Internship Report

Monitoring of *Cucujus cinnaberinus* (flat bark beetle) within different domains spread over Flanders (Belgium)

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Table of Contents

1.0 INTRODUCTION 1

1.1 Habitat and ecology 1

1.2 Habitat in Flanders 2

1.3 Importance of the species 3

1.4 Threats 3

1.5 Distribution in Europe 4

1.6 Distribution in Belgium and the Netherlands 5

1.7 Objectives 6

2.0 MATERIALS AND METHOD 7

2.1 Study area 7

2.1.1 Location of the study sites 7

2.2 Investigated dead trees 8

2.3 Larvae search method 9

3.0 STATISTICAL ANALYSIS 10

4.0 RESULTS 11

4.1 Assessment of *Cucujus cinnaberinus* distribution patterns. 11

4.1 Statistical results 17

5.0 DISCUSSION 24

6.0 CONCLUSION 26

7.0 ACKNOWLEDGEMENTS 28

8.0 REFERENCES 29

1.0 INTRODUCTION

Cucucjus cinnaberinus is one of the four Cucujidae species in Europe (Bonacci et al., 2012; Gutowski et al., 2014). Before the twenty-first century, the knowledge of the distribution and ecology of *C. cinnaberinus* was limited and the species was perceived as an extremely rare “relict beetle” associated with natural or even primaeval forests (Speight 1989; Eriksson 2000).

The species is endemic to Europe. More specifically, they are distributed from the Mediterranean to Scandinavia and from Russia to Spain. Their scattered distribution pattern was probably due to past population decline and local extinctions (Horak et al., 2009; Horák et al., 2010).

The European species *C. cinnaberinus* is strictly protected and is enclosed in Annexes II and IV of the EU Habitat Directive 92/43, to improve existing populations and bring them in a favourable state of conservation. Its status has been classified as “near threatened” in Europe by the International Union for Conservation of Nature (IUCN) organization (Bonacci et al., 2018) and “very rare” in Belgium (Thomaes & Marchand, 2019). The species is also listed in the Bern Convention and is strictly protected by law in most European countries across its distribution range (Nieto et al., 2010). Inclusion in Appendix IV means that Belgium must take protective measures for the species, their breeding sites and habitats. Also, Belgium must report on its conservation status to the European Union. Henceforth the status of its population and habitat has become crucial for the identification and monitoring of protected areas included in the Natura 2000 network across the entire European Union (Bełcik et al., 2019).

1.1 Habitat and ecology

C. cinnaberinus larvae and adults live under the bark of dead tree trunks of a variety of broad-leaved trees such as *Quercus, Robinia, Populus,* rarely on *Pinus* and *Acer* (Horák et al., 2008). This lifestyle might also be a reason for their low detection rate in the past (Horák et al., 2010).

The species has a flat body, which allows them to crawl through thin parts that open between the xylem and phloem of decaying dead trees (Bonacci et al., 2018). This allows them to live for several months under the bark, being active outside for a relatively short period. Hence the species is referred to as a saproxylic beetle, meaning a beetle that depends on deadwood. Under the bark, *C. cinnaberinus* is associated with Silvan flat beetles (*Silvanidae*), ground beetle (*Carabidae*), red cardinal beetle (*Pyrochroa coccinea*) and non-coleopteran taxa such as ants from the genus *Lasius*, mites, flies, centipedes and springtails (Horák et al., 2012).

This species is mainly found in wet forest types along rivers and streams as well as in alder forest (Eckelt et al., 2014). Several studies have shown that the species is dependent on wet forests with a high stock and a high proportion of deadwood (Bussler, 2002; Eckelt et al., 2014; Goczał & Rossa, 2017).

Similarly, the species also inhabits various types of deciduous and mixed forest, both in mountainous regions and lowlands. Other studies state that *C. cinnaberinus* prefers large and sun-exposed dead wood of various deciduous and coniferous trees characterized by a moderate to high moisture content and an intermediate stage of decay (Bonacci et al., 2012; Goczał and Rossa, 2017; Vrezec et al., 2017).

Their suitable substrate consists of deadwood parts of both lying and standing, recently died trees. Optimally, the diameter would at least be 20 cm (Palm 1941, Bussler, 2002). On a few occasions, larvae are also found on deadwood with an optimal diameter of at least 10 cm (Straka, 2006). The bark of the concerned dead trunks must be moist and affected by fungi and slowly start to detach from the underlying still hardwood (Palm 1941, Straka 2008).

1.2 Habitat in Flanders

In Flanders, after the Second World War, Poplars trees were planted in river valleys, as the agricultural value of wet soils had reduced. In the last decades, many of these Poplar plantations acquired the status of nature reserves, after buying land from private owners. They were left unmanaged and became or become over mature stands which are now characterized by a high stock of deadwood. This has made these wet forests an ideal habitat in a relatively short period for such a demanding species (Thomaes & Marchand, 2019).

1.3 Importance of the species

According to Nieto and Alexander (2010), saproxylic beetles play an important role in the proper functioning of forest ecosystems. They influence the rate of deadwood decomposition, nutrient cycling and food webs in forests (Speight, 1989).

For that reason, it is important to mention that forest management can greatly affect the habitat suitability for saproxylic beetles (Buse et al., 2010; Grove, 2002; Siitonen, 2001). Wood-inhabiting beetles have specific habitat requirements and life-history traits that make them sensitive to human-induced forest changes. Yet, the scale of this effect is dependent on the forestry activities and management practices that are applied (Müller et al., 2013; Rosenvald and Lõhmus, 2008).

Groove (2002) showed that in many cases available quantities of dead wood have a positive correlation with the biodiversity of beetles. Additionally, their occurrence is also correlated with the presence of several other indicators such as fungi (Floren et al., 2015; Linnakoski et al., 2012), other insects (Vindstad et al., 2014) and bird species like woodpeckers (Mikusiński et al., 2001; Tomiałojć and Wesołowski, 2004; Wesołowski, 1995). Besides that, saproxylic beetle species can also be used as bioindicators of ecosystem disturbance and biodiversity (Floren et al., 2015).

In Southern Italy, *C. cinnaberinus* is being used as a simple and useful tool for diversity monitoring in forest nature reserve networks (Mazzei et al., 2018). Additionally, Mazzei *et al* (2011) regard the species as an "umbrella species", protection of which serves to protect many other co-occurring species.

Finally, because *C. cinnaberinus* is particularly seen in protected areas and some populations have been found to inhabit long-term managed forest in central Europe, it is an ideal subject for studying the relations between deadwood characteristics, forest management and species occurrence in forest ecosystems (Goczał and Rossa, 2017).

1.4 Threats

The major threats faced by this species are degradation and loss of habitat, which results in fragmentation and isolation of populations (Røed et al., 2014). This would lead to a lack of sufficient deadwood and suitable wet habitats.

1.5 Distribution in Europe

In Europe, until recently *C. cinnaberinus* was considered to be a species mainly present in central Europe. Its range in north-southern Europe had declined considerably in the past. Outside of central Europe, until recently only a limited number of sightings had been reported from Spain, Scandinavia, Poland, the Baltic States and Russia (Horák et al., 2008; Horák et al., 2010).

More recently, however, this species is on the rise and is currently becoming established in regions without former reports. Firstly the species expanded strongly in central Europe, especially in river valleys (Eckelt et al., 2014) but after that, the species greatly increased its range. The original population in south-eastern Bavaria managed to emerge from 2002 and expanded to other areas in Bavaria. From 2003, there were also reports in western Germany, namely in Baden-Württemberg (Reibnitz, 2008). Since no intermediate populations have been discovered since then, it seems that this location was colonized from a great distance. This could have been possible due to transport or via distant journeys e.g. with jet streams (Jeffries et al., 2013). As of 2012, the species also appeared in neighbouring areas in Hessen (Schaffrath, 2014) and in 2016 in Reinland-Pfalz and in 2017 in North Rhine Westphalia (Thomaes & Marchand, 2019).

In northern Germany, an initial colonization took place with the first sighting in Mecklenburg-Vorpommern and later in Brandenburg (Esser & Mainda, 2016) and Schleswig-Holstein (Horren et al. 2016).

A strong recent expansion has also been noted in Austria. The species is currently observed in all states except Carinthia and Vorarlberg. Additionally, in Austria, observations have also been made along the Salzach in Salzburg and the Northern Alps (Eckelt et al. 2014). In Italy, the species has reappeared (since 1960). In May and June 2009, a sighting of *C. cinnaberinus* was reported in southern Italy (Mazzei et al., 2011). Lastly, in northern France, in an area called Alsace (Bas-Rhin), there have been confirmed cases of the species as well (Fuchs et al., 2014). Figure 1 shows a distribution map of *C. cinnaberinus* in Europe until 2010.

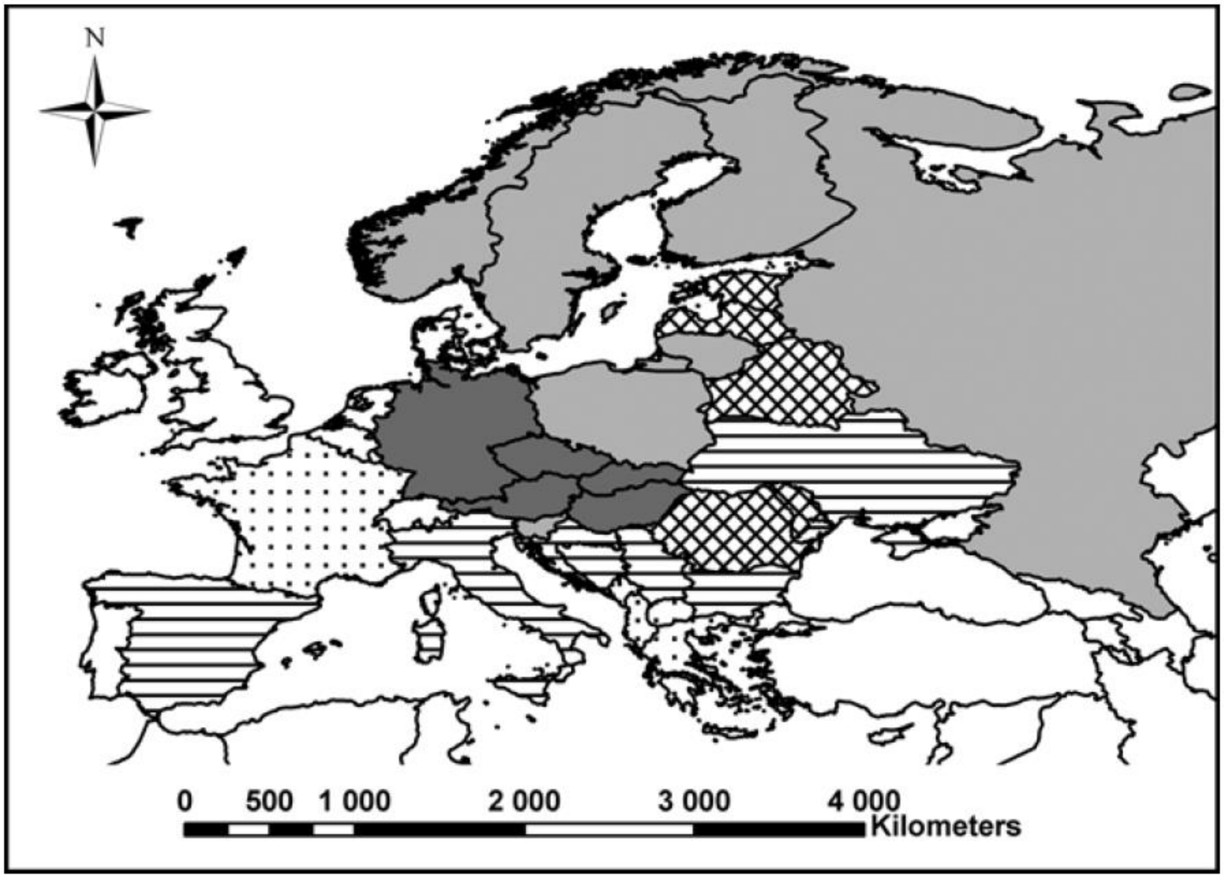


Figure 1: Distribution and status of Cucujus cinnaberinus populations in European countries based on the number of records reported since 2000, more than 50 sites (dark grey); 5–20 sites (light grey); less than 5 sites (crosshatch); based on records for the period 1950–1999, less than 5 sites (simple hatch); possible occurrence (stipple) (Horák et al., 2010).

In other parts of the European continent, knowledge about its occurrence is very little, which is due to various reasons - one of which is the difficulty of locating imagines, whose season is short (being limited to 2-3 months per year), and also the secretive lifestyle. Yet, in contrast to imagines, larvae are easy to find throughout the whole year in their breeding sites (Horák and Chobot, 2011).

1.6 Distribution in Belgium and the Netherlands

In the Netherlands, the species was first found in 2012 in an area called Dommel vallei, which is just a few kilometres from the Belgium border (Colin & Noordijk, 2012). Later on, in 2017, the species was also found in the Groene Woud, Bergsche Maas and Zuid-Limburg (Noordijk et al., 2017).

According to Thomaes & Marchand, (2019), there were no known historical observations of this species in Belgium, although the attention to such smaller beetles in entomological literature and collections did not start until the end of the 19th and the beginning of the 20th century. At that time in Flanders, there were barely any unmanaged floodplain forests or non-drained swamp forest left with lots of thick dead trees. In Flanders, *C. cinnaberinus* was first observed in 2014 in a wet poplar forest in Hamont-Achel. In 2017, the species had already spread over Limburg and several areas in Antwerp. Based on the previous study, there are 37 known validated observations from 12 different areas (see figure 2).

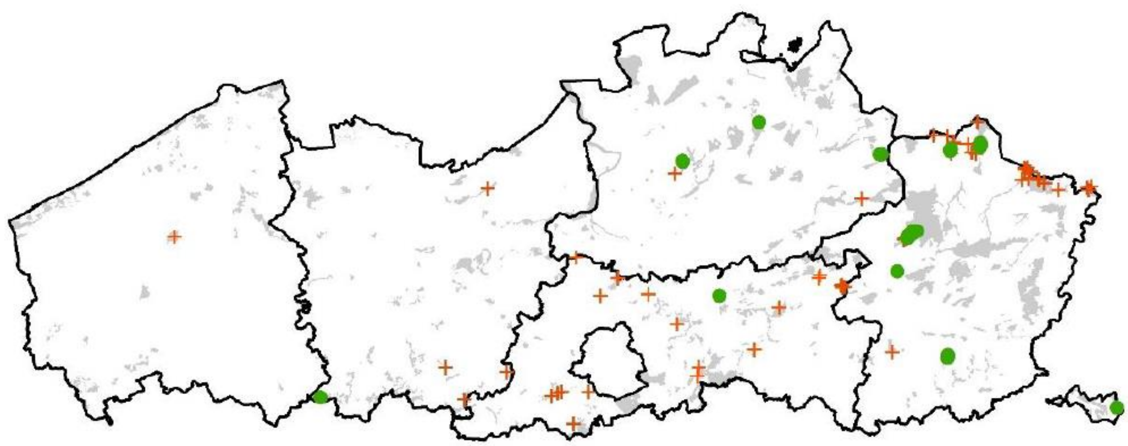


Figure 2: Previously known distribution of flat bark beetle in Flanders (Belgium), green dots show where the species is present and red cross sign where the species is absent. The Habitat directive areas are shown as a grey background. The absence areas should be interpreted primarily as places that have been searched. Based on the research by INBO. Source: Thomaes & Marchand (2019).

In 2019, an internship survey of the species, co-guided by ANB and INBO, confirmed the additional presence of the species in Zoerselbos and Vrieselhof (Kennedy, 2019).

1.7 Objectives

This study aims to further map the distribution of *C. cinnaberinus*, by monitoring its range of expansion in selected areas and to gain additional experience in setting up a monitoring network in the region of Flanders. It mainly focuses on the presence or absence in distinct areas in Flanders. Therefore, a monitoring program that gives an insight into population trends and the effectiveness of conservation measures is needed. Given that the species is expanding its range, we investigated mainly areas where the species has not yet been observed to add to existing knowledge about the species.

2.0 MATERIALS AND METHOD

2.1 Study area

2.1.1 Location of the study sites

The study was conducted during the winter period from December 2019 to early March 2020. This period was a good time to do the study because most of the shrubs, weeds and grass were dry, making it easier to access areas.

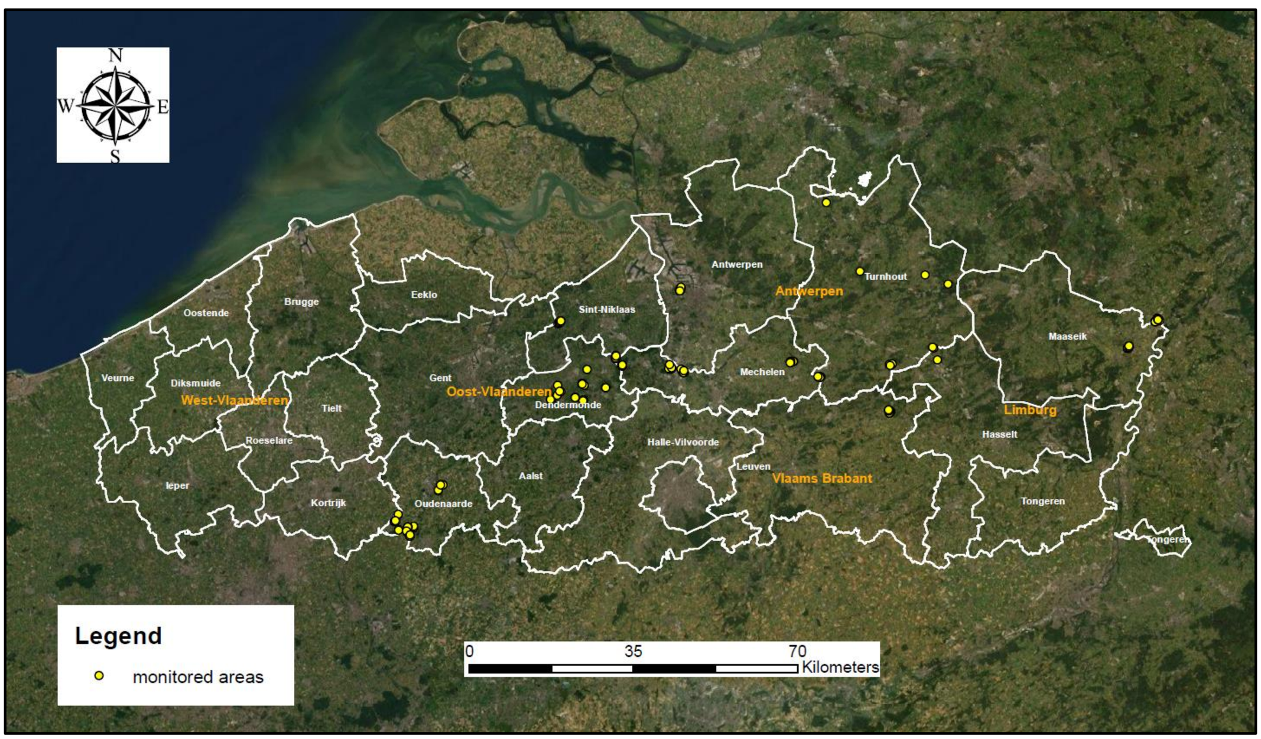


Figure 3: A map of Flanders (Belgium) which shows the forest areas which were monitored for the presence or absence of C.cinnaberinus.

Monitoring of *C. cinnaberinus* was mainly done in Antwerp and East Flanders and few areas in Flemish Brabant and Limburg as shown in figure 3. The decision to focus more on the provinces Antwerp and East Flanders was based on the expectation of new colonizations in those areas. Within these provinces, sites were selected where the species was not yet found, had potential habitats and were nearby known populations. For areas managed by ANB, a preference was included to those expected to get a new management plan during the next years.

Suitable habitats were selected based on the presence of former Poplar plantations which are now managed as a reserve and they have large quantities of recent deadwood (Thomaes & Marchand, 2019). The sites were comprised of a wet forest with Poplar (*Populus*) trees species, with an expectation of high stock of suitable dead trees. This is because the species is known to be found under the bark of Poplar tree species and in wet forests. These areas were easy to locate with the help of reference maps.

2.2 Investigated dead trees

We sampled different trees species: Poplar (hybrid as well) was the main tree species that were sampled, but we also sampled other tree species like Silver birch (Ruwe berk), Common white birch (Zachte berk), Goat Willow (Boswilg), White willow (Schietwilg), Northern red oak (Amerikaanse eik), Pedunculate oak (Zomereik), Black cherry (Amerikaanse vogelkers), Ash (Es), European silver fir (Zilverspar), Larch (Lork), Maple (Esdoorn), Norway Spruce (Fijnspar), Common alder (Zwarte els), European black pine (Zwarte den) and Scots Pine (Grove den). Investigating different tree species in a similar habitat is important to understand if other tree species can be suitable habitats for this species.

The dead trees examined included those that died one to three previous years and still had an intact bark. We were interested in naturally dead trees (by storms or raised water table) and those fallen by logging activities from foresters. Those dead trees could be in a standing, hanging or lying position as you can see in figure 4.

Figure 4: showing some examples of standing, hanging and lying dead trees that were sampled in the field (all Poplar trees)

2.3 Larvae search method

The occurrence of *C. cinnaberinus* was checked by peeling bark of suitable looking lying, hanging and standing dead trees and the inside of the removed bark was examined for the presence of larvae or adults (imagines). Under the bark there must be a red or dark discolouration indicating a fermentation process due to lack of oxygen, indicating suitable habitat for the species. This process of peeling off the bark of the dead trees was done using a claw hammer.

This search method is destructive, meaning by removing the bark, suitable habitat for *C. cinnaberinus* gets lost. Therefore, in each investigated area, we sampled at most three dead trees per plot. Once larvae or an adult (imago) was found on the dead tree, it was considered present in that area. In that case, another sample plot had to be examined to minimize the impact on barks where *C. cinnaberinus* was present. Furthermore, only a limited amount of bark was removed per tree; about 2m length and half of the circumference.

Once debarked, the length and width of the debarked area (surface area measured) and the circumference (at the middle of the debarked area) was measured. The position of the dead tree (lying, hanging or standing) was noted, a photo of the debarked area taken, and coordinates of the sampled tree specimen were also recorded. If larvae or an imago were present, the number seen (present) or absence was recorded. We also mentioned the observer. After the fieldwork was done, this data was uploaded in the database of https://waarnemingen.be.

In total, we monitored the presence or absence of *C. cinnaberinus* on 369 dead trees. This number is based on all the combined data from different observers. When larvae were found, it was important to be able to distinguish the *C. cinnaberinus* from the cardinal beetle larvae (*Pyrochroa coccinea* and *Pyrochroa serraticornis*) as they look slightly similar and are mostly found on similar tree species. Imagines (adults) are much easier to distinguish.

3.0 STATISTICAL ANALYSIS

To assess the impact of selected variables on the probability of occurrence of *C. cinnaberinus*, a generalized linear regression (GLM) with binomial distribution was used on the presence/absence of *C. cinnaberinus*. The explaining variables being used were circumference, surface area examined and position of the tree (lying or standing tree). Hanging tree position could not be included in the statistics because it did not have adequate occurrence, it only had 6 records where the species was found. The dependent variable (presence/absence of the species) was binary: presence was coded as 1, absence as 0.

The surface area of the examined surface on the trees was calculated by multiplying length x width x pi/4 (which approximates an oval with length and width of the stripped part). The abundance of the species was calculated by the number of larvae/adults found divided by the examined surface area. This number of larvae per m^2 is calculated to compare the different investigated surfaces.

An ANCOVA regression analysis was used to analyse the relationship between the abundance of *C. cinnaberinus* (larvae/m^2), the circumference of the sampled dead trees, the position of the dead trees, the province where we sampled and the location of the sampled areas.

4.0 RESULTS

In this study, a total of 369 dead trees where sampled, but only 42 dead trees had records of *Cucujus cinnaberinus*. Thus only 12% of the investigated trees had *Cucujus cinnaberinus* present. A total number of 134 individuals were found, with 127 being in the larvae stage and 7 in imago stage. In comparison to previous findings, we observed an increase in new locations where the species was present. 116 larvae were found on Populus, while 4 larvae were found on Scots pine. 1 imago was found on Pediculate oak. The other 6 imagines were found on one tree of unknown species. But for analysis, only dead trees that had complete data were used.

Taking the provinces into account, we found a total of 50 *C. cinnaberinus* individuals in Antwerp province, 53 in East Flanders, 28 specimens in Limburg and 3 specimens in West Flanders. No specimen was found in Flemish Brabant. This result can be explained by the number of samplings in each area, as more sampling was done in Antwerp and East Flanders.

4.1 Assessment of *Cucujus cinnaberinus* distribution patterns.

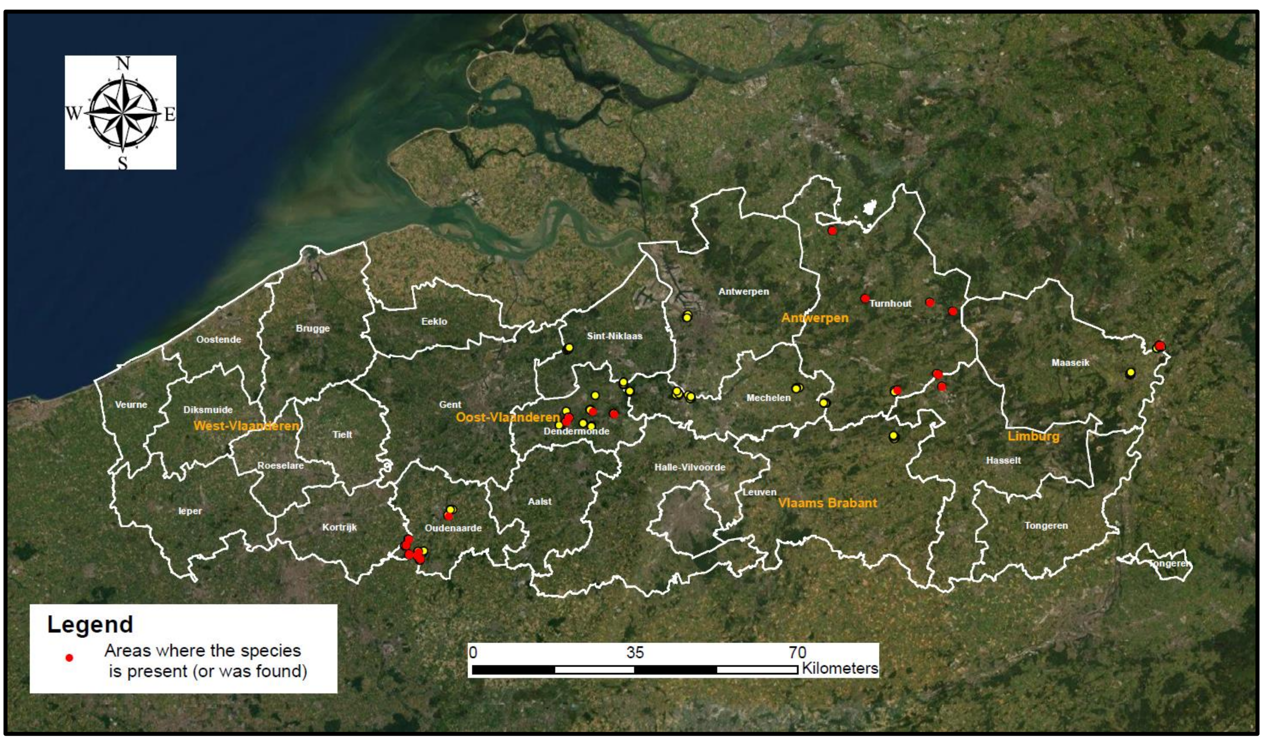


Figure 5: A maps showing the new locations where C. cinnaberinus is present (in red) or absent (in yellow).

The map in figure 5 shows the areas where the species was found (present) and where not (absent).

Additionally, table 1 gives more details about the areas where the species was found such as the municipality, the name of the location where the species was found, the research date, the number of *C. cinnaberinus* found, their life stage and the observer. The overview is sorted by municipality and location.

Table 1: the areas/sites details where the species were present and the species count, its life stage and the observers.

Municipality	Location	Date	Number of C. cinnaberinus	Stage	Observer
Balen	Olmen – Beukenbergsveld	01/01/2020	3	Imago	Obtained from waarnemingen.be
Balen	Gerhoeven - De Rammelaars	04/01/2020	1	Imago	Obtained from waarnemingen.be
Balen	Balen - De Most	13/03/2020	3	larva/nymph	Vincent Smeekens
Beerse	Vlimmeren - Hoge Bergen-Ekstergoor	05/02/2020	2	larva/nymph	Vincent Smeekens
Beerse	Beerse	05/02/2020	15	larva/nymph	Vincent Smeekens
Beerse	Vlimmeren - Hoge Bergen-Ekstergoor	05/02/2020	1	larva/nymph	Vincent Smeekens
Berlare	Berlare – Berlarebroek	26/02/2020	1	larva/nymph	Arno Thomaes, Gibson Daka
Berlare	Berlare - Berlarebroek (OV)	26/02/2020	1	larva/nymph	Arno Thomaes, Gibson Daka
Berlare	Berlare - Berlarebroek (OV)	26/02/2020	2	larva/nymph	Arno Thomaes, Gibson Daka
Dendermonde	Baasrode - Vlassenbroekse Polder en Schorren	10/01/2020	1	larva/nymph	Vincent Smeekens, Kevin Scheers
Dendermonde	Appels - Sint-Onolfspolder - 28 Roeden en Meerdam West	17/02/2020	1	larva/nymph	Arno Thomaes
Dendermonde	Baasrode - Vlassenbroekse Polder en Schorren	13/02/2020	1	larva/nymph	Arno Thomaes, Gibson Daka
Dessel	Dessel	29/02/2020	1	larva/nymph	Obtained from waarnemingen.be
Ham	Ham – Veldhovenheide	22/12/2019	1	Imago	Obtained from waarnemingen.be
Hamme	Kastel - Het Zwijn	10/01/2020	1	larva/nymph	Vincent Smeekens, Kevin Scheers
Hamme	Kastel - Het Zwijn	10/01/2020	11	larva/nymph	Vincent Smeekens, Kevin Scheers
Hechtel-Eksel	Mil. Dom. Kamp Beverlo - Schietveld (Strikt ontoegankelijk)	28/02/2020	7	larva/nymph	Vincent Smeekens
Hechtel-Eksel	Mil. Dom. Kamp Beverlo - Schietveld (Strikt ontoegankelijk)	28/02/2020	1	larva/nymph	Vincent Smeekens
Hechtel-Eksel	Mil. Dom. Kamp Beverlo - Schietveld (Strikt ontoegankelijk)	28/02/2020	5	larva/nymph	Vincent Smeekens
Hechtel-Eksel	Mil. Dom. Kamp Beverlo - Schietveld (Strikt ontoegankelijk)	28/02/2020	10	larva/nymph	Vincent Smeekens

Hoogstraten	Wortel-Kolonie - Zone Zuidwest	25/02/2020	4	larva/nymph	Obtained from waarnemingen.be
Kasterlee	Tielen - Tielenkamp (Ontoegankelijk)	01/02/2020	1	Imago	Obtained from waarnemingen.be
Kerkhove	Kerkhove - Scheldegebied (WV)	03/03/2020	3	larva/nymph	Arno Thomaes, Gibson Daka
Kinrooi	Kinrooi - Vijverbroek (LI)	05/03/2020	1	larva/nymph	Vincent Smeekens, Gibson Daka
Kinrooi	Kinrooi - Vijverbroek (LI)	05/03/2020	3	larva/nymph	Vincent Smeekens, Gibson Daka
Kluisbergen	Ruien - Kluisbos oost	04/02/2020	9	larva/nymph	Obtained from waarnemingen.be
Kluisbergen	Ruien - Kluisbos oost	04/02/2020	4	larva/nymph	Arno Thomaes
Kluisbergen	Ruien - Kluisbos oost	04/02/2020	3	larva/nymph	Arno Thomaes
Kluisbergen	Kwaremont - Kalkhovenbos (K-19) (OV)	19/02/2020	1	larva/nymph	Arno Thomaes, Gibson Daka
Kluisbergen	Kwaremont - Feelbos (K-16) (OV)	19/02/2020	2	larva/nymph	Arno Thomaes, Gibson Daka
Kluisbergen	Berchem (OV) - Weyns (OV)	19/02/2020	2	larva/nymph	Arno Thomaes, Gibson Daka
Kluisbergen	Kwaremont - Feelbos (K-16) (OV)	19/02/2020	9	larva/nymph	Arno Thomaes, Gibson Daka
Laakdal	Vorst - 't Hoeves Vorst	31/01/2020	4	larva/nymph	Vincent Smeekens, Gibson Daka
Laakdal	Vorst - 't Hoeves Vorst (AN)	31/01/2020	1	larva/nymph	Vincent Smeekens, Gibson Daka
Mol	Mol - Den Diel - West - Ten zuiden van beek	26/12/2019	1	larva/nymph	Obtained from waarnemingen.be
Olmen	Olmen – Asbeek	13/03/2020	1	larva/nymph	Vincent Smeekens
Olmen	Olmen – Asbeek	13/03/2020	1	larva/nymph	Vincent Smeekens
Olmen	Olmen – Asbeek	13/03/2020	2	larva/nymph	Vincent Smeekens
Olmen	Olmen – Asbeek	13/03/2020	1	larva/nymph	Vincent Smeekens
Oudenaarde	Oudenaarde - Bos t'Ename - Wallebos (OV)	03/03/2020	3	larva/nymph	Gibson Daka
Ranst	Oelegem - Vrieselhof (Groendomein provincie Antwerpen - regio Antw.)	23/01/2020	8	larva/nymph	Vincent Smeekens
Ronse	Ronse - Heynsdale (Prov. Dom. Oost-Vlaanderen) (OV)	03/03/2020	1	Imago	Arno Thomaes, Gibson Daka

As previously mentioned, we selected different tree species. The sampling was based on expected suitability of dead trees found on each investigated area. This resulted in a dataset where some trees species were more investigated than others. As figure 6 shows, 74% of the investigated trees were Poplar, 11% were Pedunculate Oak, 4% White willow, 3% Alder and 2% Scots pine (indicated in green).

When we analyze tree species with one or more larvae present, we found 94 % of the larvae records came from Poplar and 3 % on Pine and Oak as figure 6 shows (indicated in blue).

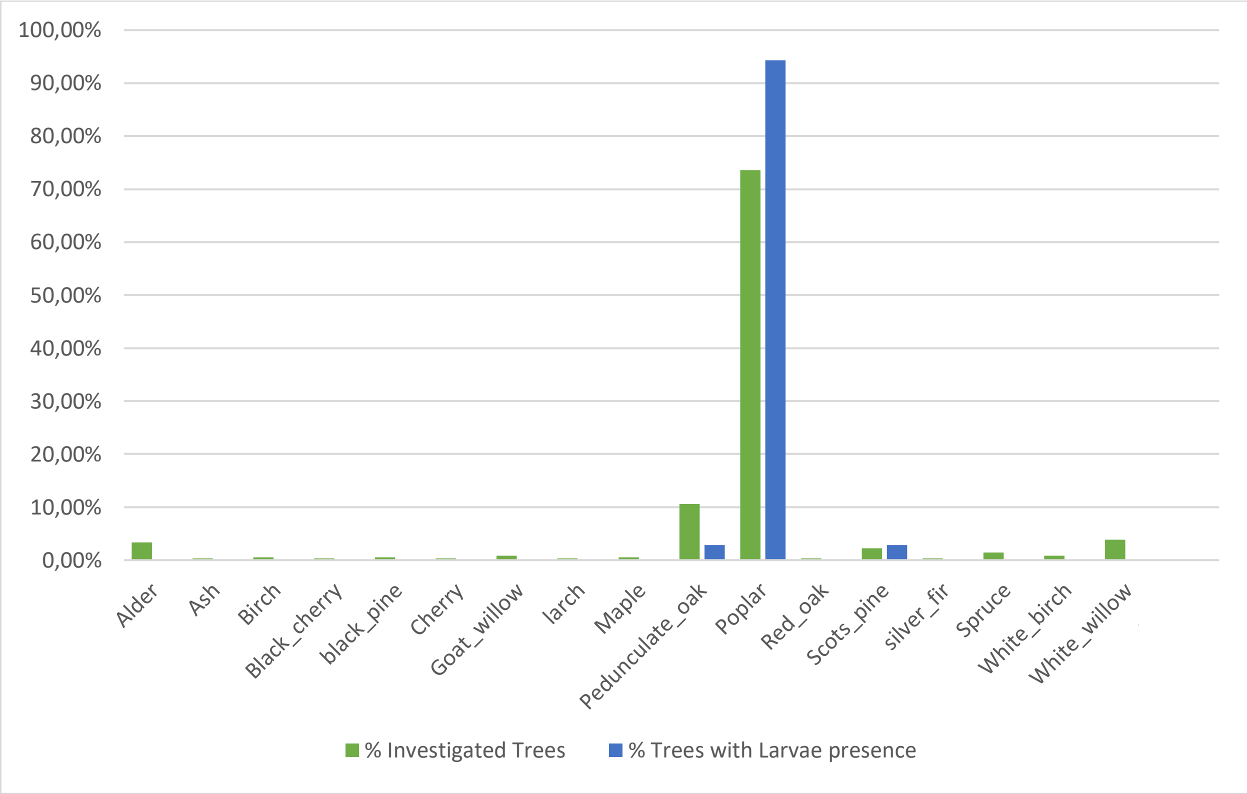


Figure 6: Showing a relative graph with the number of tree species investigated as a percentage (in green) and the percentage of tree species with C. cinnaberinus present (in blue).

Finding a relatively higher percentage of Poplar dead trees with larvae present can mainly be explained by the high % of investigated Poplars. Nevertheless, the percentage of trees with larvae records does not follow the distribution of investigated tree species. The relative % of Poplars with larvae present was found to be higher than the relative part of investigated tree species (94% and 74%) and the % of Pedunculate Oak trees with larvae present is lower than the relative part of investigated species (3% vs 11%).

The stem circumference of the different sampled tree species had a range of 15-340 cm, with an average of 121 cm.

The circumferences were categorized in ranges of 50 cm in figure 10, with the % investigated trees (blue) and % trees with records of *C. cinnaberinus* (orange). The analysis showed that 91% of the records of *C. cinnaberinus* presence was found in the circumference ranges of 50cm -199cm.

While in the circumference less than 49 cm, the presence had a lower percentage. Furthermore, with a circumference above 250 cm, there was no presence of the species.

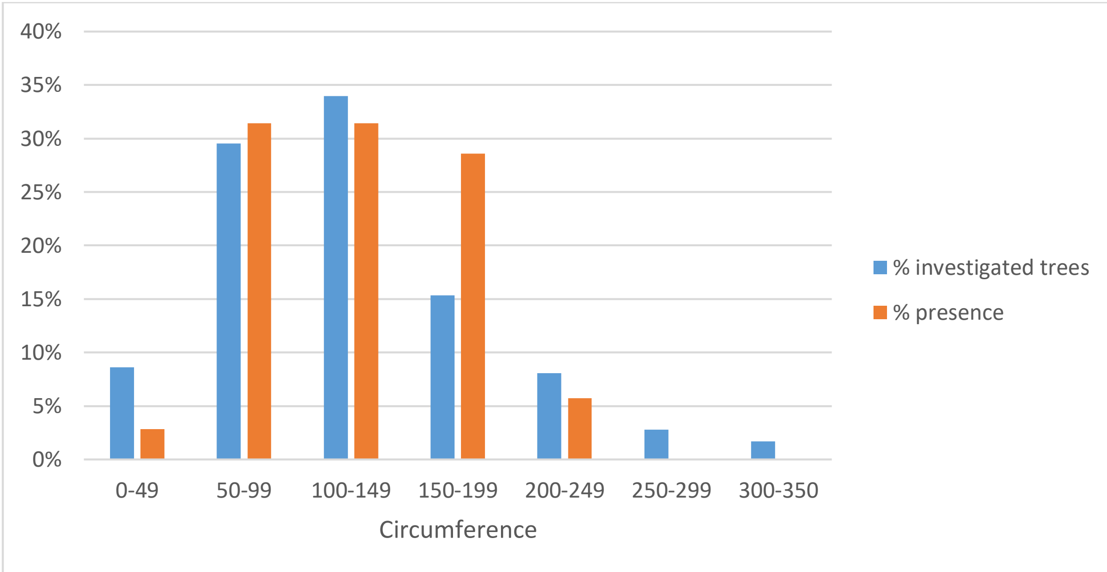


Figure 7: the percentage of investigated trees and the presence for different ranges of the circumference (in centimetres).

This means that the species prefers a dead stem circumference within the range of 50-199 cm. Trees with a smaller (less than 50) or bigger stem circumference (great than 200 cm) are less preferred.

The high % of *C. cinnaberinus* present in the circumference ranges between 50-99 cm and 100-149cm could be explained by the high % of the investigated trees. However, for the range of 150-199cm, a significant difference was seen between their relative % of all investigated trees (15%) and the relative % of trees with *C. cinnaberinus* being present (29%). Therefore the sampling is only a part of the explanation for the high %, but there could be other factors.

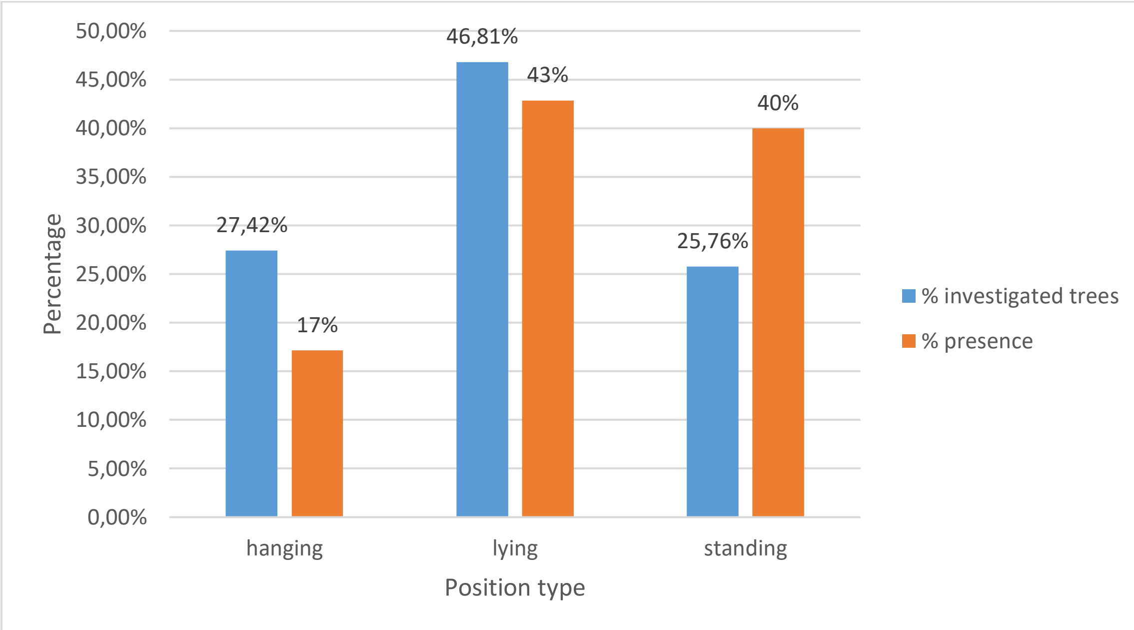


Figure 8: showing the percentage of investigated trees and presence with recorded C. cinnaberinus per position of the sampled dead trees.

Figure 8 indicates that the percentage of *C. cinnaberinus* larvae records present were mostly found on lying as well as standing trees. This was also analysed well along in the statistics.

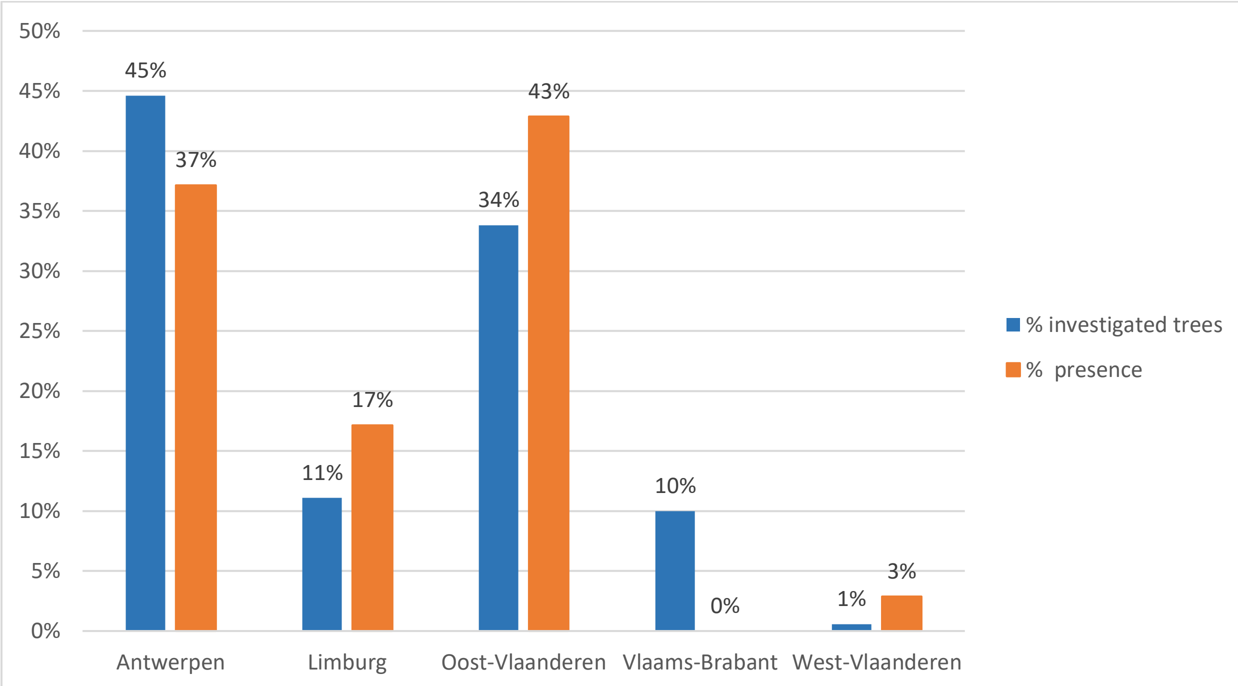


Figure 9: showing the % investigated trees and % presence from all the province that were sampled.

Figure 9 shows that most the records of species present were high in East Flanders and Antwerp followed by Limburg. West Flanders had low records because we only found it in one area, of two. Nonetheless, the high sampling in Antwerp does not necessarily give an equally high % of presence and the low sampling in Limburg does not reflect the presence. This means that there could be other factors in these areas that influenced the presence of the species. In Flemish Brabant, no species were found in the sampled areas.

4.1 Statistical results

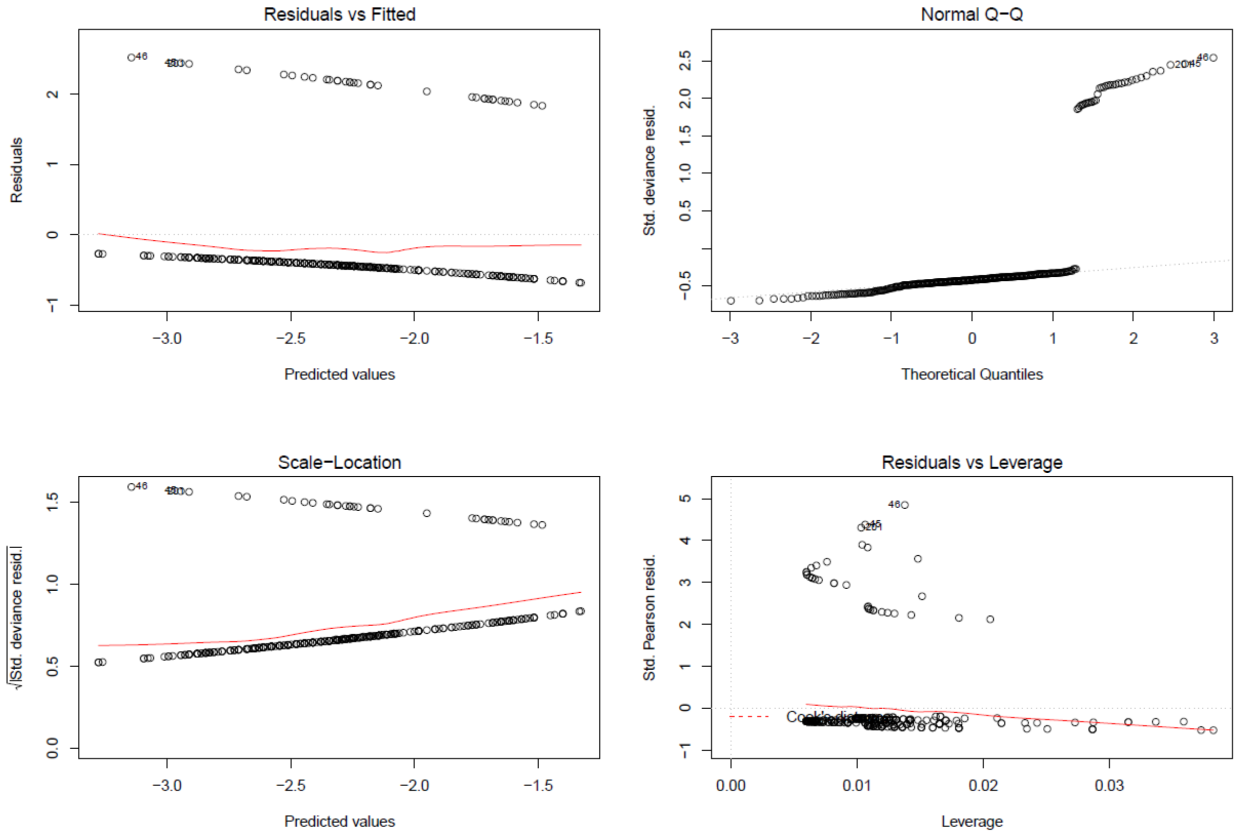
In analysing the correlation between presence/absence of *C. cinnaberinus* and stem circumference, surface area examined and position of the tree, the model revealed that surface area investigated was the most important variable at explaining the probability of *C. cinnaberinus* presence ($p<0.001 = 0.0002$). The surface area showed a significant negative relationship with the probability of *C. cinnaberinus* presence based on the slope ($\beta=-3.3167$). This can be explained by the search method used. Once the species was found on that dead tree, we stopped searching to minimize damage to the bark habitat. Meaning the surface area was smaller when we found the species, but when we didn't find the species the surface area searched was bigger because we searched more. This means the surface area was not consistent, hence it was excluded from the final model.

The final model analysed the correlation between the presence/absence of *C. cinnaberinus* and stem circumference and the position of the tree. This final model showed that stem circumference had a negative effect on the probability of *C. cinnaberinus* presence (β=-0.0033) and was not significant. Additionally, there was a significant correlation between the presence of *C. cinnaberinus* and standing trees (β=1.227, p=0.05). This means that the probability of *C. cinnaberinus* is more likely to be present in standing dead trees. These results are shown in output 1.

Lastly, a relationship between the presence and absence of the species and the location and province was analysed but there was no significant relationship hence it was excluded.

Output 1: shows the coefficient of a generalized linear model statistics with presence/absence as the dependent variable and circumference, the position of the dead trees (lying and standing) as independent variables.

```
Call:
glm(formula = presence ~ circumference + position_type, family = binomial,
    data = presenceonly)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.6859  -0.4707  -0.4213  -0.3618   2.5242

Coefficients:
                       Estimate Std. Error z value Pr(>|z|)
(Intercept)           -2.411035   0.524531  -4.597  4.3e-06 ***
circumference         -0.003329   0.003292  -1.011   0.3119
position_typelying     0.433230   0.501262   0.864   0.3874
position_typestanding  1.226950   0.554244   2.214   0.0268 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 229.43  on 358  degrees of freedom
Residual deviance: 223.91  on 355  degrees of freedom
  (2 observations deleted due to missingness)
AIC: 231.91

Number of Fisher Scoring iterations: 5
```



To examine the residuals concerning homogeneity of variance and normality, the results show that there appears to be a problem with the normality of the residuals. This can be explained by the fact that we had a lot of zero values in our data set.

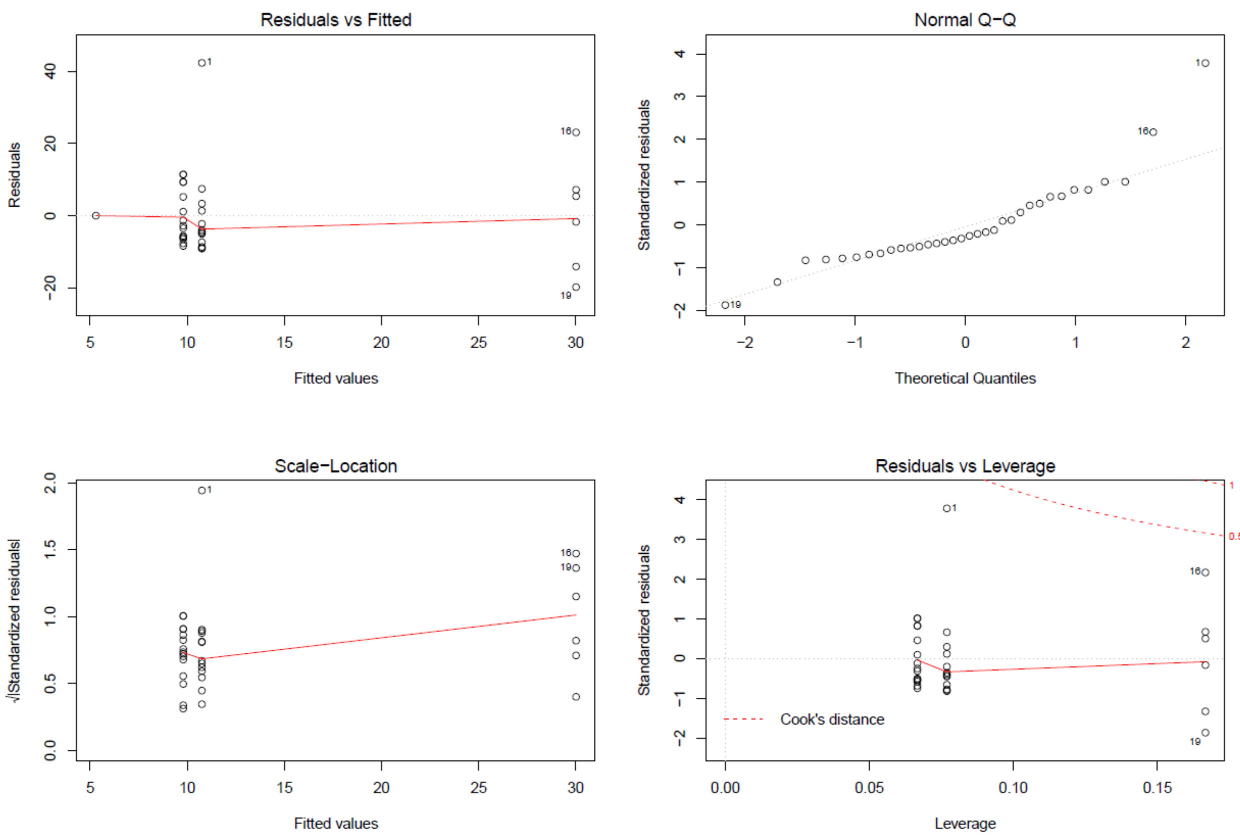
Before the second analysis was done, we first tested if there was a relationship between abundance, circumference and position type. The results showed that there was no significance. Hence, this model was excluded. However, the third model showed that there was a significant relationship between abundance, province and location as shown in the model and output 2 below:

Output 2: shows the results of an ANCOVA regression statistics with abundance as the dependent variable and province and location as exploratory variables. Each exploratory variable was tested separately for abundance.

The first model was to investigate a relationship between abundance and the provinces.

```
> result2 <- lm(abundance~province,data = abundanceonly)
> print(summary(result2))

Call:
lm(formula = abundance ~ province, data = abundanceonly)

Residuals:
    Min      1Q  Median      3Q     Max 
-19.817  -6.293  -2.793   5.295  42.346 

Coefficients:
                        Estimate Std. Error t value Pr(>|t|)   
(Intercept)              10.7538     3.2318   3.328  0.00227 **
provinceLimburg          19.2628     5.7510   3.349  0.00214 **
provinceOost-Vlaanderen  -0.9605     4.4155  -0.218  0.82922   
provinceWest-Vlaanderen  -5.4538    12.0923  -0.451  0.65512   
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 11.65 on 31 degrees of freedom
Multiple R-squared:  0.323,	Adjusted R-squared:  0.2575 
F-statistic:  4.93 on 3 and 31 DF,  p-value: 0.006487
```

The model showed that there was a significance in the province of Limburg (β=19.263, p-value= 0.002). This means that there was a higher abundance in the province of Limburg compared to Antwerp (intercept model) while West and East Flanders did not differ from Antwerp.



To examine the residuals concerning homogeneity of variance and normality, the results show that the distribution of the residuals looks okay. There are few outliers but they are not influential points.

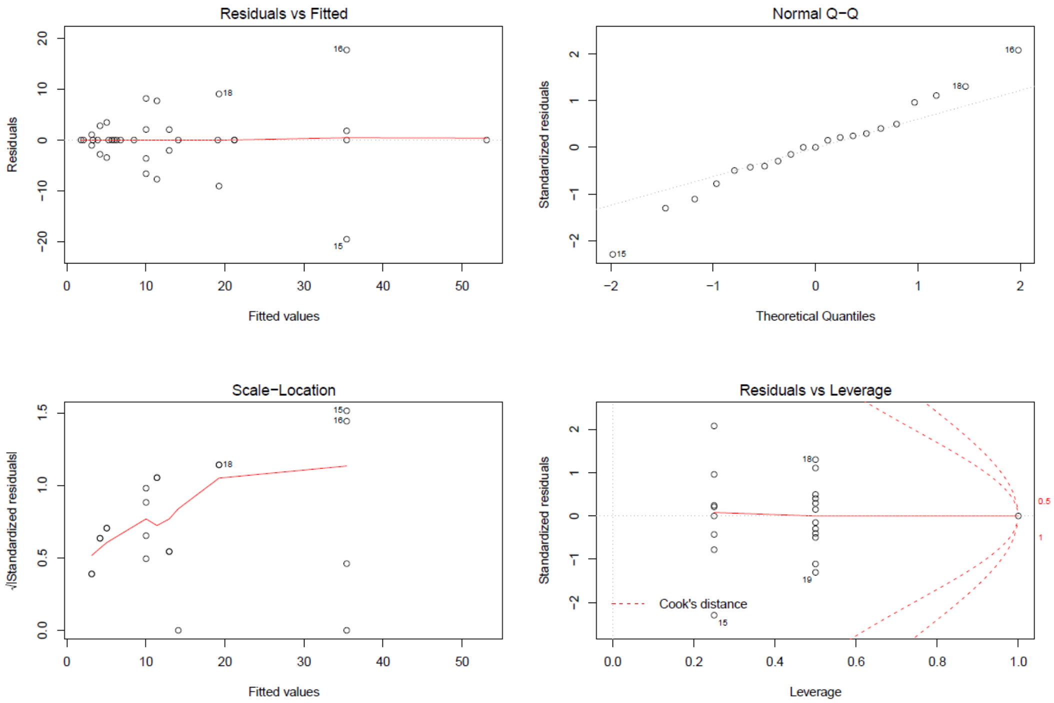
The next model investigated a relationship between abundance and location. The results are shown below:

```
> result1 <- lm(abundance~Location,data = abundanceonly)
> print(summary(result1))

Call:
lm(formula = abundance ~ Location, data = abundanceonly)

Residuals:
    Min      1Q  Median      3Q     Max
-19.500  -0.525   0.000   1.425  17.700

Coefficients:
                                          Estimate Std. Error t value Pr(>|t|)
(Intercept)                                 3.300      9.821   0.336  0.74266
LocationAsbeek                              6.725     10.980   0.612  0.55166
LocationBeerse                             10.800     13.889   0.778  0.45187
LocationBerchem (OV) - Weyns (OV)           3.000     13.889   0.216  0.83262
LocationBerlarebroek                        0.600     13.889   0.043  0.96625
```

```
LocationBerlarebroek (OV)                      -0.150     12.028  -0.012  0.99026
LocationBos t'Ename - Wallebos (OV)            17.900     13.889   1.289  0.22176
LocationDe Most                                49.800     13.889   3.586  0.00374 **
LocationDessel                                 -1.200     13.889  -0.086  0.93257
LocationHeynsdale                               5.200     13.889   0.374  0.71464
LocationHoge Bergen-Ekstergoor                  1.750     12.028   0.145  0.88674
LocationKastel - Het Zwijn                      9.650     12.028   0.802  0.43799
LocationKwaremont - Feelbos (K-16) (OV)         8.100     12.028   0.673  0.51344
LocationKwaremont - Kalkhovenbos (K-19) (OV)   17.900     13.889   1.289  0.22176
LocationMil. Dom. Kamp Beverlo - Schietveld    32.100     10.980   2.923  0.01276 *
LocationOelegem - Vrieselhof                    2.400     13.889   0.173  0.86569
LocationRuien - Kluisbos oost                  15.800     13.889   1.138  0.27750
LocationScheldegebied (WV)                      2.000     13.889   0.144  0.88789
LocationVijverbroek (LI)                       15.950     12.028   1.326  0.20951
LocationVlassenbroekse Polder en Schorren       0.900     12.028   0.075  0.94159
LocationVorst - 't Hoeves Vorst                 2.700     13.889   0.194  0.84912
LocationVorst - 't Hoeves Vorst (AN)           -1.500     13.889  -0.108  0.91578
LocationWortel-Kolonie - Zone Zuidwest          3.500     13.889   0.252  0.80531
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 9.821 on 12 degrees of freedom
Multiple R-squared:  0.8138,  Adjusted R-squared:  0.4725
F-statistic: 2.385 on 22 and 12 DF, p-value: 0.0608
```

The results showed that the location De Most was the most statistically significant (β=49.80, p-va lue=0.004). Additionally, the location Domein Kamp Beverlo-Schietveld also showed that it was also statistically significant (β=32.00, p-value=0.123). This means that you would expect to find a high abundance in the two locations (De Most and Dom Kamp Beverlo-Schietveld) compared to other locations.

Afterwards, diagnostic plots were run to check for normality of residuals.



The diagnostic plots results show that residuals seem to be fine as the residuals seem to be equally and randomly distributed.

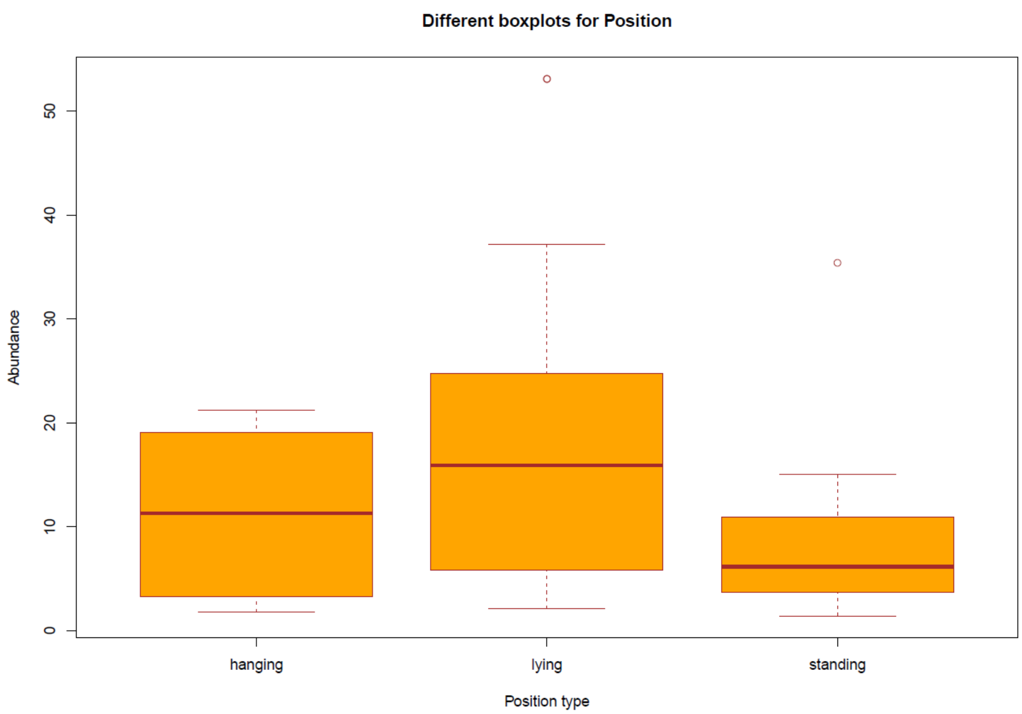


Figure 10: a boxplot showing the difference in abundance with the position type

Figure 10 shows that standing trees had often a lower abundance compared to hanging and lying trees but not significantly, based on the model.

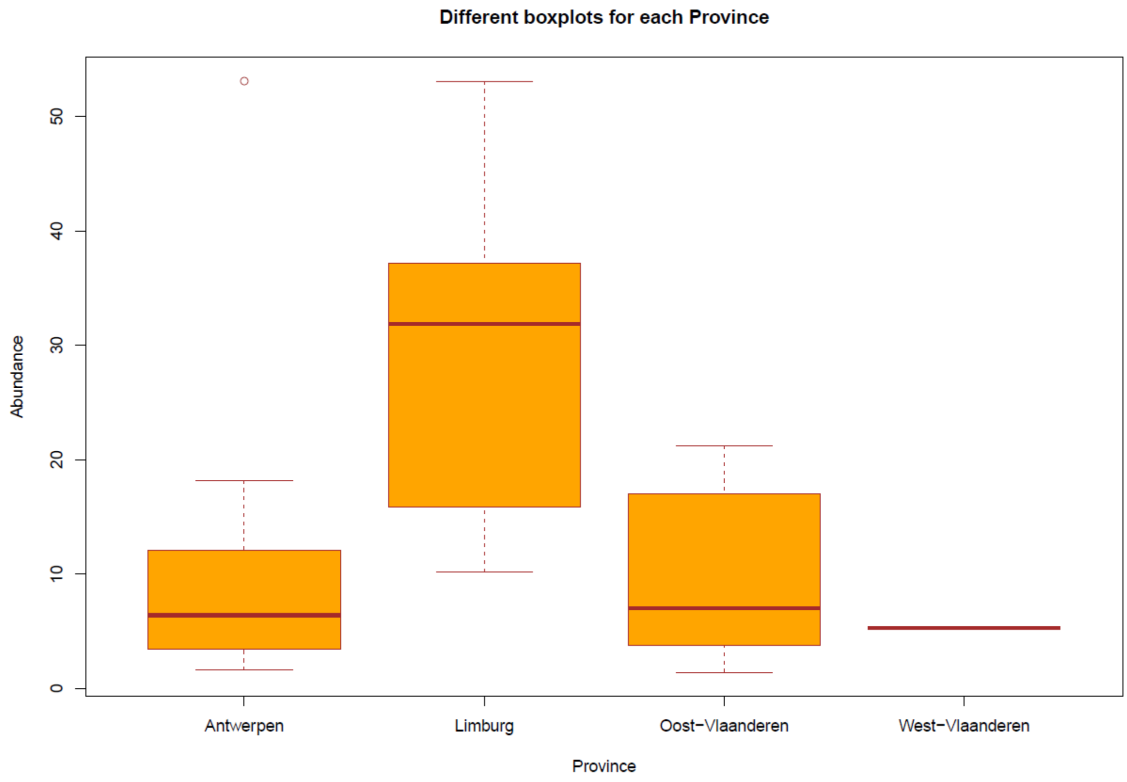


Figure 11: a boxplot showing the difference in abundance between the provinces

Figure 11 shows that the median abundance for Limburg was higher than that of Antwerp and East Flanders cf. the model. While in Antwerp and East Flanders, the median abundance is almost the same.

5.0 DISCUSSION

Our distribution maps revealed that the species is colonizing well. In comparison to the study of Thomaes and Marchand (2019), we observe an increase in the presence of *C. cinnaberinus* especially in Antwerp and East Flanders. Furthermore, we reported the first population in West-Flanders. Together with recent data, our data show that in Limburg, Antwerp and East Flanders the species has relatively well spread. It should be noted that in Limburg the species was also found right on the border between Belgium and Netherlands. The province where the distribution currently is poorly known is West Flanders. Here the species was only found in an area near the border with East Flanders. In Flemish Brabant, the species was only found in one area based on the study of Thomaes and Marchand (2019).

The movement or spread of *C. cinnaberinus* is still uncertain how the species has managed to spread over large distances and relocated in a relatively short period. Though the large-scale habitat recovery through restoration projects can also be linked to some extent. The current discoveries show that the species follows local watercourses, on a larger scale can easily colonize new river basins. In the Flanders, the species has switched from the Meuse basin to the Scheldt basin, this may mean, they could be transported through the watercourse and/or via an active flight. This assumption may mean the species have recently changed their mobility. Additionally, another possibility could be that species can detect fresh dead wood from great distances and able to reach it by active flight (Thomaes & Marchand, 2019).

However, only 10% of the investigated trees had a recorded *C. cinnaberinus*. Therefore, it is important to consider several factors that would lead to the species being absent. A first explanation can be that the species exist in low numbers or have not yet colonized those investigated areas. Their absence can also be related to the selection criteria of the sampled trees that were used. In some areas, there were hardly any or few suitable dead Poplar trees to check for the presence of the species. In some areas, it was difficult to access suitable dead Poplar trees due to a high water level. Hence, zero observations must be interpreted as an indication of relative search effort, rather than of true absence.

As the analysis has shown, the presence of *C. cinnaberinus* was found more on Poplars, as expected based on previous research and existing literature. This can only be partially explained by the fact that we sampled more Poplars. Thus, the preference for the Poplar tree of this species has been confirmed by this study.

Nonetheless, with *C. cinnaberinus* being found also on Pine and Oak tree species, it shows that the species can occur on both coniferous and deciduous trees, though they prefer deciduous trees with a thick and rough bark (Horák et al., 2010; Nieto et al., 2010; Vrezec et al., 2017). Most of the records that were found were in their larvae stage and only one imago was found on an Oak tree species. In the Netherlands, one of the first records were found under the bark of Oak and Scots Pine in a dry habitat in an area with wet forests (Thomaes & Marchand, 2019).

Our results show that the species prefers lying and standing dead Poplar trees. This too corresponds with existing literature.

The presence was mostly found on trees with stem circumference ranging between 50-199 cm. However, we found a relative preference for the range of 150-199 cm.

The conditions of the tree can explain this effect. Another study also showed that bark thickness may also be important for the saproxylic beetles, which is associated with the tree species and age of the dead tree (Blazyte-Čereškiene and Karalius, 2012). These factors were variable in the provinces we sampled.

A possible explanation as to why abundance showed no significance with the stem circumference and position but showed some significance with location and province could be that several other habitat factors may greatly affect the occurrence and diversity of patterns of saproxylic beetles. Possible factors can be the stage of decay, tree species and presence of a specific fungus or substrate moisture (Blazyte-Čereškiene and Karalius, 2012; Jacobs et al., 2007; Lindhe et al., 2005; Lonsdale et al., 2008; McComb and Lindenmayer, 2010; Ranius and Jansson, 2000). Lastly, it's important to note that the density of the specimens can be difficult to evaluate for several reasons, as is the case for most insects: the species is found mostly under the bark, with searches for the species being destructive for the habitat. Hence, the search effort is limited and usually stops when the species is found. The sampling was not systematic either but was random, yet based on expert judgement, taking into account the available number of suitable dead trees.

6.0 CONCLUSION

In conclusion, based on these findings, it is clear that the species is spreading, gaining a wide distribution throughout Flanders. Based on available distribution maps and our data, we can conclude that the species has further established itself especially in Limburg, Antwerp and East Flanders.

Though it is unclear how the species has managed to move over these distances in a relatively short period. Assumptions are that specimens follow certain watercourses, and thus can easily colonize new river basins. Also, transport as a dispersal factor cannot be excluded (for instance with wood transport), as well as active flight colonization (Thomaes & Marchand, 2019).

In future, West Flanders should be further monitored regarding the presence of *C. cinnaberinus*, to have a complete distribution map for the whole of Flanders. Additionally, if possible with enough resources, a follow-up on areas that yielded absence at a given time, can be re-examined after three years.

Lastly, forest management should pay more attention to tree species composition, e.g. Oak and Pine, but especially Poplar tree species in forest stands where the occurrence of *C. cinnaberinus* is confirmed (as well as in adjacent areas) (Goczał and Rossa, 2017). It is important to have a continuous supply of fresh dead wood, including specimens with a thick bark being available to *C. cinnaberinus.* Supplies of both cut trees, as well as spontaneously dying trees, are relevant to prevent lack of suitable habitat (Thomaes & Marchand, 2019). In future, it may also be necessary to explore the impacts of environmental factors such as climatic effects, and gaining insight to which degree these limit or positively contribute to the species distribution.

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