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Use of an insecticide in field-scale plant–herbivore studies: no side effects of synthetic pyrethrin on *Betula pendula* growth or chemistry

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Birches are ecologically and economically important trees in forest ecosystems and commonly used in studies of plant–herbivore interactions. Such studies often require manipulation of natural levels of herbivory. In field conditions, this can be executed using insecticides, but such experiments have to assume that the insecticide has no side effects on the study plant. Here we (1) provide results of a test of the effects of synthetic pyrethrin, deltamethrin, on growth, leaf and litter chemistry and litter decomposition rate of *Betula pendula* seedlings in controlled growth chamber conditions, and (2) illustrate effectiveness of the insecticide in a field study of the effects of insect herbivory on *B. pendula*. The field effects, a significant reduction in leaf damage and a corresponding increase in growth, show that we used an effective dose of deltamethrin, while the results of the growth chamber experiment show that the dose did not cause any side effects on *B. pendula* seedling growth or chemistry. We conclude that pyrethrin compounds can be safely used for reducing insect-herbivore load on *B. pendula* in field-scale studies of tree–herbivore interactions.

Introduction

Due to their significance in forestry and forest ecology (Hynynen *et al.* 2010), birch species such as *B. pendula* and *B. pubescens* ssp. *czerepanovii* are commonly used for studying plant–insect interactions (e.g. Prittinen *et al.* 2003, Haukioja 2003, Silfver *et al.* 2009, Sinkkonen *et al.* 2012), and when herbivore-induced plant responses or plant tolerance to herbivory are investigated, exclusion of insects is often required (Karban & Baldwin 1997, Strauss & Agrawal 1999). Insect colonization of plants can be prevented manually or by using sticky barriers, but in large field studies that is laborious and often impractical. Insects are therefore often excluded from their host plants using insecticides, thereby enabling comparison of host performance between infested and non-infested plants. For instance, pyrethrin sprayings have been used to show that herbivory reduces growth of *B. pendula* (Prittinen *et al.* 2003, Silfver *et al.* 2009). Such conclusions, however, implicitly assume that the insecticide has no direct effects on the study plant that could confound the effects of the insect removal.

In general, insecticides are tested for phytotoxic effects on the crops before the formulations are released to the market, but those studies are not necessarily public knowledge or easily accessible. Studies that have publicly reported the tests of the direct effects, or side effects, of insecticides on plants have mainly focused on organophosphates (Brown *et al*. 1987, Tippin & Center 2002). Those compounds are however gradually being replaced by other less toxic insecticides, like pyrethrins. A few tests of pyrethrin effects are available; in those, growth of the grass *Poa annua* and the herb *Capsella bursa-pastoris* was suppressed (Hanley & Whiting 2005), whereas growth and seed production of many other species were unaffected (Root 1996, Mauricio *et al*. 1998, Siemann & Rogers 2003, Prittinen 2005). What is common among these studies is that they all examined the effects of pyrethrin sprayings on shoot growth or seed production, while the potentially equally important effects on below-ground growth and plant chemistry were neglected. Most of those studies also reported the results of additional short-term pre-tests but the description of their design and results were often insufficient.

Insect herbivory can induce production of secondary compounds (Karban & Baldwin 1997), which may persist through leaf abscission and affect subsequent leaf litter decomposition (Findlay *et al*. 1996, Schweitzer *et al*. 2005). To better understand how this process is controlled in *B. pendula* (cf. Silfver *et al*. 2007), we carried out an extensive field study using pyrethrin sprayings of *B. pendula* seedlings. Here, we first cursorily present the results of this study to exemplify the effectiveness of the synthetic pyrethrin sprayings in reducing the herbivore load of *B. pendula* seedlings in field conditions, and then focus on the laboratory test, which was aimed at finding whether seedlings not attacked by herbivores, but sprayed either with water or pyrethrin, differed from each other. Since our primary interest is in wide, ecosystem-scale effects of insect herbivory in *B. pendula* forests, such as those on plant nutrient uptake and litter decomposition rate, we tested the potential, direct pyrethrin effects on a number of important plant attributes (root growth, leaf and litter chemistry and litter decomposition rate), for which this information is still lacking. Our grand aim was to test if synthetic pyrethrins are an efficient and experimentally justified way to reduce the insect herbivore load in field-scale *B. betula* studies.

Material and methods

Field experiment

Study site, plant material and treatments

Our field site is situated 140 m a.s.l. near the Haapastensyrjä Unit of the Finnish Forest Research Institute in Loppi, southern Finland (60°36´N, 24°24´E). *Betula pendula* seedlings that are growing at the site were produced at the Haapastensyrjä Unit using a micropropagation technique in early 2008. The seedlings were kept in a nursery during the summer 2008, overwintered in a cold room, and were planted in the field in the spring of 2009. Each of the six replicate blocks in the field was divided into 132 planting plots of 2 × 2 m and seedlings of 19 different genotypes were randomly allocated and planted in the plots to provide seedling material for different experiments. Two of the genotypes did not, however, survive in the field and were excluded from the experiments.

Of the 17 remaining genotypes, two randomly selected seedlings in each block were included in the herbivory study (altogether 204 seedlings). One seedling was assigned as a control (sprayed with water and therefore naturally colonized by the insects), while the other was subjected to insecticide sprayings using 0.1% solution of synthetic pyrethrin Decis EC25 (Bayer CropScience, Germany). Decis EC25 is an emulsifiable concentrate, which contains 25 g l^{-1} (2.8% w/w) deltamethrin and has 2-methylpropan-1-ol, calciumbis(tetrapropylenebenzenesulphonate) and solvent naphtha (petroleum, light aromatic) as inert ingredients. The seedlings were sprayed once a week during the growing seasons of 2010 and 2011 using portable garden sprayers (one for pyrethrin, the other for water), with few exceptions in both seasons due to particularly rainy weather. Wind drift of the insecticide was eliminated by using a portable shower cubicle.

Measuring growth and herbivore damage

In both years, leaf damage was recorded in the middle (late July) and the end (middle of September) of the growing season. For each seedling, the top and the second highest side branch were surveyed, and the leaf damage was calculated using a modified Schreiner-type method (Fritz *et al*. 1998), in which the index of the damage is calculated using the following two scores: one describes the average area damaged (eaten) per leaf (assumes the values of 0, 1, 5 and 25 for the damage of 0%, 1%–4%, 5%–20% and 21%–100%, respectively), the second the proportion of the leaves damaged (0 = 0%, 1 = 1%–25%, 2 = 26%–50%, 3 = 51%–75% and 4 = 76%–100%). Multiplication of these two scores gives the damage index that ranges from 0 to 100.

Seedling height was measured three times: in the spring of 2010 and 2011, and in the autumn of 2011. Using these measurements, the annual growth of the seedlings was calculated for the years 2010 and 2011. Some of the seedling tops were damaged during the winters 2009–2010 and 2010–2011 (the top was considered damaged if it was dark brown/black or dry, or if the buds were not swollen). All the seedlings were classified by the condition of their tops (damaged or not damaged) and that grouping was used as a random factor in the statistical tests.

Growth chamber experiment

Plant material and growing conditions

A sack of 5-year-old *B*. *pendula* seedlings was purchased from a local forestry society (Päijät-Hämeen Metsänhoitoyhdistys) for the growth chamber experiment. Of that material, sixteen robust seedlings having no suckers were chosen and divided into "short" and "long" seedlings (seedling height ranged from 48 to 79 cm, the average being 66 cm). Four short and four long seedlings were then randomly allocated to both the pyrethrin and the control treatments. Seedlings were planted in plastic pots (diameter 12 cm, volume 1 litre), which were filled with fertilized nursery soil (Biolan, Puutarhan Mustamulta). The pots were placed in a growth chamber (h × w × d = 125 × 50 × 50 cm, Sanyo 31r-350, Japan) in the beginning of May 2010. To isolate the treatment and control seedlings, the chamber was divided into two equal sections using a plastic wall (two rows of four seedlings in each section). The chamber was illuminated by 15 fluorescent tubes (Sanyo fl 40 ss w/37), arranged on three chamber walls, and the photosynthetically active radiation (PAR) in the chamber varied between 180 and 240 μmol m^{-2} s^{-1}. To achieve equal radiation for each seedling, the seedlings were rotated within their sections and the sections were swapped once a week in the pursuance of the insecticide sprayings.

During the active growing period, the photoperiod in the chamber was 16/8 h and the temperature 15 °C, which represent the summer (June–August) conditions in southern Finland. The seedlings were sprayed using the same utensils, schedule and pyrethrin solution as in the field. All seedlings were sprayed outdoors and were allowed to dry off for 1 h before being returned to the chamber. Due to a limited growth space in the chamber, the autumn senescence was initiated already in the middle of August by decreasing the temperature by 3 °C and the day length by 3 h per week until all leaves were abscised in the beginning of October (five seedlings showed imperfect senescence, however, and some leaves fell green and/or green leaves were still attached to the seedling at the end of the autumn period). To collect the leaf litter, the seedlings were enclosed in mesh bags when autumn started.

Measuring plant growth and chemistry

At the final harvest, for each seedling buds, leaves and branches were counted and the length of each branch was measured. Since some leaves had fallen before the seedlings were enclosed in the mesh bags, the leaf number is an estimate. Entire root systems of the harvested seedlings were washed free of soil with tap water over a set of two sieves (mesh size 1 mm and 0.429 mm). For measuring seedling biomass, the main stem, branches and washed roots were dried at 60 °C for three days. Leaf-litter dry mass was estimated using a subsample of ten leaves dried at

60 °C. Growth of the seedlings, i.e., the height and base diameter increments, were calculated using measurements made prior to and after the experiment.

For analyzing the chemistry of summer leaves and leaf litter, six full-grown leaves were collected from the seedlings in the middle of August before the autumn senescence, and another set of ten leaves was taken from the mesh bag at the final harvest. Since imperfect autumn senescence might affect litter chemistry and decomposition rate, yellowish leaves were intentionally preferred for the chemical measurements and the litter decomposition trial for those five seedlings that did not senesce properly. Leaf and litter samples were ground in liquid nitrogen and the total N and C concentrations were determined using a LECO CNS-2000 Analyzer (LECO Corporation, USA). For the extraction of secondary metabolites, subsamples of leaf and litter material were moved to Eppendorf-tubes, 1 ml of 80% MeOH was added and the material was homogenized for 30 min. The samples were then centrifuged at 13 000 *g* for 2 min and the supernatants were collected. The residues were washed with 1 ml of 100% MeOH for 10 min in a homogenizer and again centrifuged at 13 000 *g* for 2 min. The obtained supernatants were combined with the previous ones and dried in a vacuum fuge at 45 °C. Thereafter, the samples were diluted in 250 μl of 100% methanol, and finally, before the HPLC analysis, 250 μl distilled water was added to each sample.

HPLC analyses were carried out in a Waters Acquity UPLC® system (Waters, Milford MA, USA) with a sample manager and a binary solvent manager. Waters Synapt G2-S HDMS mass spectrometer (Waters, Milford MA, USA) was interfaced with the UPLC system via ESI source. Mass range was from 50 to 1200 and the samples were analyzed in positive ion mode (ESI+). The source temperature was 120 °C and the desolvation temperature set to 350 °C. The cone gas flow rate was 20 l h^{-1} and the desolvation gas flow rate 1000 l h^{-1}. The compounds were separated using an Acquity UPLC® BEH C18 column (1.7 μm, 50 × 2.1 mm, Waters, Ireland), with the column temperature set to 40 °C. The mobile phase included (A) H_2O and (B) acetonitrile (Chromasolv® grade, Sigma-Aldrich, Steinheim, Germany) both with 0.1% HCOOH (Sigma-Aldrich, Steinheim, Germany), with a linear gradient from 95% of A to 45% in 9 min, then back to 95% in 9.1 min and equilibration for 1 min, 10 min in total. The injection volume was 2 μl and the flow-rate was set to 0.6 ml min^{-1}. The tray temperature was 10 °C. The compounds were identified using retention times, UV spectra and HPLC-MS, as previously described in Keski-Saari *et al*. (2007). The relative content of the compounds was calculated as peak height per 1 g dry leaf or litter material.

Litter decomposition trial

At the final harvest, ten abscised leaves were collected from each mesh bag, weighed, placed in a 10 × 10 cm litter bag (mesh size about 4 μm) and stored in a fridge until placed on the ground surface at the Haapastensyrjä field site in the middle of October 2010. To preserve all microbes, such as endophytes, which may have colonized the seedlings in the nursery or during outdoor sprayings and which naturally grow on the falling litter, a separate subsample of ten leaves was dried and used for measuring the initial dry mass in the litter bags. Litter bags were kept in the field until the late summer of 2011, then they were dried at 60 °C for three days, weighed and the percentage mass loss was calculated for each sample.

Statistics

In the growth chamber data, the significance of differences between the treatments in growth parameters, litter mass loss and leaf and litter chemistry was tested using an independent samples *t*-test. In the secondary metabolite data, the dimensions were first reduced using the principal component analysis (PCA), and the differences between the treatments in axes scores were tested using a *t*-test. The PCA was performed separately for the green leaves and the litter. Metabolites that were beyond the detection limit in more than 30% of samples (myricetin 3-arabinopyranoside and acacetin for green leaves and acacetin for litter) were excluded from the PCA.

The compounds were scaled to equal variance to make every compound equally important in PCA and the data were log-transformed before PCA to normalize variable distributions. In the field data, the effect of the insecticide on the growth, leaf production and herbivore damage index of *B. pendula* seedlings was tested using repeated measures ANOVA. Leaf number was $\log(x + 1)$-transformed prior to analysis to meet the test assumptions. All statistical analyses were performed using the SPSS 20.0.0.1. statistical package (IBM, USA).

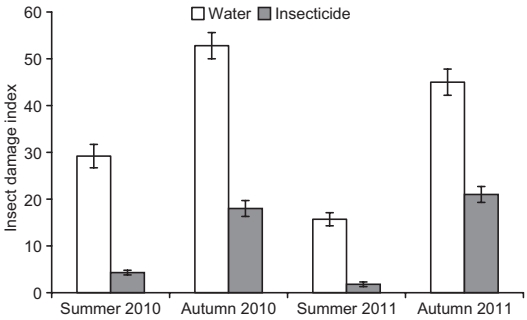

Fig. 1. The insect damage index (mean ± SE; n = 98–101) of field-grown seedlings sprayed with either the insecticide (deltamethrin) or water in the summer and autumn surveys of 2010 and 2011.

Results and discussion

Deltamethrin sprayings reduced the leaf damage of field-grown seedlings significantly in both years ($F_{1,157} = 255$, $p < 0.0001$; Fig. 1) and the reduction was more pronounced in the summer (87% decrease) than autumn (60%) surveys (date × insecticide interaction, $F_{2.9,448} = 11.2$, $p < 0.0001$; Fig. 1). These results show that although herbivores were not completely excluded from *B. pendula* seedlings, our deltamethrin concentration and spraying frequency could significantly decrease the herbivore load of the seedlings. Our results are comparable to the results in studies, where application of a corresponding synthetic pyrethrin, esfenvalerate, reduced the herbivore load on the herb *Solidago altissima* by 50%–95% (Root 1996) and the visible leaf chewing damage of the trees *Sapium sebiferum* and *Celtis laevigata* by 30%–80% (Siemann & Rogers 2003). In contrast, Mauricio *et al.* (1998) reported that esfenvalerate sprayings could eliminate virtually all visible leaf damage in *Arabidopsis thaliana*. That the efficacy of insecticide sprayings can vary between different studies even with a comparable dose is expected considering the remarkable spatial and temporal variation in herbivore load between sites and plant species. For instance, in our field site the herbivore load was exceptionally high as the damage was about ten-fold in comparison to the damage found by Prittinen *et al.* (2003) in their study of *B. pendula* herbivore resistance.

Even though the deltamethrin contains some N (1.38 ppm N in the spraying solution), the insecticide sprayings did not have a fertilizing effect on the seedlings in our growth chamber experiment: i.e., neither the growth attributes nor the shoot/root ratio differed between the control and deltamethrin treated seedlings (Table 1). These findings show that weekly sprayings using 0.1% Decis EC25 solution have neither phytotoxic nor fertilizing effects on *B. pendula* seedlings. As a result, the 38%–44% greater annual growth in the insecticide treated than control seedlings ($F_{1,156} = 13.2$, $p < 0.0001$, Fig. 2), which we recorded in the field, can be interpreted to be due to decreased herbivore consumption, not due to direct (or side) effects of deltamethrin on seedling growth. Similar interpretation is also valid for the number of leaves (date × insecticide interaction; $F_{1,159} = 13.3$, $p < 0.0001$) and the dry mass of leaf litter (insecticide main effect; $F_{1,16} = 27.1$, $p < 0.0001$), which were 25% (in 2011) and 72% greater, respectively, in the insecticide treated than in the control seedlings (Fig. 2). Altogether our data show that synthetic pyrethrin can be safely used to decrease the herbivore burden of *B. pendula* seedlings in field studies. In some herbaceous plants, like the graminoid *Poa annua* and the herb *Capsella bursa-pastoris*, pyrethrin sprayings suppressed plant growth (Hanley & Whiting 2005), and Siemann and Rogers (2003) speculated that pyrethrin altered biomass allocation between the above- and below-ground plant parts in their woody *Celtis laevigata* seedlings. In the latter study, however, the seedlings were grown for 15 weeks outside in a university campus and there were no details given of whether the herbivores were properly excluded from the non-treated study plants.

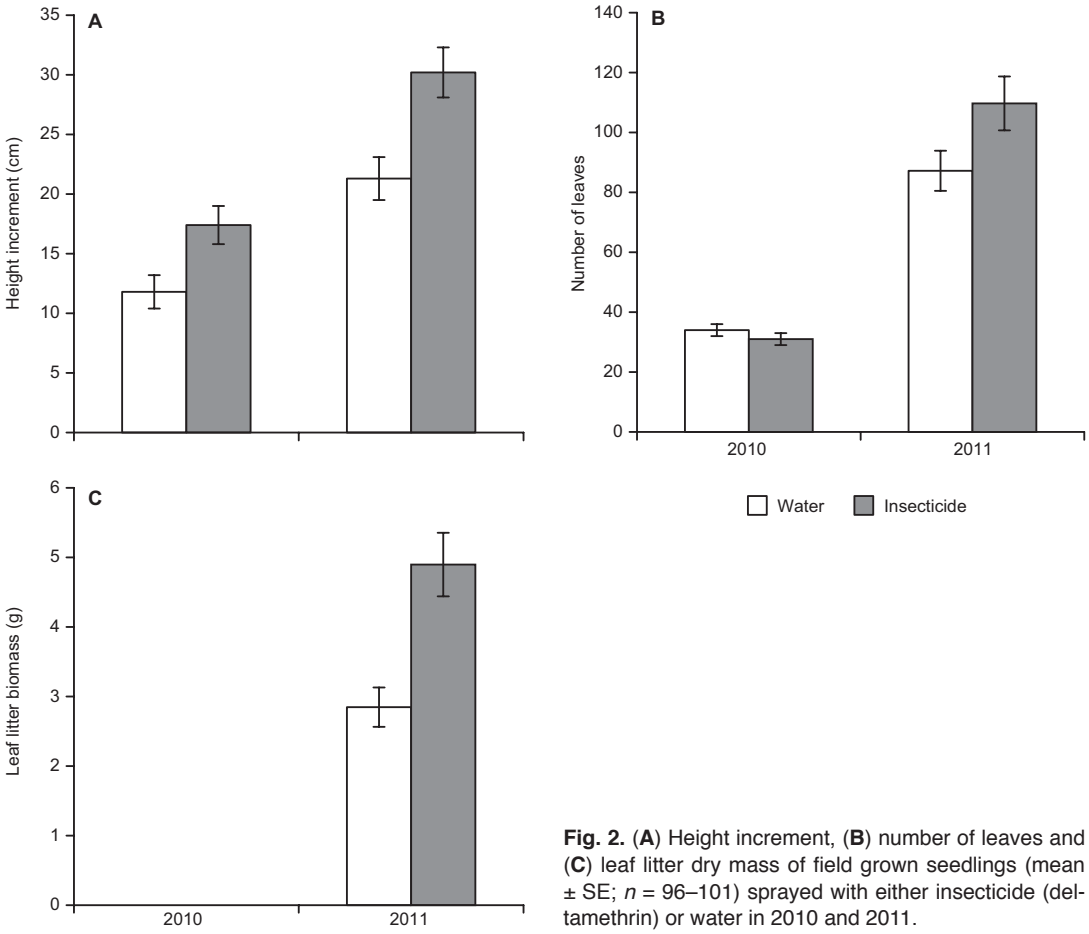

Fig. 2. (**A**) Height increment, (**B**) number of leaves and (**C**) leaf litter dry mass of field grown seedlings (mean ± SE; n = 96–101) sprayed with either insecticide (deltamethrin) or water in 2010 and 2011.

Table 1. Growth parameters of seedlings subjected to weekly water or insecticide sprayings in the 22-week growth-chamber experiment. Statistical significance of differences between the control and treatment seedlings was tested using a *t*-test; for all variables n = 8. Dry weight (d.w.) of the leaf litter and number of leaves are estimates, *see* Material and methods.

Parameter	Water control mean ± SE (min–max)	Insecticide mean ± SE (min–max)	*t*-test's *p*
Diameter growth (mm)	3.3 ± 0.7 (1.6–8)	3.1 ± 0.3 (2.0–4.5)	0.74
Height growth (cm)	17.6 ± 2.1 (8.4–25.0)	14.3 ± 0.7 (12.0–17.5)	0.17
Number of branches	9.1 ± 1.3 (5–14)	10.1 ± 0.8 (7–13)	0.51
Total branch length (cm)	91.2 ± 10.3 (44.9–122.9)	90.2 ± 4.6 (71.0–105.0)	0.93
Number of leaves produced	56.1 ± 4.4 (40–74)	65.0 ± 4.2 (54–85)	0.17
Number of buds	52 ± 4.4 (37–66)	53 ± 2.8 (40–65)	0.85
Leaf litter biomass (g d.w.)	2.68 ± 0.21 (1.28–3.21)	2.98 ± 0.26 (1.91–4.13)	0.40
Branch biomass (g d.w.)	1.20 ± 0.17 (0.54–2.14)	1.16 ± 0.06 (0.79–1.37)	0.84
Stem biomass (g d.w.)	9.06 ± 0.47 (7.28–10.89)	8.86 ± 0.61 (6.58–11.27)	0.20
Root biomass (g d.w.)	9.63 ± 0.80 (5.51–12.52)	9.78 ± 0.53 (7.81–11.58)	0.88
Shoot*/root ratio	1.40 ± 0.12 (1.06–2.13)	1.34 ± 0.04 (1.13–1.46)	0.64

* Shoot = stem + branch + leaf litter biomass.

The active ingredient of the insecticide Decis EC 25, i.e., deltamethrin, has been in use for long and it is likely that any detrimental effects on crops would have been noticed. Our finding that deltamethrin does not affect the *B. pendula* growth is therefore not unexpected. However, Decis EC 25 also contains other ingredients like solvent naphtha (i.e. petroleum) and plants may deal with such harmful substances by increasing accumulation of antioxidative secondary metabolites. For instance, *B. pendula* seedlings are known to accumulate phenolics, such as quercetin glycosides, in response to elevated UVB-radiation, heavy metals and acid rain (Keski-Saari *et al.* 2005, van Ooik *et al.* 2012). Since polyphenols are known to affect litter decomposition rates (Hättenschwiler & Vitousek 2000), increased concentrations of secondary metabolite could potentially lower litter decomposition. However, in the growth chamber experiment, the insecticide spraying had no statistically significant effects on the concentrations of water, N, C or total secondary metabolites in either the green-leaf or leaf-litter material (Table 2). Moreover, when three principal components were extracted from the green-leaf and leaf-litter data using PCA (Table 3), the axes scores (Fig. 3) were not different between the seedlings sprayed with water and the insecticide (green leaves, Fig. 3A, $p \geq 0.82$ for each axis; leaf litter, Fig. 3B, $p \geq 0.47$ for each axis). For the litter data, PCA axis 1 (explaining 31.9% of total variation) summarized the variation in flavonoid aglycones and flavonoid glycosides, axis 2 (15.3%) the variation in coumaroyl quinic acids (CouQAs) and the chlorogenic acid (CGA), and axis 3 (12.8%) the variation in triterpenes (Table 3). The loadings of these three groups on the PCA axes were more mixed in green leaves (Table 3), but clearly, the use of deltametrin in insecticide sprayings had no effect on the composition of phenolic compounds in either the active or senescent *B. pendula* leaf material. This result is in good agreement with the finding that mass loss did not differ between the litter collected from the control and the insecticide treated seedlings (Table 2).

Significant reduction in leaf damage and the equal increase in plant growth in our field experiment show that a weekly application of 0.1% Decis EC25 solution can significantly reduce tree herbivore load in field conditions. At the same time, the results of the growth-chamber experiment show that this dose is low enough to not affect *B. pendula* seedling growth, biomass allocation, leaf chemistry or litter quality and decomposition. Side effects of an insecticide that can affect performance of a plant species have not earlier been tested using equally wide selection of plant attributes, ranging from plant chemistry to ecosystem-scale attributes like litter decomposition rate. We, therefore feel, confident

Table 2. Parameters of leaf and leaf litter chemistry and the litter mass loss of seedlings subjected to weekly water or insecticide sprayings in the 22-week growth-chamber experiment. Statistical significance of differences between the control and treatment seedlings was tested using a *t*-test.

Parameter	Water control mean ± SE (min–max)	Insecticide mean ± SE (min–max)	*t*-test's *p*
Leaf			
water content (%)	60.3 ± 1.6 (56.2–70.1)	58.8 ± 1.5 (53.9–66.9)	0.51
N (%)	1.44 ± 0.08 (1.19–1.84)	1.31 ± 0.09 (1.11–1.88)	0.29
C (%)	49.4 ± 0.08 (49.2–49.9)	49.0 ± 0.18 (48.3–49.7)	0.07
C/N-ratio	34.9 ± 1.84 (26.8–41.4)	38.4 ± 2.04 (26.3–43.7)	0.23
total secondary metabolites*	8796 ± 831 (7472–10329)[a]	9465 ± 789 (6172–11824)[b]	0.62
Litter			
mass loss (%)	51.6 ± 2.1 (43.0–58.9)	53.9 ± 2.6 (45.5–68.1)	0.49
N (%)	0.77 ± 0.10 (0.47–1.26)	0.58 ± 0.07 (0.48–1.02)[c]	0.16
C (%)	49.1 ± 0.3 (47.9–50.3)	48.4 ± 0.3 (47.5–49.4)	0.12
C/N-ratio	70.5 ± 8.0 (38.0–106.8)	88.6 ± 7.2 (46.7–100.4)[c]	0.12
total secondary metabolites*	28843 ± 3249 (14856–46070)	25120 ± 1364 (19069–31674)	0.31

* Secondary metabolite unit is peak height g^{-1} dry leaf or litter material. $n = 8$, except for [a]$n = 3$, [b]$n = 6$ and [c]$n = 7$.

Table 3. Variable loadings from PCA of the secondary metabolite data of green summer leaves and leaf litter of each birch seedling. The loadings are directly proportional to the correlation between the metabolites and the PCA axes. Values set in boldface indicate a significant contribution of the metabolite to the respective PCA axis.

	PCA axes (green leaves)			PCA axes (leaf litter)		
Compound	1	2	3	1	2	3
DHPPG	0.445	**0.506**	**–0.527**	**0.589**	**0.501**	–0.021
CGA1	–0.435	0.113	–0.167	0.389	**0.616**	0.104
CouQA1	**0.625**	–0.205	–0.453	0.224	**0.539**	–0.303
CouQA2	**0.719**	–0.026	–0.124	0.178	**0.770**	0.092
CouQA3	**0.916**	–0.072	–0.174	–0.098	**0.793**	0.184
Catechin	0.209	**0.561**	–0.234	0.306	0.129	–0.408
M 3-galactoside	**0.699**	–0.251	–0.382	**0.610**	0.425	–0.415
M 3-glucuronide	**0.503**	–0.396	0.378	**0.600**	0.129	**–0.574**
M 3-glucoside	**0.630**	–0.474	–0.383	**0.524**	0.477	–0.271
M 3-arabinofuranoside	0.032	**0.617**	0.448	0.375	**–0.571**	–0.059
M 3-arabinopyranoside	–	–	–	0.400	–0.077	**–0.660**
M 3-rhamnoside	–0.080	–0.125	**–0.837**	–0.133	**0.834**	–0.148
Q 3-galactoside	0.268	**0.741**	0.324	**0.797**	–0.198	–0.060
Q 3-glucoside	**0.669**	0.355	–0.156	**0.635**	0.088	0.212
Q 3-arabinofuranoside	–0.025	–0.279	–0.357	0.049	0.148	–0.393
Q 3-arabinopyranoside	–0.083	**0.790**	0.347	0.234	**–0.661**	0.280
Q 3-rhamnoside	–0.217	**0.653**	–0.580	0.137	0.354	0.242
Q 3-glucuronide	0.059	0.134	–0.017	**0.546**	–0.069	0.028
Q 3–rutinoside	–0.200	0.475	0.233	0.255	0.288	0.389
K 3-glu/gal-galactoside	0.575	0.461	0.294	**0.798**	–0.021	–0.197
K 3-glucuronide	–0.111	–0.095	**0.782**	**0.698**	–0.020	–0.198
K 3-rhamnoside	–0.425	0.284	–0.737	–0.022	**0.615**	0.152
K 3-glucoside	0.329	–0.003	–0.018	**0.668**	0.262	0.391
cirsimarin	**0.714**	0.185	0.449	**0.847**	0.252	–0.048
apigenin	**0.786**	0.393	0.296	**0.960**	–0.098	0.106
Flav 285	**0.678**	**0.571**	0.242	**0.868**	–0.185	0.112
Flav 301_1	**0.833**	–0.032	–0.051	**0.846**	0.253	0.182
Flav 301_3	**0.650**	0.473	0.349	0.384	–0.205	0.428
Flav 315_2	–0.183	0.259	**0.763**	**0.559**	–0.217	0.244
Flav 315_3	–0.051	0.196	**0.529**	**0.640**	–0.215	0.046
Flav 331_01	**0.588**	**0.603**	0.373	**0.759**	–0.056	–0.025
Flav 331_1	**0.650**	**–0.619**	–0.240	**0.738**	0.476	–0.052
Flav 331_2	–0.003	0.018	**0.805**	**0.649**	0.354	0.196
Flav 331_3	**0.578**	0.179	0.422	**0.832**	0.101	0.088
Flav 331_4	**0.517**	0.159	**0.623**	**0.796**	–0.095	0.289
Flav345_1	0.141	**–0.544**	0.042	**0.649**	**0.623**	0.238
Flav 345_2	0.225	–0.247	**0.841**	**0.549**	0.236	0.399
Flav 345_3	0.402	**–0.758**	–0.296	**0.589**	**0.523**	–0.031
Flav 345_4	–0.087	–0.380	–0.208	**0.574**	**0.537**	0.198
Flav 345_5	0.143	–0.169	**0.950**	**0.824**	0.186	0.158
Flav 347	**0.796**	–0.027	0.274	**0.866**	0.211	0.229
Flav 361_1	–0.150	**–0.802**	0.220	**0.583**	**0.568**	–0.021
Flav 361_2	**0.809**	–0.111	0.232	**0.927**	0.045	0.023
Flav 375	–0.076	**–0.812**	0.087	**0.629**	**0.526**	–0.172
trit 279	–0.188	–0.132	–0.455	–0.291	0.496	0.358
trit 341	0.311	**0.885**	–0.244	0.271	–0.080	**0.787**
trit 515/555	–0.222	–0.327	**–0.742**	–0.332	0.440	0.126
trit 527	–0.011	**0.703**	–0.022	0.338	0.118	**0.758**
trit 545	0.438	–0.297	**0.603**	0.138	0.307	**0.780**
trit 557	0.029	–0.167	–0.042	0.128	**0.573**	**0.611**
trit 571	–0.156	0.281	–0.012	0.378	0.181	**0.610**
trit 603_1	0.075	**0.904**	0.093	–0.091	0.168	**0.743**
trit 603_2	–0.293	**0.595**	0.008	0.140	0.031	**0.789**
Variance explained (%)	20.7	20.5	18.9	31.9	15.3	12.8

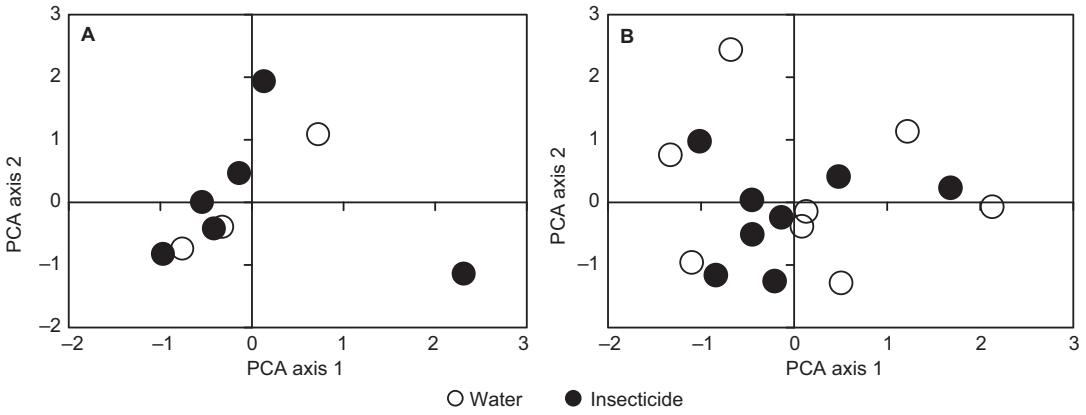

Fig. 3. Principal component analysis (PCA) graphs of the secondary metabolite data of (**A**) green summer leaves and (**B**) abscised senescent leaves for each seedling grown in the growth chamber and sprayed with either the insecticide (deltamethrin) or water. Metabolite loadings on the PCA axes are listed in Table 3.

to conclude that pyrethrin compounds are suitable for chemically reducing insect herbivore load in *B. pendula* in the field-scale studies of tree-herbivore interactions.

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