be

extremely useful for surveys in Halifax, and for early detection at sites at risk of new *T. fuscum* introductions.

Unlike most lepidoptera, few cerambycids appear to use long- range pheromones to locate mates and instead depend on mutual attraction to olfactory cues from their hosts (Hanks 1999). Positive response to monoterpenes, ethanol, and other conifer volatiles has been shown for some species of cerambycids (Chénier and Philogene 1989; de Groot and Nott 2001) but little is known of *T. fuscum*'s response to host-volatiles. In 2001, we analyzed cortical volatiles collected from *T. fuscum*-infested red spruce *in situ* to determine the relative concentrations and enantiomer ratios of chiral compounds present. This information was used to create a synthetic “spruce blend” which was field tested in June-August 2001 on McNabs Island, NS. The synthetic lure was significantly attractive, with more than 63 *T. fuscum* captured in 30 baited traps compared with 0 beetles captured in 30 unbaited traps. The cross-vane (Peter) pan trap (designed by Peter de Groot) caught about three fold more *T. fuscum* on average than Lindgren funnel traps but differences were not significant.

Our objectives for 2002 were to: 1) repeat field trials testing the attraction of the spruce blend lure and the efficacy of various trap designs in capturing *T. fuscum* and *Tetropium* spp. 2) test the attraction of ethanol by itself and in combination with either the spruce blend or racemic alpha pinene; and 3) analyze the cortical volatile profiles of red *versus* Norway spruce and stressed *versus* healthy spruce trees to identify additional components or blends that may enhance attraction of *T. fuscum*.

LIST OF DELIVERABLES FOR 2002-2003:

1. Composition of host volatiles emitted from the stems of stressed and healthy spruce.
2. A synthetic lure, composed of spruce host volatiles, that is significantly attractive to the brown spruce longhorn beetle.

3. Recommendation of the most efficacious trap for detection of the BSLB, among prototype designs and commercially available traps tested.

METHODS

Study Areas

Trapping experiments were conducted in three areas: 1) McNabs Island, Halifax, N.S.; 2) near Develier (blocks 1-10) and Mont-Crosin (blocks 11-15) in Switzerland; and 3) in compartments 496C (blocks 1-10) and 494C (blocks 11-15) in an old growth forest near Bialowieza, Poland. Host volatiles were sampled in the Acadia Experimental Forest near Chipman, N.B. The forest on McNabs Island is dominated by *Picea glauca* (Moench) Voss, *P. rubens* Sarg., and *Abies balsamea* (L.) Mill. In Switzerland, the most common trees species at Develier were *Abies alba* Mill., *Picea abies* (L.) Karst., *Pinus sylvestris* L., *Fagus sylvatica* L., *Acer pseudoplatanus* L., *Crataegus monogyna* Jacq., and *Sorbus aria* (L.) Crantz. The site at Mont-Crosin was dominated by *A. alba* and *P. abies*. In the Bialowieza forest, compartment 494C was dominated by old growth *Pinus sylvestris* and *P. abies* and sporadically some *Betula pendula* Roth; Compartment 496C was mixed deciduous forest with *P. abies*, *Quercus robur* L., *Carpinus betulus* L., *P. sylvestris*, *Populus tremula* L., and *B. pendula* the most common species.

Trapping Experiments

Three field trapping experiments were conducted in 2002. All were replicated in randomized complete block designs with 25-30 m between traps within blocks and at least 100 m between blocks. Wet traps had a 1% solution of dish detergent in water in the collecting pan or bucket; dry traps contained a dichlorvos strip. Lures were produced by Phero-Tech, and were replaced every 4 weeks. Release rates, measured at 20°C, were 130-180 mg/d from each 15 ml bottle (used for spruce blend and alpha-pinene lures), and 40-60 mg/d from the ethanol lures. Traps were checked weekly and all beetle specimens were preserved in 70% ethanol. Identifications of *Tetropium*

species and other cerambycids were made or confirmed by J. Gutowski (Poland), C. Coquempot (Switzerland), S. Laplante and G. Smith (Halifax).

Experiment 1 tested the attraction of spruce blend to *Tetropium* spp. and the efficacy of three different wet trap types: 1) cross-vane (Peter) pan trap, 2) IPM-Intercept trap, and 3) IPM-Intercept-R (with vanes coated with “rainex” to reduce friction). Each trap type was either baited with two spruce blend lures or left unbaited, for a total of six treatments. There were 15 replicates per treatment. The experiment was conducted on McNabs Island, Halifax, NS, from 14 May to 7 August, and in Switzerland from 25 May to 30 July, 2002.

Experiment 2 tested the effect of adding an ethanol lure to traps baited with either spruce blend, alpha-pinene, or nothing. We compared *Tetropium* catch in cross-vane (Peter) pan wet traps baited with one of six different treatments: 1) two spruce blend lures; 2) one racemic alpha-pinene lure (which released alpha-pinene at the same rate as from two SB lures); 3) two spruce blend lures + one ethanol lure; 4) one alpha-pinene lure + one ethanol lure; 5) one ethanol lure; 6) unbaited. This experiment was conducted on McNabs Island, NS, from 16 May to 8 August, and in Bialowieza, Poland from 27 May to 2 August, 2002.

Experiment 3 compared the catch of *T. fuscum* among four trap types: 1) “Colossus” cross-vane prototype trap (developed by Simon Fraser University and PheroTech) with dry collecting bucket; 2) Colossus trap with wet collecting bucket; 3) IPM-Intercept-R-Dry; 4) Cross-vane (Peter) pan wet trap. All traps were baited with two spruce blend lures plus one ethanol lure. This experiment was conducted from 5 June to 8 August 2002, on McNabs Island, NS.

Volatiles from girdled vs. ungirdled spruce

Cortical volatiles were sampled *in-situ* from red and Norway spruce using the solid-phase micro extraction method and analyzed by gas chromatography/mass spectrometry. On 9-10 May 2002, 20 red spruce and 20 Norway spruce were tagged at Acadia, NB, and 10 of each species randomly selected and girdled at breast height. The circumference of girdled trees was scored to the sapwood in two 2-3 cm wide bands, about 10-15 cm apart at breast height, using “the ringer” a specialized girdling tool (Canadian Forestry Equipment). Volatiles were sampled from girdled and ungirdled trees at 2, 6, 12 and 18 weeks after girdling; volatiles were collected from above and below the girdle at 6, 12, and 18 weeks post-treatment.

Data analysis

Total season’s catch of *T. fuscum* and *T. castaneum* (Europe only) per trap were transformed by square root and subjected to analysis of variance; means were compared using the Ryan-Einot-Gabriel-Welsh range test and an experiment-wise type I error rate of 5% in SAS-GLM (SAS 1999-2001). Loss of data due to trap disturbance happened infrequently in 2002. However, if a trap was found to be vandalized on a weekly check, we deleted that week’s catch data from the season totals for all treatments in the affected block. Paired t-tests were used to compare cortical monoterpene concentrations collected above and below the girdle on girdled red and Norway spruce. Standard t-tests (assuming unequal variances) were used to compare the mean percent concentration of monoterpenes between girdled and ungirdled trees within spruce species.

RESULTS AND DISCUSSION

Trapping Experiments

Experiment 1. The spruce blend was significantly attractive to *T. fuscum* on McNabs Island ($F_{1,70}=44.0$, $P<0.0001$) and to *T. castaneum* in Switzerland ($F_{1,70}=5.7$, $P=0.02$) (Fig. 1a,b). Only one *T. fuscum* was captured in Switzerland. The effects of trap design and trap x lure interaction were not significant at either location.

Experiment 2. The addition of an ethanol lure to traps baited with spruce blend significantly increased capture of both *T. fuscum* (in N.S. and Poland) and *T. castaneum* (in Poland) compared to traps baited with spruce blend alone (Fig. 2abc). Ethanol and alpha-pinene, alone or combined were not attractive to either *T. fuscum* or *T. castaneum*. Traps baited with spruce blend alone captured significantly more *T. fuscum* than unbaited control traps on McNabs Island but not in Poland (Fig. 2). In contrast to results in Switzerland, the spruce blend alone was not significantly attractive to *T. castaneum* in Poland (Fig. 2).

Experiment 3. Mean catch of *T. fuscum* was 4-fold greater in Colossus-dry traps than in either the IPM Intercept or Peter-Pan traps but means did not differ significantly among the four trap types, due in part to high variability in catch and a limited sample size (n=7) (Fig. 3). The trend for greater catch in the Colossus-dry versus Colossus-wet trap suggests that the solution of dish detergent and water may have been somewhat repellent to BSLB. This or a similar experiment should be repeated in 2003 with more replicates.

Volatiles from girdled vs. ungirdled spruce

Mean percent volatile concentration differed between samples collected above versus below the girdle in only a few cases. In all cases, the terpene concentrations sampled above and below the girdle were averaged and used to compare with those from ungirdled trees. With the exception of the 12-week post-girdling sampling date, there was an increase in the relative percent concentration of (-) β -pinene, (+) 3-carene, and bornyl acetate emitted from girdled trees (Appendix 1). Differences were significant ($P < 0.05$) or near significant ($P < 0.10$) for both spruce species when sampled 6 and 18 weeks after girdling but not at 12 weeks after girdling. Changes in the cortical terpene profile may have resulted from wounding, moisture stress, or both. Because girdling slowly starves the roots of nutrients, girdled trees may not have suffered a lot of

moisture stress in 2002. For this reason, volatiles should be sampled again in the spring of 2003, one year after girdling, to confirm whether trends observed in 2002 are repeated. From these results, a terpene blend that simulates stressed spruce will be synthesized and tested for attraction of *Tetropium* spp. in field trapping experiments.

Attraction of other cerambycid species to spruce volatiles

Conducting trapping experiments in Europe provided the opportunity of testing the attraction of spruce volatiles to other wood-boring species that may be at risk of introduction to North America. A total of 36 cerambycid species and two buprestid species were captured in the experiments in Europe. Besides *T. fuscum* and *T. castaneum*, only four cerambycid species were captured in high enough numbers (>50) to warrant statistical analysis. Mean catch of *Aredolpona rubra* L. and *Alosterna tabacicolor* (DeGeer) did not differ significantly among treatments; the latter species attacks severely decayed wood of conifers and hardwoods. *Spondylis buprestoides* (L.)¹, a secondary pest of pines, often found in fresh pine stumps in Europe, was attracted to spruce blend (Fig. 4a). *Monochamus urussovi* (Fischer), a secondary pest of spruce and fir, was attracted to the combination of spruce blend plus ethanol (Fig. 4b). Adults of *M. urussovi* live about one month and feed on bark and sapwood on the lower surfaces of shoots in the crowns of spruce and fir, causing dieback. Although *M. urussovi* is not a serious pest in Poland, an outbreak affecting over a million hectares was reported in the Krasnodar region of Russia in the 1950's (Rozhkov 1976).

Conclusions

Our results confirm the attraction of the spruce blend to *T. fuscum* in Halifax and to *T. castaneum* in Switzerland, but spruce blend alone was not significantly attractive to either *Tetropium* species in Poland. Traps baited with both spruce blend and ethanol lures were significantly attractive to *T. fuscum* in Halifax and Poland, and to *T.*

¹ *Spondylus buprestoides* were captured only on blocks 11-15, so data for blocks 1-10 were omitted from the analysis

castaneum in Poland. We recommend that surveys for *T. fuscum* use cross-vane traps baited with spruce blend + ethanol lures, as this should significantly increase the probability of detecting *T. fuscum* where it is present, compared to baiting with spruce blend alone.

High (but variable) catches in the prototype Colossus dry-traps suggest that it or similar trap designs have potential for increasing mean catch of (and ability to detect) *T. fuscum*, and for reducing trap maintenance (dry vs. wet traps) and this should be tested in 2003. Girdled spruce trees emitted greater concentrations of (-) beta-pinene, (+) 3-carene, and bornyl acetate than ungirdled trees at 6 and 18 weeks after girdling. These changes in the terpene profile of stressed trees should be confirmed in May 2004 and tested for response of *Tetropium* spp. in trapping bioassays in 2003.

SUMMARY

The brown spruce longhorn beetle, *Tetropium fuscum* Fabr., (Coleoptera: Cerambycidae), is a quarantine pest recently found established near the port of Halifax, Nova Scotia. In its native Europe the *T. fuscum* mainly attacks weakened Norway spruce but in Nova Scotia it is infesting and killing apparently healthy red spruce. An attractant-baited trap capable of detecting the presence of *T. fuscum* at low densities would be extremely useful for surveys and monitoring of the eradication effort in Halifax and also for early detection of *T. fuscum* at other sites at risk of new introductions. In 2001, we analyzed cortical volatiles collected *in situ* from *T. fuscum*-infested red spruce to determine the relative concentrations and enantiomer ratios of chiral compounds present. This information was used to create a synthetic "spruce blend" lure which was tested with and without the addition of an ethanol lure in various trap designs for efficacy in capturing *T. fuscum* and other *Tetropium* species.

In 2002 field trials, traps baited with spruce blend captured significantly more *T. fuscum* (in Nova Scotia) and *T. castaneum* (in Switzerland) than unbaited traps. The addition of an ethanol lure to spruce blend-baited traps significantly increased capture of *T. fuscum* (in N.S. and Poland) and *T. castaneum* (in Poland). The prototype Colossus-dry traps caught the most *T. fuscum* but mean catch did not differ significantly among the various trap types tested.

Cortical volatiles were sampled *in-situ* at regular intervals from May to September 2002 from girdled and ungirdled red and Norway spruce at Acadia, NB, to determine the effects of wounding and moisture stress on the relative concentration of terpenes. Although results were not consistent among all sampling dates, girdled trees of both species emitted relatively greater percentages of (-) β -pinene, (+) 3-carene, and bornyl acetate and lower percentages of sesquiterpenes than did ungirdled trees. These changes in cortical volatile profiles may be exploited by *T. fuscum* as indicators of host stress. Cortical volatiles will be sampled again in May 2004 (one year after girdling) to

confirm differences and a “stress” lure or lures synthesized and tested in field trapping experiments.

Our results indicate that the addition of an ethanol lure synergizes attraction of the spruce blend to both *T. fuscum* and *T. castaneum*, another wood borer on the Canadian Food Inspection Agency’s list of unwanted quarantine pests. The probability of detecting *T. fuscum* and *T. castaneum* in areas where either is present will be increased by the addition of an ethanol lure to cross-vane traps baited with spruce blend.

ACCOUNTING OF EXPENDITURES – 2002-2003

Materials, supplies, shipping	12.0
Contribution agreement (Sandy Smith, U. Toronto) for European field trials	14.1
Travel expenses	19.5
Student salaries	9.7
Total	55.3

Source of funds

Fundy Model Forest	6.0
Forest Protection Limited	6.0
Ontario Ministry of Natural Resources	6.0
Canadian Food Inspection Agency	37.3
Total	55.3

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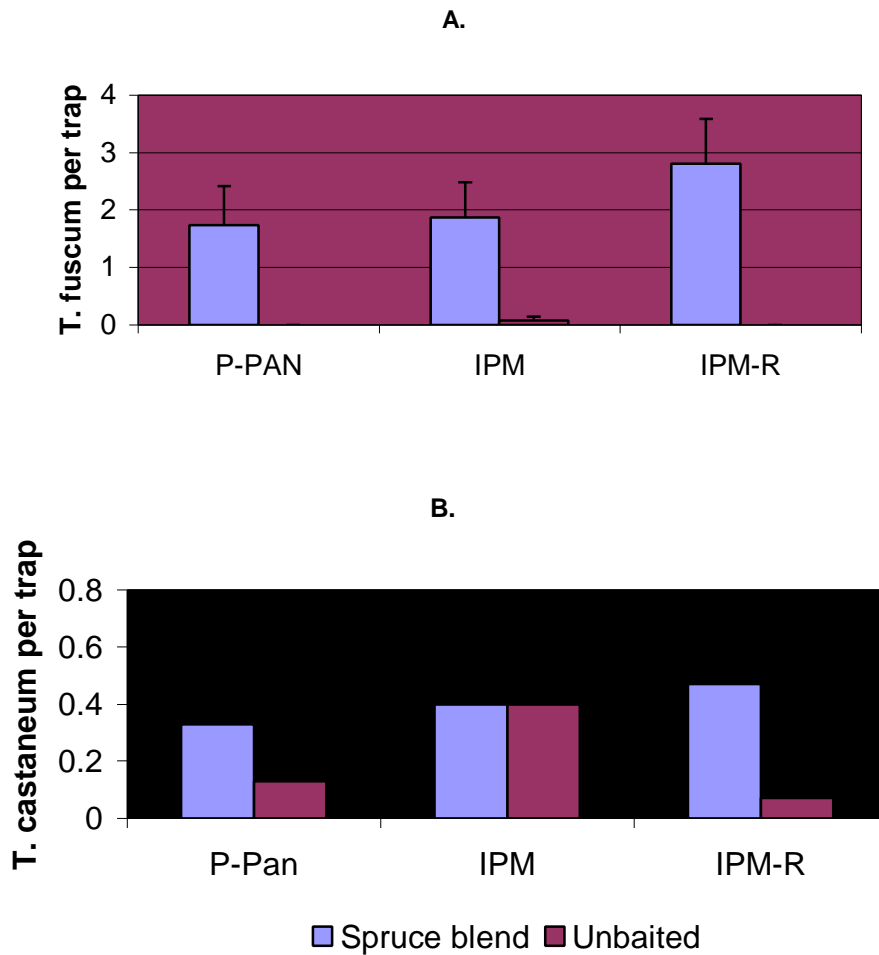


Fig. 1 Mean catch per trap (SE) in 2002 in cross vane traps either baited with spruce blend or unbaited, of A) *Tetropium fuscum* on McNabs Island, NS and B) *T. castaneum* in Switzerland. Mean catch differed significantly between baited and unbaited traps ($P < 0.05$) but not among trap types. Data transformed by square root.

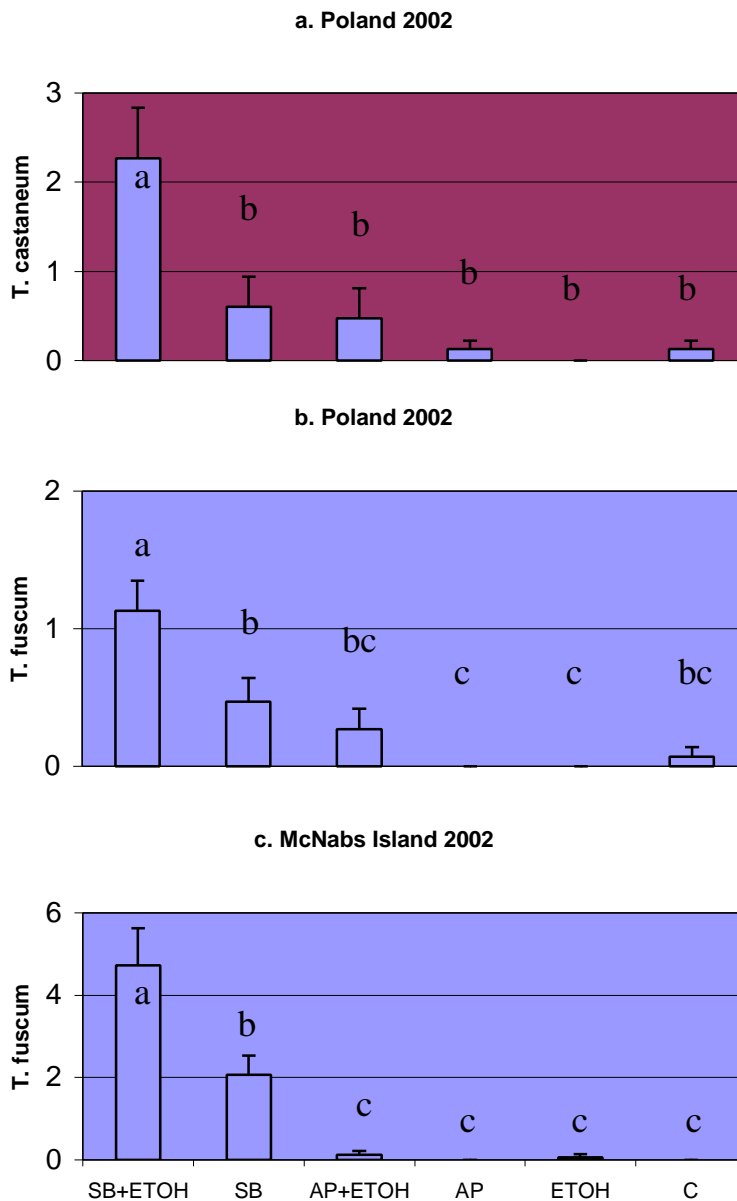


Fig. 2 Mean catch per trap (SE) in 2002 in cross vane (Peter) pan traps baited with different lures (SB=two spruce blend lures, ETOH=one ethanol lure; AP=one alpha pinene lure, C=unbaited) of: a) *Tetropium castaneum* in Poland, b) *T. fuscum* in Poland and c) *T. fuscum* on McNabs Island, NS. Within each graph, means with different letters were significantly different ($P < 0.05$). Data transformed by square root.

McNabs Island 2002

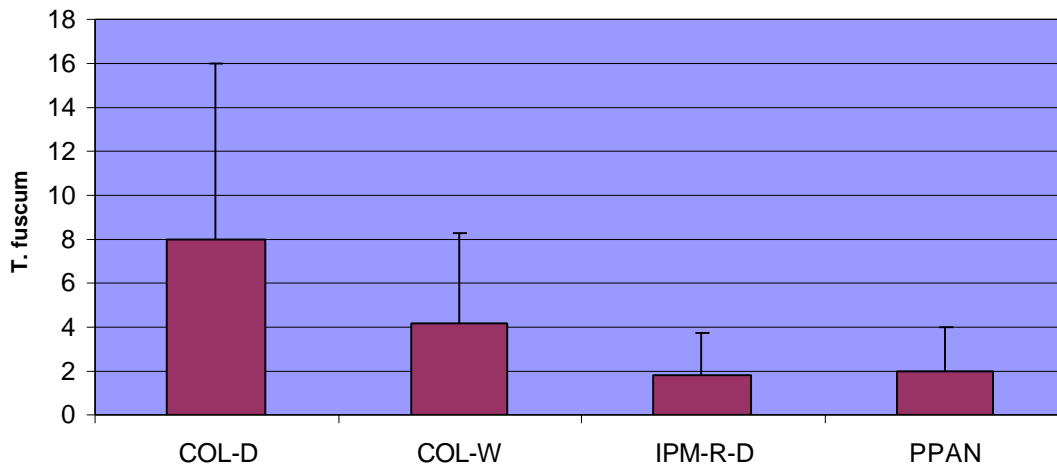
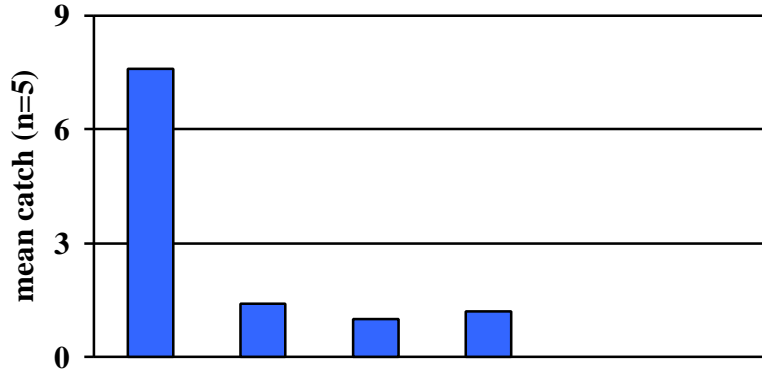


Fig. 3. Mean catch (SE) of *Tetropium fuscum* in traps baited with spruce blend plus ethanol. COL=Colossus trap; IPM-R = IPM intercept trap treated with Rainex; PPAN=cross vane (Peter) pan trap; W=wet, water/dish detergent in collecting bucket; D=dry, dichlorvos strip in bucket. Catch did not differ significantly among trap types ($P>0.05$, $n=7$).

a. *Spondylus buprestoides*



b. *Monochamus urossovi*

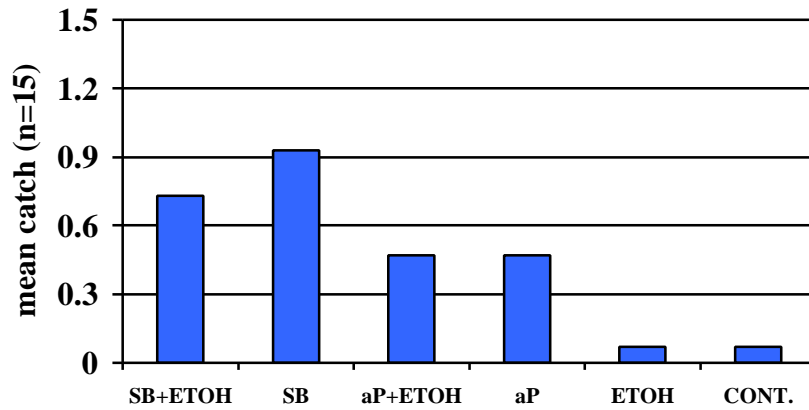
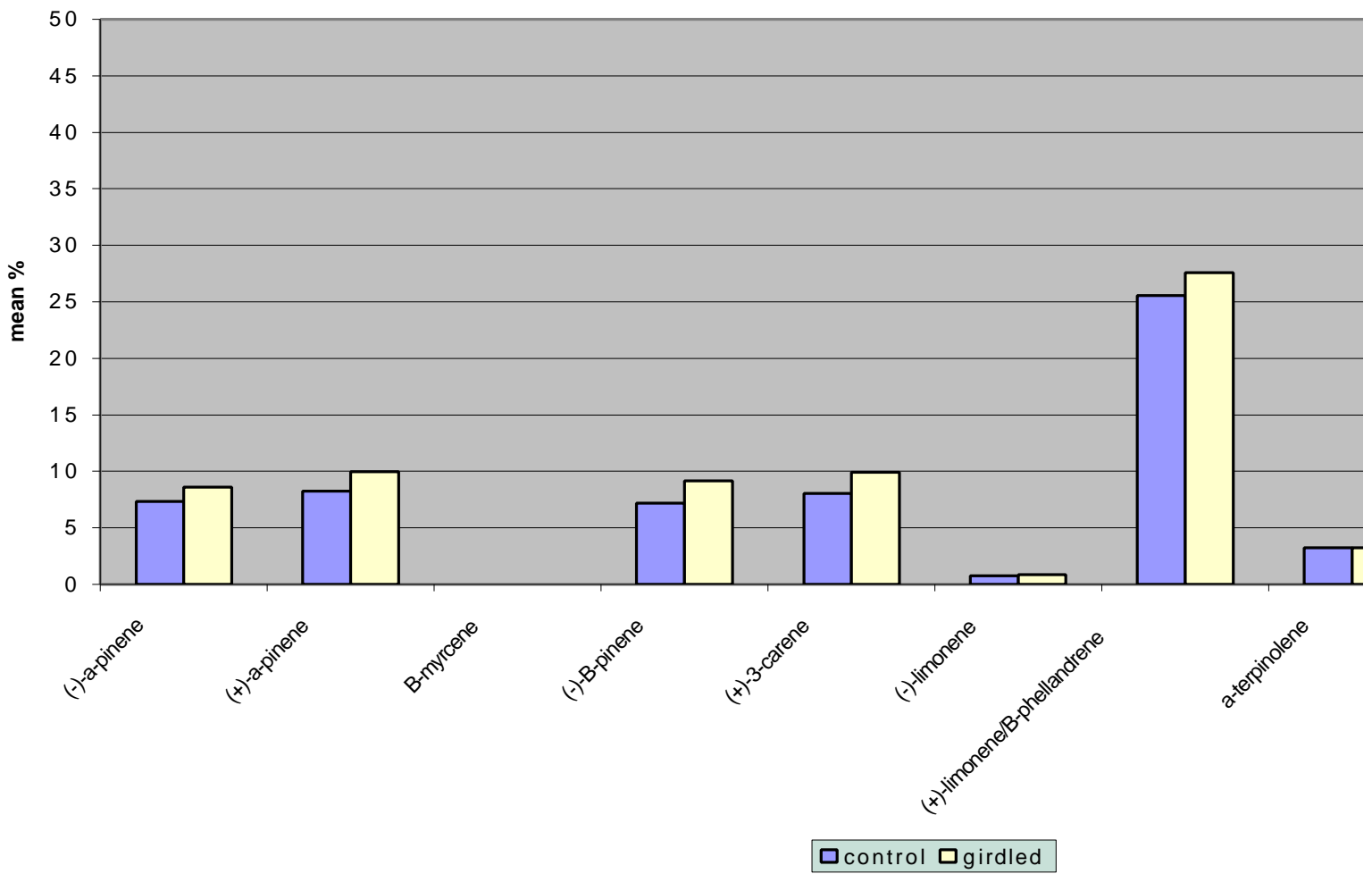


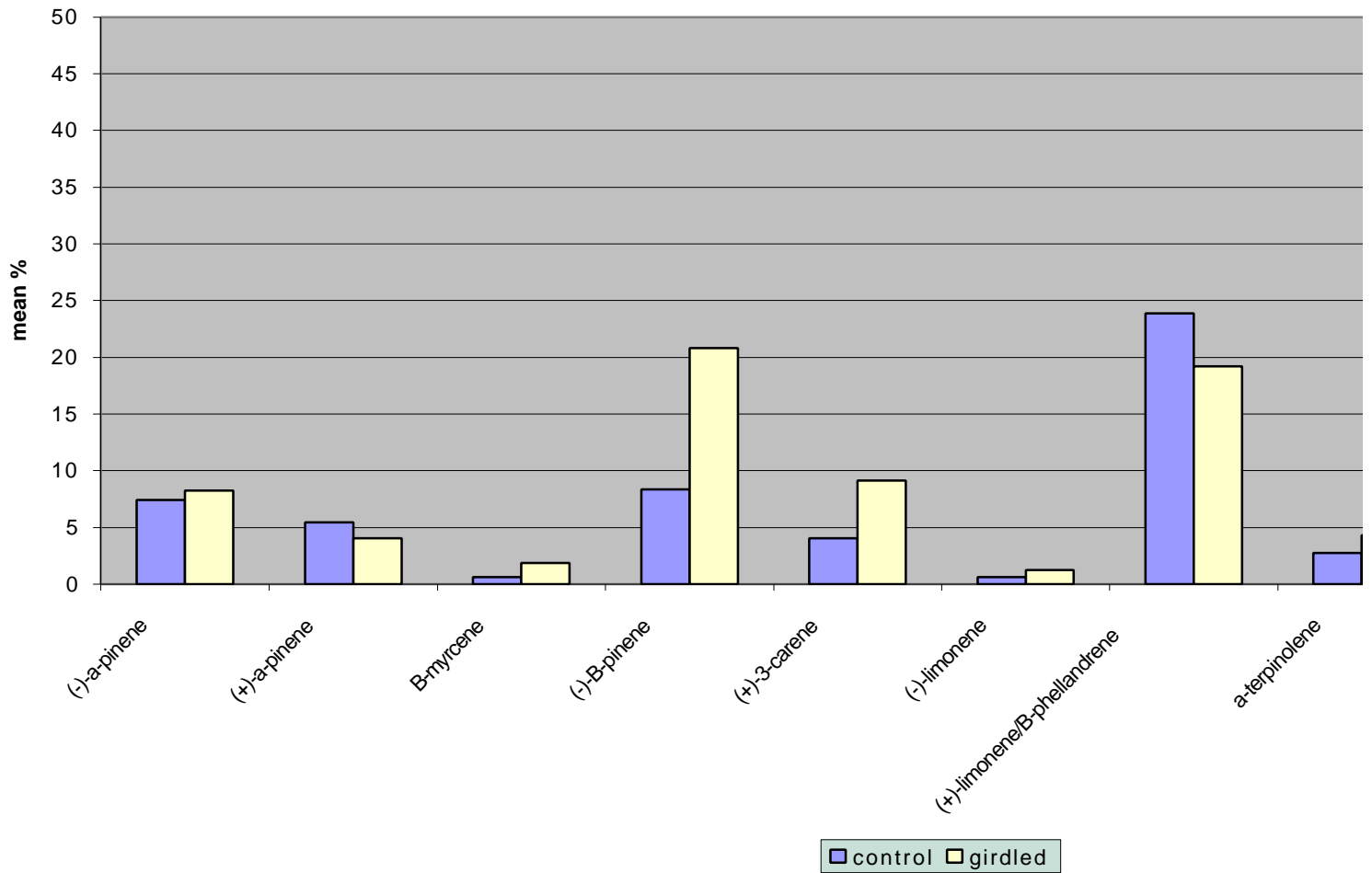
Fig. 4 Mean catch of a) *Spondylus buprestoides* (Col: Cerambycidae) and b) *Monochamus urossovi* (Col: Cerambycidae) in cross-vane (Peter) pan traps baited with various combinations of spruce blend (SB), ethanol (ETOH), racemic alpha-pinene (aP), or unbaited (CONT), in Bialowieza forest, Poland, 2002.

APPENDIX I Mean percent concentration of cortical volatile terpenes sampled *in situ* from red and Norway spruce, Acadia, NB, from May to September 2002. For each species, 10 trees were girdled at breast height on 9-10 May 2002, and 10 trees were left ungirdled. (8 pages follow)

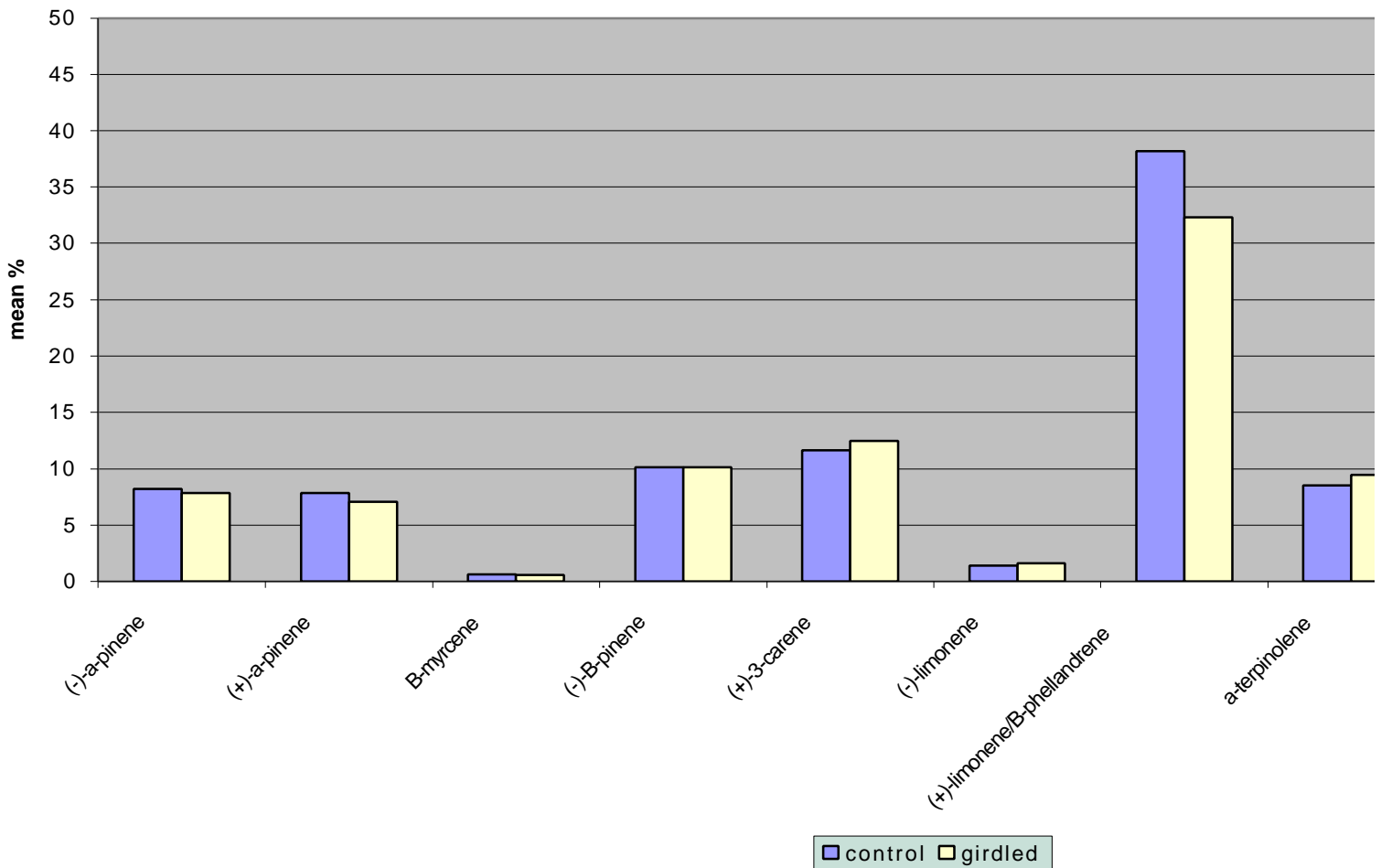
Red spruce Acadia, NB 24 May 2002, 2 weeks after girdling



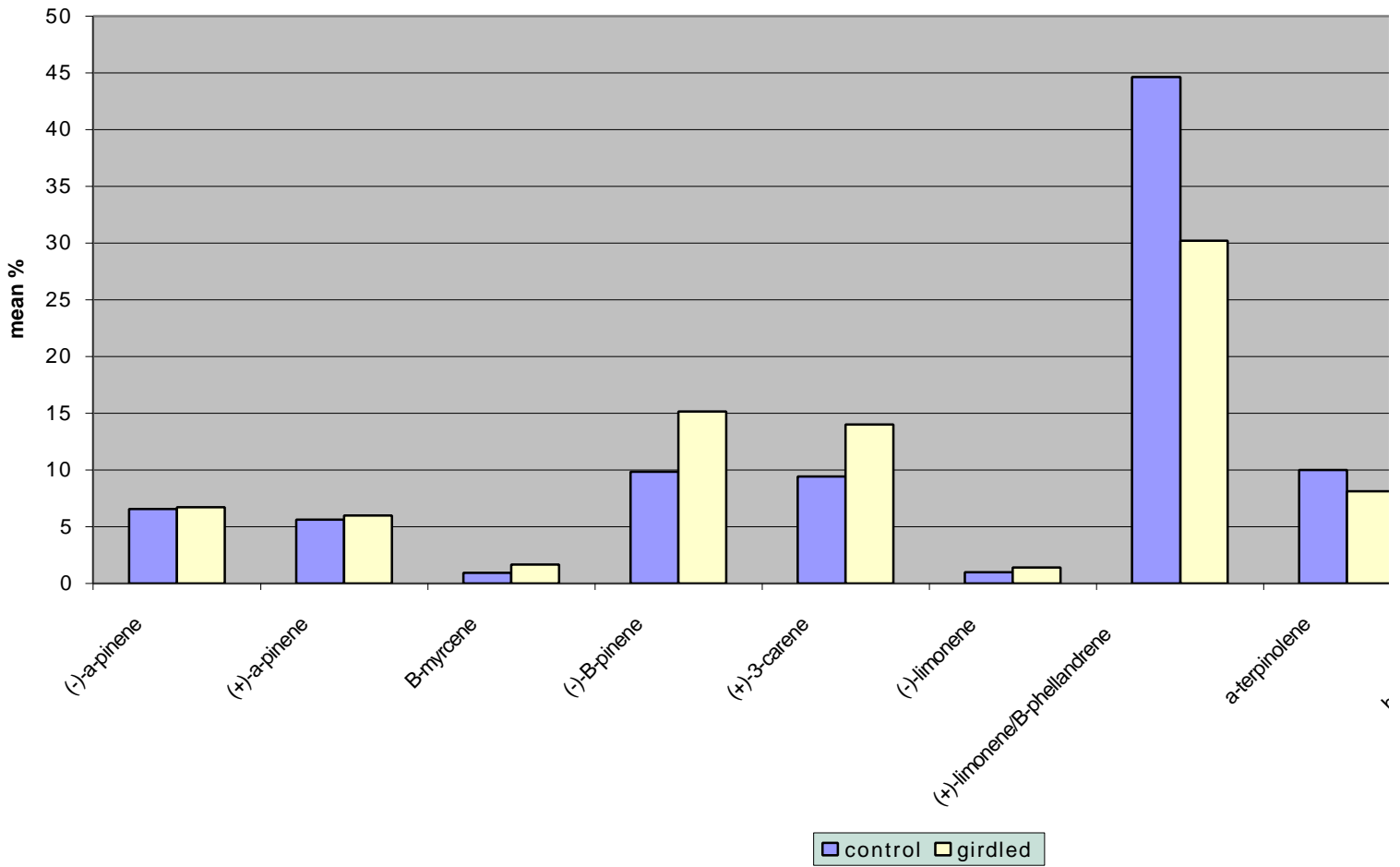
Red spruce, Acadia, N.B. 20 June 2002, 6 weeks after girdling



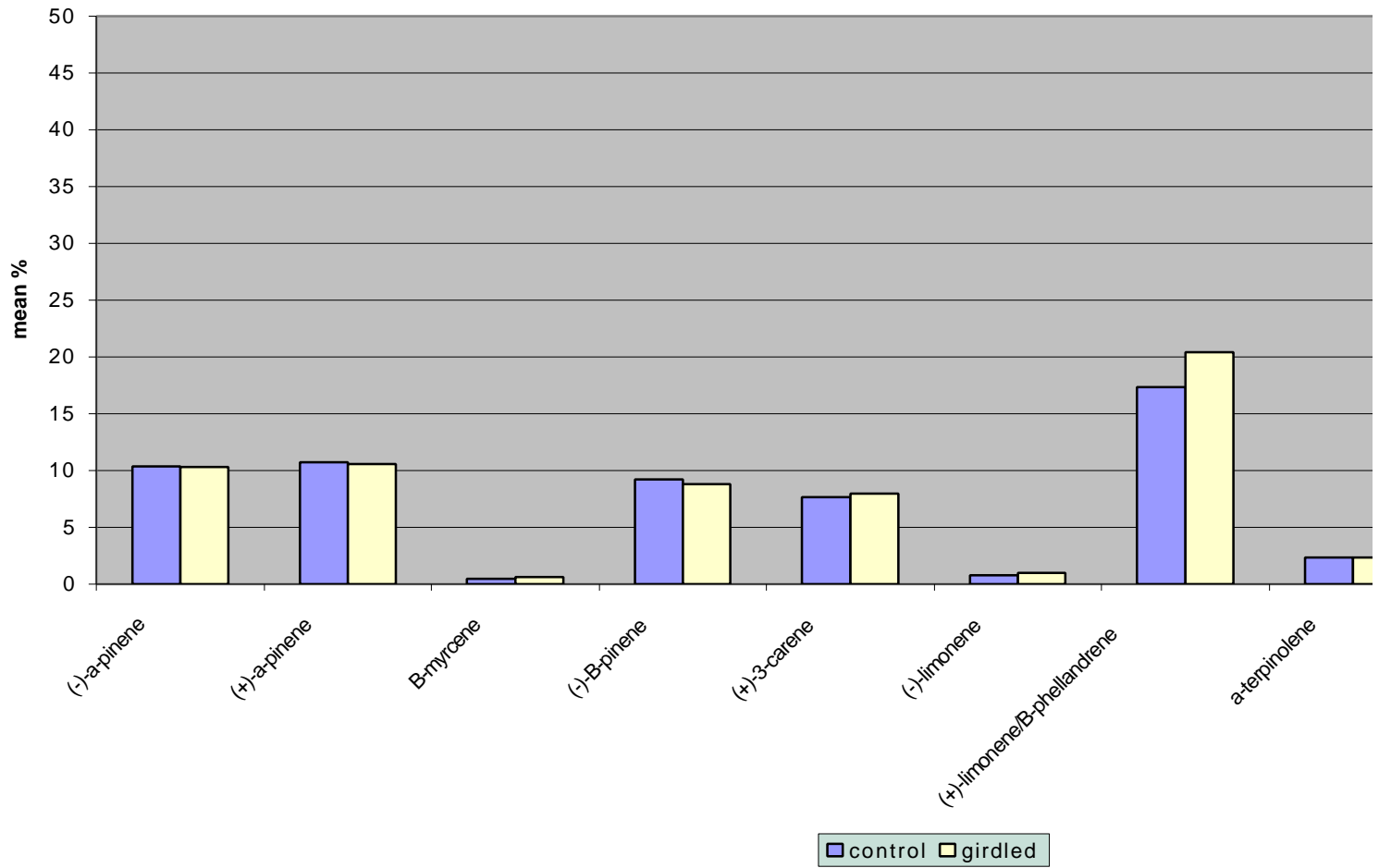
Red spruce Acadia, NB, 2 August 2002, 12 weeks after girdling



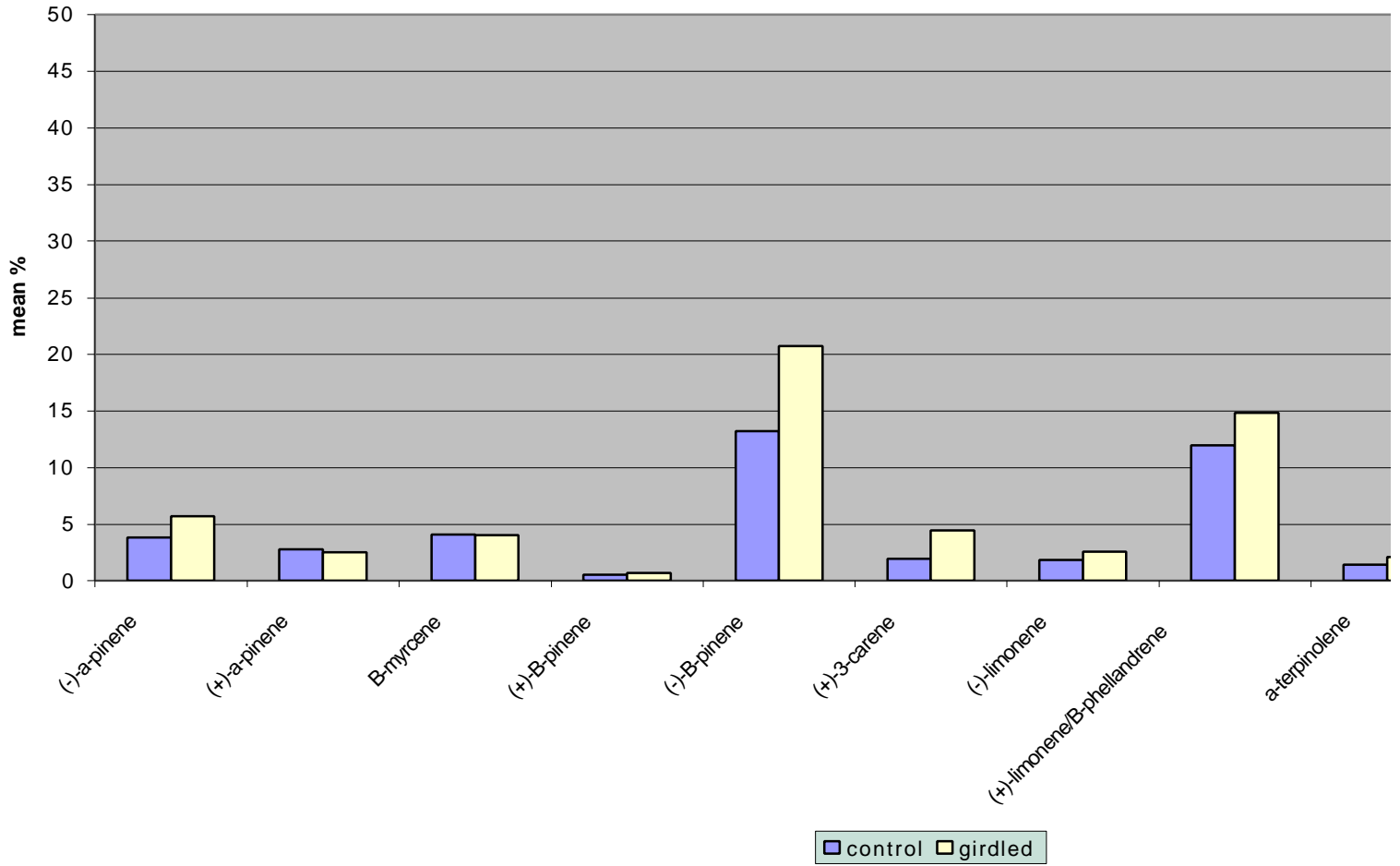
Red spruce Acadia, NB, 13 September 2002 18 weeks after girdling



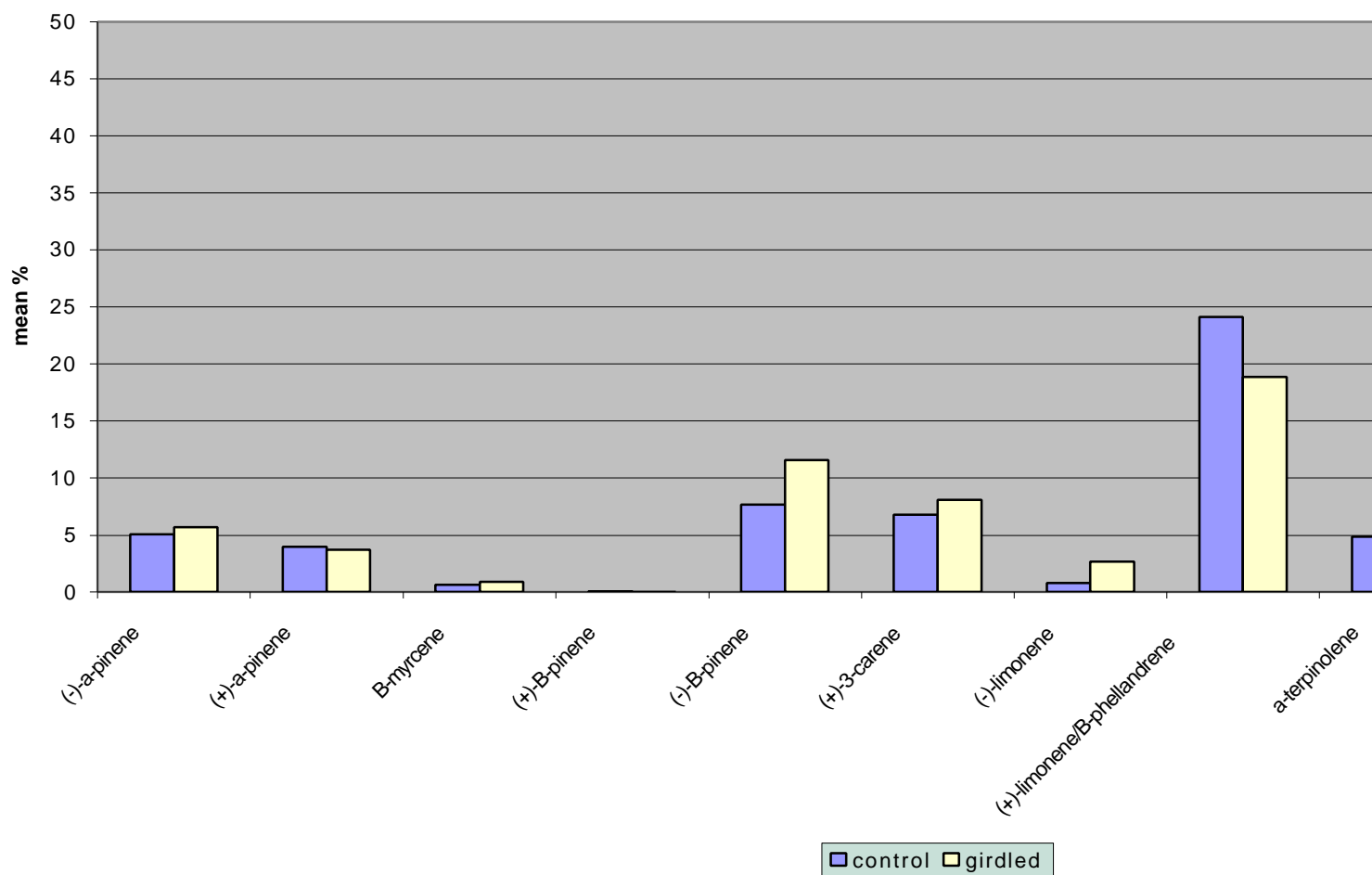
Norway spruce, Acadia, NB, 22 May 2002 2 weeks after girdling



Norway spruce Acadia, NB, 20 June 2002, 6 weeks after girdling



Norway spruce Acadia, NB, 1 August 2002, 12 weeks after girdling



Norway spruce Acadia, NB, 12 September 2002, 18 weeks after girdli

