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Updates on the invasive oak lace bug, *Corythucha arcuata*, in Italy

Matteo Bracalini[1], Domenico Rizzo[2], Tiziana Panzavolta[1]
[1]*Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology Section, University of Florence, Italy*
[2]*Laboratory of Phytopathological Diagnostics and Molecular Biology, Plant Protection Service of Tuscany, Italy*

Abstract

The sap-sucking insect *Corythucha arcuata* (Say) (Hemiptera Tingidae), also known as the oak lace bug (OLB), is an invasive pest mainly associated with oaks. Native to North America, it was introduced in Europe in 2000 (Northern Italy). The OLB is now reported in several other European countries, mostly from the Balkan region. At the time of its first record in Italy, the OLB had already invaded an extensive area between Lombardy and Piedmont. New reports were published in the following years for other north-eastern Italian regions: Veneto, Friuli-Venezia Giulia, and Emilia Romagna. The object of this study was to determine the presence of the OLB in Tuscany (central Italy). A survey of *Quercus* trees was carried out from late summer to early autumn 2021. All Tuscan provinces were included. In addition, during the survey also few sites in Latium, on the border with Tuscany (province of Viterbo), were included in this study, for a total of 50 survey points (SPs) located in urban and in peri-urban areas. In each SP, the number of attacked trees, as well as the infestation level of each oak species were recorded. Both morphological and molecular approaches were adopted to identify the pest. *C. arcuata* was found in 29 out of 50 SPs (28 in Tuscany and one in Latium). About 79% of the urban SPs had been invaded, as opposed to 32% of peri-urban invaded ones. This concurs with the agreed-upon hypothesis that the dispersal of this invasive pest is mainly human-mediated. *Quercus robur* and *Quercus cerris* were the most infested species (72% and 69% of attacked trees, respectively), while only 8% of *Quercus pubescens* trees were found to be infested. First records of OLB often are in urban environments, however, this insect is not only an urban problem, but also a forestry problem, in fact reports from Eastern Europe account for over 1.7 million hectares of OLB-infested forests. Thus, the OLB is a concern for oak forest health, and it is worthy of further research in order to help overcome the progressing European oak decline.

Key words: *Quercus*, *Corythucha arcuata*, sap-sucking insect, non-native species.

Introduction

The invasive oak lace bug (OLB), *Corythucha arcuata* (Say) (Hemiptera Tingidae), is a Nearctic sap-sucking insect native to the Eastern USA and Southern Canada and mainly associated with oaks; however, it can also exploit, for feeding and overwintering, non-oak hosts. More specifically, it has been recorded on 27 deciduous *Quercus* species in Europe (both native and exotic species), as well as 28 non-oak hosts (e.g. *Acer campestris* L., *Castanea sativa* Mill., *Fagus sylvatica* L., *Carpinus betulus* L., *Corylus avellana* L., *Rosa canina* L., *Crataegus monogyna* Jacq., *Ulmus minor* Mill., *Tilia cordata* Mill.) (Bernardinelli, 2006; Csóka *et al*., 2020). OLB adults overwinter in bark crevices or under raised bark. The species completes 2 or even 3 generations per year, both in its native range (Connell and Beacher, 1947; Csepelényi *et al.*, 2017) and in northern Italy (Bernardinelli, 2007), where it was accidentally introduced in 2000 for the first time in Europe (Bernardinelli and Zandigiacomo, 2000). The females lay groups of 10-100 eggs on the underside of the host leaves. Both nymphs and adults feed here by sucking cell content from the lower leaf surface, where they remain for a large part of the warm season. As a consequence, here their excrements, small black droplets, markedly accumulate.

Damage caused by OLB feeding becomes visible on the upper side of the leaves. Nymphs' and adults' sucking causes small chlorotic spots (1-3 mm), which later increase in number and size, ultimately merging into larger chlorotic areas. Total discoloration of the leaves can be observed in case of heavy OLB populations as early as the beginning of summer (Csepelényi *et al*., 2017; Paulin *et al*., 2020). Heavy infestations of several generations repeated on the same host plant can lead to discoloration of the whole crown in July-August, which may cause the abscission of the most damaged leaves a few months earlier than usual. Although few studies have been carried out on the impact of OLB on tree health, especially in new European introduction areas, it is likely that the pest may have repercussions on the overall health of the host tree, especially after several consecutive years of infestations (Nikolić *et al*., 2019; Paulin *et al*., 2020). Large forested areas, when severely infested by OLB, are easily spotted and they may even be detected/monitored by remote sensing, as the entire canopy suddenly changes colour before the end of the growing season. Aerial or satellite imaging have already been used to assess OLB infestations in Europe (Paulin *et al*., 2020; Kern *et al*., 2021).

After its first discovery Europe in Northern Italy (Bernardinelli and Zandigiacomo, 2000), in a time-span of 20 years OLB spread in several European countries, including European Russia (Csóka *et al*., 2020; Paulin *et al*., 2020). However, most of the recordings were reported more than a decade after the initial sighting in Italy, suggesting that OLB populations are not immediately observable upon their arrival in a new area (Paulin *et al*., 2020). Perhaps they remain initially at low infestation levels, and then they can explode demographically if favourable weather conditions occur (Csóka *et al*., 2020).

This was true also in Italy where, at the time of its first recording in Lombardy (2000), the insect had already invaded a vast area between Lombardy and Piedmont (Jucker *et al.*, 2008; Faraci, 2019).

After more than two decades since the first record of the insect in Italy, few papers investigated the further spread of OLB in other Italian regions, such as Lombardy, Piedmont, Friuli-Venezia Giulia, Veneto, and Emilia Romagna (Bernardinelli, 2001; Reggiani and Bonifazi, 2009; Faraci, 2019). More specifically, Faraci in 2019 reported that the OLB had yet to be observed south of the Po Valley. Considering the rapidly increasing concern about the potential impact of OLB on European oaks, the aim of this paper was to survey OLB's spread further south in Italy, namely, to the oaks of mainland Tuscany and partially Latium, both in urban and peri-urban environments.

Materials and methods

Investigation sites

A survey of the Tuscany region was carried out from August 30th to October 30th 2021, when OLB signs and symptoms are most clearly visible on plant hosts (Williams *et al*., 2021). All Tuscan provinces (Arezzo, Florence, Grosseto, Livorno, Lucca, Massa-Carrara, Pisa, Pistoia, Prato, and Siena) were included in our survey, as well as the province of Viterbo (Latium), for a total of 40 municipalities randomly selected along a network of communication routes including both main and secondary roads (37 municipalities in Tuscany and three in Latium). In each municipality selected, based on the availability of oaks, one or more sites were considered, giving a total of 50 survey points (SP), each measuring about 1 hectare (table 1): 28 were in city parks and boulevards of urban areas; 22 were in peri-urban areas along roads. Where forested areas were considered, the infestation was observed only along the forest margin, where the probability of finding the insect is higher.

Insect survey

All deciduous oaks present inside the borders of the SP were examined; however other known OLB hosts were not checked. The minimum number of trees to be inspected per site was fixed at 10, though if fewer trees were available they were examined nonetheless. A total of at least 100 leaves per tree were closely examined for attack by OLB, in some cases after a preliminary observation with the help of a binocular, followed by their collection using a long-reach pruner. Only oaks showing both OLB adults/egg clusters and feeding damage were considered as positive observations. No trees were counted as having been attacked by the OLB until a clear correlation between leaf discoloration and the presence of the insect could be verified. When only juvenile stages were observed, not being as easily distinguishable from other lace bugs, the survey on the same trees was continued until a confirmation of OLB could be made with both egg clusters and OLB adults.

Table 1. Site and tree infestation levels by *C. arcuata*, recorded during the survey carried out in the Tuscany region and Viterbo province during the year 2021.

Province	Municipality	*Quercus spp.*	Urban/ Peri-urban (u/p)	Survey Point	Elevation m a.s.l.	Site infestation level[a]	Tree infestation level[b]
Arezzo	Laterina Pergine Valdarno	*Q. cerris*	u	28	244	3	Heavy
	Laterina Pergine Valdarno	*Q. robur*	u	29	251	3	Heavy
	Ortignano Raggiolo	*Q. pubescens*	p	21	472	0	
	Ortignano Raggiolo	*Q. pubescens*	p	23	362	0	
	Poppi	*Q. pubescens*	u	22	355	1	Light
	Pratovecchio	*Q. pubescens*	p	20	716	0	
Florence	Barberino Tavarnelle	*Q. robur*	p	25	393	x	
	Barberino Tavarnelle	*Q. pubescens*	p	26	228	1	Sporadic
	Barberino Tavarnelle	*Q. pubescens*	u	27	356	0	
	Capraia e Limite	*Q. pubescens*, *Q. robur*	u	13	30	3	Severe (*Q. robur*)
	Capraia e Limite	*Q. pubescens*, *Q. cerris*	p	14	67	2	Severe (*Q. cerris*)
	Florence	*Q. robur*, *Q. cerris*	u	17	38	4	Heavy on both
	Florence	*Q. robur*	u	18	53	4	Severe
	Montelupo Fiorentino	*Q. robur*, *Q. petraea*	u	15	36	4	Severe (*Q. robur*)
	Sesto Fiorentino	*Q. robur*	u	16	39	2	Severe
	Sesto Fiorentino	*Q. pubescens*	p	19	662	1	Heavy
Grosseto	Civitella Paganico	*Q. pubescens*, *Q. robur*, *Q. cerris*, *Q. petraea*	p	41	185	1	Sporadic (*Q. pubescesns*)
	Follonica	*Q. pubescens*, *Q. cerris*, *Q. robur*	u	40	2	1	Heavy (*Q. pubescens*), Sporadic (*Q. cerris*)
	Grosseto	*Q. robur*, *Q. cerris*	u	42	3	2	Sporadic on both
	Grosseto	*Q. robur*	u	43	6	1	Light
	Grosseto	*Q. pubescens*	u	44	22	0	
	Massa Marittima	*Q. pubescens*	p	33	598	0	
	Orbetello	*Q. pubescens*, *Q. cerris*	p	45	7	x	
	Pitigliano	*Q. pubescens*, *Q. cerris*	p	46	156	0	

(Continued)

(Table 1 continued)

Province	Municipality	*Quercus spp.*	Urban/ Peri-urban (u/p)	Survey Point	Elevation m a.s.l.	Site infestation level[a]	Tree infestation level[b]
Grosseto	Pitigliano	*Q. pubescens*, *Q. cerris*, *Q. petraea*	u	47	346	2	Light on both
Livorno	Cecina	*Q. robur*	u	30	19	0	
	Montescudaio	*Q. pubescens*	u	31	15	0	
	Piombino	*Q. pubescens*	p	39	45	0	
Lucca	Forte dei Marmi	*Q. robur*	u	2	4	+	Heavy
	Viareggio	*Q. robur*	u	3	6	4	Heavy
Massa Carrara	Massa	*Q. pubescens*, *Q. robur*	u	1	1	x	
Pisa	Calcinaia	*Q. pubescens*. *Q. cerris*	p	6	21	3	Severe (*Q. cerris*)
	Pontedera	*Q. robur*	u	5	14	2	Light
	Santa Maria a Monte	*Q. cerris*	p	7	41	1	Sporadic
	Vecchiano	*Q. pubescens*	p	4	43	0	
Pistoia	Montecatini Terme	*Q. robur*	u	8	45	4	Heavy
	Pieve a Nievole	*Q. robur*	p	9	16	+	Heavy
	Pistoia	*Q. robur*	u	10	71	4	Severe
	Agliana	*Q. robur*	u	11	43	4	Severe
Prato	Montemurlo	*Q. cerris*	p	12	96	0	
Siena	Buonconvento	*Q. pubescens*, *Q. robur*	u	36	146	2	Light on both
	Chiusdino	*Q. pubescens*	p	34	310	0	
	Montalcino	*Q. pubescens*, *Q. cerris*	p	37	161	0	
	Monteroni d'Arbia	*Q. pubescens*	p	35	166	0	
	Poggibonsi	*Q. pubescens*	u	24	178	0	
	San Quirico d'Orcia	*Q. pubescens*	u	38	285	1	Light
	Siena	*Q. pubescens*, *Q. robur*, *Q. cerris*, *Q. petraea*	u	32	300	4	Light (*Q. pubescens*), Heavy (*Q. petraea*), Severe (*Q. robur*), Severe (*Q. cerris)*
Viterbo	Acquapendente	*Q. pubescens*, *Q. cerris*	u	49	439	2	Severe (*Q. cerris)*
	Onano	*Q. cerris*	p	48	551	0	
	Proceno	*Q. pubescens*	p	50	274	0	

[a] Infestation levels of sites with more than five oaks present: "0" = no attacked oaks (AO); "1" = 0 < AO ≤ 25%; "2" = 26% ≤ AO ≤ 50%; "3" = 51% ≤ AO ≤75%; "4" = 76% ≤ AO ≤ 100%. Symbols used for sites with less than five oaks present: "x" OLB absence; "+" OLB presence.

[b] Infestation classes of attacked trees: "Sporadic" = few eggs clusters or OLB specimens on less than 10% of examined leaves, observable only after intensive search, and without conspicuous discoloration spots on the upper side of leaves; "Light" = OLB specimens and eggs easily found on more than 10% of examined leaves, with noticeable signs of discoloration on the upper side of leaves; "Heavy" = many OLB specimens and egg clusters on the majority of examined leaves with, at close inspection, clear signs of discoloration on the upper side of most of the attacked leaves; "Severe" = large amount of OLB specimens and egg clusters affecting the whole crown, whose discoloration may be easily spotted from afar.

Damage classification

Considering the total number of oak trees, the infestation level of each site was assessed according to five classes of symptom presence: "0" = no signs of OLB; "1" = OLB present on 1-25% of examined oak trees at that SP; "2" = OLB present on 26-50% of examined trees; "3" = OLB present on 51-75% of examined trees; "4" = OLB present on 76-100% of examined trees. Also, for every attacked SP, an assessment of damage intensity was carried out based on four classes of damage describing the average attack on each oak species per site: "Sporadic" = few eggs clusters or OLB individuals on less than 10% of examined leaves, observable only after intensive search, and without conspicuous discoloration spots on the upper side of the leaves; "Light" = OLB individuals and eggs easily found on more than 10% of examined leaves, with noticeable signs of discoloration on the upper side of the leaves; "Heavy" = OLB individuals and egg clusters on the majority of examined leaves (more than 50%) with, at close inspection, clear signs of discoloration on the upper side of most of the attacked leaves; "Severe" = large amount of OLB specimens and egg clusters affecting the whole crown, whose discoloration may be easily spotted from afar. If a SP had less than five oaks available for the observations, no class of infestation was assigned; nevertheless, the data on OLB presence/absence were recorded. Number of attacked and unattacked trees per SP were also recorded separately per oak species to compare the infestation level of the different *Quercus* spp. involved. To this aim, we considered only urban sites (where it is more likely to find higher infestation levels) infested by OLB. Percentages of attacked trees of different oak species were compared using the G test.

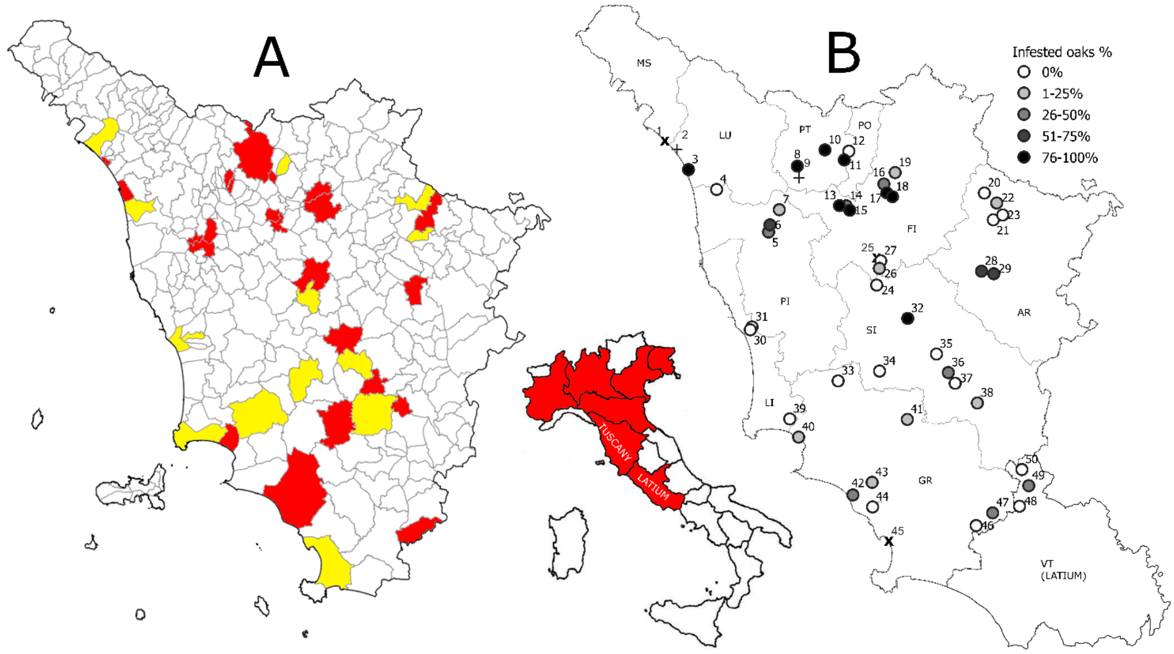

Figure 1. Tuscan municipalities (**A**) surveyed for the oak lace bug (*C. arcuata*) and the survey points selected (**B**) in Tuscany (SPs 1-47) and Latium (SPs 48-50). Figure 1A: yellow = OLB not observed; red = OLB present; Figure 1B = infestation levels are shown in the legend as percentage of infested oaks per SP. In sites with less than five oaks present, the following symbols were used: "x"= OLB absence; "+" = OLB presence. AR = Arezzo; GR = Grosseto; FI = Florence; LI = Livorno; LU = Lucca; MS = Massa Carrara; PI = Pisa; PO = Prato; PT = Pistoia; SI = Siena; VT = Viterbo (Latium).

Insect sampling and identification

A sample of about 50 OLB adults was collected from all sites for morphological and molecular identification. Morphological identification of adults from every SP was carried out in the lab following the keys available in Gibson (1918) and Forster *et al*. (2005). To confirm morphological identification, molecular analysis was also carried out by extracting and sequencing DNA from a sub-sample of 25 OLB adults from SP 10. DNA was extracted from insect samples according to the protocol described by Rizzo *et al*. (2020) and modified with CTAB 2% extraction buffer and use of an automated MaxWell 16 purificator (Promega, Madison, WI, USA). To test the quality of the extracted DNA from the insect samples, an aliquot of each extract was diluted in double distilled water (1 : 20) and tested in a qPCR reaction, using a dual-labeled probe and targeting a highly-conserved region of 18S rDNA (Ioos *et al*., 2009). This quality test allowed to check for inhibitors potentially affecting both the quantification cycle (Cq values) and the slope of amplification curves. A portion of the COI mitochondrial gene of OLB (DNA barcode) was amplified with LCO1490 and HCO2198 primers (Folmer *et al*., 1994). Amplicons of the organisms were purified and sequenced.

Results and discussion

An average of 10 oak trees was recorded in each SP. OLB's presence was recorded in seven Tuscan provinces, as well as in the Viterbo province, the only investigated province of Latium (figure 1); only in three Tuscan provinces (Livorno, Massa Carrara, and Prato) was OLB not observed. All sampled adults were identified as *C. arcuata* by morphological analysis, then confirmed via barcoding (GenBank accession number: SUB11546025). DNA extraction from insect adults had Cq values ranging from 15 to 20, showing a satisfactory performance of the molecular protocol. The presence of OLB was confirmed in 23 out of the 37 Tuscan municipalities (figure 1A) and in one out of three Latium municipalities. The insect was recorded in 29 SPs out 50 (58% of the total) (figure 1B). It was more frequently recorded in urban sites (79% of SP) than peri-urban sites (32% of SP). This is in accordance with the most widely-accepted assumption suggesting passive transport with motor vehicles as main dispersion mean of OLB. Although OLB adults can colonize new areas by flying or exploiting wind currents, most of their long-distance dispersal is supposed to be human-mediated. For this reason, most OLB recordings in Europe are located along main roads or within towns (Dobreva *et al*., 2013; Simov *et al*., 2018; Tomescu *et al*., 2018).

The province of Florence was the most colonized one. Here, the insect was found in all the municipalities investigated, with three sites (2 in Florence and 1 at Montelupo Fiorentino) having the overall highest infestation level, with OLB present on 94% of the examined oaks (table 1, figure 2). Moreover, in most SPs from Florence province, oaks had a "heavy" or "severe" infestation level (table 1). In addition, such high infestation levels were recorded also in urban parks of other five municipalities of Tuscany where 94% of the surveyed oaks showed large populations of OLB. *Quercus robur* L., *Quercus cerris* L., *Quercus petraea* (Matt.), and *Quercus pubescens* Willd.

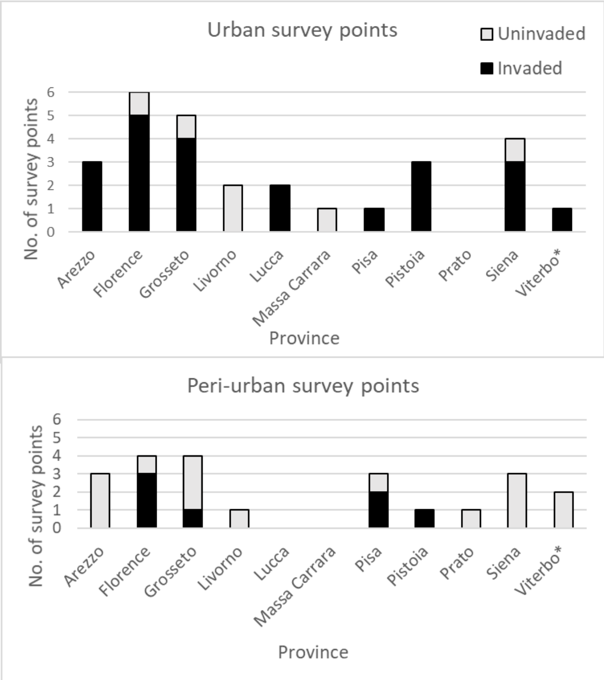

Figure 2. Comparison of numbers of invaded and uninvaded (by *C. arcuata*) survey points in urban and in peri-urban areas of the Tuscan and Latium municipalities surveyed in this study.

were the four oak species found during the survey (table 2). *Q. pubescens* was the most represented species, with about 46% of all trees examined, and it was quite evenly distributed in urban and peri-urban SPs. *Q. robur* was the second-most-frequent oak species (about 33%); however, it was found almost entirely in city parks (about 97% of the examined trees), where it is the main oak species. *Q. cerris* (about 19%) was found both in urban and in peri-urban sites; finally, *Q. petraea* was the least-represented oak species (about 2%), with only eight oaks in urban environments and two found in peri-urban areas. Also, 70% of SPs were constituted by a single oak species, while only 30% of SPs had two or more oak species.

To compare the infestation level of the different *Quercus* spp. involved, *Q. petraea* was not taken into account because of the low number of trees found. The three remaining oak species were not equally attacked (G test, df = 2, G = 105.244, $P < 0.001$). *Q. pubescens*, with only 8% of specimens infested and percentages of trees attacked per site that have never exceeded 40%, resulted the least attacked (G test, df = 1, G = 96.656, $P < 0.001$). This finding is in disagreement with previous observations, which indicated *Q. pubescens* as one of the most heavily-attacked species in Eurasia, together with *Q. robur* (Csóka *et al.*, 2020). On the contrary, our observations about the English oak (*Q. robur*) are in accordance with Csóka *et al.* (2020) and with other studies (Bernardinelli and Zandigiacomo, 2000; Bernardinelli, 2006) which report it to be one of the favoured hosts in Europe. In fact, half of the invaded sites where *Q. robur* was present had 100% of the trees of this species attacked. In addition, this species in our study had the highest percentage of infested trees (72%) (figure 3). Nevertheless, this percentage was not statistically different from that of *Q. cerris*, whose percentage of infested trees (69%) was only slightly lower than that of *Q. robur*. However, only in 20% of the invaded sites, all specimens of *Q. cerris* resulted attacked. Also, in this case the result is in agreement with previous studies (Csóka *et al.*, 2020). Finally, 87.5 % of *Q. petraea* trees showed signs of OLB infestation, but our overall sample included only 8 trees belonging to this species.

Conclusions

Despite OLB having been recorded in Italy since 2000, the present study reports the first formal recordings in Tuscany and Latium, besides a few reports from a citizen-science project (iNaturalist) listing OLB both in Tuscany (Pisa, Prato) and Latium (Frosinone), as well as Umbria (Perugia) (even if in this region the pest is not officially reported yet) during the year of our observations. In our study the insect was observed in 29 sites out of 50; in addition, half of the attacked sites had a heavy or severe infestation level. Thus, the high rate of observations across Tuscany supports the hypothesis that the tingid may be widely spread at least in the bordering Regions (i.e. Liguria, Latium, Umbria and Marche). In our study, urban sites resulted more infested than peri-urban sites. Further studies would be needed to ascertain whether higher temperature in urban sites could affect the development of OLB infestations (e.g. the role of the 'urban heat island' on insect pests). In fact, OLB damage may indeed reflect the added effects of both higher temperatures and the human driven spread of the species in urban settings.

Even though OLB recordings are more likely to happen in urban settings or road networks, as confirmed by our results, the dispersal of newly introduced populations could affect entire oak forests. In Italy no cases of infestation in wide forested areas have been reported yet. Also, trees which are more exposed in the open have been found to be more susceptible to OLB (e.g oaks in forest margins, parks, roads, etc.) (Tomescu *et al.*, 2018). However, surveys carried out in Europe have already shown

Table 2. Number of surveyed oaks in Tuscan sites and in Viterbo province (Latium).

	Number of surveyed oaks				
SP setting	*Q. robur*	*Q. cerris*	*Q. petraea*	*Q. pubescens*	Total
Urban	164	36	8	97	305
Peri-urban	5	59	2	134	200
Total	169	95	10	231	505

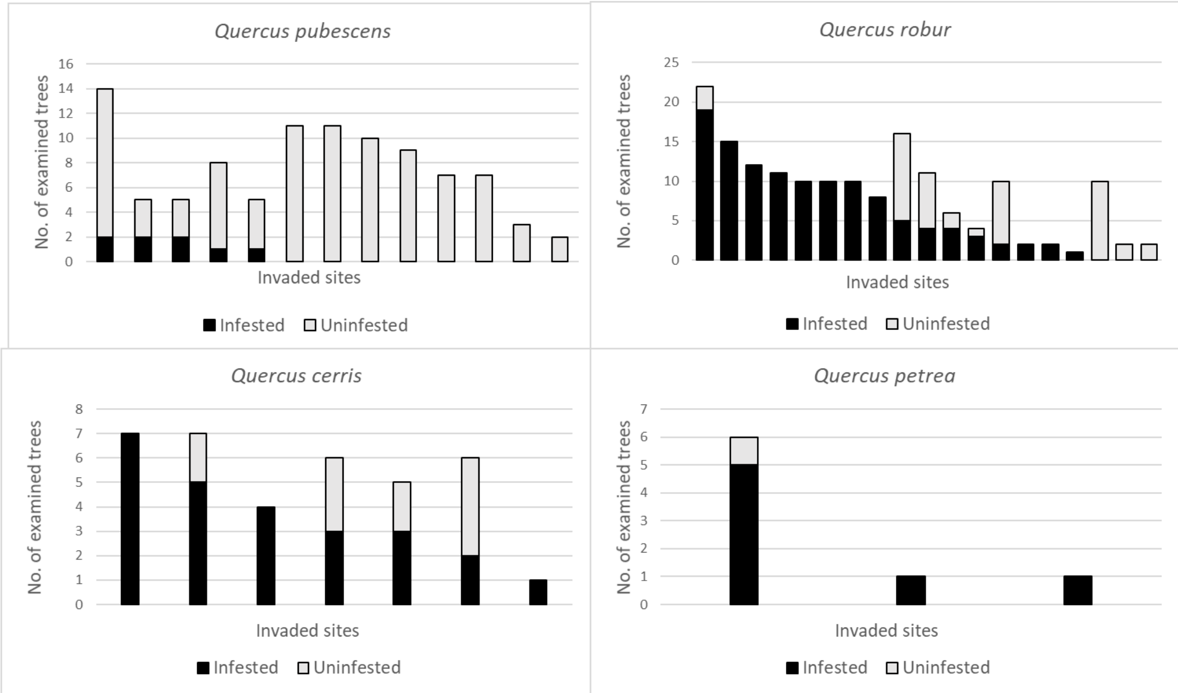

Figure 3. Infested and uninfested trees per species in each urban site invaded by *C. arcuata*.

extensive forest-wide infestations (Mutun *et al*., 2009; Neimorovets *et al*., 2017; Paulin *et al*., 2020). For example, Paulin *et al*. (2020) reported an estimated area of more than 1.7 million hectares of infested *Quercus* spp. forests in just the five countries where OLB has been introduced (i.e. Croatia, Hungary, Romania, European Russia, and Serbia). Finally, Kern *et al*. (2021), who studied the dynamics of OLB outbreaks in Central Europe over a period of seven years via space-borne remote sensing, estimated a radial spread of OLB infestations of 6-50 km every year, depending on the age of the infestation, and on the intensity of human activity and transportation. Thus, OLB populations observed in this study are likely to grow in the future, with potentially dire consequences for central Italy broad-leaved forests.

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Authors' addresses: Matteo BRACALINI (corresponding author: matteo.bracalini@unifi.it), Tiziana PANZAVOLTA, Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology Section, University of Florence, via Maragliano 77, 50144 Florence, Italy; Domenico RIZZO, Laboratory of Phytopathological Diagnostics and Molecular Biology, Plant Protection Service of Tuscany, via Ciliegiole 99, 51100 Pistoia, Italy.

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