Are secondary bark beetles a problem? – A case study of *Cryphalus piceae* (Ratzeburg, 1837) (Coleoptera, Scolytinae) in relation to *Heterobasidion annosum* (Fr.) Bref. (Russulales, Bondarzewiaceae) root rot

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Bark beetles (Scolytinae) that only attack weakened trees can have a significant economic impact if they kill trees that would have recovered in their absence. On the other hand, some bark beetles only target trees that are irreversibly stressed and have no chance of survival, regardless of the presence of bark beetles. Distinguishing between these two types of bark beetles is important for optimal pest management, and thus economic profitability.

This study focused on *Cryphalus piceae* (Ratzeburg, 1837), a bark beetle species reported as economically problematic, attacking weakened or healthy trees. We evaluated the damaging capabilities of *C. piceae* by monitoring its activity, tree-health appearance, and tree moisture levels in an *Abies procera* Rehder, 1940 stand weakened by *Heterobasidion annosum* s.str. We compared *A. procera* trees attacked by *H. annosum* to healthy *A. procera* trees, allowing us to assess the degree of tree weakening before *C. piceae* colonization. Additionally, wood core samples were taken to evaluate tree health prior to *C. piceae* colonization.

Our results suggest that *C. piceae* can only locate and colonize very weak or recently dead trees, thereby confirming that the species has had little to no effect on the survival of the studied *A. procera* trees. This highlights the importance of future studies on the damaging properties of secondary bark beetles.

Key words: Tree moisture, weakened trees, tree health, pathogen, secondary pests, semi-aggressive bark beetles, Coleoptera, Curculionidae, Scolytinae.

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Introduction

The impact of pest insects on host trees has been a topic of interest since the beginning of modern forestry. Especially outbreaks of semi-aggressive or tree-killing bark beetles (Curculionidae, Scolytinae) that damage living tissues and affect tree survival. Usually bark beetle outbreaks are triggered by environmental factors such as drought, forest fires, or floods. However, the scale of an outbreak also depends on other factors such as stand heterogeneity, bark beetle density, availability of suitable hosts within the stand, and proximity to other host stands (Raffa et al. 2008). Current research suggests that the magnitude of bark beetle outbreaks are increasing (Raffa et al. 2008), causing significant financial losses (Ebata 2004, Grégoire & Evans 2004, Hlásny 2019).

Most tree-killing bark beetles attack conifer trees and naturally, this has prompted conifers to develop highly complex defences, to avoid getting killed (Franceschi 2005, Krokene 2013, Krokene 2015). The bark beetles' ability to overcome these host defences define their aggressiveness. Bark beetles capable of attacking and killing vigorous trees with active host defences during epidemics (high population densities) are defined as "aggressive" or "primary", whereas bark beetles restricted to weakened, dying, or recently dead trees, with compromised or latent host defences are termed "secondary" or "semi-aggressive" (Figure 1) (Graham 1929, Ryan & Amman 1996, Paine et al. 1997, Martikainen et al. 1999, Lindgren & Raffa 2013, Krokene 2015). These two categories overlap, as bark beetles in both definitions can only overcome host defences of weakened or recently dead trees at low population densities, but at epidemic conditions, only the primary bark beetles kill vigorous host trees.

Because some bark beetles complete their



Host condition

FIGURE 1. Conceptual illustration of the terms primary and secondary -bark beetles and the bark beetle *Cryphalus piceae* (Ratzeburg, 1837), in relation to host condition. The figure is modified from Lindgren and Raffa (2013).

life cycle in living trees without killing them, more strict definitions describing bark beetles' relationship with host condition have been proposed by Paine *et al.*, (1997), Krokene *et al.*, (2013), Lindgren & Raffa (2013).

The negative adverse effects caused by primary bark beetles during landscape-scale eruptions are easily acknowledged, as they can sustain outbreak conditions on vigorous trees, even after any abiotic mass weakening has stopped (Krokene 2015). However, it is more challenging to recognize the adverse effects of secondary bark beetles. This is because different secondary bark beetle species have different tolerances towards host defences (Figure 1). All secondary bark beetles are dependent on relatively fresh substrates, but some tolerate lowered active host defences (semiaggressive) and can therefore colonize living but stressed trees, while others are only able to breed in recently dead trees, solely containing latent host defences. Adding to the complexity of studying adverse effects of secondary bark beetles, is the fact that denoting exactly when a tree is physiologically dead (irreversibly stressed) is very difficult, even more so, because a physiologically dead tree can appear healthy with green foliage for weeks or months (Krokene 2015).

The difficulty of identifying which secondary bark beetles are aggressive and which are not, makes studies of beetles in this category very important, as unnecessary resources could be wasted trying to control bark beetles with insignificant or no effect on host tree survival.

Anderegg *et al.* (2015) introduced a two-axis framework to highlight adverse effects caused by different actors in a mortality event. The specific framework was created in relation to drought and revolves around two central questions; How many trees would die in a given mortality event with drought but without insects? Conversely, how many trees would die in a given mortality event with insects but without drought? We adopted this framework to fit our context of evaluating adverse effects of secondary bark beetles; How many trees would die from stress inducing factors in a given mortality event without secondary bark beetles? Conversely, how many trees would die in a given mortality event with stress inducing factors and secondary bark beetles? Answering these questions can be cumbersome, but similar to Gaylord *et al.* (2013) and Netherer *et al.* (2014), we attempted to answer the question by monitoring the health of trees prior to bark beetle attack. Specifically, we choose to investigate the bark beetle *Cryphalus piceae* (Ratzeburg, 1837).

Cryphalus piceae has been subject to several studies, for instance, studies of the fungal associates of C. piceae (Jankowiak & Kolařík 2010), statistical population size estimations (Podlaski & Borkowski 2009), detailed life cycle studies (Toper 2002, Justesen et al. 2020). Additionally, the species is mentioned in numerous bark beetle inventories and life cycle studies. Several of these studies classify C. piceae as a semi-aggressive problematic species attacking weakened Abies trees (Chararas 1975, Capecki 1982, Klein 1984, Sekendiz 1987, Cerchiarini & Tiberi 1997, Faccoli 2000, Toper 2002, Yıldız et al. 2007, Akinci et al. 2009, Podlaski & Borkowski 2009, Jankowiak & Kolařík 2010, John 2011, Ertugrul et al. 2017). Other authors are unsure of the role of C. piceae (Hrašovec et al. 2008). In an European questionnaire, 3 out of 19 countries evaluated C. piceae as a financially problematic bark beetle (Grégoire & Evans 2004). However, no empirical evidence of the negative effect of C. piceae exists.

Additionally, a few studies highlight the fact that most of the C. piceae population overwinters as adult beetles in healthy trees, which could affect the growth of trees or vector diseases (Klein 1984, Cerchiarini & Tiberi 1997, Justesen et al. 2020). Cryphalus piceae recently started occurring in dead A. procera Rehder trees, in Danish greenery plantations (Ravn & Justesen 2013, Hansen & Jørum 2014). Greenery plantations aim to generate profits by harvesting tree branches during autumn and early winter, and selling them for wreaths and decorations. Initially, C. piceae was thought to be the main cause of the thousands of dead A. procera in these plantations, but subsequent studies found fruitbodies of Heterobasidion annosum s.str. present at or in the vicinity to all attacked trees (Justesen et al. 2020).

Two *Heterobasidion* species are present and widespread in Denmark, *H. annosum* s.str. and *Heterobasidion parviporum* Niemelä & Korhonen, 1998 (Woodward *et al.* 1999). The latter is mainly found in *Picea* species, usually, *P. abies*, whereas *H. annosum* has a broader host spectrum, and is found on various conifer genera such as *Picea, Pinus, Abies, Larix* and *Pseudotsuga* (Woodward *et al.* 1999). The third European species, *Heterobasidion abietinum* Niemelä & Korhonen, 1998, which occurs on fir (*Abies*) and spruce (*Picea*) in southern and central Europa has not been documented in Denmark.

In general, Heterobasidion root rot only causes direct mortality in young trees, whereas older trees are mostly affected by decay in the roots and the stem. This decay causes loss of timber value and may destabilize trees, occasionally entire stands, leading to wind throw and thus indirect mortality (Woodward et al. 1999). However, highly susceptible tree species can also die while standing due to Heterobasidion root rot (Woodward et al. 1999, Chastagner et al. 2002, Chastagner & Dart 2006). The biotic stress induced in these standing trees creates a situation similar to abiotic drought stress. Among the Abies species a large variation in susceptibility to H. annosum root rot is observed, with even old trees of A. procera being very susceptible and A. alba and A. nordmanniana less susceptible (Woodward et al. 1999).

In this specific study, we utilized that *H. annosum* infected trees were present in large numbers, together with high population densities of *C. piceae* (Justesen *et al.* 2020). These circumstances were used to examine the degree of stress in trees, at the point in time when *C. piceae* were able to overcome host defences. Our focus was limited to the potential damages caused by *C. piceae* during the outbreak period from spring to autumn, and did not address the issue of *C. piceae* overwintering in healthy trees (Justesen *et al.* 2020).

Materials and methods

Experimental design and study site. A two hectare *A. procera* greenery plantation near Jyderup, Denmark, was chosen as study site (55°36'57.4"N 11°25'28.4"E). The area is influenced by a

temperate seasonal climate with clearly marked vegetative and non-vegetative seasons. The annual mean temperature is 9°C and the average annual precipitation is 598 mm (DMI 2023). Initially, 13.000 trees were planted in 1994, but standard thinning practices led to 4149 trees and 4375 visible stumps at the time of inventory. A complete inventory of the 4149 trees and 4375 stumps was conducted in 2017 (Figure 2b).

For each tree or stump, the presence of large fruitbodies and pustules of *Heterobasidion annosum* (Fr.) Bref. sensu lato (s.l.) was recorded and used to determine infected trees and to map the general presence of *H. annosum* in the plantation. Due to the nature of the fungus, which spreads from tree to tree via the root systems, adjacent trees or stumps could be infected despite not having a fruitbody. The information about the presence of *H. annosum*, was used to create the experimental design.

Ten identical blocks each measuring 20 meters in length and 10 meters in width, were established (Figure 2b). In the opposing ends of a block, H. annosum was either present (Rot) or absent (Control) in the surrounding trees or stumps (Figure 2a). At the end of the block where H. annosum root rot was present, one living tree with a visible H. annosum fruitbody was selected and monitored (henceforth Rot/Infected tree type). In both the Rot and the Control part, an apparently healthy-looking tree without visible fruitbodies of H. annosum was monitored (henceforth Control/ Healthy or Rot/Healthy tree type). Similarly, using a chainsaw, a tree in each of the opposing ends was girdled in the width of the chainsaw (approx. 1 cm) 10 cm above ground level and thereafter monitored (henceforth Control/Ringed or Rot/Ringed tree type). In total our design consisted of five tree types replicated in 10 blocks (Figure 2a), so in total 50 trees were monitored. The reason for each of the five tree types are described in detail below:

- **Control/Healthy:** No treatment. No visible presence of *H. annosum* within a 10 m radius of the tree. This tree was assumed to be healthy and functioned as a control tree for comparison.
- Control/Ringed: Girdled with a chainsaw. No



FIGURE 2. Experimental design. **a.** Illustration of the five tree types investigated in each block. **b.** Inventory made in 2017 and placement of the 10x20m blocks. Legends at the top indicate all *Heterobasidion annosum* (Fr.) Bref. infected trees and stumps and the trees used in the experiment in 2018.

visible presence of *H. annosum* within a 10 m radius of the tree. This was done to compare trees with a controlled weakening with the *H. annosum* weakened trees.

- **Rot/Healthy:** No treatment. Presence of *H. annosum* within a 10 m radius of the tree. It was assumed that these trees could be weakened by *H. annosum*, but to a lesser degree than the trees with a visible fruitbody.
- Rot/Ringed: Girdled with a chainsaw. No visible presence of *H. annosum* within a 10 m radius of the tree. It was assumed that these trees could be weakened by *H. annosum*, but to a lesser degree than the trees with a visible fruitbody and that they would die faster by the girdling than the Control/Ringed tree type.
- **Rot/Infected:** No treatment. Presence of *H. annosum* within a 10 m radius of the tree and a visible fruitbody or pustule. These trees were assumed to have a highly compromised

root system and to be more weakened than the Rot/Healthy trees.

Measurements and collection. Fifty trees were included in the experimental design (5 tree types X 10 blocks) and they were visited 19 times approximately two weeks apart, during 2018. At each visit, we measured tree moisture, evaluated tree health and bark beetle attack on each tree. Lastly, we visited the plantation one year after the experiment ended, and reevaluated whether the trees had survived.

We used time-domain reflectometry (TDR) to estimate the moisture content in trees (see e.g. Constantz & Murphy 1990). Specifically, we used the premanufactured Hydromette HT 85 T from GANN. Temperature settings were applied to fit the ambient temperature. At each visit, the moisture content of each of the 50 trees was measured around 1m, 1.35m, and 1.70m from

ground level. At each measurement, we kept the device measuring for five seconds, before noting the calculated moisture content. In an attempt to standardize the method, all measurements were taken on the same side of the tree (north-facing), and the tree was never re-sampled in the same spot in subsequent collections. The temperature settings on the Hydromette HT 85 T were changed between each collection time to fit ambient temperature, therefore the relative measurements between collection times were incomparable. To account for this, we calibrated the moisture measurements. The calibration was done by taking the average moisture of each collection time (not including moisture measurements from trees that were visibly dead) and subtracting this value (e.g. 94.27 in collect 1), from the calibrating value, which was the average moisture of the 10 trees in the healthy/control treatment measured at collection 10 (78.57). This difference (15.69 in collect 1) was subtracted from all moisture measurements in collect 1, and so forth for all collection times. Statistics were performed on the calibrated moisture, but also checked on noncalibrated moisture measurements.

Based on the presence and colouration of needles, each tree was evaluated as living (needles attached to tree and green foliage), almost dead (part of foliage beginning to turn yellow) or dead (defined as 100% needle browning) at each visit. Most of the trees infected with H. annosum had short and light green needle growth, but only when part of the foliage began to turn yellow were they evaluated as almost dead. The succesful attack of bark beetles was evaluated based on exit holes at the end of the year. Also, to pin point the exact time of C. piceae attack, trees were inspected for frass and entry holes at each visit. In case of attack, ten bark beetles were excavated from 10 different galleries and identified to species based on morphological characteristics (Justesen 2023). One year after the experiment took place (16 October 2019) we remeasured the moisture in the trees, evaluated the presence and colouration of needles, and attack of C. piceae based on exit holes.

In March 2019, we used a Pressler increment borer (5 mm diameter), to sample one wood core

from each tree in the experiment. Within the plantation a small part of the trees were placed on a slight slope, so to avoid uneven ring widths in these wood cores, all samples were taken perpendicular to the inclination of the slope. Each sample was allowed to dry for two months and was then glued to a square wooden beading (1 x 1 cm), then grounded (grit 50) and polished (grit 120 & 300) with sandpaper. Afterwards, the wood cores were scanned on an EPSON scanner at high-resolution (1200 dpi). The WinDENDRO software (Regent Instruments Inc. 2009), was used to measure the annual ring widths. The year 2007 had exceptionally high growth rates and was used to calibrate the samples, so we could assign calendar years to the remaining growth seasons (2005–2017). To make the tree growth (width of year ring) in 2016 and 2017 comparable between trees, we divided the tree growth in 2016/2017 with the trees total growth from 2005 to 2015. Statistics were conducted on this relative tree growth.

Statistics. All analyses were conducted with the statistical software R version 4.0.3 (R Core Team, 2020), and graphs were created using the 'ggplot2' package (Wickham *et al.* 2016). The ringed trees began dying much later than expected, so the 20 ringed trees were not included in the analyses of moisture and tree growth.

To test for differences in wood moisture content and tree growth among tree types (Control/Healthy; Rot/Healthy; Rot/Infected), we used linear mixed-effects models (LMM) from the 'lme4' package (Bates *et al.* 2009) with block as a random factor. Data was tested for normality using a Shapiro-Wilk's test, and log-transformed if necessary. Significance was assessed using the 'lmerTest' package (Kuznetsova *et al.* 2017), and in case of significance followed by a Tukey's post hoc test using the 'emmeans' package (Lenth *et al.* 2019). The 'cld' function with an appropriate alpha and confidence level (alpha = 0.05 and confidence level = 0.95) was used.

In total, four different models for wood moisture were conducted: (1) comparing tree types across all collection events; (2) comparing tree types across the first three collection events prior to *C. piceae* attack; (3) comparing nonattacked trees with trees attacked in 2018 and 2019 across the first three collection events; and (4) comparing moisture levels in the attacked and non-attacked Rot/Infected trees. Similarly two different models for tree growth were tested: (1) comparing tree types; (2) comparing nonattacked trees with trees attacked in 2018 and 2019.

Results

The ringed trees died later than expected and were therefore excluded from moisture and wood core results. Including these results did not affect any of the main conclusions from 2018, but because several of the ringed trees died and were attacked in 2019 (Supplement 1), they naturally influenced the 2019 attack projections. Since we only had one collection from 2019, we decided to exclude the ringed trees data altogether. The measurements of these tree types are shown in the supplementary figure (Supplement 2).

Moisture data. There was a significant difference in moisture between the three tree types LMM F(2, 589.4) = 84.57, p = 0.0001). With an alpha level of 0.001 and a confidence level of 0.999. The tree types Rot/Healthy and Control/Healthy showed significantly higher moisture content than the Rot/Infected tree type. The estimated (means) calibrated moisture in each tree type were 78.7% (Control/Healthy), 75.5% (Rot/Healthy) and 65.4% (Rot/Infected) (Figure 3).

There was also a significant difference in moisture between the three tree types (LMM F(2, 78) = 6.56, p = 0.0023) when only including collection event 1-3 i.e. before beetle attack (Figure 4b). With an alpha level of 0.01 and a



Moisture in trees

FIGURE 3. Calibrated moisture in the 10 trees belonging to the three tree types investigated in 2018. The dotted line represents an almost dead tree (part of foliage beginning to turn yellow). The dashed line represents a dead tree (100% needle browning).



FIGURE 4. Calibrated moisture and ring-width measured from wood cores. **a**. The % of the total ring-width in 2016/2017 in the different tree types and in relation to attacks of *C. piceae* (Ratzeburg, 1837) **b**. Calibrated moisture in collection event (1–3), in the different tree types and in relation to attack-year of *C. piceae* (Ratzeburg, 1837).

confidence level of 0.99, the tree type Rot/Healthy constituted one group with significantly higher moisture content than the Rot/Infected tree type. Control/Healthy was placed in both groups. The estimated (mean) calibrated moisture in each tree type was 78.3% (Control/Healthy), 78.7% (Rot/Healthy) and 77.0% (Rot/Infected).

Finally, there was a significant difference in moisture between (LMM F(2, 86.4) = 9.01, p = 0.0003) non attacked trees and trees that would be attacked later in 2018 and 2019, when only looking at moisture before beetle attack (Figure 4b). With an alpha level of 0.001 and a confidence level of 0.999, trees attacked in 2018 constituted

one group with significantly lower moisture than the non-attacked group. Trees attacked in 2019 were placed in both groups. The estimated (mean) moisture in each group was 76.1% (attacked later in 2018), 78.5% (attacked later in 2019), 78.5% (not attacked).

When only looking at the Rot/Infected tree type before beetle attack (n=30), there was a tendency (LMM F(1, 8) = 4.24, p = 0.074) that moisture was different between trees attacked later in 2018 and trees not attacked in 2018 (Figure 4b). With an alpha level of 0.08 and a confidence level of 0.92, trees attacked in 2018 constituted one group with lower moisture than non attacked trees. The estimated (means) moisture in each group was 76 (attacked later in 2018) and 78 (not attacked later in 2018).

Wood core data. There was a significant difference in tree growth between the three tree types (LMM F(2, 48) = 11.98, p = 0.0001) (Figure 4a). With an alpha level of 0.001 and a confidence level of 0.999, Rot/Healthy and Control/Healthy constituted one group with significantly higher tree growth than the Rot/Infected tree type. The estimated (means) tree growth in 2016/2017 of the total tree growth, was 5.63% (Control/Healthy), 5.03% (Rot/Healthy) and 3.37% (Rot/Infected).

There was also a significant difference in tree growth between (LMM F(2, 54.3) = 5.22, p = 0.0084) trees attacked in 2018 or 2019 and non attacked trees (Figure 4a). With an alpha level of 0.05 and a confidence level of 0.95, trees attacked in 2019 constituted one group with lower tree growth than non-attacked trees. Trees attacked in 2018 were placed in both groups. The estimated (means) tree growth in 2016/2017 of the total tree growth was 3.73% (attacked later in 2018), 3.18% (attacked later in 2019), 5.16% (not attacked).

Dead and attacked trees. In total 26 of the 50 investigated trees died between March 2018 – October 2019 (Supplement 1).

Eleven trees died during 2018, of which five belonged to the tree type that had visible fruitbodies at the end of 2017 (Rot/Infected), two were in the Rot/Healthy tree type (root rot fruitbodies were observed in October 2019), and four of these were ringed trees in the control (n=1) and rot (n=3) tree types. Six of these 11 trees were attacked by C. piceae (100+ entry holes) in 2018 between April 12 - 25th, of which five belonged to the Rot/Infected tree type and one to the Rot/ Healthy tree type. In December 2018 we evaluated the number of exit holes in all 11 trees that died. Besides a few exit holes (below 20) on two trees in the Rot/Ringed category, none of the other trees that died in 2018 were attacked. One Rot/Healthy tree died in the autumn of 2018 without any beetle attack and with root rot fruitbodies in 2019.

In 2019, 15 trees died. Twelve of these belonged to the Control/Ringed (n=7) and

Rot/Ringed (n=5) tree types and the last three belonged to the Rot/Infected tree type. Nine trees were attacked by *C. piceae* in 2019 (100+ exit holes). Seven ringed trees in the rot (n=4) and control (n=3) tree types were attacked, and the last two trees were in the Rot/Infected tree type. One Rot/Infected tree died during the winter of 2018/2019 and was not attacked by *C. piceae*.

Discussion

The study provides empirical evidence that trees attacked by *C. piceae* were severely weakened or borderline dead, with measurably lower moisture content before the beetle attack, and significantly lowered tree growth up to three years before the attack. This raises questions about the actual damaging properties of *C. piceae*, and highlights the need for similar studies on other secondary bark beetles considered aggressive and problematic.

In the experimental design there were five tree types with ten replicates to investigate the interaction between C. piceae and H. annosum weakened Abies procera trees. The design was considered robust, as no trees died or were attacked in our healthy control, and most trees (n=7) were attacked in the infected tree type (Supplement 1). The ringed part of the design did not work as intended. The trees ringed at the beginning of March 2018 were intended to be attacked by C. piceae in 2018 for comparison, but they died later than expected, and none of these trees were attacked during 2018 (Supplement 1). The calibration of moisture had a significant effect on the results because the variation between collection events was pronounced. This difference between collection events was explained by the settings on the Hydromette, to fit the ambient temperature. The way the moisture was calibrated (subtracting the same value from all trees in a collection event) only affected the variation between collection events and not within them.

Differences between tree types and attacked trees

Moisture. Time-domain reflectometry (TDR) was used to estimate the moisture content in trees. This technique has been used to monitor

treemoisture in other studies (Constantz & Murphy 1990, Irvine & Grace 1997, Nadler et al. 2006, Hernández-Santana et al. 2008). However, other measures such as bole resin flow (Lorio 1993), production of resin in terminal twigs (Mopper et al. 1991), hydraulic conductivity (Hietz et al. 2008), relative water content (Netherer 2014) could be argued to better capture the weakened state of a trees, but to stress trees as little as possible with the repetitive samplings throughout the year, TDR was used. When comparing moisture in the tree types from all collection events during 2018, moisture was lowest in the Rot/Infected tree type. This is partly explained by the fact that 5 of 6 trees that died before July 2018 belonged to this tree type, and the death of a tree naturally lowers the moisture content (Figure 3). However, the same result was found when comparing the three tree types before C. piceae attacked (collect 1-3). Again we found significantly lower moisture in the Rot/infected tree type (Figure 4b). This difference between Control/Healthy and Rot/Infected even before beetle attack was also expected, as the presence of an H. annosum fruitbody on a living tree indicates a compromised root system, resulting in water-stressed trees (Woodward et al. 1999). However, an even better predictor than tree type, for the moisture levels before bark beetle attack, was whether the tree would be attacked later in 2018, by C. piceae (Figure 4b and Supplement 3). In all five blocks with attacked trees, the lowest moisture was always found in the trees attacked later in 2018 (Supplement 3). It is noteworthy that even when comparing trees attacked in 2018, to the other infected trees with visible fruitbodies (non-attacked trees in the Rot/Infected tree type) the moisture was lower in the attacked trees (Figure 4b). However, the difference was not significant, as the sample size was low (n=10), and the variation between blocks (described above) was high.

Tree growth. When comparing tree growth (wood cores samples) across tree types, significantly lowered growth in 2016/2017 in the Rot/Infected tree type was observed (Figure 4a). This effectively means that the trees with *H. annosum* fruitingbodies in 2017, have had such a compromised root system, that it has led to

14

significantly lowered growth two years prior to experiment start. Additionally, seven of the eight trees attacked by C. piceae in 2018 or 2019 (see Supplement 3) clearly had lowered tree growth two years prior to attack, again emphasizing the weakened state of the attacked trees. The trees attacked in 2018 was not significantly different from non-attacked trees. This is explained by the low sample size (n = 6) and the fact that one tree (B7.rr.inf) did not have lowered tree growth (Supplement 3), before attack. Despite this single tree not showing a lowered growth, a fruiting body on the stem of the tree was observed, so undoubtedly this tree have had a compromised root system. The tree also had lower moisture prior to beetle attack when compared to the healthy tree in the same block.

Other relevant observations. The observation that trees attacked in 2018 were extraordinarily weakened, is supported by several observations. A Rot/Infected tree in Block 9 died during the winter of 2019 but was never attacked by C. piceae (no frass or exit holes). This tree had low growth in 2016/2017 (Supplement 3) and was visibly weakened. Even so, it survived two entire C. piceae seasons without any attack attempts (no resin or frass was observed). This indicates that H. annosum is perfectly capable of killing A. procera trees, as also stated in literature (Woodward et al. 1999), and highlight the weakened state of the H. annosum infected trees. This point was further emphasized by the ringed trees. The second generation of adult C. piceae searching for suitable host material, is active during July and the beginning of August (Justesen et al. 2020). Data on moisture and time of death (Figure 3 & Supplement 2, dashed line) showed that the ringed trees had 100% needle browning by the end of August and that they were beginning to wilt (Figure 3 & Supplement 2, dotted line) around mid-August. Despite Justesen et al. (2020) observing trees attacked by the second generation in 2018, which was an exceptionally warm and dry summer, none of the ringed trees dying in 2018 were attacked. The fact that several of the ringed trees dying in 2019 were attacked (Supplement 1.) confirms that they can function as suitable hosts. This indicates that such a tree, despite dying the following month, was too unattractive for the beetles to attempt an attack.

The only indication we found that C. piceae could have a negative effect on tree survival, is the rejected attacks on visibly weakened trees i.e. root rot infected trees with short needle growth and a light green colour. A handful of trees in the plantation (one living and four dead) have been observed with a shotgun pattern of resin coming out of the stem. In one of these attacks (in 2017) on a living tree, beetles and eggs were observed within the resin, so undoubtedly a rejected attack on a tree with an active defence. This tree survived the 2017 season and died in 2018 with a new colonization of C. piceae (not part of the experiment). In light of the other results of the study, these rejected attacks could also be seen as evidence that even very weak trees with impaired host defences can reject C. piceae, and only hosts with latent or extremely compromised active host defences are suitable hosts for C. piceae.

Cryphalus piceae - a problem or not? In a European questionnaire, 3 out of 19 countries evaluated *C. piceae* as a problematic bark beetle species (Grégoire & Evans 2004) and several studies have reported *C. piceae* as a semi-aggressive bark beetle (Chararas 1975, Capecki 1982, Klein 1984, Sekendiz 1987, Cerchiarini & Tiberi 1997, Faccoli 2000, Toper 2002, Yıldız *et al.* 2007, Akinci *et al.* 2009, Podlaski & Borkowski 2009, Jankowiak & Kolařík 2010, John 2011, Ertugrul *et al.* 2017).

The moisture and wood core measurements only allow for speculation of how weakened the trees were at the time of attack. However, the fact that attacked trees have such a comprised root system, that it has resulted in lowered growth two years prior to C. piceae attack, is strong evidence that the trees are almost dead at the time of colonization. Especially combined with the fact that A. procera is highly susceptible to H. annosum and that the mortality rate caused by H. annosum is high in this tree species (Woodward et al. 1999, Chastagner et al. 2002, Chastagner & Dart 2006). Observations of trees dying during winter, without any C. piceae attack confirms the ability of H. annosum to kill A. procera trees at the study site. The very weakened state of attacked trees was further supported by the fact that trees attacked in 2018 had lowered moisture content before beetle attack (Figure 4b), even when compared to other trees with visible *H. annosum* fruitbodies (Figure 4a), suggesting internal processes in the attacked trees to be different from the non-attacked trees.

The results and observations from our study all indicate that the absence of C. piceae from the A. procera plantation would have very little (if any) effect on the number of dead trees. Additionally, Justesen et al. (2020) discussed how C. piceae has been abundant in several greenery plantations throughout Denmark, but never in plantations where H. annosum is absent. The only observation indicating potential damaging properties of the beetle was the rejected attacks discussed in the section above. Based on these results, we placed C. piceae in the conceptional figure (Figure 1). Cryphalus piceae was placed as a possible coup de grâce (Franklin 1987) able to overcome very compromised host defences (hence presence on the right side of the line denoting a physiologically living tree), but mostly as an opportunistic bark beetle effective at locating trees and branches with latent host defences. The problematic nature described by previous authors is likely explained by the high densities of C. piceae in very stressed (or recently dead) trees, still visually appearing healthy. In fact, much of the evidence for the positive relationship between insect attack and drought, is based on these correlative observations of insect activity and tree mortality after drought (Gaylord et al. 2013).

The results highlight that, possibly, several secondary bark beetles are regarded as problematic species, simply because they occur in recently dead or irreversibly weakened trees, and not because their effect significantly alters the two-axis framework (host survival) adopted from Anderegg et al. (2015). This highlights the importance of studying secondary bark beetles, as resources might be spent managing bark beetle populations with insignificant financial impacts. This is especially relevant as studies indicate that all aspects of insect outbreak behaviour will intensify as the climate warms, and the correct use of resources will be critical (Dale et al. 2001, Logan et al. 2003, Raffa et al. 2008).

It is important to underline that even though the results find *C. piceae* as a non-problematic bark beetle, this does not translate to all secondary insects, as other secondary organisms could affect the outcome of the two-axis framework negatively. The study merely emphasizes that even though an organism is found in large numbers in weakened trees, it should not automatically be identified as problematic.

This study cannot prove that *C. piceae* attacks during outbreak conditions are never of significant importance. As mentioned in Anderegg *et al.* (2015), the development time and thereby potential harmful properties, are affected by climate, so additional studies in different climatic zones, and on different host species, are needed to confirm if these results correspond during outbreak conditions caused by e.g. drought in warmer climates. This study does however bring strong evidence that *C. piceae* in the Danish *A. procera* plantations should not be regarded as a tree-killing agent.

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Received: 23 May 2023 Accepted: 23 November 2023 **SUPPLEMENT 1**. Bar chart showing how many of the ten in each of the five tree types trees that died or were attacked in 2018 and 2019.



SUPPLEMENT 2. Calibrated moisture in the 10 trees belonging to the two ringed tree types investigated in 2018. A dotted line represents an almost dead tree () a dashed line represent a dead tree ().



Moisture in trees

SUPPLEMENT 3. Calibrated moisture and ring-width measured from wood cores, in each block. a) The % of the total ringwidth in 2016/2017 in the different tree types and in relation to attacks of *C. piceae* b) Calibrated moisture in collection event (1-3), in the different tree types and in relation to attack-year of *C. piceae*.

