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1 Yield and compositions of bark phenolic extractives from three commercially  
2 significant softwoods show intra- and inter-specific variation

3 Maree Brennan<sup>a</sup>, Clément Fritsch<sup>a</sup>, Sylvain Cosgun<sup>a</sup>, Stéphane Dumarçay<sup>a</sup>, Francis Colin<sup>b</sup>, Philippe  
4 Gérardin<sup>a\*</sup>

5 <sup>a</sup>Université de Lorraine, INRAE, LERMAB, Faculté des Sciences et Technologies, Boulevard des  
6 Aiguillettes, 54506 Vandoeuvre-lès-Nancy, France

7 <sup>b</sup>INRAE, AgroParisTech, Université de Lorraine, SILVA, Route d'Amance, 54280, Champenoux, France

8 \*Corresponding author at: LERMAB, Faculté des Sciences et Technologies, Université de Lorraine,  
9 Boulevard des Aiguillettes, 54506 Vandoeuvre-lès-Nancy, France

10 *E-mail address:* philippe.gerardin@univ-lorraine.fr

11

12

## 13 **Abstract**

14 Tree bark is rich in commercially valuable secondary metabolites such as polyphenolic compounds  
15 like flavonoids and tannins. The yield and composition of bark extractives from *Abies alba* varies  
16 longitudinally within the stem. *A. alba* bark above the crown had the greatest extractive content, but  
17 the concentration of polyphenolic compounds was greatest below the crown. Here, we use a  
18 nonlinear model describing how bark extractive yields of *A. alba*, *Picea abies* and *Pseudotsuga*  
19 *menziesii* change with height, where differences among species are accounted for using different  
20 model coefficients. For all species there is longitudinal variability in extract yield. For *P. abies* and *P.*  
21 *menziesii*, the form of that variation differs depending on whether the bark was located at the same  
22 position in the stem as branches. Although the relationship form of total extractive yield differs  
23 between branched and un-branched samples, the relationship forms for individual compound yields  
24 does not change depending on branch presence. Despite trees from thinned stands having longer  
25 crowns and faster growth rates, indicative of greater photosynthetic activity, there was no evidence  
26 that thinning had affected either the yield or composition of extractives in these species. In *P. abies*,  
27 the proportions of flavonoids was higher in bark from the top of the tree, whereas epi-gallocatechin  
28 gallate was found in high proportions at the stem base. In *P. menziesii* bark extracts, taxifolin was the  
29 dominant compound, present in significantly higher proportions in bark towards the base of the  
30 stem.

31

## 32 **Keywords**

33 Bark extractives; Douglas fir; Norway spruce; Silver fir; tannin; within-tree variability

34

## 35 **1. Introduction**

36 Bark comprises the outer tissues surrounding the cambium of tree trunks, branches and roots, and  
37 has diverse roles within the tree. As the outermost organ, it provides a mechanical barrier against  
38 pathogens and grazing animals, and also against transpiration. Not just a mechanical barrier, bark  
39 contains a range of secondary metabolites that help resist pathogen attack by arresting the growth of  
40 pathogenic fungi, and that can also make the bark less palatable for grazing animals and bark-beetles  
41 (Evensen et al., 2000; Franceschi et al., 2005, 2000). There are two bark tissues, the “inner” bark  
42 which includes the cells of the secondary phloem including the polyphenolic parenchyma cells which  
43 are responsible for synthesis of tannins and other phenolic compounds (Krekling et al., 2000), and  
44 the “outer” bark (the periderm) which forms a highly effective protective barrier due to its suberised  
45 cells impregnated with tannins and lipidic compounds.

46 The secondary metabolites present in bark largely comprise tannins, which can make up 10 to  
47 39% of dry softwood bark biomass depending on species, tissue, and the method of quantification  
48 (Hernes and Hedges, 2004; Kempainen et al., 2014; Trivelato et al., 2016; Vieira et al., 2011).  
49 Tannins are polyphenolic compounds classed as either hydrolysable or condensed. Hydrolysable  
50 tannins are esters of gallic acid, whereas condensed tannins (proanthocyanidins) are oligomers of  
51 flavan-3-ol, and are either procyanidins (oligomers of catechin or epicatechin, Fig. 1) or  
52 prodelphinidins (oligomers of gallo catechin). B-type procyanidins are dimers which have C4-C8  
53 interflavan linkages (procyanidins B1 to B4) or C6-C8 interflavan linkages (procyanidins B5 to B8). C-  
54 type procyanidins are trimers of these and A-type procyanidins contain additional ether-type  
55 interflavan linkages between units (He et al., 2008). They are the products of the flavonoid pathway,  
56 and therefore it can be reasonably assumed that the availability of precursors might be directly  
57 affected by changes in available photoassimilate, due to differences in light interception caused by  
58 canopy size for example.

59 Tannins are useful not just for tanning, but condensed tannins contain a large proportion of  
60 hydroxyl groups which renders them useful for the production of resins, foams and adhesives (Feng

61 et al., 2013; García et al., 2014; Lacoste et al., 2015). Tannins are hydrophilic compounds and can be  
62 efficiently extracted with water, or a mixture of water and ethanol (Bianchi et al., 2015; Markom et  
63 al., 2007), solvents which can feasibly be used and recovered in industrial processes with low  
64 environmental impact (Capello et al., 2007). The compounds present in such extracts can be  
65 separated, quantified and identified using UV high-performance liquid chromatography (HPLC), gas  
66 chromatography or matrix-assisted laser-desorption ionisation followed by mass spectroscopy (MS)  
67 (Benković et al., 2014; Bianchi et al., 2015; Karonen et al., 2004; Krogell et al., 2012; Ohara et al.,  
68 2003).

69       Currently, tannins used commercially in Europe are obtained mainly from the heartwood of the  
70 exotic species quebracho (*Schinopsis lorentzii*), or the bark of black wattle (*Acacia mearnsii*). Ideally, a  
71 more local supply of tannins from currently available sources would both ensure sustainability and  
72 reduce the current environmental costs associated with their production and transport. More locally,  
73 European softwood barks are used in the horticulture sectors, energy sectors (for burning) and  
74 directly as a material (e.g. cork). It has recently been shown that the bark of European softwood  
75 species contains valuable compounds, and that the tannins of *A. alba* may even have better rates of  
76 condensation reactions than those of currently used industrial species due to longer oligomers and  
77 higher proportions of hydroxyl groups (Bianchi et al., 2015, 2014; Chupin et al., 2013; García et al.,  
78 2014; Jablonsky et al., 2017; Lacoste et al., 2015). However, there is limited information on how the  
79 diversity and availability of these compounds from local supply sources varies within trees of  
80 different softwoods. Such information would allow identification of co-product fractions of the most  
81 value for the chemical industries, ensuring better efficiency and therefore sustainability in the supply  
82 chain by allowing the most effective use of the primary resource for specific markets. It has been  
83 shown that the proportion of polyphenolic compounds in bark of silver fir (*Abies alba*) is greatest  
84 below the base of the crown where the highest proportion of outer bark is found, and that stand  
85 thinning does not have an effect on the yield of ethanol:water extracts in that species (Brennan et al.,

86 2020). Consequently, intra-specific variation in extractives should be taken into account in order not  
87 to waste energy and consumables by extracting low-value bark fractions.

88 In this study, the relationships between bark extractive yield and height were investigated among  
89 three economically-important European softwood species, *A. alba*, *Picea abies* and *Pseudotsuga*  
90 *menziesii*. We tested the hypotheses that extractive yield changes longitudinally along the stems of  
91 all three species, and that this relationship may be influenced by the stand management practice of  
92 thinning, and/or whether the bark was taken from discs at the same height as branches, or discs at  
93 heights without branches. Further, we tested the hypothesis that the diversity and quantity of  
94 phenolic compounds present in the bark extracts of *P. abies* and *P. menziesii* differed depending on  
95 longitudinal sample height within the stem, as was previously reported for trees of *A. alba* (Brennan  
96 et al., 2020).

97

## 98 **2. Materials and Methods**

### 99 1. *Tree growth and disc sampling*

100 The *A. alba* trees were grown as described in Brennan et al. (2020). Trees of *P. abies* were grown in  
101 the Mas-Dorier forest, Puy-de-Dôme, at an elevation of 770 m and latitude north 45°75,360',  
102 longitude east 3°6,540', and were harvested at 48 years of age. Trees of *P. menziesii* were grown in  
103 the Domaniale de Mélagues forest, Aveyron, at an altitude of 800 m and latitude north 43°71,330',  
104 longitude east 3°0,580', and were harvested at 53 years of age. Two management programmes were  
105 used: un-thinned and thinned management. Final stand densities for these treatments were 3233  
106 and 591, 1370 and 308, 972 and 141 trees per hectare for the un-thinned and thinned management  
107 type, for *A. alba*, *P.abies* and *P. menziesii*, respectively. Eight trees were harvested from each species.

108 As described in Brennan et al. (2020), up to 13 discs with height of ~10 cm were harvested from  
109 the base of the trees (30 cm above the ground), to the height where the trunk has a diameter of 10

110 cm, which defines the limit of wood suitable for industrial use. The samples harvested for each tree,  
111 their height, tree heights, ages and management are shown in Table 1. Samples at the base of the  
112 stem were harvested at 30, 80 and 130 cm above the ground, and did not have branches present.  
113 The remaining samples were harvested with branches (where they were present), and 10 cm above  
114 these positions samples without branches were harvested. These positions were the following: limit  
115 of construction wood (20 cm stem diameter), mid-height between stem base and the limit of  
116 construction wood, the first green branch, crown base and the limit of industrial wood (10 cm stem  
117 diameter).

118

## 119 2. *Milling and extraction*

120 The total extractive yield was calculated as the weight percentage of dry bark as described in  
121 Brennan et al. (2020). Briefly, bark from each disc was milled at 1100 rpm in a Fritsch pulverisette 9  
122 mill (Fritsch, Idar Oberstein, Germany) for 90 s. Some samples of *P. menziesii* bark at the base of the  
123 stem required milling for 180 s because the outer bark of this species contains layers of cork and the  
124 texture made milling less efficient, as reported in Trivelato et al. (2016). In triplicate, 2.00 g samples  
125 ( $\pm 0.01$  g) were extracted in a Thermo Scientific™ ASE™ 350 Accelerated Solvent Extractor with  
126 water:ethanol (50:50, v/v), three cycles at 1600 psi and 100°C, with 60% fresh solvent for each cycle.  
127 The extract was quantitatively transferred to a flask of known weight ( $\pm 0.0001$  g), the ethanol  
128 removed by rotary evaporation, and the water removed by freeze-drying before weighing to  
129 calculate the extract mass. This mass was used to calculate yield as a percentage of dry weight of the  
130 bark.

131

## 132 3. *Compositional analysis*

133 For analysis of the extract composition, a subset of four trees was chosen for each species. In each  
134 species, the subset comprised two trees of each management type, and for each management type  
135 one taller and one shorter tree was chosen, in order to maximise variability. The trees chosen were  
136 the following for each species: *A. alba*, trees 1, 2, 5 and 8; *P. abies*, trees 2, 4, 6 and 8; *P. menziesii*,  
137 trees 1, 3, 5 and 6.

138 The polyphenolic compounds in the extracts were assigned a number and quantified using  
139 UV-HPLC as described in Brennan et al. (2020). Briefly, to 10 mg of dry extract 1 ml of water:ethanol  
140 (50:50, v/v) was added and the sample was sonicated for 2 min before analysis. Samples (5  $\mu$ l) were  
141 analysed on a Shimadzu LC-20A ultra-HPLC system (Shimadzu, Kyoto, Japan) equipped with an  
142 autosampler interfaced with an SPD-20A photodiode-array (PDA) UV detector. Separation was done  
143 using a Luna C18 analytical column (i.d. 150 mm  $\times$  3 mm, Phenomenex, Torrance, CA, USA), and  
144 mass spectrometry was done with a Shimadzu LC-MS 8030 triple-quadrupole mass spectrometer.  
145 The profile was as follows: 0.0 to 10.0 min 5% (v/v) acetonitrile ( $\geq$ 99.9%, LC-MS Chromasolv™) in  
146 water (LC-MS Chromasolv™), 10.0 to 18.0 min 30% acetonitrile, 18.0 to 19.0 min 80% acetonitrile,  
147 19.0 to 20.0 min 5% acetonitrile containing 0.1% (v/v) formic acid, at a flow rate of 0.4 ml min<sup>-1</sup>. UV-  
148 visible spectra were recorded from 190 to 800 nm. Positive and negative ion electrospray mass  
149 spectrometric analyses were done with unit resolution from 100 to 2000 m/z and scan speed of  
150 15000 U/s and ion spray voltage +/- 4500 V. Heatblock and desolvation line temperatures were  
151 400°C and 250°C. Nitrogen gas was used for drying (15 litres min<sup>-1</sup>) and nebulizing (3 litres min<sup>-1</sup>).  
152 Data were acquired and analyzed with LabSolutions software (5.42SP4, Shimadzu). Peaks visible at  
153 the phenolic absorption maximum 280 nm were numbered according to their mass spectra, and  
154 identified by comparing their mass spectra to the literature and standards of (+)-catechin hydrate  
155 and (-)-epicatechin. Numbered peaks, their retention times and characteristic ions in both positive  
156 and negative mode used for identification are given in Table 2. All quantities (mg) of polyphenolic  
157 compounds represented by each peak are expressed in catechin equivalent weights (using the mean



158 yield-area relationships of the two standards) and converted to a yield of mg compound per g dry  
159 bark.

160

#### 161 4. *Data analysis*

162 Statistical analyses were done using R (R Development Core Team., 2008). For total extractive yield,  
163 samples with and without branches were modelled separately. To examine how total extractive yield  
164 changes along the height of the tree for all three species, a linear mixed effects model was built with  
165 total yield (% by weight) as the response variable, and using the interaction between species and the  
166 second-degree polynomial of % total height as the predictor variables, with total tree height as the  
167 random effect. To examine how the amount of individual compounds present in the bark changes  
168 with respect to tree height for the species *P. albies* and *P. menziesii*, the raw data were plotted and a  
169 model built to test the significant of height using an appropriate functional form depending on the  
170 form in the plot; only the linear form was tested where there was no detectable relationship with  
171 height. Model forms tested were linear ( $y = \alpha x + c$ ), exponential ( $y = \alpha \beta^x [+ c]$ ) or exponential decay ( $y$   
172  $= \alpha \beta^x$ ), where  $y$  is the response variable,  $\alpha$  and  $\beta$  are calculable or estimatable parameters, and  $c$  is an  
173 intercept which is generally zero. Final model parameters are given in Table S1. There was no  
174 evidence that either thinning management or branch presence had an influence on the form of the  
175 relationship between yield and height of any individual compound, for either of these species. For  
176 the nonlinear decay relationships, the parameters  $\alpha$  and  $\beta$  were first estimated then iteratively  
177 tested and chosen to maximise correlation and covariance with  $y$ .

178

### 179 3. **Results**

#### 180 1. *Tree form*

181 For each of the three species, up to 13 samples were harvested along the height of a total of eight  
182 trees. For each tree, the lowest sample was 30 cm above the ground, and the highest sample was  
183 taken where the diameter of the trunk was 10 cm. The height of all samples for each tree is shown in  
184 Table 1, with the information about thinning management type, tree height, age, and diameter at  
185 breast height (DBH).

186 The trees of *A. alba* have previously been described in Brennan et al. (2020). Briefly, these  
187 trees had similar mean heights between the thinned and un-thinned stands, however, the trees from  
188 the thinned stand had a greater mean DBH (32.3 cm) than those from the un-thinned stand (18.7  
189 cm), with the thinned-stand trees also having greater crown depth. Similarly, *P. abies* trees had  
190 similar mean tree heights between the un-thinned (2749 cm) and thinned (2747 cm) stands, but the  
191 mean DBH of trees from the un-thinned stand (31.4 cm) was less than that of the thinned-stand trees  
192 (39.5 cm). The trees of *P. menziesii* differed slightly in that the trees of the un-thinned stand had a  
193 lower mean height (3020 cm) compared to the trees from the thinned stand (3125 cm). However,  
194 like the other species examined, the mean DBH of trees of the un-thinned stand was less (33.8 cm)  
195 compared with the thinned-stand trees (55.4 cm). Both *P. abies* and *P. menziesii* had greater crown  
196 depth in trees from the thinned management stand compared with the un-thinned stand, and  
197 consequently the height of the first green branch was also lowest in the thinned-stand trees.  
198 Together, these results indicate that across all species, trees in the thinned stands had a greater  
199 capacity for photosynthesis compared to trees from the un-thinned stands, which consequently  
200 resulted in better radial growth.

201

## 202 2. Extractive yields

203 The coefficients for the modelled relationships between extractive yield (% dry weight) and the %  
204 total height of samples is given for each species, without and with branches, in Table S2. The  
205 relationships are plotted in Fig. 2A for samples without branches, and in Fig. 2B for samples with

206 branches. For samples without branches, the use of total tree height as the random effect gave a  
207 small improvement over the use of diameter at breast height (DBH) (each explaining 34.6 and 32.7%  
208 of the total variance, respectively), and the inclusion of the random effect improved the model fit  
209 compared to not using any random effect (AICs (Aikaike information criterion) were 1012 and 1080,  
210 respectively). Similarly for samples with branches, total tree height was the slightly better random  
211 effect compared with DBH (each explaining 25.1 and 21.6% of the total variance, respectively), with  
212 inclusion of the random effect improving the model fit compared to not using any random effect (AIC  
213 559 and 607, respectively). Because for all species, the crown depth was greater in trees from the  
214 thinned management stand, the mean height of the base of the crown occurred at a lower height in  
215 these trees, although there was no significant effect of management type on the relationship form ( $p$   
216  $< 0.3$ ,  $p < 0.8$ , without and with branches, respectively). Although the management type did not have  
217 a significant effect on the relationship, the points in Fig. 2 are shown filled (un-thinned management)  
218 and open (thinned management) to illustrate this point.

219         Across the three species, the mean height of the base of the crown for the un-thinned group  
220 was 69.5% of total height, whereas the mean height of the base of the crown for the thinned group  
221 was 42.2% of total height. Between these two heights is a transitional zone where, for samples both  
222 without and with branches, the direction of the correlations between yield and height changes in a  
223 species-dependent manner, giving rise to the significant polynomial form of the relationship. For  
224 samples without branches, *A. alba* and *P. menziesii* have a similar relationship (Fig. 2A). The mean  
225 yield increases with sample height until the transition zone around the base of the crown is reached.  
226 After this point, the mean yield tends to remain stable, giving a reasonably flat polynomial curve. In  
227 *P. abies* on the other hand, the mean extractive yield tends to be relatively stable until the transition  
228 zone is reached, after which yield increases rapidly with sample height reaching its peak at the  
229 highest-harvested sample, resulting in a polynomial with a positive leading coefficient. For samples  
230 without branches (Fig. 2B) as previously reported (Brennan et al., 2020), *A. alba* has a similar form of  
231 relationship as found in samples with branches. In *P. abies*, the relationship is different for samples

232 with branches compared to those without, having a negative leading coefficient caused by an  
233 increase in extractive yield with height which peaks during the transitional zone, after which it  
234 stabilises. *P. menziesii* has a positive coefficient on the other hand, although extractive yield only  
235 increased with height after the transitional zone.

236

### 237 3. Extractive compositions

238 Example chromatograms show the retention times of the 15 quantified compounds from *P. abies*  
239 extracts (Fig. 3A), and the 20 quantified compounds from *P. menziesii* extracts (Fig. 3A). Compound  
240 masses and intensities used for identification from their mass spectra are given in Table 2, and  
241 correspond to the peak numbers in Fig. 3. It is clear that extracts from *P. abies* contain a large  
242 proportion of condensed tannins with a degree of polymerisation of three or more; these are  
243 evidenced by the rising baseline caused by these molecules which are unable to be well resolved  
244 (Cadahia et al., 1997; Mueller-Harvey et al., 1987; Putman and Butler, 1989). The extracts of *P*  
245 *menziesii* however, are dominated by a large peak at eight minutes (comprising a single compound,  
246 taxifolin), which obscures the rising baseline caused by unresolved tannins. Although oligomeric  
247 tannins are clearly present in quantifiable proportions in *P. menziesii*, they comprise less of the total  
248 extract than those present in *P. abies*.

249 Individual plots of the yield ( $\text{mg g}^{-1}$  bark) of all compounds against sample height are shown  
250 for *P. abies* in Supplementary Figure S1. Few of the compounds quantified from the *P. abies* extracts  
251 had a significant relationship with sample height. Compound 5 and epigallocatechin gallate were  
252 present only at the base of the stem, with epigallocatechin gallate fitting an exponential decay curve  
253 (Fig. 4A, see Table S1). Other compounds that had a relationship between yield and height typically  
254 had a growing rate of increase from zero  $\text{mg g}^{-1}$  bark at the base of the stem, towards a higher yield  
255 at the top of the stem, making an exponential model appropriate. This relationship was only  
256 significant for compounds 13, isorhamnetin and circimaritin (Fig. 4B, 4C and 4D, respectively). Both

257 samples without and with branches present had an exponentially increasing yield for compound 13,  
258 isorhamnetin and circimaritin, despite samples with branches typically having a stable total extract  
259 yield after the crown base transition zone. Although the samples without branches had a significant  
260 yield increase for total extractives after the crown base transitional zone, branch presence was not a  
261 significant factor influencing the yield of individual compounds along the height of the tree. For the  
262 two most abundant compounds, piceatanol-3-glucoside ( $4.7 \text{ mg g}^{-1}$ ) and compound 12 ( $1.2 \text{ mg g}^{-1}$ ),  
263 some samples without branches did have higher yields towards the top of the tree which are likely to  
264 have contributed to the observed increase in total extractive weight from higher samples in this  
265 species. However, other samples without branches retained near-zero yields for these compounds.  
266 Identified compounds with stable yields along the length of the stem include protocatechuic acid ( $0.3$   
267  $\text{mg g}^{-1}$ ), protocatechuic aldehyde ( $0.8 \text{ mg g}^{-1}$ ), catechin ( $0.1 \text{ mg g}^{-1}$ ), dihydroquercetin-3-O-  
268 rhamnoside ( $0.8 \text{ mg g}^{-1}$ ) and taxifolin ( $0.8 \text{ mg g}^{-1}$ ).

269 Individual plots of the yield ( $\text{mg g}^{-1}$  bark) of all compounds against sample height are shown  
270 for *P. menziesii* in Supplementary Figure S2. In contrast to the composition of *P. abies*, 16 out of the  
271 20 quantified compounds present in *P. menziesii* have a significant relationship between their yield  
272 and the height of the sample. Seven compounds have an increase in yield with sample height,  
273 generally approximating to an exponential relationship although compound 3 is linear (Fig. 5A),  
274 whereas the other nine have a decreasing relationship with sample height approximating to an  
275 exponential decay curve except for compound 2 which was linear. Although the relationship between  
276 total extract yield and sample height was different for samples without and samples with branches,  
277 there were no significant differences in the relationships of individual compounds between samples  
278 with opposing branch presence. The increasing total extract yield for samples with branches that  
279 occurs after the crown base transition zone in this species must therefore be due to the presence of  
280 non-UV visible compounds. Those compounds with an exponential relationship had very low  
281 proportions of the compounds towards the base of the stem (e.g. compound 10, a procyanidin C1,  
282 Fig. 5B), typically with lower variance than those towards the top of the stem as compound yield

283 increased. Some of the compounds with this relationship had values close to zero mg g<sup>-1</sup> bark at the  
284 base of the stem, whereas others such as compound 5, a procyanidin B2 (0.25 mg g<sup>-1</sup>), had a higher  
285 minimum yield. Taxifolin (Fig. 5C) and eriodictyol (Fig. 5D) were the two most abundant compounds  
286 found in *P. menziesii*, and their abundance decreased from 200 and 10 mg g<sup>-1</sup> down to zero mg g<sup>-1</sup>  
287 respectively. All such compounds with exponential decay relationships tended towards a value of  
288 zero for samples at the top of the trees. A procyanidin B1 (compound 7, 1.0 mg g<sup>-1</sup>), epicatechin (0.6  
289 mg g<sup>-1</sup>) and compounds 11 (0.5 mg g<sup>-1</sup>) and 14 (0.4 mg g<sup>-1</sup>) had stable yields along the stem height.

290

## 291 1. Discussion

292 Previously, it was shown that the total extractive yield of *A. alba* bark increased from the base of the  
293 stem up to the base of the crown, and stayed reasonably stable within the crown, only increasing  
294 slightly and with no effect of branch type (Brennan et al., 2020). Here, this relationship between  
295 extractive yield and height was expanded to include a further two economically significant softwood  
296 species, *P. abies* and *P. menziesii*. Like *A. alba*, a polynomial model was able to describe how  
297 extractive yield changes along the length of the tree, although the model coefficients are dependent  
298 on species. However, for these two additional species, it was clear that the form of the relationship  
299 changed depending on whether the disc was taken at heights where branches were present. The  
300 polynomial form is necessary to describe the variation in extractive yield due to the change at the  
301 transitional zone at the base of the crown, from a stable relationship to a positive relationship, or  
302 vice versa, depending on species and branch presence. This indicates that for *P. abies* and *P. menziesii*  
303 at least, either synthesis or transport of the extractives differed when branches were present. It is  
304 possible that samples taken from discs containing branches had more immediate access to  
305 photoassimilate for metabolite synthesis. There is little information in the literature about how the  
306 total bark extract yield changes longitudinally within the tree for these species, although it has been  
307 shown that the yield of extractives in knot wood tends to be higher at the base of the crown than at

308 the top (Kebbi-Benkeder et al., 2017; Piispanen et al., 2008). Studies that compare extractive yields  
309 from inner and outer bark tissues can be used to infer how yields might be expected to change along  
310 the tree stem, as there is a greater proportion of outer bark in the mature (basal) parts of the stem  
311 compared to that of the younger (apical) stem parts. Inner bark generally contains higher proportions  
312 of extractives (Eberhardt, 2013; Krogell et al., 2012), and the results of the present study indicate  
313 that bark from higher samples does indeed contain the largest proportion of extractives in all three  
314 species.

315 Changes in bark properties along stem height were apparent during milling of *P. abies* and *P.*  
316 *menziesii* that were not previously found for *A. alba* in the study of Brennan et al. (2020). The bark  
317 from the top of the *P. abies* trees contained a greater proportion of sticky resin compared to samples  
318 from the upper stem portions of *A. alba* or *P. menziesii*, and the bark from the lower portions of *P.*  
319 *menziesii* contained a large amount of cork not present in the other two species. The resinous quality  
320 of the upper bark likely explains the sharp increase in yield observed in *P. abies* samples without  
321 branches after the transitional zone at the base of the crown, compared to the other species which  
322 had only a gradual increase in extractive yield, although it is not clear why this was not observed for  
323 bark samples taken at the level of branches. The presence of cork layers in bark of the lower stem  
324 portion of *P. menziesii* has been indicated previously, and it has been shown that the yield of  
325 extractives from phloem interspersed in between the cork layers changes radially (Trivelato et al.,  
326 2016). Although the management type resulted in different crown sizes, there was no difference in  
327 extractive yield between management type, indicating that trees from these un-thinned stands do  
328 not have a reduced ability to synthesise extractable secondary metabolites, although we cannot rule  
329 out that this is due to the small sample size analysed between treatments. It is known that the  
330 proportions of extractives in the different bark tissues also changes with height (Vedernikov et al.,  
331 2011), and it would be interesting to know whether specifically the inner bark extractives in the  
332 crown change with management or at the transitional zone, as differences in secondary metabolite  
333 synthesis may be obscured by analysing both bark tissues together. That the presence of branches

334 changed the relationship form in *P. abies* and *P. menziesii* however, suggests that branch position  
335 affects transport of photoassimilates in these species more than in *A. alba*.

336 Previously, bark extracts of *P. abies* have been shown to contain tannins, stilbenes, lignans,  
337 flavonoids and the stilbene glucosides piceid, astringin and isorhapontin (Bianchi et al., 2014; Jyske et  
338 al., 2014; Pietarinen et al., 2006; Royer et al., 2013). Co-elution of some compounds occurred, where  
339 two or more molecules have the same retention times, complicating the precise identification of  
340 some peaks in the present study. In this study, astringin was also identified, and was sometimes (but  
341 not often) present in large quantities. Flavonoids identified include isorhamnetin and cirscimaritin,  
342 which are compounds which have anti-inflammatory effects. No lignans, piceid or isorhapontin were  
343 identified in *P. abies* extracts, although these have previously been identified . These may co-elute as  
344 one or part of the unidentified numbered compounds, as masses of 302 m/z were found, indicating  
345 that piceid may have been present. The composition of *P. menziesii* bark has been less well-studied,  
346 and it is clearly not as complex a mixture as *P. abies*. As in the study of Trivelato et al. (2016), the  
347 extracts were dominated by taxifolin, and oligomeric tannins. Large amounts of sugars have also  
348 been found in *P. menziesii* bark extracts (Pietarinen et al., 2006), however the present methodology  
349 only identified compounds visible in UV, therefore sugars were not identified. Epi-catechin has been  
350 shown to be the typical repeating unit of *P. abies* tannins, which are procyanidins, and have a degree  
351 of polymerisation up to 13 (Bianchi et al., 2014), although we only found it in minor quantities in our  
352 studies. Although the degree of polymerisation of the oligomeric tannins in the present study was  
353 not measured, it has been demonstrated that longer tannin oligomers elute later than shorter ones  
354 (Cadahia et al., 1997; Mueller-Harvey et al., 1987; Putman and Butler, 1989). Procyanidin units were  
355 the most abundant tannins that resolved well in chromatograms of *P. menziesii*, indicating that the  
356 tannins of this species are largely procyanidins. The later elution of *P. menziesii* oligomeric tannins  
357 compared to that of *P. abies* indicates that in *P. menziesii*, the oligomers are likely to have a mean  
358 degree of polymerisation even greater than 13.



359 That the extractive yield increased so dramatically with height for samples without branches but  
360 within the crown of *P. abies*, and as it was noted that higher bark samples were more resinous, it  
361 could be expected that a higher proportions of resin acids and terpenes would be identified and  
362 change significantly with bark height. The most commonly identified resin acid of *P. abies* is abietic  
363 acid (Norin and Winell, 1972), which was not identified in the present study. Protocatechuic acid was  
364 identified, but was present in stable proportions across the different heights, rather than increasing  
365 with height. Only two flavonoids and an unidentified compound increased with bark height in fact,  
366 therefore it is possible that the increased extractive yield observed was potentially due to oligomeric  
367 tannins which were not quantified here, or due to sugars or fatty acids which are not UV-visible and  
368 thus were not identified. Starch granules are known to be present in the polyphenolic parenchyma  
369 cells of *P. abies* for example (Krekling et al., 2000), and may also be present in the extracts and  
370 contribute to yield. Other sugars are known to be present in bark extracts, either alone or conjugated  
371 to phenolic compounds, and it is not easy to separate them whilst quantitatively leaving only the  
372 phenolics (Kemppainen et al., 2014; Krogell et al., 2012; Le Normand et al., 2012). Epi-gallocatechin  
373 gallate (0.1 mg g<sup>-1</sup> bark), an important anti-cancer polyphenol of green tea (Du et al., 2012), was  
374 higher in bark at the stem base of *P. abies*, but was not often present in bark above 1.3 m, making  
375 this an unlikely source specifically of epi-gallocatechin gallate for commercial markets. Previously,  
376 stilbene glucosides including astringin were shown to decrease with height in the bark of *P. abies*  
377 (Jyske et al., 2014). No significant change in astringin abundance with height was observed in the  
378 present study; Jyske et al. (2014) examined trees with no differences in stem diameter or mean  
379 annual growth, and random effects such as stem diameter were not included when modelling  
380 changes in compound quantity here. The flavonoid taxifolin was the most abundant compound in *P.*  
381 *menziesii* bark as previously reported, but towards the top of the tree it is present in only minor  
382 proportions, therefore bark from the lower portions of the stem only could be an important source  
383 of this compound. Catechin and procyanidin units tended to augment with height, indicating that the  
384 building blocks of condensed tannins are more abundant in younger bark in this species. The smaller

385 proportions of these compounds in the lower part of the trunk could be explained if they are become  
386 part of tannin oligomers with higher degrees of polymerisation in older bark.

387

## 388 **2. Conclusions**

389 The bark extractive yields of *A. alba*, *P. abies* and *P. menziesii* do change longitudinally along the  
390 stems, with yields typically higher at the top of the tree. This indicates that synthesis of such  
391 compound is unlikely to be uniform along the stem, perhaps depending on distance from the crown.  
392 The form of the relationship between yield and height is a second-degree polynomial, with the sign  
393 (positive or negative) of the coefficient depending on species. The stand management did not  
394 significantly affect extractive yields, but branch presence or absence changed the form of the yield-  
395 height relationship in *P. abies* and *P. menziesii*. The diversity and quantity of phenolic compounds in  
396 *P. abies* and *P. menziesii* does differ depending on height. This is most evident for flavonoids in *P.*  
397 *abies* extracts, which have higher abundance with increasing height of the bark within the tree. In *P.*  
398 *menziesii* however, taxifolin is most abundant in extracts from bark lower in the trunk.

399

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413

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- 522

**Table 1.** Height (cm) of discs<sup>a</sup> sampled within each tree, tree height, age and management type.

Species	Treatment	Tree	30	80	130	MILAB	MILSB	DEC20AB	DEC20SB	H1BVAB	H1BVSB	HBHAB	HBHSB	DEC10AB	DEC10SB	Height	Age	DBH <sup>b</sup>	
<i>Abies alba</i>																			
Un-thinned	1	30	80	130	480	490		955		1130	1140	1365	1375	1685	1705	2240	50	28.0	
	2	30	80	130		330				1136	1150	1350	1361	617	629	1575	44	13.9	
	3	30	80	130	265	277	465	479		950	965	1095	1107	1509	1523	2050	51	22.6	
	4	30	80	130						947	958	947	958			1360	57	10.4	
Thinned	5	30	80	130	600	605				720	732	780	793	1147	1154	1699	48	19.6	
	6	30	80	130	697		1350	1365	495	505	795	800	1745	1765	2140	43	41.2		
	7	30	80	130	435	445	870	885	515	525	765	775	1540	1525	2010	43	29.5		
	8	30	80	130	685		1318	1340	703	713	890	905	1720	1740	2120	44	38.9		
<i>Picea abies</i>																			
Un-thinned	1	30	80	130	1040	1055	2036	2065	1540	1552	1965	2003	2567	2588	3065	53	40.9		
	2	30	80	130	995	1010			1785	1804	2014	2028	2580	2595	3055	53	34.7		
	3	30	80	130	860	872	376	388	1470	1480	1868	1880	1595	1605	2203	53	22.3		
	4	30	80	117	662	674	1224	1239	1535	1547	1827	1837	2100	2110	2672	53	27.7		
Thinned	5	30	80	130			1535	1552	744	760	1148	1160	2050	2061	2500	53	33.8		
	6	30	80	130	836	850	1710	1730	915	930	1295	1310	2250	2265	2693	53	39.3		
	7	30	80	130	966	982	1914	1927	760	772	1050	1065	2488	2496	2952	53	44.1		
	8	30	80	130			1857	1884	932	955	1414	1446	2396	2412	2844	53	40.9		
<i>Pseudotsuga menziesii</i>																			
Un-thinned	5	30	80	130	225	245	445	465	1780	1790	2412	2423	225	245	2719	48	23.2		
	6	30	80	130	1103	1125	2211	2233	1882	1908	2059	2080	2716	2733	3175	48	47.7		
	7	30	80	130	785	762	1530	1548	1815	1832	2040	2053	2408	2420	3065	48	33.3		
	8	30	80	130	785	815	1512	1527	1767	1780	1918	1930	2452	2474	3120	48	31.4		
Thinned	1	30	80	130			2142	2155	1112	1150	1421	1455	2590	2615	3072	48	57.0		
	2	30	80	130	1274	1300	2500	2525	1045	1065	1415	1436	2725	2740	3413	48	46.5		
	3	30	80	130	925	952	1880	1902	810	840	1065	1095	2200	2225	2655	48	52.5		
	4	30	80	130	1300	1335	2530	2550	900	928	1215	1262	2865	2885	3360	48	65.6		

523 <sup>a</sup>30 cm = 30 cm above ground; 80 cm = 80 cm above ground; 130 cm = 130 cm above ground; MILAB = mid-height of the tree, with branches; MILSB = 10 cm above MILAB,  
524 without branches; DEC20AB = limit of wood for use in construction, with branches; DEC20SB = 10 cm above DEC20AB, without branches; H1BVAB = first green branch above

525 the ground; H1BVS<sub>B</sub> = 10 cm above H1BV<sub>A</sub><sub>B</sub>, without branches; HBH<sub>A</sub><sub>B</sub> = base of the crown; HBHS<sub>B</sub> = 10 cm above HBH<sub>A</sub><sub>B</sub>, without branches; DEC10<sub>A</sub><sub>B</sub> = limit of wood for  
526 industrial use, with branches; DEC10<sub>S</sub><sub>B</sub> = 10 cm above DEC10<sub>A</sub><sub>B</sub>, without branches.

527 <sup>b</sup>DBH = Diameter at breast height (130 cm).

528

529



**Table 2.** Compound assignments based on mass spectra.

Species	Compound	m/z (Intensity)
<i>Picea abies</i>	Compound 1	[M+H] <sup>+</sup> 158.15 (100), 140.00 (12), 156.15 (11)
		[M-H] <sup>-</sup> 195.15 (100)
	Protocatechuic acid	[M+H] <sup>+</sup> 105.25 (100), 122.75 (100), 248.05 (74), 102.45 (73), 114.85 (37), 188.25 (36), 174.00 (34), 187.50 (24), 180.35 (22), 197.90 (16), 290.15 (15), 161.00 (15), 155.40 (14), 209.05 (13), 260.10 (13), 319.00 (13), 207.55 (12), 249.20 (12), 138.95 (11), 225.25 (10), 204.30 (10)
		[M-H] <sup>-</sup> 153.25 (100), 197.15 (41)
	Protocatechuic aldehyde	[M+H] <sup>+</sup> 194.95 (100), 367.10 (97), 579.30 (58), 379.00 (56), 105.15 (51), 371.15 (39), 139.10 (35), 239.00 (35), 369.20 (24), 389.15 (22), 122.75 (20), 580.30 (18), 441.00 (18), 218.70 (18), 376.10 (17), 331.10 (15), 177.15 (15), 304.55 (13), 351.20 (12), 114.40 (12), 172.25 (12), 425.10 (12), 212.30 (11), 102.25 (11)
		[M-H] <sup>-</sup> 401.25 (100), 137.30 (99), 355.30 (79), 577.35 (49), 389.35 (40), 578.30 (19), 402.30 (19), 307.35 (15), 391.40 (14), 356.30 (14), 169.25 (12), 361.30 (12), 193.25 (11), 275.15 (11), 423.25 (10)
	Catechin	[M+H] <sup>+</sup> 291.00 (100), 105.25 (17), 381.05 (16), 122.50 (11), 226.55 (11)
		[M-H] <sup>-</sup> 289.25 (100), 335.25 (21), 352.25 (15), 290.30 (15)
	Compound 5	[M+H] <sup>+</sup> 102.40 (100), 441.05 (57), 104.95 (56), 122.80 (45), 276.85 (39), 439.15 (28), 114.85 (19), 476.85 (15), 667.35 (14), 179.65 (14), 145.90 (13), 196.40 (13), 461.10 (12), 291.35 (10), 167.05 (10)
		[M-H] <sup>-</sup> 437.25 (100), 665.55 (26), 463.40 (15), 577.30 (15), 505.00 (10), 721.05 (10)
	(-)-Epigallocatechin gallate	[M+H] <sup>+</sup> 102.20 (100), 105.15 (95), 667.40 (56), 122.85 (35), 299.05 (33), 499.25 (33), 668.40 (29), 114.85 (25), 317.25 (18), 207.85 (15), 278.55 (15), 291.15 (14), 428.20 (14), 198.40 (11), 197.45 (11)
		[M-H] <sup>-</sup> 521.40 (100), 665.50 (51), 538.45 (32), 522.35 (24), 666.50 (24), 553.35 (16), 539.30 (12), 475.35 (10)
	Astringin (piceatannol-3-glucoside)	[M+H] <sup>+</sup> 245.00 (100), 407.05 (41), 369.10 (10)
		[M-H] <sup>-</sup> 405.30 (100), 451.30 (49), 468.30 (37), 811.55 (33), 406.30 (22), 812.50 (13), 452.30 (11)
	Epimer of Astringin	[M+H] <sup>+</sup> Epimer of compound 7
		[M-H] <sup>-</sup> Epimer of compound 7
	Dihydromyricetin 3-O-rhamnoside	[M+H] <sup>+</sup> 305.00 (100), 105.15 (17), 306.05 (16), 169.10 (10)
		[M-H] <sup>-</sup> 465.30 (100), 466.30 (24), 375.35 (20), 528.35 (10)
	Compound 10	[M+H] <sup>+</sup> 105.05 (100), 667.40 (35.73), 123.00 (32.31), 169.05 (28.25), 102.55 (26.92), 353.10 (24.25), 114.95 (21.99), 122.20 (19.88), 515.15 (17.8), 152.95 (17.29), 173.70 (16.15), 208.20 (14.61), 547.35 (12.63), 165.25 (11.86), 598.70 (11.23), 305.05 (11.04),
[M-H] <sup>-</sup> 665.45 (100), 465.30 (57), 271.20 (37), 193.20 (31), 335.30 (24), 981.40 (20), 309.25 (18), 809.45 (17), 195.30 (16), 537.10 (16), 173.45 (15), 375.35 (14), 377.30 (13), 1235.70 (12), 523.45 (11), 333.35 (11),		

			720.20 (10)
	Taxifolin (dihydroquercetin)	[M+H] <sup>+</sup>	305.05 (100), 317.15 (57), 105.05 (42), 233.55 (28), 529.30 (21), 306.15 (16), 318.25 (12), 371.15 (10)
		[M-H] <sup>-</sup>	303.20 (100)
	Compound 12	[M+H] <sup>+</sup>	259.05 (100), 421.10 (35), 401.15 (30), 271.10 (13), 105.00 (11)
		[M-H] <sup>-</sup>	465.35 (100), 419.30 (57), 482.30 (31), 466.30 (23), 420.30 (13), 257.25 (10)
	Compound 13	[M+H] <sup>+</sup>	297.10 (100), 313.10 (84), 331.10 (67), 277.10 (59), 333.15 (50), 355.15 (33), 687.55 (32), 315.15 (30), 298.20 (30), 317.20 (20), 299.10 (20), 105.10 (18), 275.15 (17), 688.55 (14), 318.20 (13), 334.20 (12), 351.15 (12)
		[M-H] <sup>-</sup>	331.35 (100), 329.35 (32), 349.40 (26), 332.30 (21), 275.35 (20), 315.35 (12)
	Isorhamnetin (t) <sup>a</sup>	[M+H] <sup>+</sup>	317.15 (100), 339.15 (72), 655.55 (55), 299.15 (43), 315.15 (36), 261.15 (27), 656.55 (24), 340.10 (17), 301.15 (16), 309.25 (14), 289.15 (13), 319.15 (12), 380.15 (11), 105.10 (10)
		[M-H] <sup>-</sup>	315.35 (100), 316.35 (24)
	Cirsimaritin (t)	[M+H] <sup>+</sup>	315.15 (100), 317.15 (24), 105.15 (21), 289.20 (15), 629.50 (14), 356.20 (12), 261.15 (12), 337.10 (11), 339.15 (11)
		[M-H] <sup>-</sup>	313.35 (100), 315.40 (39), 359.40 (12), 316.40 (11), 345.40 (10)
<i>Pseudotsuga menziesii</i>	Compound 1	[M+H] <sup>+</sup>	102.35 (100), 122.70 (86), 105.00 (83), 127.00 (48), 187.90 (46), 214.45 (35), 208.45 (27), 179.70 (26), 123.90 (25), 172.65 (21), 114.05 (19), 195.30 (14), 391.10 (12), 213.45 (12), 265.00 (12), 193.25 (12), 180.85 (10), 167.10 (10), 371.00 (10)
		[M-H] <sup>-</sup>	1043.70 (100), 213.75 (99), 457.80 (77), 146.90 (74), 113.20 (32), 175.15 (29), 259.35 (17), 130.65 (16), 322.25 (15), 261.10 (14), 239.00 (14), 242.70 (14), 205.40 (12), 295.10 (11), 208.80 (11), 243.50 (11), 141.00 (10), 641.75 (10)
	Compound 2	[M+H] <sup>+</sup>	101.90 (100), 122.80 (95), 105.05 (86), 114.80 (25), 179.85 (21), 188.10 (21), 180.70 (19), 213.20 (15), 110.55 (15), 226.90 (14), 1385.60 (14), 173.30 (13), 275.30 (13), 198.60 (13), 240.10 (12), 259.55 (12), 234.60 (12), 207.85 (11), 302.95 (11), 214.80 (10), 289.15 (10), 391.15 (10)
		[M-H] <sup>-</sup>	153.15 (100), 197.15 (32), 154.40 (12)
	Compound 3	[M+H] <sup>+</sup>	250.95 (100), 102.30 (98), 105.05 (89), 122.65 (88), 114.85 (47), 188.10 (43), 187.35 (32), 391.15 (32), 198.40 (31), 274.25 (24), 167.50 (24), 208.20 (23), 173.20 (19), 224.95 (19), 331.05 (18), 171.20 (17), 252.95 (15), 403.10 (15), 241.35 (15), 212.70 (14), 226.25 (13), 127.05 (13), 103.80 (13), 329.10 (12), 134.80 (12), 211.00 (11), 245.10 (11), 255.10 (11), 232.85 (11), 189.40 (11), 279.60 (11), 361.15 (11), 345.05 (10)
		[M-H] <sup>-</sup>	211.20 (100), 389.25 (77), 343.20 (59), 147.05 (49), 289.35 (39), 181.35 (35), 379.55 (30), 242.60 (22), 113.70 (21), 459.10 (19), 411.50 (18), 175.50 (17), 255.25 (17), 390.45 (15), 344.60 (15), 413.35 (14), 194.90 (14), 130.05 (14), 112.45 (14), 345.35 (14), 155.45 (13), 406.10 (13), 478.30 (13), 281.55 (13), 239.30 (13), 407.10 (13), 180.50 (13), 238.35 (13), 278.60 (12), 158.50 (12), 391.35 (12), 514.60 (11), 421.10 (11), 174.40 (11), 461.15 (11), 473.80 (11), 246.95 (11), 331.60 (11), 473.05 (11), 204.90 (10)

Procyanidin B1	[M+H] <sup>+</sup>	579.20 (100), 105.15 (38), 580.25 (30), 338.95 (21), 350.85 (20), 102.30 (19), 331.10 (12), 123.00 (11), 501.20 (11), 218.95 (10), 329.55 (10)
	[M-H] <sup>-</sup>	577.35 (100), 578.35 (36), 361.15 (35), 378.40 (14), 579.40 (11)
Procyanidin B2	[M+H] <sup>+</sup>	102.25 (100), 122.80 (92), 105.15 (77), 579.20 (52), 188.25 (45), 208.40 (36), 115.00 (33), 139.20 (22), 580.20 (22), 166.60 (18), 305.00 (18), 198.40 (18), 179.50 (16), 367.10 (15), 109.80 (14), 289.00 (13), 103.85 (12), 200.60 (11), 274.25 (11), 172.55 (11), 466.35 (11), 131.95 (11), 170.70 (10), 294.10 (10)
	[M-H] <sup>-</sup>	577.35 (100), 465.25 (74), 137.00 (59), 147.15 (36), 307.05 (22), 466.25 (21), 303.25 (21), 175.40 (19), 389.55 (18), 1959.35 (17), 535.45 (17), 361.10 (16), 533.40 (15), 391.25 (14), 528.30 (14), 507.45 (11), 191.70 (11), 208.90 (11), 159.30 (11), 662.35 (10)
Catechin	[M+H] <sup>+</sup>	291.00 (100), 381.00 (52.48), 395.10 (30.45), 105.25 (28.83), 102.25 (23.74), 226.55 (17.76), 114.70 (13.18), 122.60 (11.45)
	[M-H] <sup>-</sup>	289.25 (100), 352.30 (29), 290.30 (20), 335.20 (15), 357.30 (12), 865.45 (11)
Procyanidin B1	[M+H] <sup>+</sup>	579.20 (100), 580.25 (35), 441.05 (18), 105.00 (16)
	[M-H] <sup>-</sup>	577.35 (100), 578.40 (32)
Compound 8	[M+H] <sup>+</sup>	307.05 (100), 102.10 (93), 105.30 (69), 122.55 (56), 203.10 (28), 288.95 (16), 369.15 (15), 114.75 (15), 579.20 (12)
	[M-H] <sup>-</sup>	329.25 (100), 577.30 (37), 346.35 (34), 449.30 (22), 283.30 (20), 330.40 (17), 391.35 (16), 720.80 (11), 865.35 (11), 147.50 (10)
Epicatechin	[M+H] <sup>+</sup>	291.00 (100), 105.05 (21), 102.30 (14), 226.55 (13), 317.20 (12), 531.25 (10)
	[M-H] <sup>-</sup>	289.25 (100), 352.30 (28), 290.25 (17), 335.25 (17)
Procyanidin C1	[M+H] <sup>+</sup>	105.15 (100), 102.30 (69), 122.40 (69), 867.55 (54), 453.15 (40), 473.70 (25), 868.50 (24), 114.85 (23), 579.20 (23), 290.90 (18), 559.85 (17), 288.55 (15), 208.60 (12), 198.30 (11), 466.10 (11), 425.25 (10)
	[M-H] <sup>-</sup>	865.45 (100), 866.45 (44), 289.25 (15), 576.35 (14), 721.00 (13), 867.55 (13), 345.35 (11), 495.45 (10)
Compound 11	[M+H] <sup>+</sup>	105.10 (100), 102.10 (84), 153.05 (39), 122.70 (28), 114.80 (19), 188.35 (16), 198.40 (14), 180.25 (13), 208.15 (12), 279.30 (10)
	[M-H] <sup>-</sup>	146.85 (100), 767.40 (81), 151.35 (72), 113.20 (63), 345.15 (38), 247.40 (34), 555.50 (33), 375.40 (27), 391.25 (25), 768.55 (23), 515.30 (21), 272.50 (21), 202.60 (20), 377.00 (20)
Compound 12	[M+H] <sup>+</sup>	105.10 (100), 102.10 (91), 453.15 (64), 304.95 (53), 122.25 (48), 255.45 (39), 114.70 (35), 187.90 (28), 123.15 (17), 454.15 (16), 341.70 (15), 234.95 (15), 487.30 (14), 294.00 (14), 307.05 (13), 314.60 (13), 369.05 (13), 448.15 (12), 991.00 (12), 247.25 (11), 208.20 (11), 439.15 (10)
	[M-H] <sup>-</sup>	465.30 (100), 475.40 (72), 528.40 (23), 492.50 (17), 476.40 (16), 176.30 (13), 537.55 (11), 1008.95 (10)
Compound 13	[M+H] <sup>+</sup>	105.15 (100), 102.05 (63), 122.60 (48), 194.75 (19), 198.25 (18), 188.10 (16), 114.85 (13), 279.15 (11)
	[M-H] <sup>-</sup>	193.10 (100), 345.35 (51), 346.35 (23), 174.90 (19), 173.40 (17), 335.20 (16), 146.65 (16), 301.15 (16), 334.40 (14), 309.15 (14), 179.40 (13), 391.25 (12), 720.45 (12), 291.10 (12), 112.85 (11), 112.10 (11), 553.25 (10)

Compound 14	[M+H] <sup>+</sup>	105.15 (100), 102.15 (62.66), 122.7 (56.09), 336.8 (12.82), 198.35 (12.19), 114.85 (11.26), 194.95 (11.09), 187.95 (10.57), 208.35 (10.37)
	[M-H] <sup>-</sup>	335.15 (100), 317.25 (19.02)
Taxifolin	[M+H] <sup>+</sup>	305.00 (100), 628.35 (16), 105.10 (14), 401.15 (10)
	[M-H] <sup>-</sup>	303.20 (100), 607.35 (22), 366.25 (10)
Compound 16	[M+H] <sup>+</sup>	105.10 (100), 102.15 (60), 305.00 (55), 122.90 (34), 233.55 (22), 114.85 (19), 225.40 (13), 198.30 (12), 289.05 (10)
	[M-H] <sup>-</sup>	303.20 (100), 287.20 (28), 366.30 (11)
Compound 17	[M+H] <sup>+</sup>	105.15 (100), 102.25 (90), 375.10 (84), 397.10 (77), 357.10 (74), 122.85 (57), 248.05 (36), 198.25 (23), 398.20 (19), 114.95 (19), 305.00 (16), 379.00 (11), 240.30 (11)
	[M-H] <sup>-</sup>	373.30 (100), 303.20 (36), 317.25 (33), 374.30 (25), 605.40 (11)
Eriodictyol ( <i>t</i> )	[M+H] <sup>+</sup>	289.00 (100), 105.10 (54), 102.20 (41), 122.70 (20), 225.55 (16)
	[M-H] <sup>-</sup>	287.20 (100), 350.30 (21), 333.20 (14)
Compound 19	[M+H] <sup>+</sup>	341.10 (100), 105.05 (44), 102.25 (39), 122.65 (24), 359.15 (16), 114.85 (11)
	[M-H] <sup>-</sup>	605.35 (100), 301.35 (35), 299.20 (33), 606.40 (28), 350.15 (26), 284.95 (25), 675.40 (17), 453.30 (16), 607.35 (16), 112.70 (15), 633.45 (14), 174.45 (13), 287.25 (12), 147.55 (11), 601.45 (10)
Compound 20	[M+H] <sup>+</sup>	105.15 (100), 102.25 (68), 122.75 (34), 198.40 (17), 661.35 (15), 114.90 (11)
	[M-H] <sup>-</sup>	659.50 (100), 299.20 (42), 660.50 (41), 603.35 (41), 301.20 (22), 307.20 (17), 112.90 (13), 604.35 (13), 481.20 (13), 661.40 (13)

530 <sup>a</sup>"t" = tentative assignment

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**Figure captions**

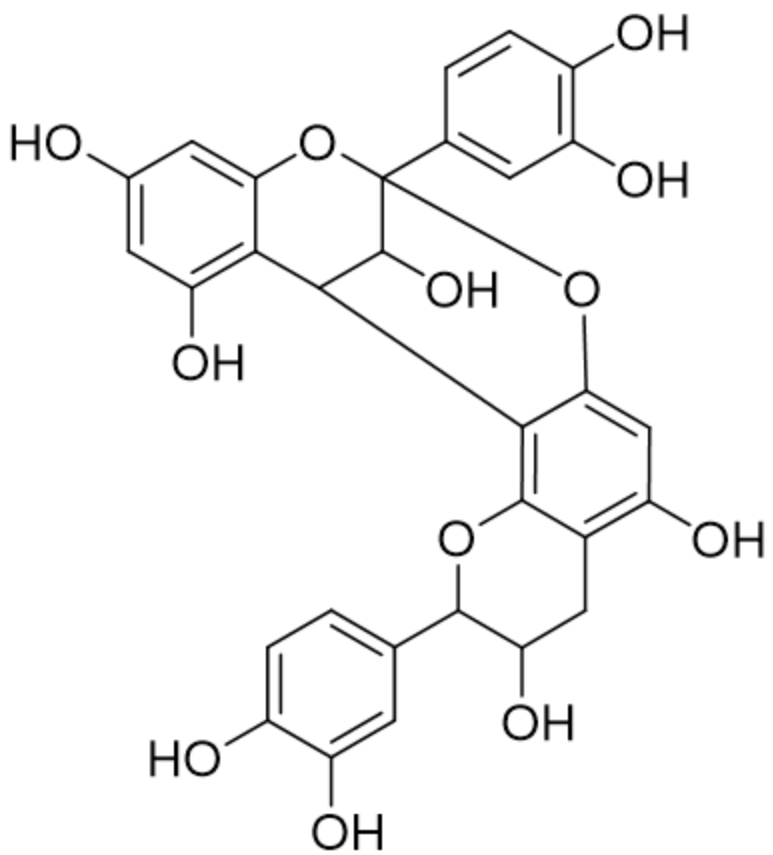
**Fig. 1** Examples of procyanidin structures. These can be A-, B-, or C-type depending on the different linkages between flavanol units.

**Fig. 2** Modelled yield (% dry weight) for samples (A) without branches, circles, and (B) with branches, triangles, as a function of % total height, with total tree height as a random effect. The two plots each show one model and are shown conditional on species. There is no difference in relationship form between the un-thinned-management samples (filled shapes) and the thinned-management samples (open shapes), despite the difference in the mean height at which the crown begins.

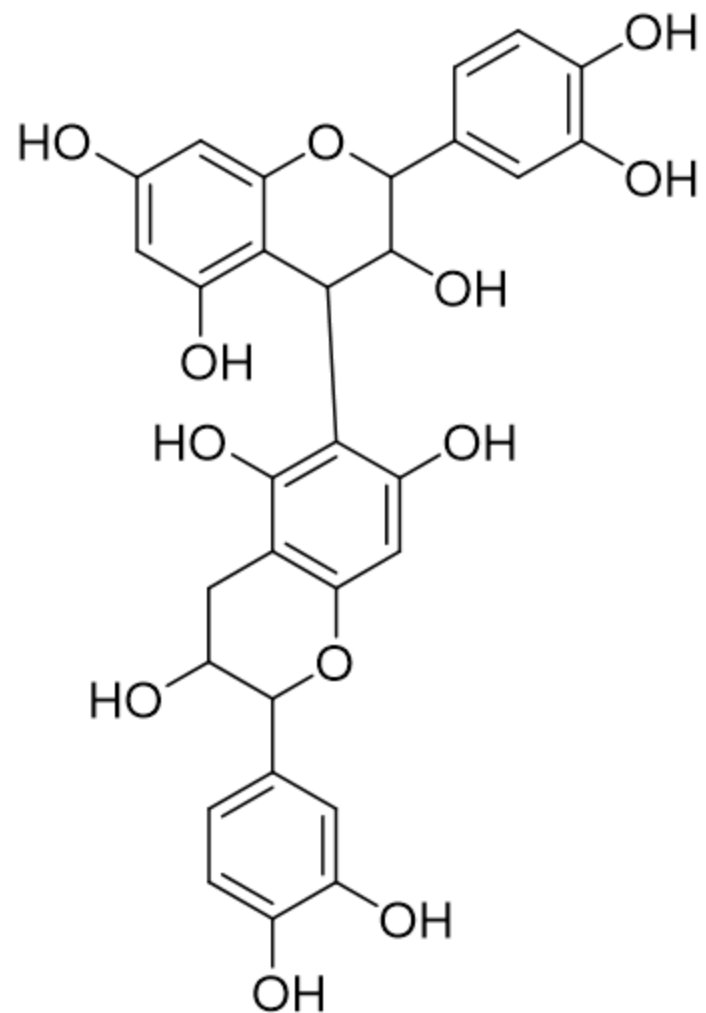
**Fig. 3** UV-chromatograms of samples from (A) *P. abies* and (B) *P. menziesii* with the positions of compounds that were quantified. The rising baseline indicates the relative proportion of oligomeric tannins with degree of polymerisation above three that can not be well resolved.

**Fig. 4** *P. abies* compounds that have a relationship between yield and sample height. A, Epigallocatechin gallate. B, compound 13. C, Isorhamnetin. D, Cirsimaritin. Samples with branches are shown with triangular shapes, whereas samples without branches are circles. There is no clear effect of branch presence or management type (un-thinned management = filled shapes, thinned management = open shapes).

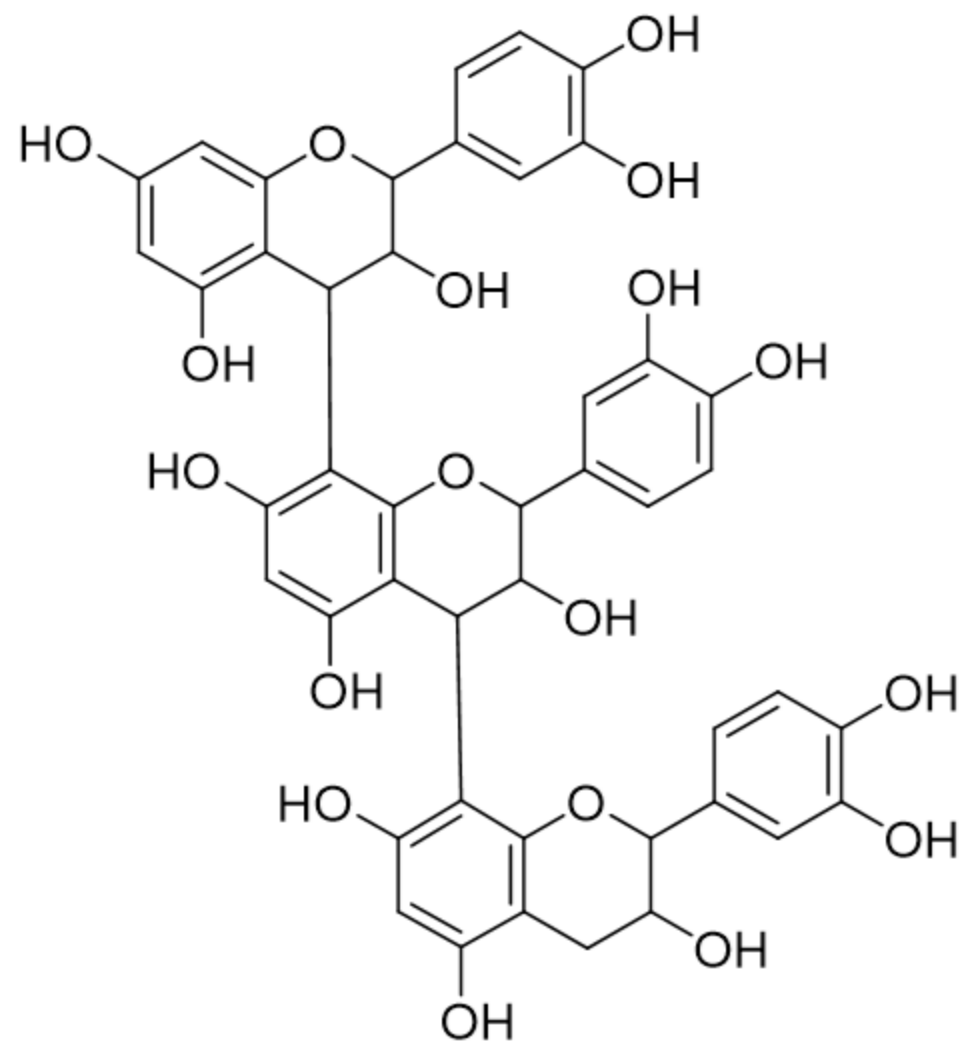
**Fig. 5** Selected *P. menziesii* compounds that have a relationship between yield and sample height. A, compound 2. B, Procyanidin C1. C, Taxifolin. D, Eriodictyol. Samples with branches are shown with triangular shapes, whereas samples without branches are circles. There is no clear effect of branch presence or management type (un-thinned management = filled shapes, thinned management = open shapes).



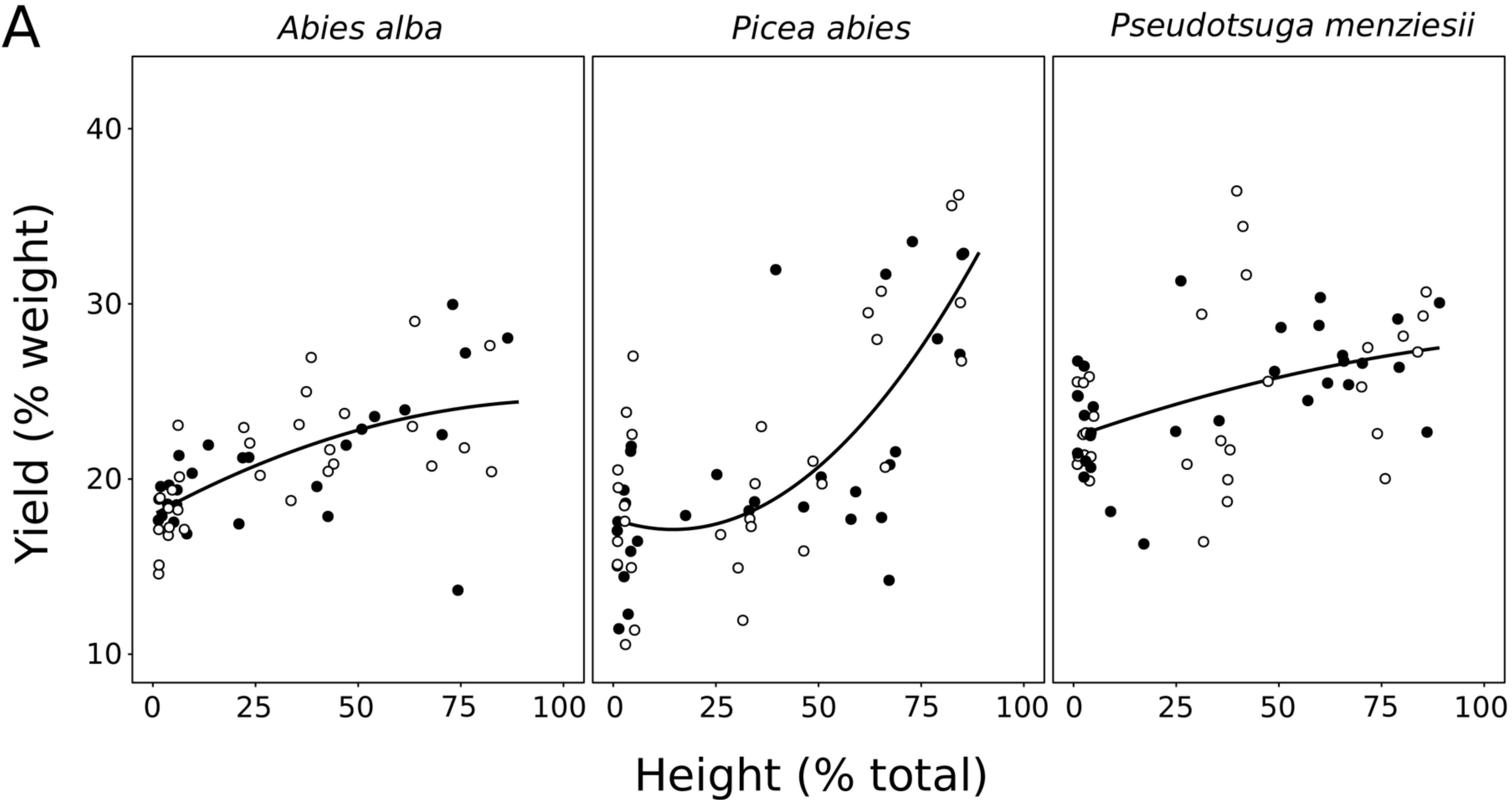
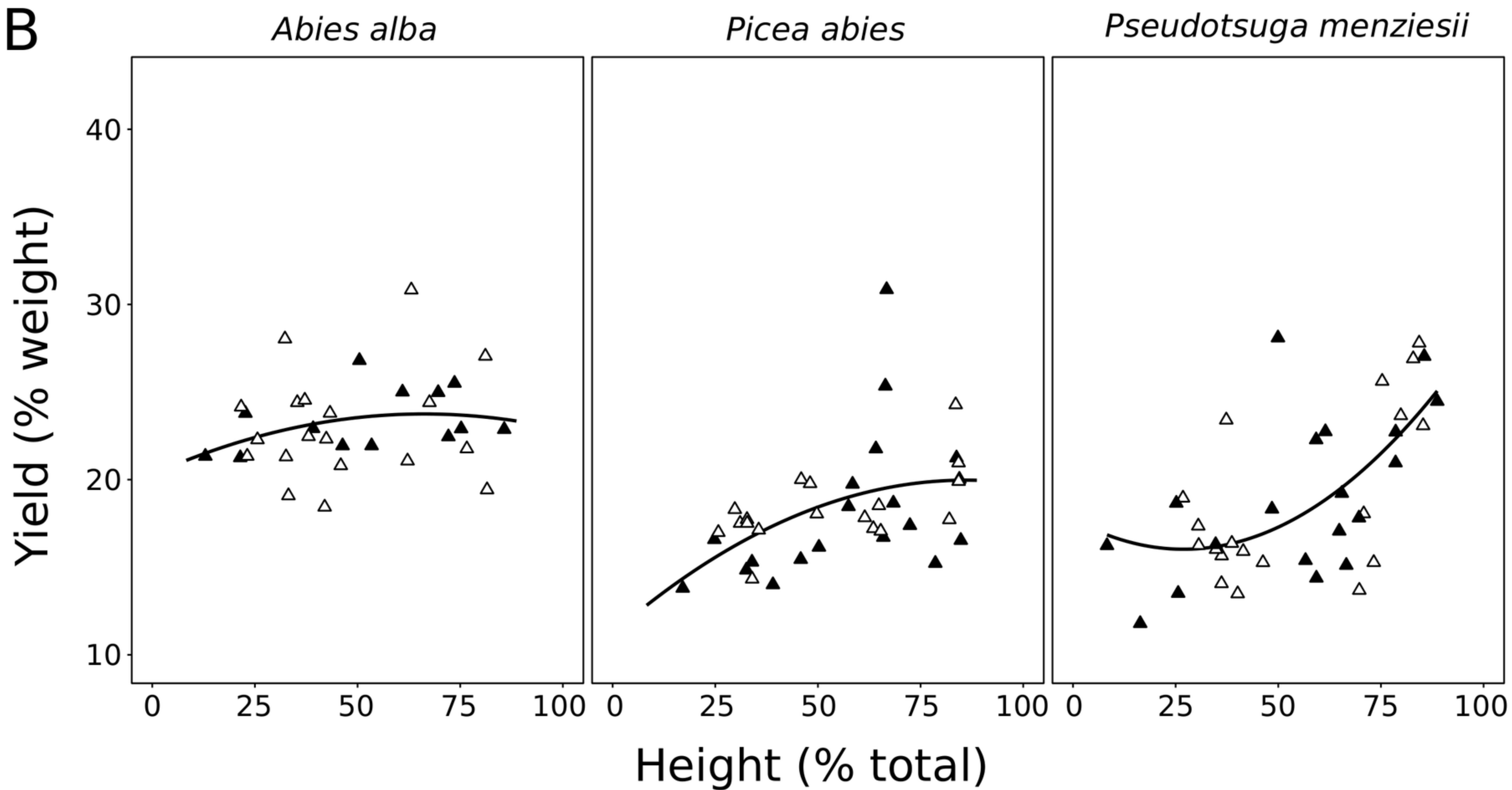
Procyanidin A

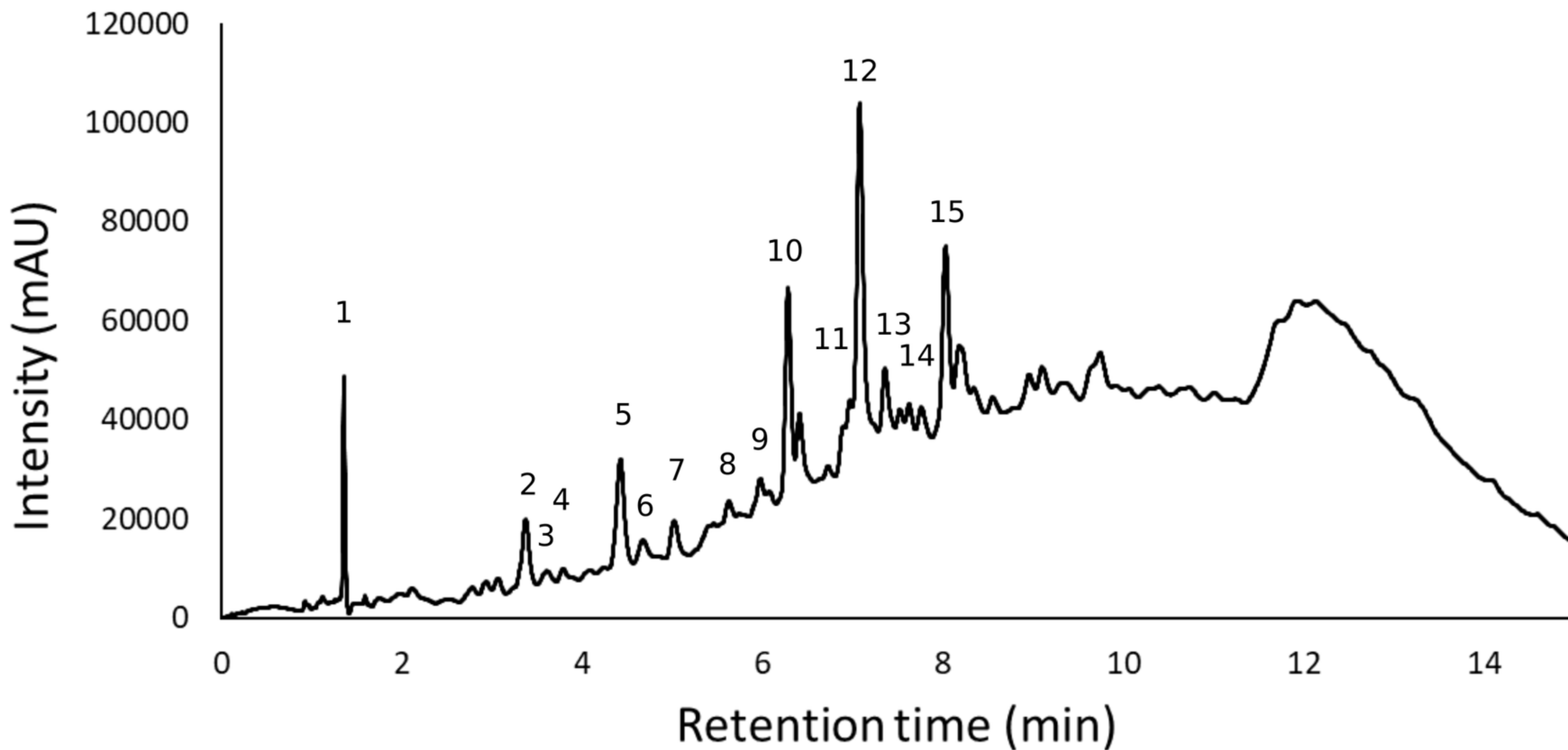
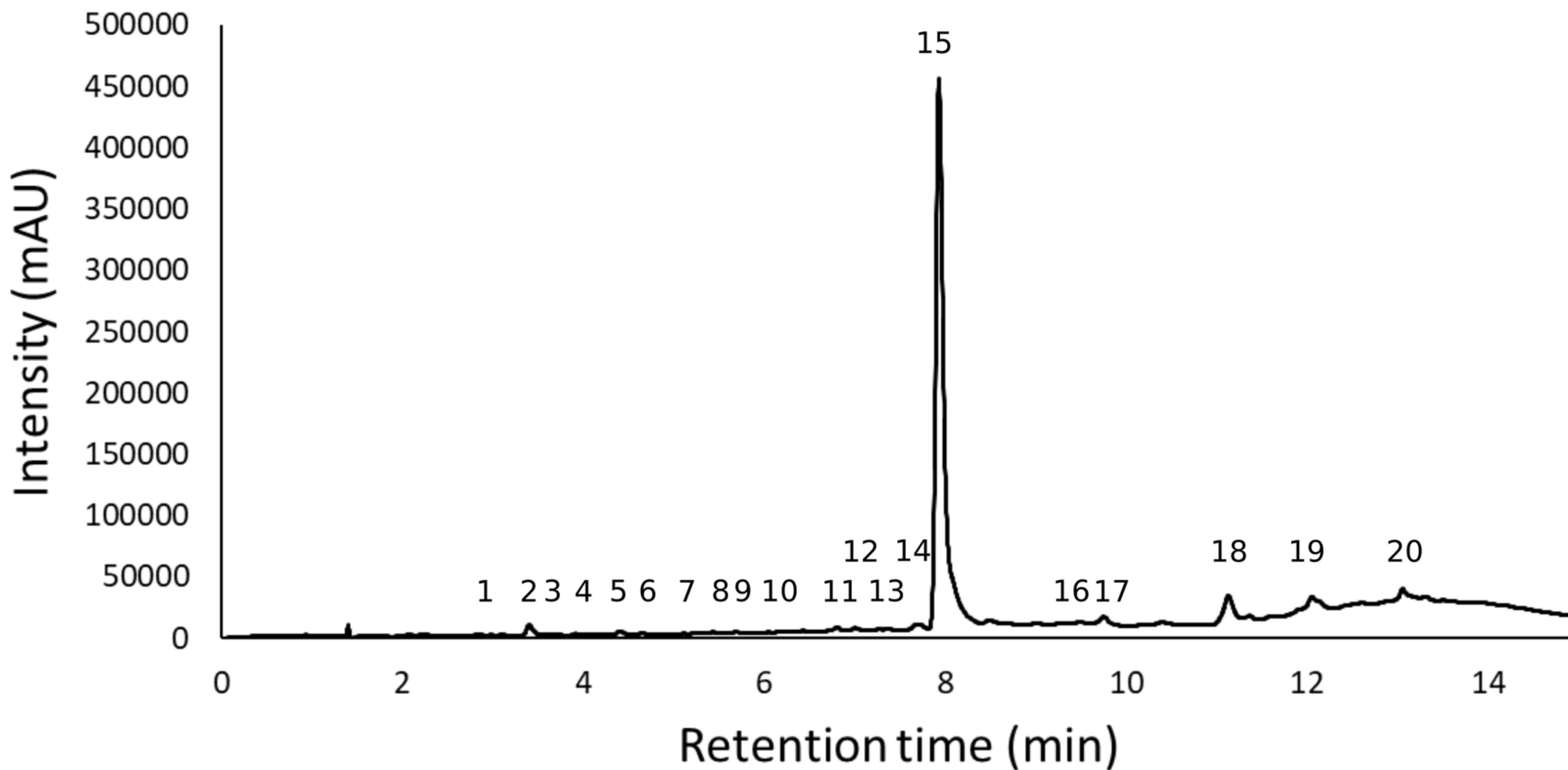


Procyanidin B

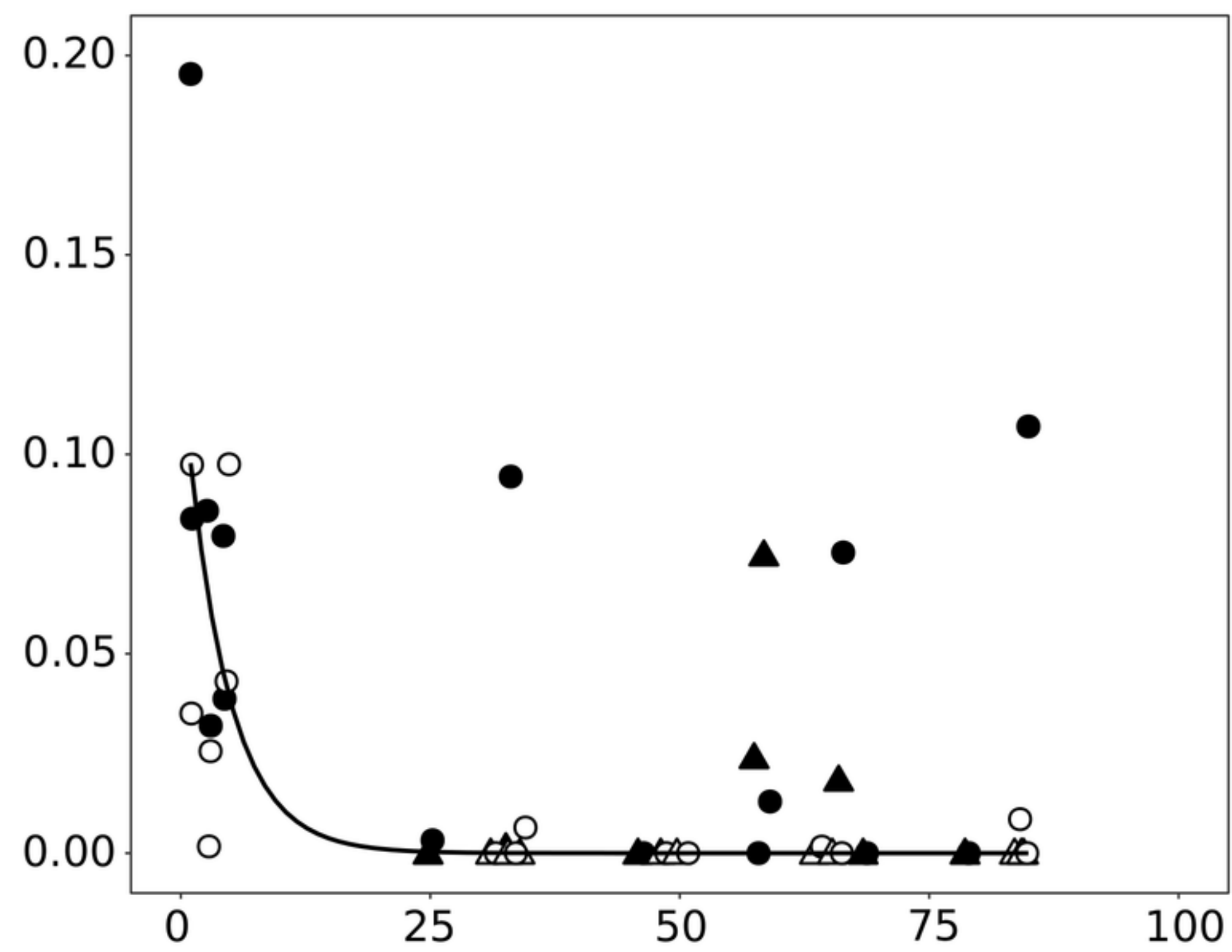
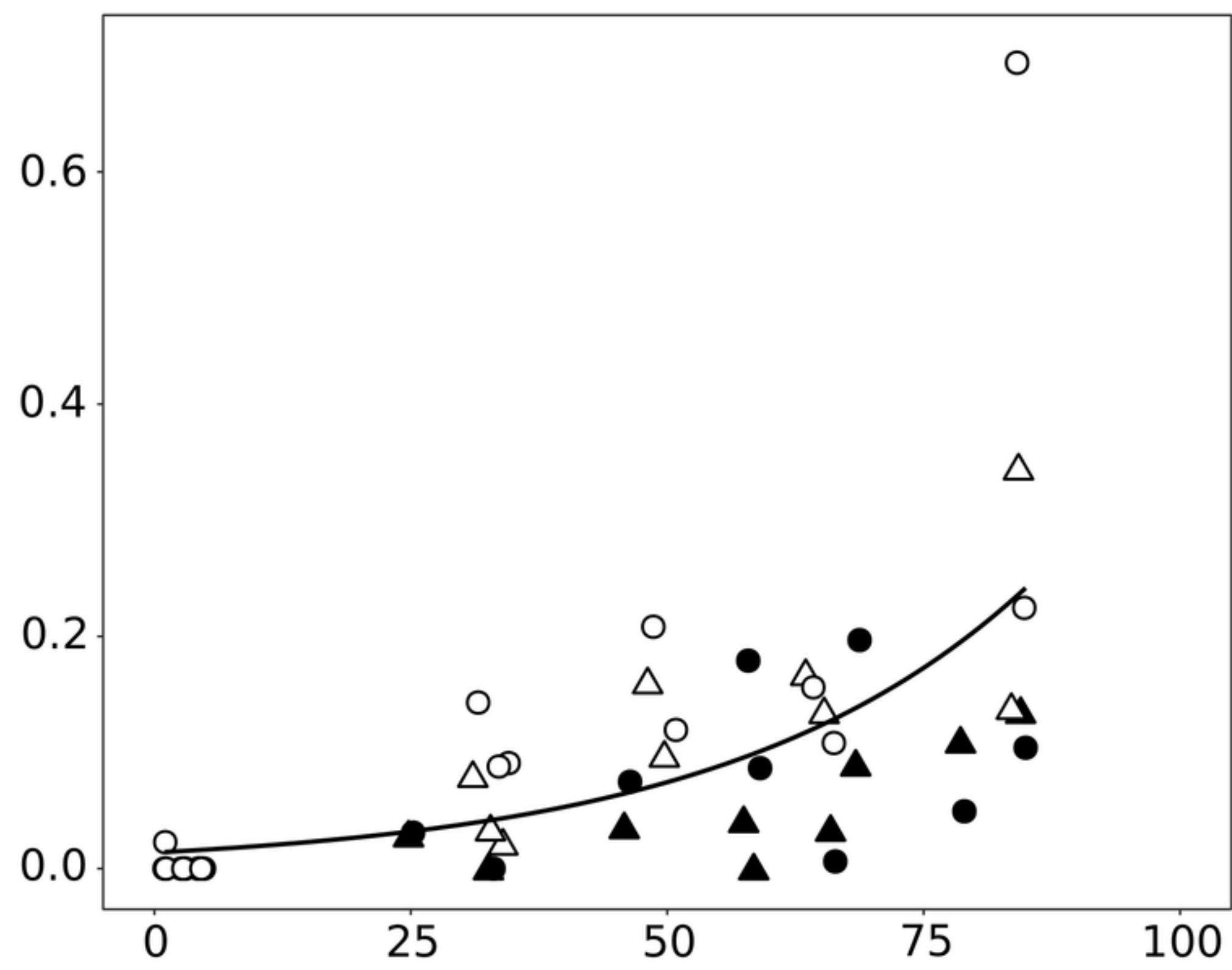
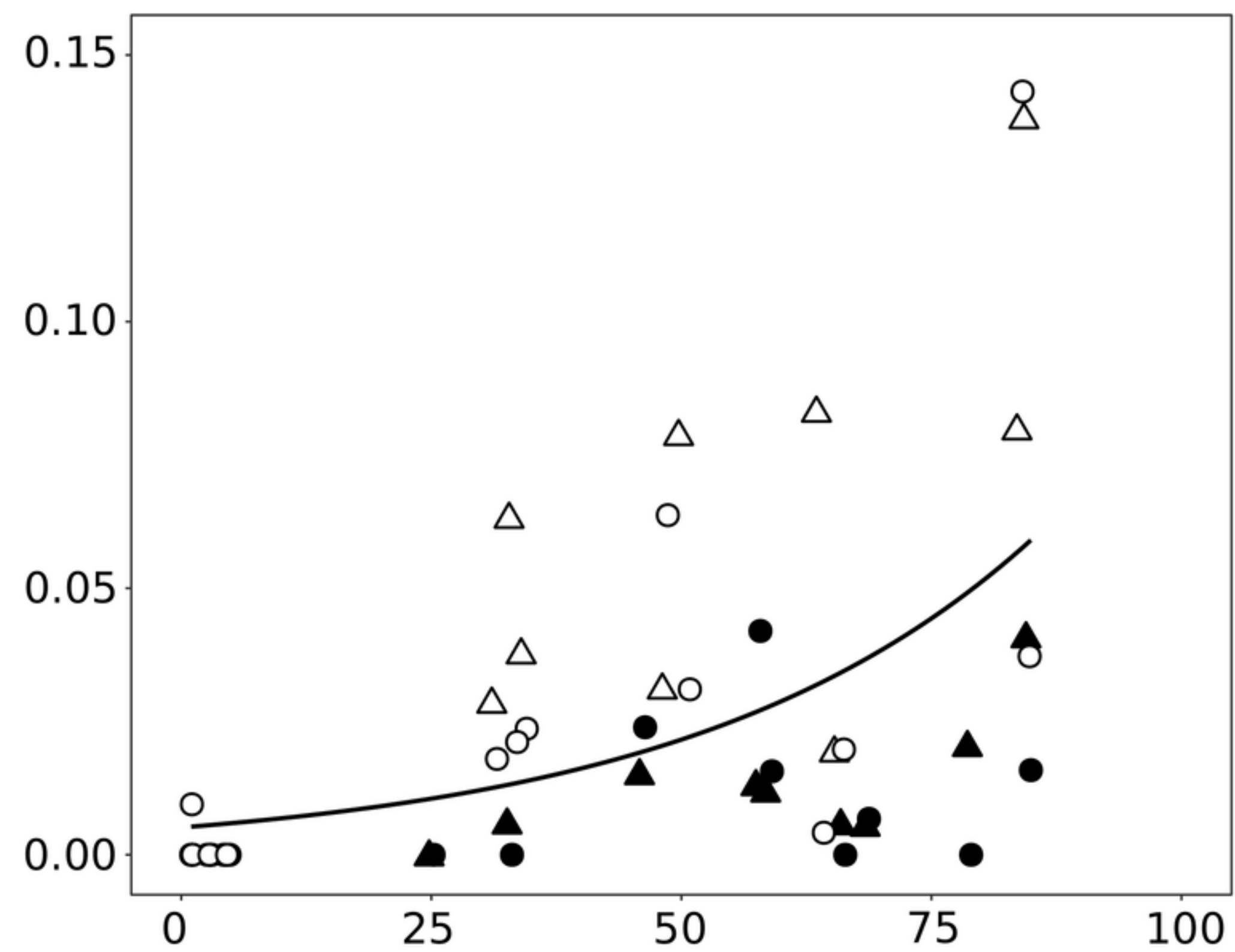
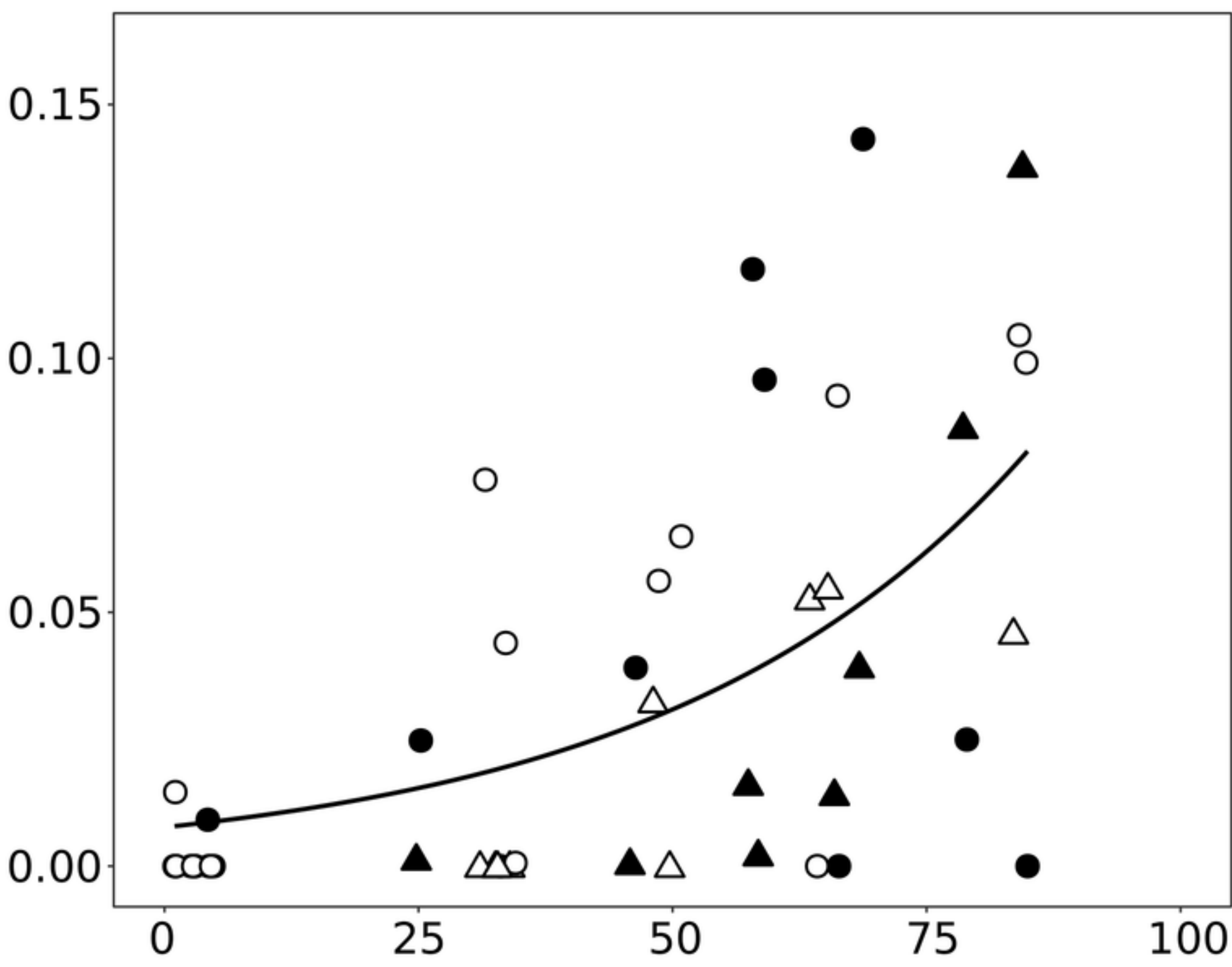


Procyanidin c

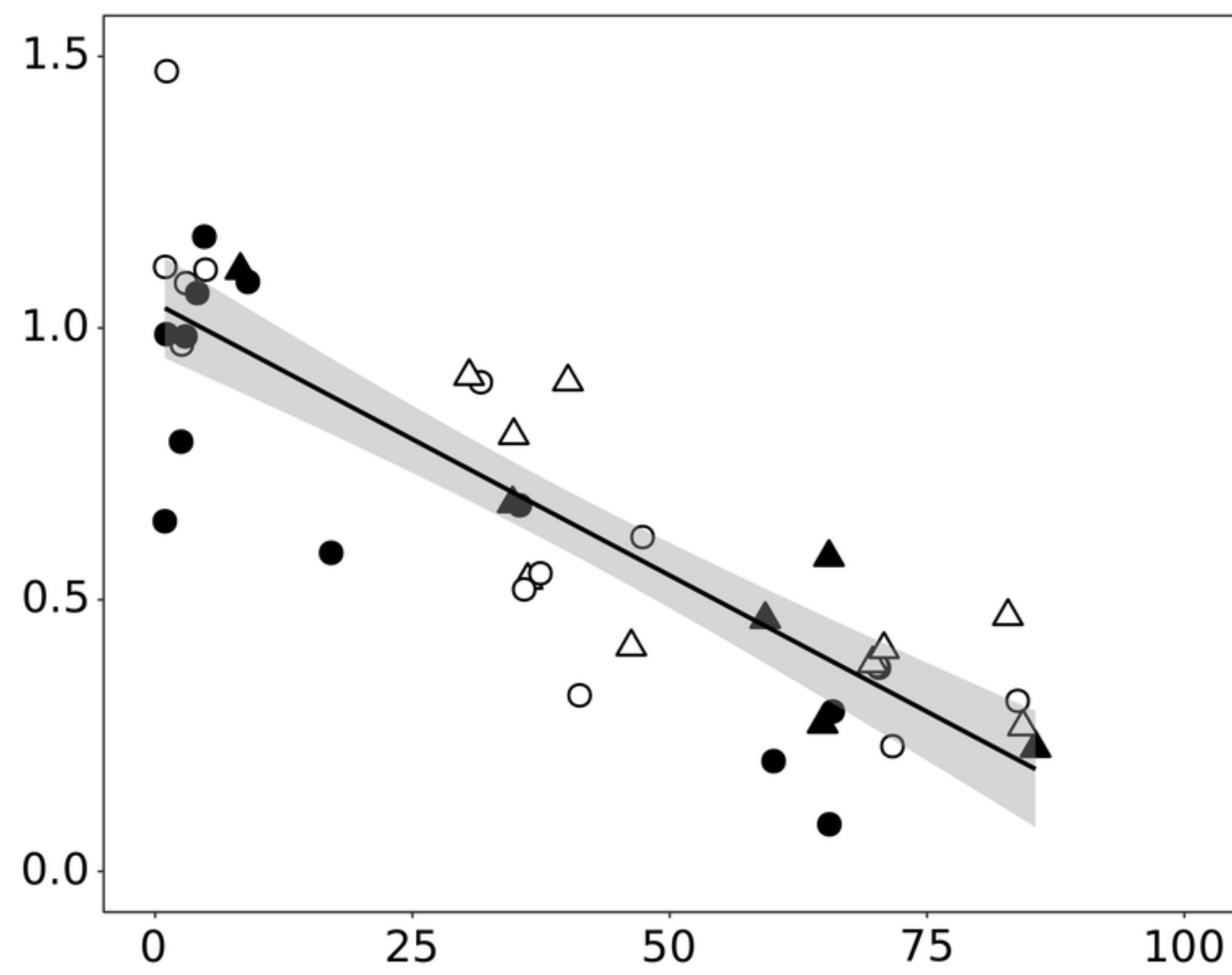
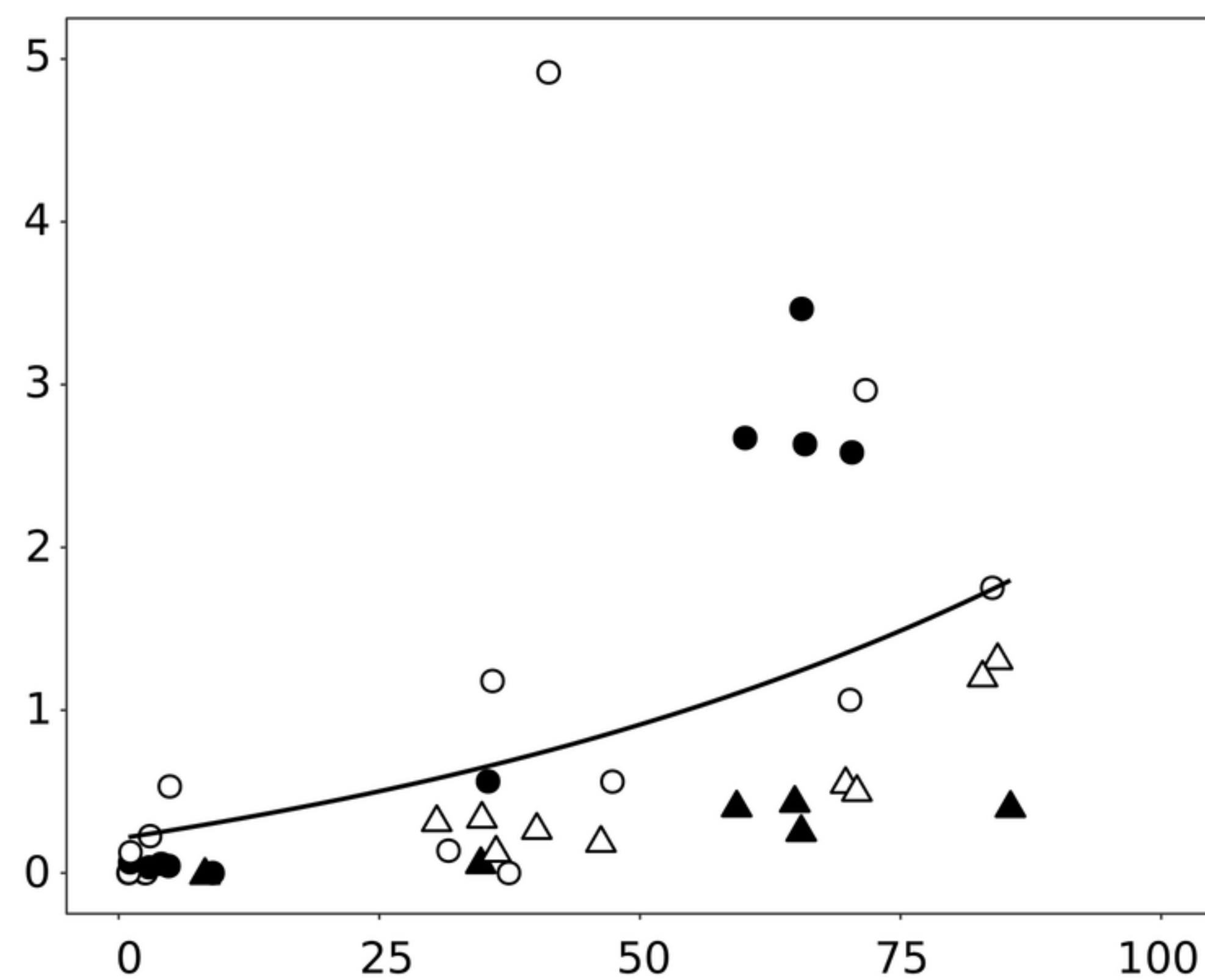
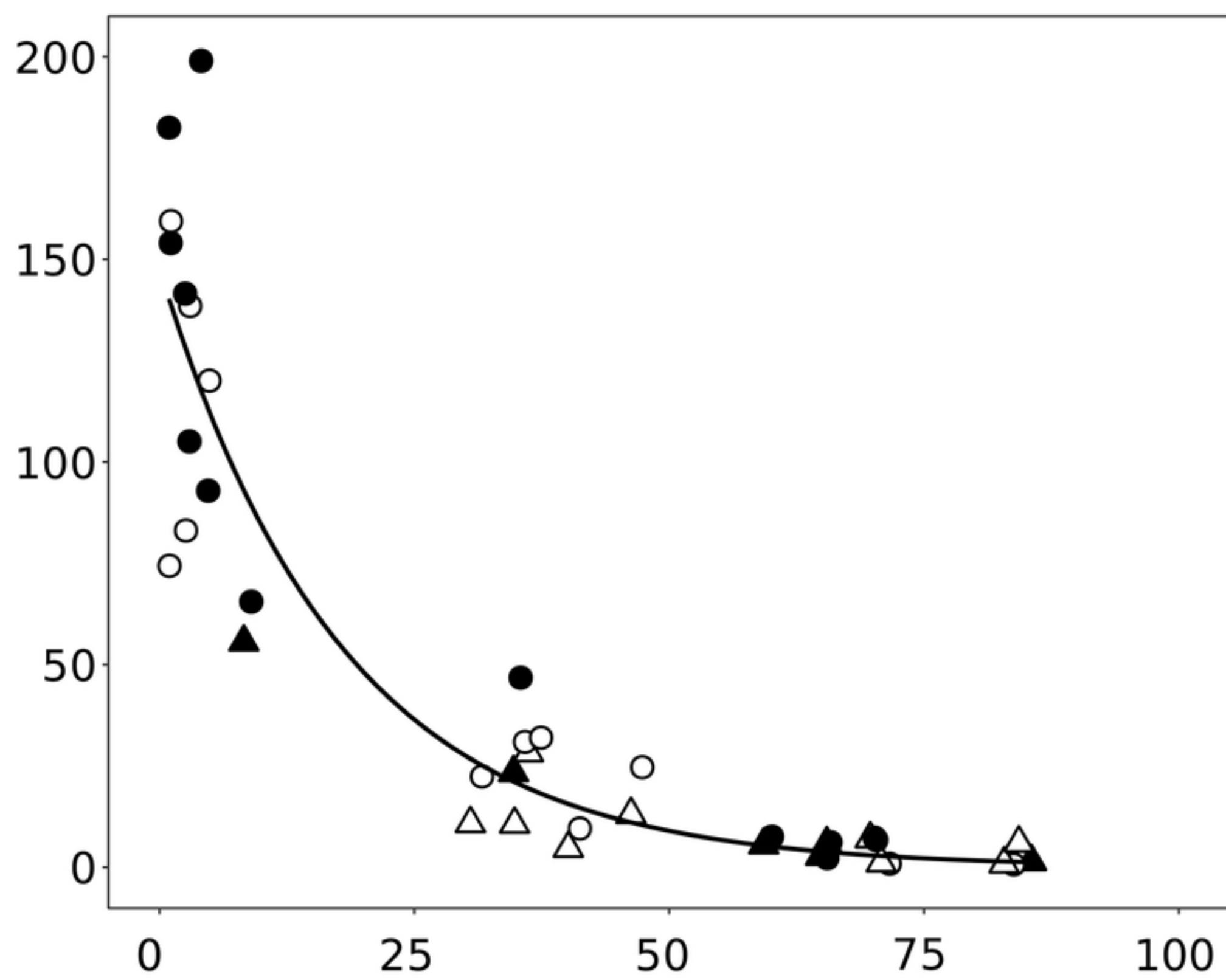
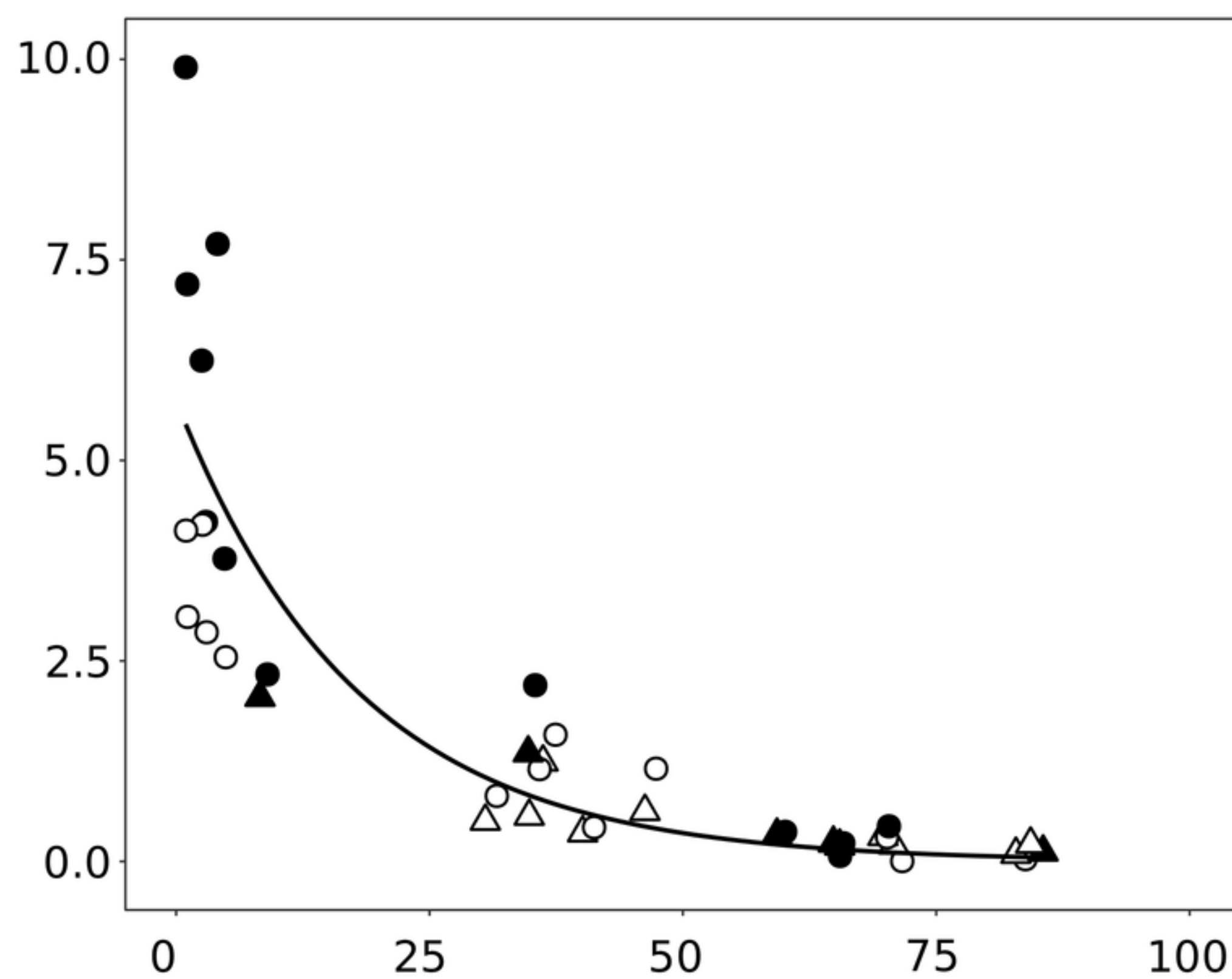
**A****B**

**A****B**



**A****B****C****D**Yield ( $\text{mg g}^{-1}$  bark)

Height (% total)

**A****B****C****D**Yield (mg g<sup>-1</sup> bark)

Height (% total)



- No branches
- - Branches

