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Geographical Distribution of Ljungan Virus in Small Mammals in Europe

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1 **Title**

2 Preventing invasions of Asian longhorn beetle and citrus longhorn beetle: Are we on the right track?

3

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35 **Abstract**

36

37 Two Asian longhorn beetles, *Anoplophora glabripennis* and *Anoplophora chinensis* are among the most
38 serious alien invasive species attacking forest and urban trees, both in North America and Europe. Major
39 efforts have been put into preventing further entry and establishment of the two species as well as
40 promoting their successful eradication. Here we review these efforts, their progress and outcome, and
41 scientific advancements in monitoring and control methods. The combined international activities and
42 harmonizing legislative changes in detection and eradication methods have proven worthwhile, with
43 more than 45% of eradication programmes successful in the last 12 years. Some countries were able to
44 completely eradicate all populations and others managed to reduce the area affected. Although the costs
45 of the eradication programmes can be very high, the benefits outweigh inaction. Attempts to eradicate
46 *A. chinensis* have been more challenging in comparison with those targeting *A. glabripennis*. For both
47 species, efforts are hampered by the ongoing arrival of new beetles, both from their native regions in
48 Asia and from other invaded regions via bridgehead effects. The methods used for eradication have not
49 changed much during the last decade, and host removal is still the method most commonly used. On the
50 other hand, detection methods have diversified during the last decade with advances in semiochemical
51 research and use of detection dogs. The next decade will determine if eradications continue to be
52 successful, particularly in the case of *A. chinensis*, which has been targeted in some countries for
53 containment instead of eradication.

54

55 **Keywords:** Biological invasions; *Anoplophora* spp.; eradication; management strategies; pest
56 detection; surveillance

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72 **Declarations**

73

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83 **Availability of data and material:** The datasets generated and analysed during the current study are
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85 **Ethics approval:** This study does not contain any experiments using any animal species that require
86 ethical approval.

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89 Science.

90

91 **Key Message**

- 92 • *Anoplophora glabripennis* and *Anoplophora chinensis* are invasive wood borers native to Asia
- 93 • Both species are serious pests in their invaded range, attacking healthy forest and urban trees
- 94 • We analyze data from 2008-2020, regarding interceptions, establishments and eradications
- 95 • In Europe and North America more than 45% of eradication programmes were successful
- 96 • Innovations on management strategies and recent scientific achievements are reviewed

97

98 **Author Contribution Statement**

99 MB and HJ had the idea for the review. MB, HJ, EB and ND set the main structure of the work. SB,
100 MF and GR conducted the literature search. SB conducted the data analysis and drafted the manuscript.
101 All authors contributed to writing the final version of the manuscript and critically revised the work.

102 **1. Introduction**

103 In the last decades, increasing international trade resulting from globalisation has facilitated the
104 introduction of non-native species to new environments and thus boosted the problems with biological
105 invasions worldwide (Brockerhoff and Liebhold 2017; Liebhold and Kean 2019; Lesieur et al. 2019;
106 Zhao et al. 2020). Invasive species have considerable ecological and economic impacts on agricultural,
107 urban and forest systems, compromising their sustainability and the ecosystem services they provide
108 (e.g., Boyd et al. 2013; de la Vega et al. 2020; Gugliuzzo et al. 2021). The Asian longhorn beetle (ALB)
109 *Anoplophora glabripennis* (Motschulsky) and the citrus longhorn beetle (CLB) *Anoplophora chinensis*
110 (Förster) (synonym *Anoplophora malasiaca* (Thomson)) (Lingafelter and Hoebeke 2002) are two
111 emblematic examples of such alien invasive species.

112
113 Both ALB and CLB are highly polyphagous wood borers developing in dozens of deciduous tree
114 species, with CLB having a wider host range than ALB (Lingafelter and Hoebeke 2002; Haack et al.
115 2010; Van der Gaag et al. 2010; Van der Gaag and Loomans 2014; Sjöman et al. 2014; EFSA et al.
116 2019a,b). In Europe, *Acer* is the most commonly attacked genus by both species (e.g. EFSA et al.
117 2019a,b). However, the two species differ regarding plant part on which oviposition and larval
118 development take place. In ALB, oviposition and larval development occur on the upper trunk and main
119 branches, whereas CLB mainly oviposits on the lower trunk, root collar region and on exposed roots,
120 and larvae develop in the lower trunk and roots. This crucial difference translates into different pathways
121 of introduction. ALB introductions are largely associated with the use of solid wood packing material
122 (WPM) in international trade of goods, whereas CLB is rarely introduced with cut wood. CLB
123 introductions are mainly associated with imports of live plants such as small maple trees and bonsais
124 (e.g. Hérard and Maspero 2019).

125
126 ALB is native to China and the Korean Peninsula (Lingafelter and Hoebeke 2002; Williams et al. 2004a).
127 Non-native breeding populations of ALB have been reported in many locations in the USA, Canada,
128 Europe and Japan (Makihara 2002; Takahashi and Ito 2005; Hu et al. 2009; Haack et al. 2010), making
129 ALB one of the most successful and most feared invasive insect species worldwide. CLB is native to
130 eastern Asia, where it is widely distributed in China, Korea, and Japan. CLB has also been reported from
131 Indonesia, Malaysia, Philippines, Taiwan, and Vietnam (Lingafelter and Hoebeke 2002; EFSA et al.
132 2019a). Contrary to ALB, established populations of CLB outside its native range have only been
133 reported in a few countries in Europe. Both species have accidentally arrived in North America and
134 Europe several times independently as documented by molecular genetic studies, numerous
135 interceptions, and infestation hotspots (e.g., Haack et al. 2010; Hérard and Maspero 2019). These
136 successive arrivals may hamper eradication attempts in a given region. Due to their potential impacts on
137 ecosystems and many economically important tree species, these two species have been regulated as

138 priority quarantine pests in Europe, the United States and other countries (EU 2019; USDA-APHIS
139 2020a).

140

141 Haack et al. (2010) reported an extensive analysis of interceptions, establishments, eradications and
142 management strategies used to deal with ALB and CLB in the invaded range, covering the period up to
143 2008. The authors also challenged the scientific community to respond to the needs identified by the
144 difficulties associated with mitigating the threat posed by these beetles and with eradicating local
145 established populations. Since then, 12 years have passed, but the two beetles still remain a menace for
146 an increasing number of countries, and a large number of eradication programmes are still in progress.
147 The aims of the present work are (i) to update the interception records which indicate ongoing transport
148 with international trade, (ii) to review the eradication programmes carried out during the last 12 years,
149 and (iii) to analyse the current status at the country level in order to understand the successes and failures
150 of measures to mitigate invasions by the two beetles. Further objectives are to analyse the scientific
151 achievements that occurred in the last 12 years, especially with regard to efforts in developing novel
152 tools and methods for detection, monitoring and control, and to understand how the scientific community
153 and managers have dealt with the challenges posed by these two species

154

155 *1.1. Terminology and data sources*

156 Interception. We follow the definition of interception provided in Haack et al. (2010), which further
157 differentiates *entry interceptions* from *post-entry interceptions*. For the period prior to 2008, data from
158 Haack et al. (2010) were used. After this period, interception data were retrieved from EPPO via
159 Europhyt for Europe, thus representing the EU member states and Switzerland (data kindly provided by
160 Françoise Petter, assistant director of EPPO) and for North America via USDA-APHIS (see Turner et
161 al. 2020, 2021).

162

163 Establishment. The International Standard for Phytosanitary Measures (ISPM) No. 5 definition of
164 establishment was adopted (FAO 2019). We consider a new establishment when located at least 5 km
165 distant from infested trees detected in previous delimiting surveys or when findings occurred in a
166 previously infested area, but where the population was officially declared eradicated by the relevant
167 authorities (e.g., Toronto in 2013).

168

169 Demarcated area. The *demarcated area* corresponds to the area legally established by each national
170 plant protection organization (NPPO) as subject to eradication and containment measures, and usually
171 comprises an infested zone, where the pest is present, and a buffer zone around the infested zone (FAO
172 2019).

173

174 In order to obtain the temporal and geographical data of ALB and CLB establishments, demarcated
175 areas and buffer zones, the main sources consulted were the EPPO Global Database (<https://gd.eppo.int>),
176 GERDA - Global Eradication and Response DAtabase (Kean et al. 2015) and the USDA-APHIS website
177 (<https://www.aphis.usda.gov/>). This information was complemented by a search of the scientific and
178 grey literature, including works published in scientific journals, conference proceedings, presentations,
179 and eradication reports and other technical reports of national and regional plant protection
180 organisations. When only distribution maps were available, affected areas were extrapolated using
181 ArcGis online tools. For data regarding the period up to 2008, this information was retrieved from Haack
182 et al. (2010).

183
184 For most analyses we used two similar twelve-year periods, comparing data from 1997 to 2008 and from
185 2009 to 2020. For interceptions we used data from 1998 until 2019 (i.e., two eleven-year periods).

186

187 **2. Interceptions and preventive measures**

188 *2.1 Regulation and legislation*

189 In international trade, the International Standards for Phytosanitary Measures No. 15 (ISPM-15), which
190 was adopted in 2002 and revised in 2009, provides treatment standards for WPM to be used in
191 international trade and was intended “*to reduce significantly the risk of introduction and spread of most*
192 *quarantine pests that may be associated with that material*” (IPPC 2009). Nevertheless, several factors
193 can theoretically impact the effectiveness of ISPM 15: i) possibility of colonization after treatment, ii)
194 insect tolerance to treatment, iii) fraudulent use of the ISPM 15 mark; and iv) unintentional
195 noncompliance, which may occur when operators attempt to treat WPM according to ISPM 15, yet the
196 minimum required doses of fumigant or heat are not achieved (Haack et al. 2010; Haack et al. 2014).
197 Still, ALB and CLB are highly unlikely to colonise sawn timber as in WPM, and survival of
198 appropriately applied ISPM 15-compliant treatment is also very unlikely (e.g., Myers and Bailey 2011).
199 So, in most cases, ISPM 15 failure can probably be attributed to fraudulent use of the ISPM 15 mark
200 and unintentional noncompliance (factors iii and iv).

201
202 Regarding introductions in association with live plants, a new EU regulation was adopted in October
203 2016 and implemented since December 2019 (regulation (EU) 2016/2031), on protective measures
204 against pests of plants (repealing Council Directive 2000/29/EC), which completely bans the import of
205 high-risk plants and selected plant products from countries outside of the EU (EU 2016). This regulation
206 is expected to reduce the number of introductions/interceptions of *Anoplophora* spp., particularly of
207 CLB.

208

209 Emergency measures to prevent the introduction into and the spread within the EU of ALB and CLB
210 are defined in Commission Implementing Decisions 2015/893/EU and 012/138/EU, respectively (EU
211 2012, 2015). These include mandatory annual surveys to be conducted by each member state.

212 In the last decade, changes to protocols for inspection at ports of entry have also been adopted: the
213 standard “Methodologies for Sampling of Consignments” (ISPM 31) was adopted in 2008. This standard
214 outlines different types of sampling methods that NPPOs may use to verify compliance of consignments
215 with phytosanitary requirements and the sample sizes required for general phytosanitary inspection
216 (IPPC 2008). It complements ISPM 23 “Guidelines for Inspection”, adopted in 2005, where the general
217 procedures for inspection of consignments are described (IPPC 2005).

218 *2.1. Interceptions*

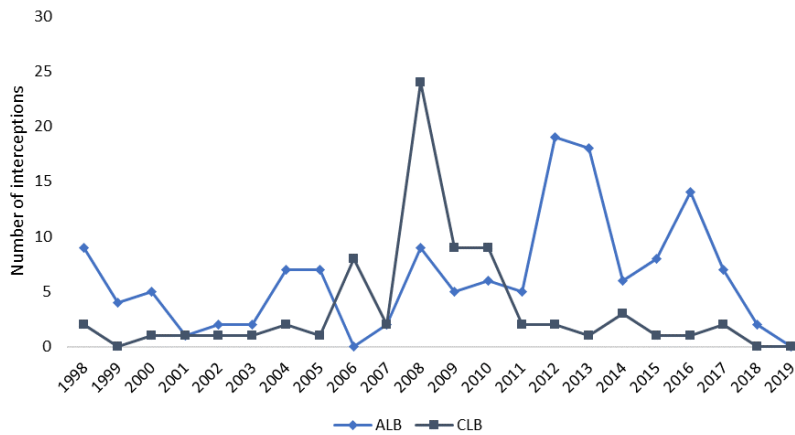
219 In Europe, ALB and CLB were intercepted 140 and 95 times, respectively, from 1980 until 2019.
220 Considering the periods from 1998 to 2008 and from 2009 to 2019, the number of CLB interceptions
221 decreased, with 48 vs 30 cases, whereas the number of ALB interceptions almost doubled (48 vs 90
222 cases) (Fig. 1).

223
224 A sharp difference was observed between time periods for both species regarding the site of interception,
225 i.e., whether the interceptions occurred at “entry” or “post-entry” such as nurseries, warehouses, private
226 residences, etc. For ALB, during 1998-2008, 97% of interceptions occurred “post-entry”, whereas
227 during 2009-2019 these proportions reversed, with 94% of interceptions reported during “entry”
228 inspections. This increase in interceptions during border inspections is possibly a result of changes in
229 legislation, namely the implementation of ISPM 31 in 2008. For CLB, the percentage of interceptions
230 at “entry” also increased during 2009-2019, albeit more moderately (19% vs 57%).

231
232 For the period from 2009-2019, information on the origin of the infested material arriving at EPPO
233 region was available in 88% of the cases (98% for ALB and 60% for CLB), mostly obtained during
234 border inspections. For ALB, all infested consignments arrived from China while for CLB, in addition
235 to China (83%), infested material originating from Japan was intercepted twice (11%) and an infested
236 bonsai of unknown origin was shipped from the Netherlands. ALB interceptions were associated with
237 wood packaging material (WPM) in 96% of cases (mostly linked to stone and tile products) and once to
238 an object with wooden parts (1%). On the other hand, CLB was found in WPM only once. In 20% of
239 cases, CLB was found in bonsais and in 70% it was found in other trees for planting. In two cases, each
240 of ALB and CLB, only adults were found and the associated material could not be identified.

241
242 Excluding border inspections, 87% of ALB detections outside of their native range occurred after
243 establishment (54/62). In the remaining 13%, which corresponded to “post-entry” interceptions, only
244 adults and/or infested WPM were found. Contrasting this with CLB, the corresponding value is much

245 lower, with only 44% (20/45) of detections relating to established populations. These values reflect the
246 different introduction pathways of each species: CLB is usually introduced in imported live plants
247 whereas ALB is introduced in association with wood packaging materials (e.g. Eyre and Haack 2017).
248 Live plants are subject to more intense inspection or incidental observation, either at nurseries or by the
249 final consumer. Of the 25 post-entry interceptions of CLB approximately half (48%) occurred in
250 nurseries, 40% at private residences, and three cases were detected in public parks and street trees.
251 Despite the adoption of ISPM 15 in 2002 which set strict standards for heat treatment and fumigation of
252 WPM to be used in international trade (IPPC 2009), the number of reported interceptions with wood
253 packaging in Europe has increased. Although this may be related to changes in inspection practices
254 resulting from the implementation of ISPM 31, it still emphasises that ISPM 15 does not provide a
255 guarantee that WPM is entirely pest-free, and that further improvements may be needed, especially to
256 ensure prescribed treatments are indeed carried out (Haack et al. 2014).
257 In North America, a sharp decrease was observed in the number of interceptions of both ALB and CLB
258 from 2009 to 2019 (18 ALB, zero CLB, Table 1), when compared to the period from 1998 to 2008 (72
259 ALB, 5 CLB, Haack et al. 2010).
260
261 In Europe, since 1998, three countries alone account for 70% *Anoplophora* spp. interceptions: The
262 United Kingdom, the Netherlands and Germany. These three countries also ranked highest in
263 international trade with East Asian countries during this period (WITS 2021), which may partially
264 explain these results. In the United Kingdom, the number of interceptions of both species decreased in
265 the last decade, by 88% for ALB and by 50% for CLB. By contrast, in Germany both increased (ALB
266 by 108% and CLB by 40%). In the Netherlands, the number of ALB interceptions increased by 267%
267 whereas CLB interceptions decreased by 55%. In Austria and Switzerland, while there were no ALB
268 interceptions in the period 1998-2008, in the last decade, 18 and 17 cases were reported, respectively
269 (Table 1). The different interception frequencies reported for each EU importing country are likely to
270 reflect differences in inspection practices and differences in the reliance on ISPM 15 having solved the
271 problem. Eyre and colleagues (2018) observed that the highest detection rates were achieved in Austria
272 and France, whereas in Spain and Poland, despite the inspection of more than 500 consignments, no
273 harmful organisms were detected. The authors suggested that harmonizing the inspection procedures to
274 the most effective methodology may lead to an approximate sevenfold increase in the number of
275 interceptions of invasive pests across all member states (Eyre et al. 2018). A study on relationships
276 between interceptions and establishments of Cerambycidae (including ALB and CLB) found that there
277 is a significant positive relationship overall between these parameters (Brockerhoff et al. 2014), which
278 highlights the potential usefulness of recording interception data from inspections of relevant imports.



279

280 Figure 1 Temporal trend of the number of interceptions of *Anoplophora* spp. in Europe from
 281 1998 to 2019.

Table 1 Interception data for *Anoplophora* spp. from 2009 to 2019

Year	2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		Total	
	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C
Region/country																								
North America			5		1		6		3		2										1		18	
USA			5		1		6		3		2										NA		17	
Canada																					1		1	
Europe	5	9	6	9	5		19	2	18	1	6	4	8	1	14	1	7	2	2				90	30
Austria									4		1		3		5		4		1				18	
Belgium					1			1															1	1
Czech Republic									2														2	
Cyprus		1																					1	
Denmark						1*																	1	
Estonia													1		1				1				3	
Finland													2				1						3	
France		2									1				1								2	2
Germany		1/2*	4	1	3		10	1	7		1	1*	1		1			1				27	7	
Netherlands	3	3	1	4			3		1		1			1	2	1						11	9	
Slovakia									1*														1	
Sweden	2																						2	
Switzerland					1*		6		3	1*	2	1*	1		3		1					17	2	
Turkey												1*						1*					2	
United Kingdom			1*	4*		1*						1*			1		1					3	5	
Total	5	9	11	9	6	2	25	1	21	1	8	4	8	1	14	1	7	2	2	0	1	0	108	30

A=ALB, C=CLB. Numbers indicate interceptions at ports of entry or transitional facilities (“entry” interceptions). “Post-entry” interceptions are indicated by “*”. Specimens identified as *Anoplophora* spp. from wood packaging material were designated ALB and those from live plants were designated CLB.

282

283 3. Establishments

284 3.1. Spatial and temporal patterns of establishments

285 At the continental scale

286 Since the first detection of an established population in New York in 1996 until the end of 2020, 56
 287 ALB and 20 CLB established populations were reported worldwide. From 2009 to 2020, 37 ALB and
 288 10 CLB establishments were detected in North America and Europe (Fig. 2). More recently, an
 289 established population has also been reported from Hyogo Prefecture in Japan (Akita et al. 2021). Until
 290 now, CLB breeding populations outside their native range were detected only in Europe. For CLB, the
 291 number of new detected establishments was identical to the previous period (1997- 2008). However, a
 292 sharp difference was observed in the number of ALB establishments, which have more than doubled
 293 from 2009 to 2020. Furthermore, out of the 37 ALB establishments detected in the last 12 years, 62%
 294 were detected between 2012 and 2016.

295

296 A summary table with all the identified establishments of ALB and CLB by detection date and
297 geographical location is shown in Supplement S1. For ALB, a brief description of the last decade of
298 establishments by region and country is presented in Supplement S2. The detailed invasion history of
299 CLB in Europe has recently been reviewed by Hérard and Maspero (2019) and is thus not covered in
300 detail in the present work.

301

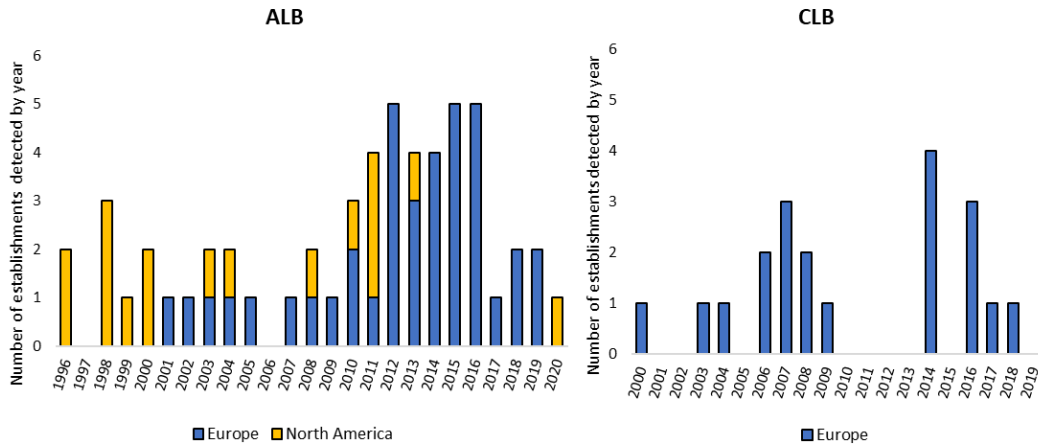
302 For ALB, the number of new establishments detected in Europe has increased more than fourfold in the
303 period 2009-2020 relative to the period from 1997 to 2008. Out of the 37 ALB establishments detected
304 in the last period, 84% were in Europe (Fig. 3, 4 and 5). The increase in the geographical distribution of
305 ALB establishments in Europe mainly reflects the high number of establishments detected in Germany
306 (9) and Italy (8). In contrast, in North America, until 2020 only six new establishments were detected
307 since 2009 (three in Ohio, one in Boston, one in South Carolina and one in Ontario), which is
308 approximately half of the number reported from 1997 to 2008. New CLB establishments were detected
309 in Italy, Turkey, Croatia, France and the Netherlands (Fig. 4). Despite the high number of establishments
310 detected in Italy, no interceptions have ever been reported there (see above).

311

312 By the end of 2020, the total demarcated area in Europe affected by ALB was about 630 km². This area
313 corresponds to a 10-fold increase compared with the area affected by 2008 (62 km²). This expansion
314 reflects the large increase in the number of active establishments. By comparison, the total affected area
315 changed little in North America, with an increase from 580 km² in 2008 to 770 km² by 2020. Still,
316 despite the number of total ALB establishments detected in Europe being higher than those in North
317 America, the current demarcated areas are similar in the two regions. The demarcated areas in Europe
318 are mainly concentrated in three countries, Italy, Germany and France (Supplement S1).

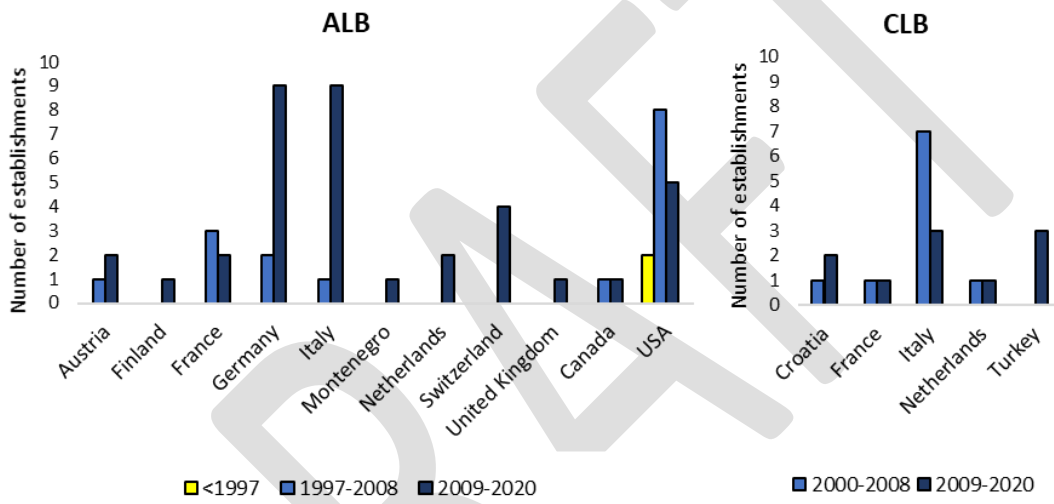
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320 For CLB, although the number of detected establishments was identical from 1997-2008 to 2009-2019
321 (10 establishments, Fig. 4), the total demarcated areas of all active establishments (including
322 establishments detected before 2009), almost quadrupled in Europe (150 km² in 2008 vs 590 km² in
323 2020). The demarcated areas by country expanded mostly in Italy (from 140 to 510 km²), Croatia (from
324 0 to 55 km²) and France (from 3.1 to 8.9 km²) (Supplement S1).



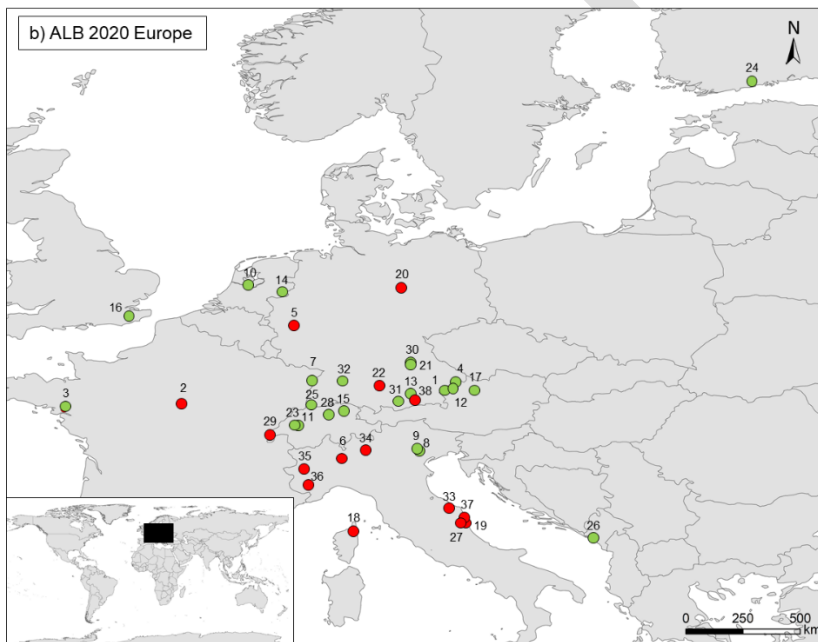
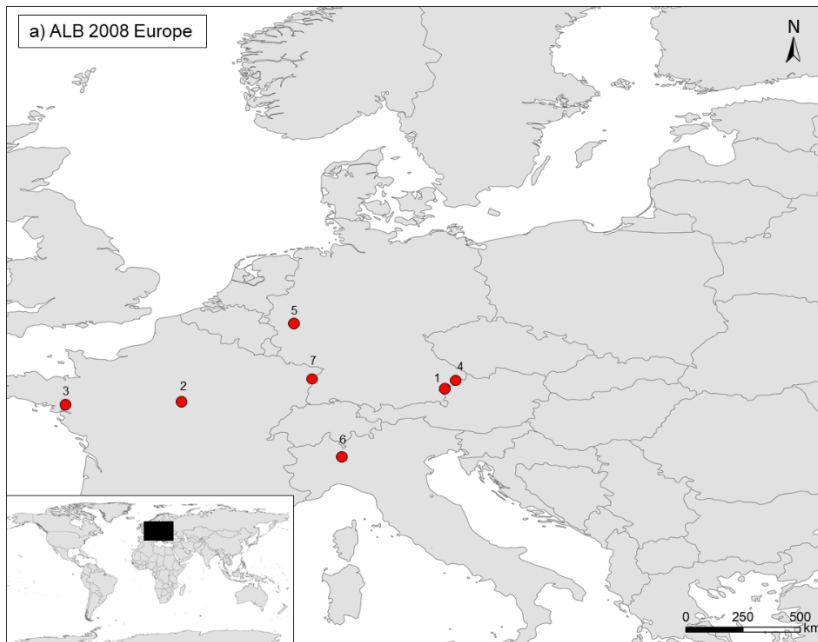
325

326 Figure 2 Number of *Anoplophora* spp. establishments detected by year, from 1996 to 2020.



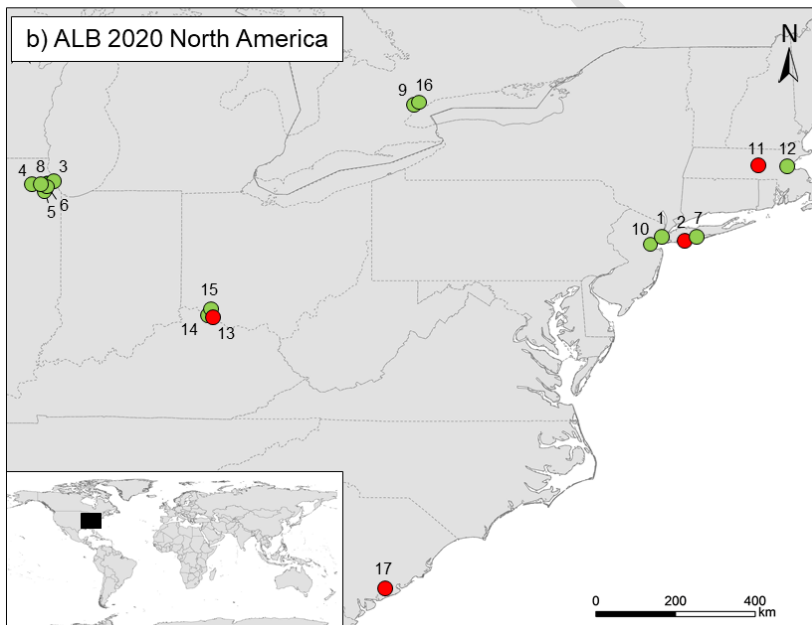
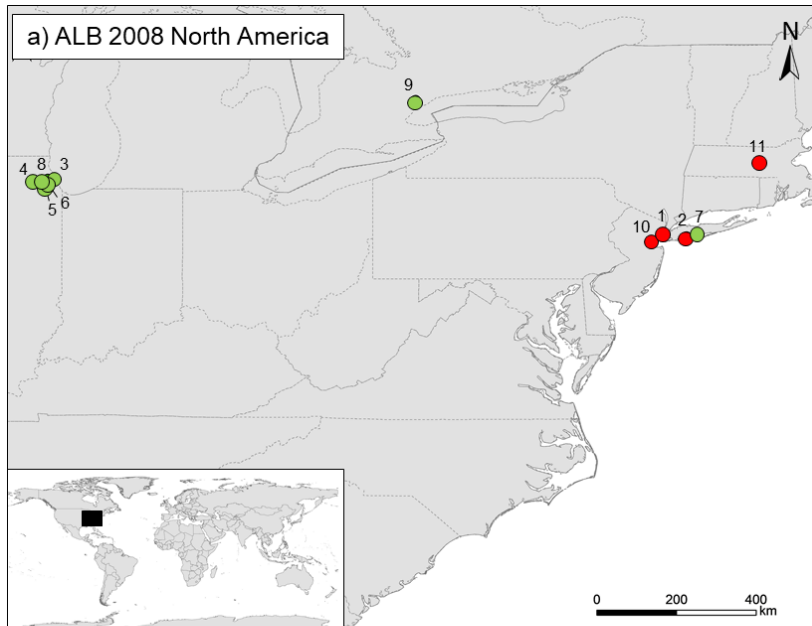
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328 Figure 3 Number *Anoplophora* spp. establishments detected by time period and country.



329

330 **Figure 4** Geographical distribution of established populations of ALB in Europe by year of detection.
 331 a) Status of establishments up to 2008, b) status of establishments from 2009 to 2020. Red dots
 332 represent active establishments, green dots eradicated establishments (as of April 2021): 2001:
 333 Braunau, Austria (1); 2002: Gien, France (2); 2003: Sainte-Anne-sur-Brivet, France (3); 2004:
 334 Neukirchen, Germany (4); 2005: Bornheim, Germany (5); 2007: Corbetta, Italy (6); 2008: Strasbourg,
 335 France (7); 2009: Cornuda, Italy (8); 2010: Maser, Italy (9), Almere, Netherlands (10); 2011:
 336 Brünisried, Switzerland (11); 2012: Geinberg, Austria (12), Feldkirchen, Germany (13), Winterswijk,
 337 Netherlands (14), Winterthur, Switzerland (15), Paddock Wood, UK (16); 2013: Gallspach, Austria
 338 (17), Furiani, France (18), Grottazzolina, Italy (19); 2014: Magdeburg, Germany (20), Neubiberg,
 339 Germany (21), Ziemetshausen, Germany (22), Marly, Switzerland (23); 2015: Vantaa, Finland (24),
 340 Grenzach-Whylen, Germany (25), Budva, Montenegro (26), Porto San Giorgio, Italy (27), Berikon,
 341 Switzerland (28); 2016: Divonne-les-Bains, France (29), Kelheim, Germany (30), Murnau, Germany
 342 (31), Hildrizhausen, Germany (32), Ostra and Senigalia, Italy (33); 2017: Trescore Balneario, Italy
 343 (34); 2018: Vaie, Italy (35), Cuneo, Italy (36); 2019: Civitanova, Italy (37), Miesbach, Germany (38).
 344



345
 346 Figure 5 Geographical distribution of established populations of ALB in North America, by year of
 347 detection. a) Status of establishments up to 2008, b) status of establishments from 2009 to 2020. Red
 348 dots represent active establishments, green dots eradicated establishments (as of April 2021) 1996:
 349 Brooklyn, New York, USA (1), Long Island, New York, USA (2); 1998: Chicago, Illinois, USA (3),
 350 Addison, Illinois, USA (4), Summit, Illinois, USA (5); 1999: Park Ridge, Illinois, USA (6); 2000:
 351 Islip, New York, USA (7), Chicago O'Hare, Illinois, USA (8); 2003: Vaughan, Ontario, Canada (9);
 352 2004: Carteret and Linden (2006), New Jersey and Prall and Staten Island (2007), New York, USA
 353 (10); 2008: Worcester, Massachusetts, USA (11); 2010: Boston, Massachusetts, USA (12); 2011: Tate
 354 Township, Ohio, USA (13), Monroe Township, Ohio, USA (14), Batavia/Stonelick Townships, Ohio,
 355 USA (15); 2013: Mississauga, Ontario, Canada (16); 2020: Hollywood, South Carolina, USA (17).
 356

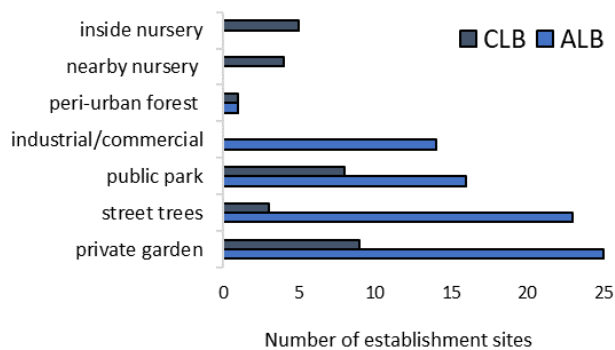
357
 358 At the local scale

359 All established populations of *Anoplophora* spp. were initially detected in urban/peri-urban
 360 environments (Fig. 6). For both species, infested trees and live beetles were initially detected in private

361 gardens in approximately half of the establishments (52% for ALB and 50% for CLB). Detections in
 362 public parks and street trees were also common, whereas detection in peri-urban forests was rare and
 363 occurred only once in one ALB and one CLB establishment, during official surveys.

364

365 In its native range in South Korea, ALB has been reported to be a riparian species adapted to the long
 366 edges of these habitats (Williams et al. 2004a). If this is the case, it might explain its adaptability to
 367 hedgerows (along roads, gardens, and parks) typical of urban habitats (Williams et al. 2004a; Faccoli et
 368 al. 2016). This is in accordance with the infestation pattern in Cornuda (Italy), where, although part of
 369 the quarantine area fell within a natural hardwood forest, infested trees have only been found along its
 370 edges (Faccoli et al. 2016). Similarly, in Chicago (USA) hundreds of *Acer* spp. were found infested
 371 along the edge of a 50-ha woodlot but not in the interior, suggesting a strong edge effect during the
 372 invasion (Sawyer et al. 2011). The infestation of hardwood stands in a large outbreak observed in
 373 Massachusetts (USA) has been pointed out as an exception (Dodds and Orwig 2011). However, the
 374 small size of the infested stands in Massachusetts, surrounded by city outskirts and streets, makes them
 375 comparable to urban parks and small rural stands (Faccoli et al. 2016).



376

377 Figure 6 Sites of initial detection(s) of *Anoplophora* spp. establishments. This information was available
 378 for 49/55 and 18/20 of ALB and CLB establishments, respectively.

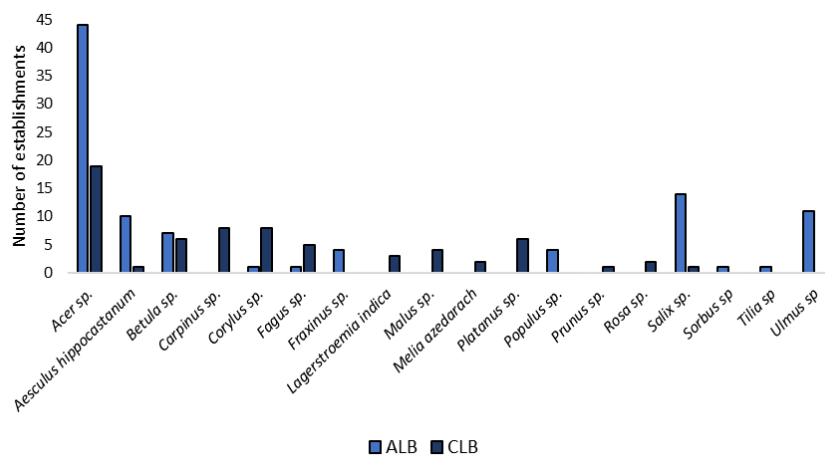
379

380 Differences between the two species can be seen which are related to their pathways. For ALB,
 381 industrial/commercial sites (areas that are likely to receive imports in WPM or live plants from potential
 382 source regions) are commonly affected and detections at such sites occurred in 30% of establishments.
 383 For CLB, 50% of detections involved sightings of insects or infested trees at plant nurseries (28%) or
 384 near plant nurseries (22%).

385

386 Considering how detections occurred initially, 73% of ALB establishments (33/45) were detected by
 387 passive surveillance and 24% were detected during official surveys. For CLB, 76% (13/17) of cases
 388 were reported during official surveys and the remaining 24% were the result of either passive
 389 surveillance (one case) or detected during scientific research activities (3 cases). Passive surveillance

390 corresponded mostly to citizens who reported symptoms or sightings of adult insects to phytosanitary
 391 authorities, operators of nurseries and city parks and landowners. For most establishments, the first trees
 392 infested were maples (*Acer* spp.), corresponding to 90% and 95% of ALB and CLB cases, respectively
 393 (Fig. 7). However, while ALB was found infesting mostly local trees of *A. platanoides* and *A.*
 394 *pseudoplatanus*, CLB was mostly found infesting *A. palmatum* and *A. negundo*. For ALB, *Salix* sp.,
 395 *Ulmus* sp. and *Aesculus hippocastanum* were also commonly found infested. For CLB, in addition to
 396 maples, the most common infested tree genera were *Carpinus*, *Corylus*, *Betula* and *Platanus* (Fig. 7).
 397 The host trees affected at each site are expected to be influenced by the host species available.
 398 Nevertheless, affected hosts may also reflect the origin of the local populations: in South Korea, for
 399 example, ALB riparian forest populations appear to display a different host usage when compared to
 400 urban populations, and the latter have been shown to result from recent invasions from China (Lee et al.
 401 2020).



402
 403 Figure 7 Tree species or genera in which establishments of ALB and CLB were detected either
 404 during passive surveillance or official or scientific surveys. This information was available for
 405 49/55 and 20/20 of ALB and CLB establishments, respectively.

406
 407 **3.2. Pathways of introduction: reconstruction of invasion routes**

408 **ALB**

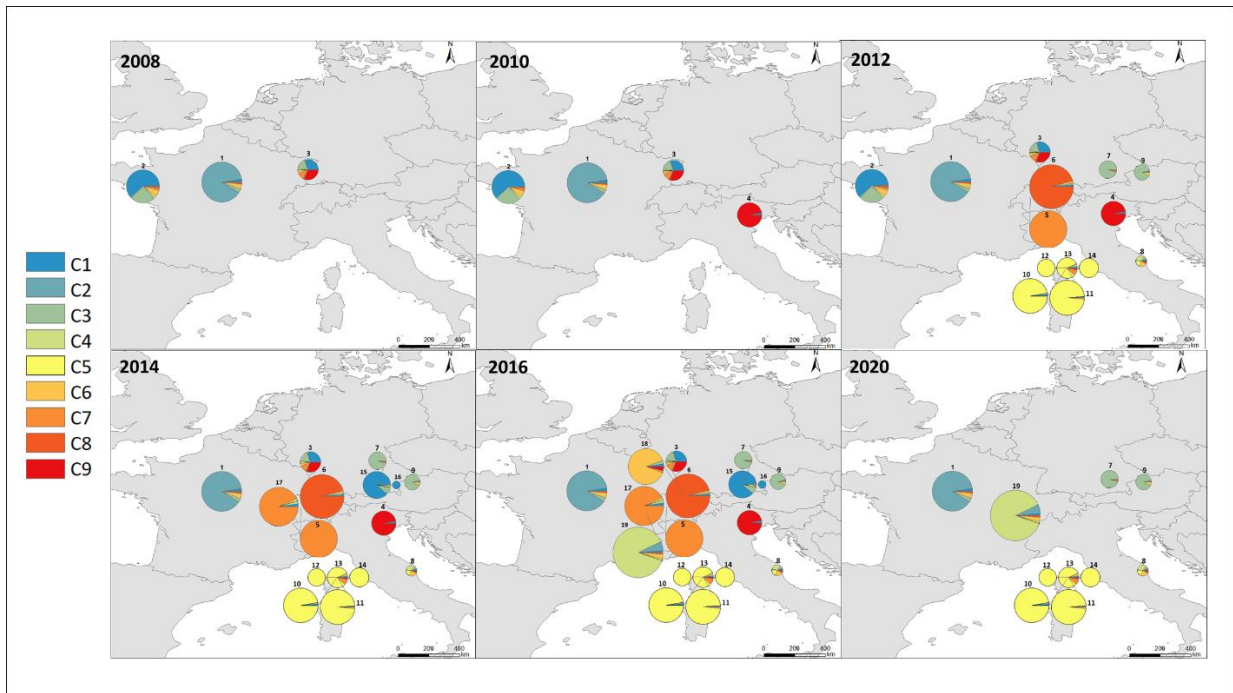
409 The first studies on the intraspecific genetic diversity have focused mainly on the native Asian regions
 410 (An et al. 2004; Carter et al. 2009a). They reported that, although the Asian populations clustered
 411 roughly into two major groups, the population structure has been influenced by movement of beetles
 412 and consequent genetic admixture (Carter et al. 2009a). Javal et al. (2019a,b) highlighted signs of an
 413 ancestral structure in NE Asia, and a strong differentiation among most of the populations following a
 414 north-south gradient. These studies also considered human-mediated population translocations at large
 415 scale, especially those linked to afforestation projects initiated by the Chinese government since the
 416 1960s in northern and eastern China (Li 2004; Haack et al. 2010).

417

418 Studies of North American establishments revealed a reduced genetic diversity within populations in
419 either the USA or Canada due to genetic bottlenecks (Carter et al. 2009b, 2010). Separate introduction
420 events were responsible for most North American populations, the founders of which likely originated
421 from populations invasive within China (Carter et al. 2010; Javal et al. (2019a,b). In addition, some
422 subsequent human-mediated regional spread occurred in the USA (e.g. New York City, Carter et al.
423 2010) and in Canada (Turgeon et al. 2015).

424

425 In Europe, mitochondrial DNA and microsatellite marker studies revealed a complex worldwide
426 invasion scenario involving recurrent introductions coupled with a bridgehead event. The genetic
427 structure observed suggests that European establishments originated mostly from multiple independent
428 introductions from the native area in Asia (Fig. 8). The resulting genetic differentiation among European
429 establishments may indicate limited gene flow between populations once established, mostly due to the
430 poor dispersal behaviour of this species. A fine-scale study in Switzerland (Tsykun et al. 2019) showed
431 that only one or a maximum of two genetic clusters were found within a given tree, suggesting that most
432 ALB individuals remain in proximity to the tree from which they emerged when suitable host trees are
433 available (Smith et al. 2001 and 2004; Javal et al. 2019a,b). Low levels of genetic diversity, high levels
434 of inbreeding, small numbers of founders and large differences in the severity of bottlenecks
435 encountered by introduced populations have shaped the genetic structure of invasive populations (Javal
436 et al. 2019a,b). Natural dispersal and/or human-mediated transportation (e.g. hitch-hiking) at a small
437 spatial scale were observed in some regions as in Corsica and in Switzerland (Javal et al. 2019a,b;
438 Tsykun et al. 2019). Bridgehead events may have contributed to the worldwide spread of ALB (Javal et
439 al. 2019). This appears to be the case for the French population in Gien that may have resulted from a
440 bridgehead population from North America (Javal et al. 2019a,b) rather than Asia as suggested in
441 previous publications (Cocquempot et al. 2003).



442
 443 Figure 8 Distribution of some European ALB populations and their defined genetic clusters inferred
 444 by structure analysis. The numbers correspond to the sampled locations used in Javal et al. (2019a): 1
 445 Gien, France; 2 Sainte-Anne-sur-Brivet, France; 3 Strasbourg, France; 4 Cornuda, Italy; 5 Brünisried,
 446 Switzerland; 6 Winterthur, Switzerland; 7 Feldkirchen, Germany; 8 Rapagnano, Italy; 9 Gallspace,
 447 Austria; 10 Arenau, Corsica; 11 Colast, Corsica; 12 Conouv, Corsica; 13 MCarlo, Corsica; 14 Costad,
 448 Corsica; 15 Neubiberg, Germany; 16 Ebersberg, Germany; 17 Marly, Switzerland; 18 Berikon,
 449 Switzerland; 19 Divonne les Bains, France. Each colour corresponds to a haplotype cluster.

450

451 CLB

452 The genetic structure and invasion pathways of CLB have not been studied as intensively as for ALB.
 453 Strangi et al. (2017) conducted a mitochondrial DNA analysis on native populations from East Asia and
 454 three Italian establishments. In Italy, a total of five haplotypes were identified in Lazio, Lombardy and
 455 Tuscany. Three of these haplotypes were only found in Tuscany, and these were closely related to
 456 haplotypes found in Chinese populations. The remaining two haplotypes, found in Lazio and Lombardy,
 457 corresponded to populations from North and Central Japan (Strangi et al. 2017). These results suggest
 458 that the Italian establishments originated from at least two separate events. CLB is known to show
 459 phenotypic polymorphism that allow for the distinction of two forms: *A. chinensis chinensis* and *A.*
 460 *chinensis malasiaca* (Ohbayashi et al. 2009). In the Lazio and Lombardy establishments, where the
 461 origin was traced back to Japan, specimens were identified as *A. chinensis malasiaca*, whereas in
 462 Tuscany, where the population origin was traced back to China, specimens were shown to be *A.*
 463 *chinensis chinensis*. In the recent establishment detected in Royan, the *A. chinensis chinensis* subspecies
 464 has been detected, and further studies are currently underway to uncover the origin of the specimens
 465 recovered at this location (ANSES 2019).

466

467 These recent studies have started to unravel the complexity of ALB's and CLB's invasion histories:
468 multiple introductions have occurred, originating from several regions of Asia (China, Korea, Japan)
469 and in some cases these appear to have included bridgeheads effects. Furthermore, studies have also
470 shown that even genetic populations of extremely low genetic diversity can multiply to outbreak
471 proportions in urban areas (Carter et al. 2009b).

472

473 **4. Eradications**

474 *4.1. Methods of eradication*

475 In the European Union, CLB and ALB infestations that affect a Member State are subject to specific
476 management procedures defined by the European Commission (EC 2012 and 2015 respectively) and
477 transcribed in national and regional decrees with the aim to eradicate all active infestations. Each country
478 where an infestation is detected usually develops an eradication programme that incorporates activities
479 focused on detecting infested trees, removing pest populations and limiting pest movement and spread,
480 i.e. every eradication programme includes monitoring, control and containment components (see section
481 6 for their description). The first step is to delineate a demarcated area and forbid movement outside the
482 demarcated area of infested or potentially infested wood material and host trees (EPPO 2013a,b).
483 Whenever a new establishment is detected, an initial, intensive delimiting survey must be conducted to
484 determine the extent of the infested area. Demarcated areas are then established including the infested
485 area and a surrounding buffer zone of typically 2 km radius. Depending on the extent of the infestation
486 and the site-specific characteristics, such as the local distribution of host plants, this buffer zone may be
487 reduced to a radius of 1 km. The prescribed survey methodology is similar across all EU member states.
488 Once trees are found to be infested by ALB/CLB, they are recorded and submitted to specific protocols
489 aimed to eliminate insect populations, based on different types of measures including tree destruction,
490 chemical and physical treatments (EPPO 2013a,b).

491

492 In North America, annual surveys to actively search for new infestations are, to our knowledge, not
493 mandatory. However, once an infestation is detected, similar protocols apply: establishment of regulated
494 areas consisting of a core area (0.8 km radius) and a buffer zone (1.6 km beyond the boundary of the
495 core area). USDA APHIS (2020b) further outlines that the buffer zone should expand to a minimum of
496 4 km from areas of high ALB density (defined as presence of a cluster of trees with many exit holes or
497 one or more trees with >100 exit holes). In North America, where only ALB establishments have been
498 detected to date, the eradication procedures have been similar to those applied in Europe (Haack et al.
499 2010; USDA-APHIS 2014; Fournier and Turgeon, 2017). In the USA specific ALB response guidelines
500 were published providing the technical and general information needed to implement any phase of an
501 ALB eradication programme and the Federal Quarantine authority for ALB according to the US Federal
502 Regulations 7 CFR 301.51 for eradication programs (USDA-APHIS 2014, 2020b).

503

504 4.2. Spatial and temporal pattern of successful eradications

505 ALB

506 Globally, as of December 2020, approximately half of all detected ALB establishments have been
507 eradicated successfully (53%, since the first successful eradication of the establishment of Addison,
508 Illinois, USA, in 2004) (Fig. 9). However, eradication success rates varied considerably among countries
509 and continents. In Europe, all ALB establishments in Austria, the Netherlands, Switzerland and the UK
510 have been declared eradicated. Austria, which had the highest affected area in 2008, succeeded to
511 eradicate its largest establishment, with only a small area of active cases remaining in 2020, which was
512 finally declared eradicated in January 2021 (Supplement S1). By contrast, in Italy, which ranks first in
513 the number of active establishments, 80% of detected ALB establishments remain active.

514

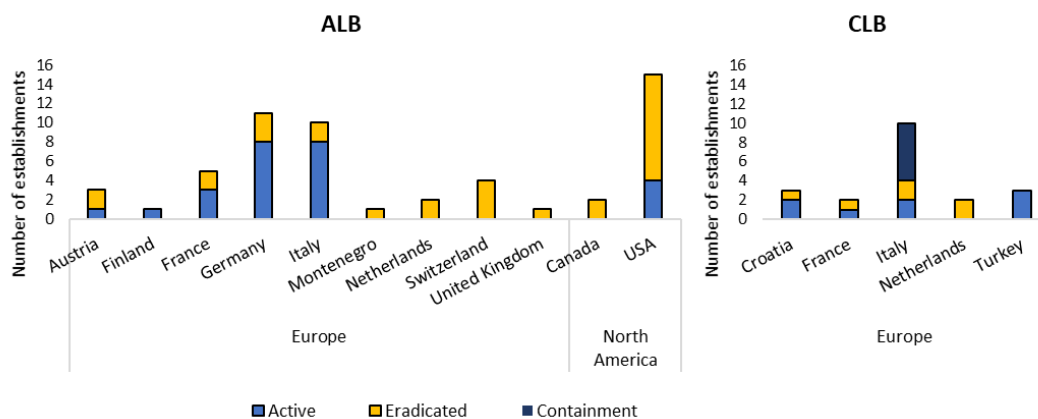
515 A high rate of successful eradications was also achieved in North America. In the United States, where
516 the highest number of ALB establishments (15) has been reported to date, 67% of eradication attempts
517 have been successful so far. A few successful eradications have been achieved before 2008, in Jersey
518 City and Illinois. The total area for which successful eradication of ALB was achieved in North America
519 during the period from 2008 to 2020 was about double that in Europe (Fig. 10). A decrease in the area
520 affected was achieved in both the USA and Canada.

521

522 CLB

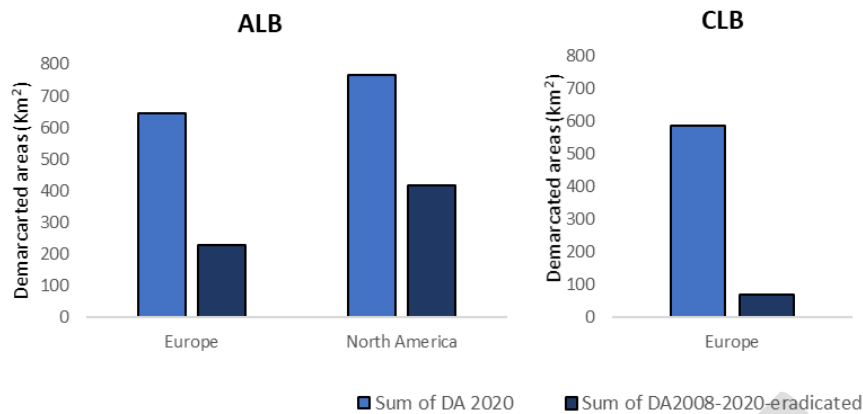
523 Eradication of CLB establishments appear to be more challenging, as only 30% of detected
524 establishments have been declared eradicated (until December 2020) (Fig. 9). Only six out of 20
525 established populations outside its native range have been eradicated successfully since 2008.

526 Furthermore, eradication of most CLB establishment sites in Lombardy has recently been declared as
527 unachievable. Currently, of the eight active establishments in Italy, only two are still under eradication.
528 With the exception of the small establishment in Sirmione, which is still under eradication, a policy of
529 containment is thus now in place at all other sites in Lombardy (SFRL 2020).



530

531 Figure 9 *Anoplophora* spp. eradication attempts by country and their respective status, as of December
532 2020 (active or eradicated).



533

534 Figure 10 *Anoplophora* spp. demarcated areas (DA) by world region, Europe and North America: active
 535 in 2020 and eradicated in the period 2008-2020.

536

537 5. Spread

538 5.1. Methods to monitor and predict the spread

539 Information on how the invasive population will likely spread across the landscape is fundamental to
 540 delineate cost-effective monitoring and control strategies. Currently, certain distances from a discovered
 541 infestation of *Anoplophora* spp. are used in eradication programmes to define the boundaries of
 542 delimiting survey areas mandatory by law (EU 2012, 2015; USDA 2019). However, those boundaries
 543 need to be adjusted according to the available scientific knowledge on the beetles' dispersal ability. A
 544 number of dispersal studies have been published for ALB (e.g. Bancroft and Smith 2005; Li et al. 2010;
 545 Sawyer et al. 2011; Turgeon et al. 2015) whereas for CLB the information is scarce (Adachi 1990;
 546 Cavagna et al. 2013). Due to their morphological resemblances one may assume the dispersal ability of
 547 the two species to be similar.

548

549 When analysing different studies, we distinguish those based on insect dispersal ability and observations
 550 of population spread. Potential dispersal ability does not always match the observed spread due to
 551 landscape features and aspects of insect behaviour. Insect dispersal ability was studied by mark-release
 552 studies, flight mills and modelling whereas population spread is accessed by analysis of historical
 553 infestation cases, genetic analysis, and different kinds of models (see below).

554

555 5.2. Patterns of spread at local scale

556 For ALB, mark-release-recapture studies conducted in China reported mean dispersal distances during
 557 one season of 100 m to 270 m, with a 98% probability of beetle recapture within 560 m to 920 m and a
 558 maximum dispersal potential of 2,600 m (Wen et al. 1998; Smith et al. 2001, 2004; Williams et al.
 559 2004b; Bancroft and Smith 2005; Li et al. 2010). Studies conducted with computerized flight mills have
 560 shown that some beetles can fly considerably longer distances, up to 14 km (Lopez et al. 2017; Javal et
 561 al 2018), although only 5% of individuals travelled more than 8 km within a 24-h period (Lopez et al.

562 2017). These extreme specimens may lead to infestations outside of quarantine zones (Javal et al.
563 2018b). However, it is important to note that the beetles' ability to fly long distances in flight mills does
564 not necessarily translate into long distance flights in the field. There is some evidence that suggests ALB
565 is reluctant to fly far even though they are physically able to do so in a flight mill situation.

566

567 By examining historical infestation cases, spread rates were seen to be highly variable both between
568 infested sites and from one period to another within a given infested area. For example, Sawyer et al.
569 (2011) observed in urban areas at Carteret (NJ; USA) and Chicago (IL, USA) that ALB spread slowly,
570 concentrated within a few hundred metres during the first 5-6 years. Yet, in another location, in Linden
571 (NJ, USA), the infestation spread much faster, about 3.2 km within five years. In a study conducted in
572 southern England, it was estimated that ALB remained restricted to a small area for approximately 10
573 years near a heavily infested sycamore tree (Straw et al. 2016). Similar patterns of infestation, with the
574 beetles remaining at or close to the natal tree have been observed in the early phases of infestation at
575 other sites (Haack et al. 2010; Sawyer et al. 2011; Turgeon et al. 2015). The discrepancies between sites
576 may in part be attributable to differences in the time until an established population was discovered,
577 while landscape heterogeneity may also play a role. Some land cover types may offer lower resistance
578 to beetle movement and low availability of suitable host trees, favouring longer dispersal flights (Keena
579 2018). ALB adults are assumed to move by walking in the vicinity of the natal tree and disperse by
580 flying only when conditions become less favourable. Still, in North America long-range dispersers of
581 up to ~1,400 m, were reported, even before the originally infested host trees were fully exploited (Hull-
582 Sanders et al. 2017).

583

584 Climatic conditions may also play a role in the dispersal of ALB. The apparently lower rate of population
585 increase and spread of ALB in southern England (Straw et al. 2016), when compared to Cornuda in Italy
586 (Favaro et al. 2015) or Jersey City and Linden in the US (Sawyer et al. 2011), has been attributed to
587 lower summer temperatures resulting in longer insect developmental times (Straw et al. 2016; Trotter
588 and Keena 2016). In northern Italy, ALB dispersal was shown to be influenced by the distance of suitable
589 hosts from the nearest infested trees ($p < 0.01$ for distances above 510 to 1,040 m, which varied among
590 years) and the number of infested trees around uninfested ones (Favaro et al. 2015). In that study,
591 although the probability of dispersing farther than 1,900 m from a previously attacked tree was very low
592 ($p < 0.001$), one dispersal occurrence was registered at 2,224 m. The dispersal pattern was shown to be
593 density-dependent, in accordance with previous mark-release studies.

594

595 Several modelling approaches have been developed to describe ALB dispersion patterns. Trotter and
596 Hull-Sanders (2015) and Trotter et al. (2019) used graph theory to determine the topological connections
597 between infested trees, which was then used to calculate dispersal patterns across the landscape in
598 Massachusetts. Two scenarios were used in this study: one in which beetles only left the natal tree when

599 it was overcrowded (strict scenario) and one under which all infested trees could act as sources of
600 dispersing beetles (relaxed scenario). The longest dispersal distance, within a 99th percentile, was over
601 2.3 km for the strict scenario, and 1.3 km under the relaxed scenario. Fragnière et al. (2018) used data
602 from establishments in Switzerland to develop a density-dependent model that relies on field
603 observations of beetles and infested trees to provide a risk index (RI) of the presence of ALB in a given
604 location. The output for Marly, for example, resulted in $RI > 0.001$ up to about 600 m of the centre of
605 the highly infested area and $RI > 0.0001$ up to about 820 m. Elmes et al. (2019) modelled dispersal
606 pathways using circuit theory. Their results showed that ALB tends to use non-habitat land-cover types
607 to connect suitable habitat patches and that for this species, circuit theory was a better predictor of
608 dispersal spatial patterns than least-cost dispersal models. The non-habitat land-cover type that displayed
609 the lowest resistance was sealed surfaces (such as roads) followed by bare soil, grassland, trees,
610 buildings, and water, in increasing resistance order. Recently, Huang et al. (2020) used a geographically
611 weighted regression model to analyse the spatial differentiation of environmental drivers on the
612 occurrence of ALB in China. Temperature, wind speed, precipitation and population density were shown
613 to affect ALB occurrence in China, yet a high spatial heterogeneity was reported on the influence of
614 these factors.

615
616 Studies on CLB dispersal are scarce compared with the information available for ALB. Its spread
617 capacity is reported to be low (EFSA et al. 2019a). Similar to ALB, most adults are assumed to disperse
618 by walking and remain in the vicinity of their natal tree unless conditions are unfavourable, although
619 some adults were shown to be able to travel distances of 2 km (Adachi 1990). In Lombardy, Italy, the
620 maximum distances between infestations in urban and agricultural areas were calculated to be about 500
621 m and 663 m, respectively (Cavagna et al. 2013). However, 97.0% and 99.2% of new cases were found
622 within 200 m and 400 m, respectively. EFSA et al. (2019a) estimated the maximum distance of natural
623 spread in one year to be approximately 194 m (with a 95% uncertainty range of 42–904 m), for a
624 population with a 2-year life cycle (EFSA et al. 2019a).

625
626 As mentioned above, human-mediated dispersal related to commerce and transport of infested plants,
627 wood and other materials is the major route for spread of both species at the continental scale. However,
628 even at shorter distances, human-mediated dispersal is an important component that needs to be
629 considered as a cause of satellite infestations, as has been shown, for example, in Switzerland, the USA
630 and Canada (Turgeon et al 2015; Tsykun et al. 2019).

631

632 **6. Control and Containment - current and future perspectives**

633 *6.1. Monitoring methods*

634 In Europe, a survey is carried out in each demarcated area at least once per year to detect and monitor
635 infested trees (EC 2012, 2015). The methods used have been quite similar among countries and mainly
636 based on visual surveys. Advancements in alternative monitoring methods are described below.

637

638 *Visual surveys*

639 Despite the advances in new detection methods in the last decade, visual surveys remain the standard
640 procedure for *Anoplophora* spp. monitoring (EFSA et al. 2019a,b). These surveys are generally based
641 on examination of potential host trees looking for signs of infestation (i.e., exit holes, larval frass on the
642 ground, oviposition pits and adult feeding, plant and branch dieback). CLB infestation signs are searched
643 on the lower part of the trunk (usually the basal 50 cm, but infestations up to two meters high have been
644 documented, Doris Hölling, Pers. Commun.), the root collar zone, and roots exposed above ground,
645 while searches for ALB symptoms are focussed on the upper part of the trunk and the main branches
646 (EFSA et al. 2019a,b). ALB surveys are usually conducted by observers on the ground equipped with
647 binoculars to detect known signs and symptoms of attack. Turgeon et al. (2010) demonstrated that the
648 efficacy of ground inspections is higher when the density of oviposition is higher, when signs are located
649 lower on the tree, and when they are positioned on the main trunk. Furthermore, the authors observed
650 that most infested trees were detected within the first 2 min of survey, and that using a team of inspectors
651 to survey each tree would be more time effective than the use of a single inspector per tree (Turgeon et
652 al. 2010). The type of environment on which the trees are located also affects detectability: infested
653 street trees are more easily detected than those located in parks or woodland, therefore affecting the time
654 required for tree inspection at different sites (Yemshanov et al. 2019). In addition to surveys carried out
655 inside the demarcated area, specific surveys are usually conducted also randomly outside the demarcated
656 area at high-risk sites such as commercial and industrial areas that receive imports from potential source
657 regions, particularly those receiving wood packaging material or live plants (EFSA et al. 2019a,b).

658

659 *Semiochemicals*

660 For ALB, pheromone-based trapping systems have been developed (Nehme et al. 2014). Males of ALB
661 are known to emit a sex pheromone composed of equal parts of 4-(n-heptyloxy)butan-1-ol and 4-(n-
662 heptyloxy)butanal (Zhang et al. 2002; Nehme et al. 2009). Intercept panel traps baited with a
663 combination of the pheromone and a mixture of selected host plant volatiles, namely linalool, linalool
664 oxide, cis-3-hexen-1-ol and trans-caryophyllene, proved attractive to females (primarily virgin females)
665 in field trials (Nehme et al. 2010, 2014). CLB males were shown to emit the same two functionalized
666 dialkylethers as ALB males. In field bioassays both sexes were attracted to 4-(n-heptyloxy)butan-1-ol,
667 suggesting that this compound is an important component of the CLB sex pheromone (Hansen et al.
668 2015). However, the effectiveness of these male pheromone-based trapping systems for monitoring
669 *Anoplophora* spp. is thought to be limited (EFSA et al. 2019a,b), not only because the lures used
670 primarily attract only virgin females but it is also likely that at close range mate finding includes

671 additional visual and chemical cues, including those coded in specific host phytochemicals (particularly
672 sesquiterpenes) which require further research (Nehme et al. 2014; Hoover et al. 2014; Xu and Teale
673 2021).

674
675 New possibilities may arise from the identification of female-produced pheromones. For ALB, female-
676 produced aggregation (Wickman et al. 2012; Xu et al. 2020a,b), contact (Zhang et al. 2003) and trail
677 pheromones (Hoover et al. 2014) have been reported. Wickham et al. (2012) identified an ALB female-
678 produced aggregation pheromone composed of a blend of heptanal, nonanal and hexadecanal, which
679 proved attractive when combined with host volatiles. Xu et al. (2020a) showed that α -longipinene is a
680 major component in extracts of virgin ALB female genitalia and that in olfactometer bioassays, both
681 sexes were attracted to this sesquiterpene. Although α -longipinene is also released by males and host
682 twigs, the authors suggest that the ratios released by these different sources may encode information
683 pertaining to multiple purposes such as aggregation, mate and host location, and that identification of
684 the naturally produced enantiomer in ALB and its hosts is also needed (Xu et al. 2020b).

685
686 For CLB, the sesquiterpenes b-elemene, b-caryophyllene, a-humulene, and a-farnesene, released both
687 by the beetles and by the host plant, *Citrus unshiu*, after beetle feeding or after mechanical wounding,
688 proved attractive to males and are thought to act both as kairomones and sex pheromones (Yasui et al.
689 2007, 2008; Yasui 2009). A female-produced contact sex pheromone of CLB has also been described
690 (Fukaya et al. 2000; Akino 2001; Yasui et al. 2003, 2007).

691
692 *Sniffer dogs*

693 Recently, “sniffer dogs” have been trained and used in several European countries to identify infested
694 trees through the specific odours released by ALB/CLB larvae and their frass. The use of sniffer dogs
695 specifically trained for the detection of *Anoplophora* spp. was pioneered in 2009 by the Austrian Federal
696 Forest Office (Bundesforschungszentrum für Wald (BFW)) in Vienna (Hoyer–Tomiczek and Sauseng
697 2013). These detection dogs proved effective at detecting all developmental stages of ALB/CLB in wood
698 packaging materials, imported plants and standing trees in areas where establishment had occurred
699 (Hoyer–Tomiczek and Sauseng 2013). In field experiments, trained dogs displayed high levels of
700 sensitivity in the order of 75–88% (correct positives out of all positives) and specificity of 85–96%
701 (correct negatives out of all negatives) (Hoyer–Tomiczek et al. 2016). This method is already being used
702 in addition to visual surveillance in several areas in Europe and good results have been obtained in
703 Austria, France, Italy, Switzerland and Germany (Hoyer–Tomiczek et al. 2016; EFSA et al. 2019a,b).
704 New dog training teams have now been established in Austria and Switzerland (EFSA et al. 2019a,b).
705 In the US, canine detector units were also evaluated with success in Worcester, Massachusetts (Errico
706 2012). The downside of this method is that in order to maintain a high-performance level, these dogs

707 must continuously be stimulated with *Anoplophora* material such as frass and live or dead larvae that
708 are still relatively fresh, and they can only be used for limited periods per day so that a large number of
709 trained dogs is necessary to inspect all relevant imports and potentially infested sites (Hoyer–Tomiczek
710 et al. 2016; EFSA et al. 2019a,b).

711

712 *Other detection methods*

713 Bioacoustic detection methods use portable detectors attached to trees to record the sounds and
714 vibrations produced by larvae (Mankin et al. 2008; Sutin et al. 2019). The potential use of acoustic
715 methods for *Anoplophora* spp. detection has been acknowledged by the international EPPO standards.
716 However, so far, the use of acoustic sensors in the field is difficult and the sensitivity and measuring
717 accuracy of these devices are strongly influenced by the nature of the sensor-substrate interface. These
718 factors limit these methods practical applications (Zorović and Čokl 2015; Hérard and Maspero 2019).
719 More recently, laser vibrometry has been developed for this purpose. With this method, a laser beam is
720 used to detect the vibrations produced by larvae. Recording is carried out directly from the vibrating
721 surface avoiding the need to mount detectors on the tested materials (Zorović and Čokl 2015). Although
722 only laboratory tests have been conducted to date, the methods displayed high sensitivity and a high
723 signal to noise ratio (Zorović and Čokl 2015; Hérard and Maspero 2019). However, a major drawback
724 is that eggs, pupae, and diapausing insects cannot be detected by these methods.

725

726 Citizens' involvement in monitoring and surveillance have been proposed and carried on in a few
727 countries, namely in Austria (EC 2010), France (EPPO RS 2017/005), Italy (Jucker et al. 2007),
728 Germany (StMELF 2020) and Switzerland (EFSF 2020).

729

730 *6.2. Control and Containment*

731 *Tree destruction and physical treatments*

732 Eradication programmes include the removal (felling) and destruction (chipping or burning) of infested
733 trees and possibly their replacement with non-host tree species. Whereas many countries fell and destroy
734 only infested trees, other countries apply preventive tree destruction of all host plants, even if healthy,
735 within in a certain radius around infested trees. This radius usually ranges between 20 m and 100 m
736 (EPPO 2013a,b) (Supplement S3). Under current EU legislation preventive tree destruction of high-risk
737 hosts trees is now mandatory (EC 2012, 2015). Other differences among countries in the management
738 of the CLB infestations concern the treatment of stumps, which could be uprooted and destroyed,
739 covered with metal nets to avoid adult emergence, or treated with herbicide to prevent regrowth
740 (Supplement S3) (EPPO 2013a). These measures are effective in reducing ALB/CLB populations and
741 can contribute to eradication, although they are very laborious, expensive, and time-consuming.

742

743 *Chemical methods*

744 In the past, trunk or soil injections with imidacloprid, a neonicotinoid systemic insecticide, were applied
745 in the USA and Japan to each potential host tree growing within an 800 m radius from infested trees to
746 reduce ALB population density and prevent infestation spread (Hu et al. 2009; Haack et al. 2010).
747 Chemical treatments of healthy trees were combined with removal of infested trees, which proved to be
748 effective. In China, ALB populations were controlled by spraying pyrethroids (cypermethrin) in the tree
749 canopies or coating trunks of host trees to kill adults. Another strategy was inserting wooden sticks
750 containing aluminium phosphide (generating phosphine) into larval galleries to kill ALB larvae, or
751 injecting trunks with organophosphate insecticides such as methamidophos (Wang et al. 2005; Hu et al.
752 2009). Most systemic insecticides were found to persist at lethal levels for several months after injection,
753 but they require new treatments year after year, and their uniform distribution within trees is still
754 uncertain. A potential alternative might be the use of emamectin benzoate trunk injections. In a study
755 recently conducted in an infested willow forest in Beijing, China, this compound proved effective at
756 reducing ALB larval populations by 89% in the first spring after application and by >99% during the
757 second year. Only in the third year after application did re-infestation occur (Wang et al. 2020).
758 Nevertheless, insecticides are costly and their use is labour intensive, making chemical control
759 economically and environmentally expensive (Hu et al. 2009).

760

761 In Europe, the use of chemical treatments has been rare (Supplement S3); it has long been acknowledged
762 that insecticides may cause significant negative externalities including biodiversity loss, ground and
763 surface water contamination (including off-field habitat contamination), impacts on non-target
764 organisms including biocontrol agents, pollinators and earthworms, bio-amplification of toxic
765 substances within the food web with potential effects on human and animal health and development of
766 resistance (Pimentel 2005; Pelosi et al. 2021). The severity of these impacts will depend on the
767 specificity and toxicity levels of the substances used. Therefore, in case eradication fails, additional
768 management options such as biological control are required.

769

770 *Genetic and cultural methods*

771 Research on the identification of tree species or clones resistant to ALB and CLB has not been successful
772 in the last decade. However, the increased use of non-host trees would be suitable for reducing new ALB
773 and CLB infestations. Under current EU legislation the planting of high-risk species in the infested areas
774 is prohibited (EC 2012, 2015).

775

776 *Biological control*

777 Many studies have been carried out on natural enemies that could be used as potential biocontrol agents
778 of ALB, including pathogens (bacteria, fungi, and nematodes), parasitoids and predators (reviewed by
779 Brabbs et al. 2015). Virulent strains of *Beauveria brongniartii* (Sacc.) (Hypocreales: Cordycipitaceae),

780 *Beauveria asiatica* Rehner and Humber, and *Metarhizium brunneum* Petch (formerly *M. anisopliae*
781 (Metschnikoff) (Hypocreales: Clavicipitaceae) are under development for control of ALB (Goble et al.
782 2014, 2016; Meng et al. 2015; Clifton et al. 2020a). *Beauveria brongniartii* has already been developed
783 into a commercial product in Japan, and *M. brunneum* is available for commercial use in the US, both
784 inducing high mortality rates (Brabbs et al. 2015, Clifton et al., 2020a,b). *Beauveria brongniartii*
785 (Hypocreales: Cordycipitaceae) and *M. brunneum* have also been shown to infect CLB (Brabbs et al.
786 2015). Exposure to *M. brunneum* fungal infection synergize with neonicotinoid insecticides
787 (Imidacloprid) used for tree protection resulted in accelerated host death (Fisher et al. 2017). However,
788 the fungal virulence of *M. brunneum* is limited by unsuitable environmental conditions and its
789 effectiveness is affected by adult age (Fisher and Hajek 2014, 2016).

790
791 Entomopathogenic nematodes belonging to the genera *Steinernema* and *Heterorhabditis* were also
792 tested against ALB (Fallon et al. 2004; Pan 2005). Strains of *Steinernema carpocapsae* and *S. feltiae*
793 have proven to be capable of infecting both *Anoplophora* species and they have potential for use as
794 biopesticides as an alternative to chemical treatments. Of the different application methods tested, the
795 most effective included using sponges or gauze to block or cover larval tunnels for CLB (90%-91%
796 mortality rate) and directly spraying into tunnels for ALB (86%). Simple trunk applications were also
797 effective when tested against CLB, albeit more moderately (60 to 77%) (Brabbs et al. 2015).

798
799 Two woodpecker species native to Eurasia, *Dendrocopos major* Beicki and *Picus canus* Gmelin, are the
800 major predators of ALB in China (Brabbs et al. 2015) and they have been shown to be effective at
801 controlling ALB in Chinese forests where nesting has been encouraged (Pan 2005, Golec et al. 2018).
802 Nevertheless, the low levels of mortality attained (less than 16%) are unlikely to provide population
803 control on their own. No detailed information on insect predators of ALB is available.

804
805 The main ALB parasitoids in Asia are larval ectoparasitoids in the genera *Dastarcus* (Coleoptera:
806 Bothrideridae) and *Scleroderma* (Hymenoptera: Bethyilidae) (Golec et al. 2018; Wang et al. 2021a).
807 Nevertheless, more than 20 parasitoid species associated with ALB have been reported in China and
808 Korea (Wang et al. 2021a). *Dastarcus helophoroides* (= *D. longulus*) is an important natural enemy of
809 ALB, CLB and other long-horned beetles in China, Japan, and Korea (Golec et al. 2018). However,
810 *Dastarcus* and *Scleroderma* species native to Asia that attack ALB and CLB have broad host ranges,
811 and their release as biological control agents is unlikely to be approved in Europe or North America
812 (Meng et al. 2015; Gould et al. 2018). In a recent survey using sentinel logs with ALB larvae, *Oxysychnus*
813 sp. (Hymenoptera: Pteromalidae) and *Bracon planitibiae* Yang, Cao et Gould (Hymenoptera:
814 Braconidae) were the most abundant parasitoids species recovered (Li et al 2020). Further studies are
815 underway to assess their potential as biological control agents against ALB.

816

817 Regarding parasitoids of non-Asian origin, Lupi et al. (2017) tested the reproductive performance of
818 *Sclerodermus brevicornis* (Kieffer), a bethylid wasp native to Europe, reared on ALB and CLB larvae.
819 Based on their results, the authors suggest that *S. brevicornis* has the potential to be efficiently mass-
820 reared and actively deployed in the biological control of these two longhorn beetles (Lupi et al. 2017).
821 Also in Europe, eight species of idiobiont ectoparasitoids were discovered attacking both CLB and ALB,
822 all of which were already known from other cerambycid hosts (Hérard et al. 2013; Maspero, 2015). The
823 two species most frequently found were *Spathius erythrocephalus* Wesmael (Hymenoptera: Braconidae)
824 and *Trigonoderus princeps* Westwood (Hymenoptera: Pteromalidae) (Hérard et al. 2013; Brabbs et al.,
825 2015). Their mass release was so far not considered due to their wide host range (Hérard et al. 2013). In
826 North America, several groups of native braconid parasitoids were found to be capable of attacking ALB
827 larvae in laboratory trials (Duan et al. 2016). *Ontsira mellipes* Ashmead was shown to be the most
828 promising species: it can be reared continuously with short generation times and produces a high female-
829 biased progeny with rapidly maturing eggs (Duan et al. 2016; Golec et al. 2016; Wang and Aparicio
830 2020; Wang et al. 2020). In a study conducted to assess the potential host range and preferences of *O.*
831 *mellipes*, this braconid successfully attacked ALB and CLB as well as three of six tested longhorned
832 beetles native to North America (Wang et al. 2019). Field trials to assess the potential of *O. mellipes* to
833 effectively reduce ALB populations are being carried out in Worcester, Massachusetts (USDA-APHIS
834 2021).

835
836 An egg parasitoid native to Asia that attacks CLB, *Aprostocetus fukutai* (Hymenoptera: Eulophidae),
837 was detected in Northern Italy in 2002 and initially described as a new species, *Aprostocetus*
838 *anoplophorae* (Delvare et al. 2004; Hérard et al., 2017). The parasitoid is thought to have accidentally
839 been introduced in Italy from Japan with bonsais containing parasitized CLB eggs (Brabbs et al. 2015,
840 Hérard et al., 2017). So far, *Aprostocetus fukutai* is regarded as the most promising biological control
841 against CLB because i) it attains high rates of parasitism in the field of up to 72% of CLB eggs (Hérard
842 et al. 2005a, 2013), ii) it is CLB specific and not able to parasitize ALB or the Italian native cerambycid
843 *Saperda carcharias* L. (Coleoptera: Cerambycidae) (Herard et al. 2005 a,b), iii) it does not show
844 specificity in terms of the host plant (Hérard et al. 2005a), iv) it is socially gregarious which facilitates
845 the rearing procedures (Maspero 2015), and v) the host and its parasitoid have a high degree of
846 developmental synchronicity (Hérard et al. 2013; Maspero 2015). Furthermore, the parasitoid persists
847 even at the very low host densities that resulted from the extensive eradication efforts conducted in
848 Northern Italy (Hérard et al., 2017; Wang et al. 2021b). For ALB, no egg parasitoids have been identified
849 (Golec et al. 2018; Wang et al. 2021a). Under such circumstances, it has been suggested that biological
850 control programmes should resort to the use of natural enemies native to regions where ALB has been
851 introduced via novel associations and augmentative releases (e.g. Wang et al. 2019; Wang et al. 2021a)

852

853

854 **7. Conclusion and future outlooks**

855 Major efforts have been put into achieving successful eradication of establishments of ALB and CLB.
856 International collaborative activities translated into legislative changes to harmonize detection and
857 eradication as well as prevention methods towards a common goal. We conclude that these efforts have
858 resulted in considerable success as more than 45% of eradication programmes were successful (and
859 some are still ongoing). Several countries were able to completely eradicate all ALB and/or CLB
860 populations, and other managed to reduce the area affected. Still, these efforts are hampered by the
861 ongoing arrival of new beetles, both from their native regions in Asia and in some cases apparently also
862 from other invaded regions via the bridgehead effect.

863
864 Several biological traits of ALB and CLB may have favoured eradication success, such as long-life
865 cycles, relatively low fecundity, low spread rate and their tendency to remain in the vicinity of the natal
866 trees unless conditions are unfavourable (Haack et al. 2010). Detectability has been identified by Tobin
867 et al. (2014) as another factor relevant for the success of eradication programmes. Thus, the fact that
868 ALB develops mostly in the upper part of trees and CLB in the lower trunk and roots, may translate into
869 a higher relative detectability of ALB, which in some cases might facilitate early detection and
870 consequently its eradication success.

871
872 Eradication campaigns have hitherto been expensive. For example, just for Lombardy in Italy, the costs
873 of CLB eradication campaigns between 2008 and 2013 totalled almost 20 million Euros (Cavagna 2014,
874 in Hérard and Maspero 2019). Nevertheless, although the costs of these eradication programmes can be
875 extremely high, the benefits still outweigh inaction in most cases. For ALB, the costs of eradication
876 campaigns undertaken between 1996 and 2013 in the USA were estimated to have exceeded US\$537
877 million (Eyre and Haack 2017). However, estimations of potential economic loss in compensatory value,
878 resulting from a widespread ALB outbreak could exceed US\$670 billion (over one trillion US dollars,
879 if adjusted to 2021 values) and a potential loss of approximately 35% of urban tree cover across the
880 United States (Nowak et al. 2001). For the small ALB outbreak in Cornuda, Italy, Faccoli and Gatto
881 (2016) estimated that during the first year of the eradication program, the ornamental value of the saved
882 trees was six times higher than the eradication costs. Pedlar et al. (2020) estimated that the annual costs
883 of inaction in an ALB outbreak in Eastern Canada could exceed CDN\$12 billion (considering street
884 tree-related costs, standing timber value and maple food products), which contrasts with an annual
885 control expenditure of approximately 5% of this value (CDN\$0.5 billion).

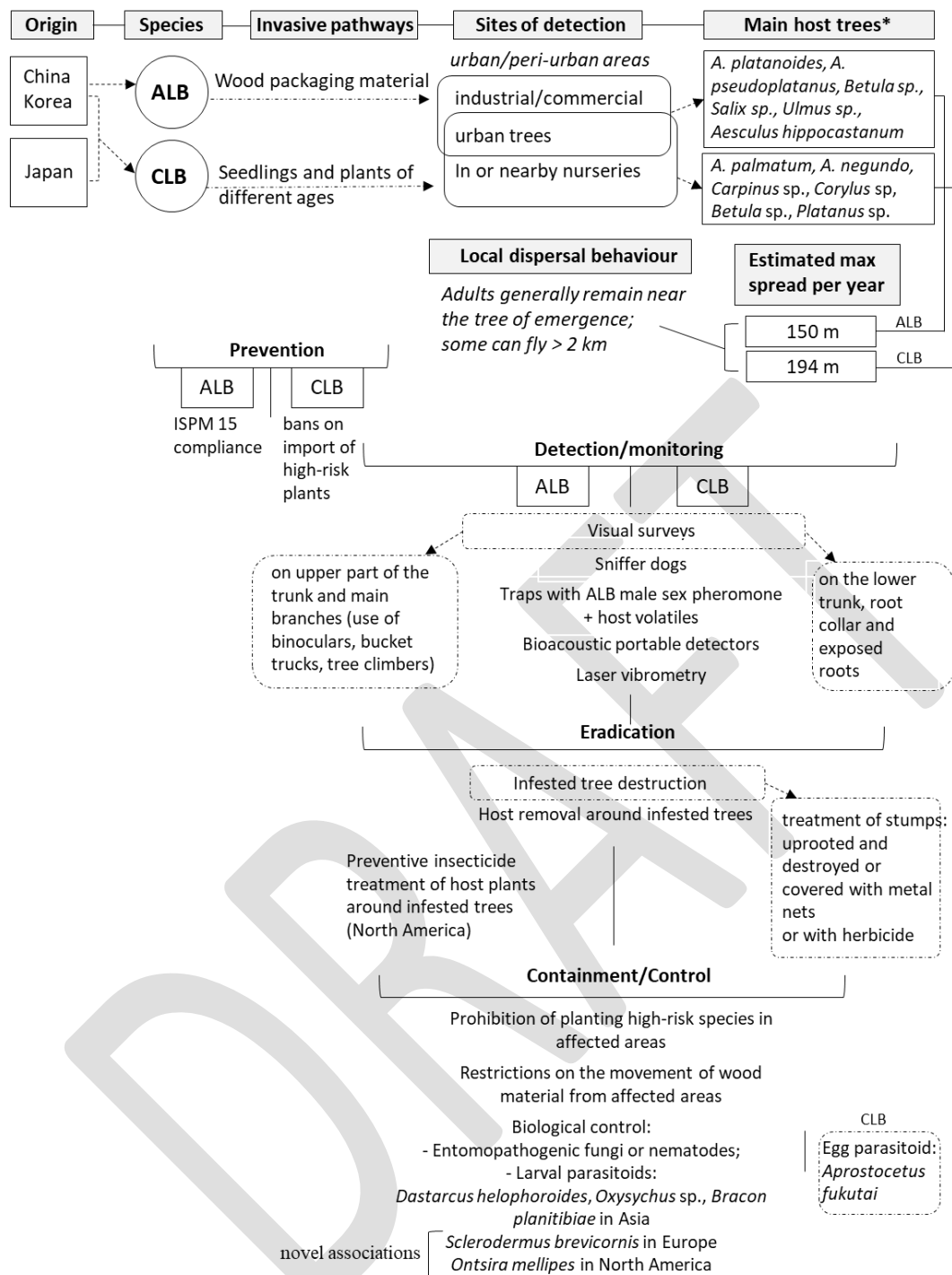
886
887 The methods used for eradication have not seen many changes during the last decade, and host removal
888 is still the method most commonly used, with or without preventive felling. In North America, the use
889 of preventive chemical treatment may have yielded good results in containing the spread of established
890 populations and facilitating their eradication, yet the externalities arising from large-scale use of most

891 insecticides may outweigh the benefits of their use. On the other hand, detection methods have evolved
892 significantly during the last decade, even if visual surveys remain the “gold standard”. In Fig. 11, a
893 summary of the known steps of invasions by the two longhorned beetles and the available management
894 strategies is presented.

895

896 Despite the advances of the last decade, prevention and management of ALB and CLB is still
897 challenging but not impossible. Research avenues that could be pursued further to improve prevention,
898 eradication and management include technical solutions such as sensors in containers to detect
899 infestations based on acoustic signals or VOCs signals, improving trapping methods based on the use of
900 semiochemicals, new models to predict spread particularly in urban areas, diversification of tree species
901 in urban and peri-urban areas, and citizen science programmes to improve detection and responses.

DRAFT



902

903 Figure 11 Summary of the steps of invasion and management strategies of *Anoplophora* spp.

904 * in invaded range.

905

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916

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