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THE ROLE OF INVASIVE SPECIES IN URBAN FOREST PLANNING

Skopje, North Macedonia
04-06 June 2024

Phoenix Rising from Ashes: Safeguarding Ash Trees Against Invasive Threats

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Swedish University of Agricultural Science



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Introduction and expansion two invasive species



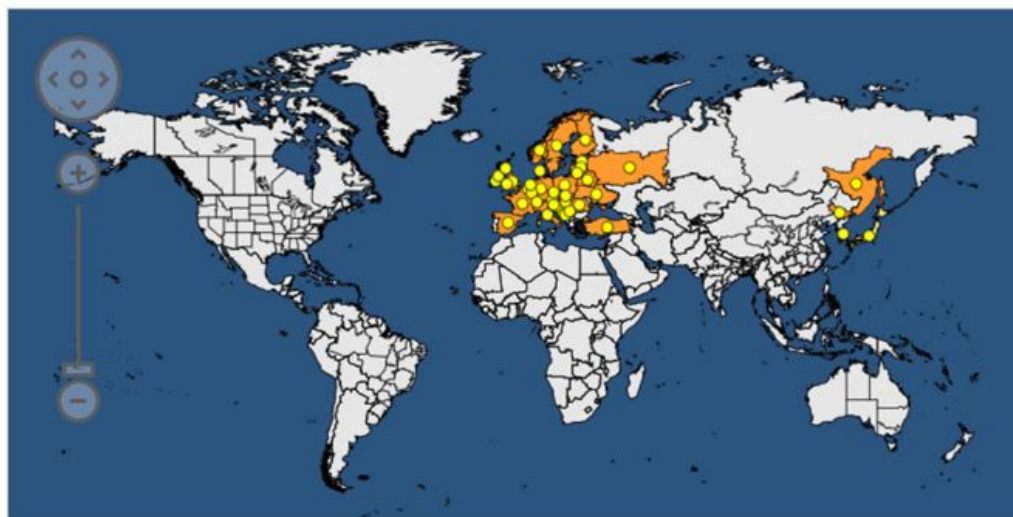
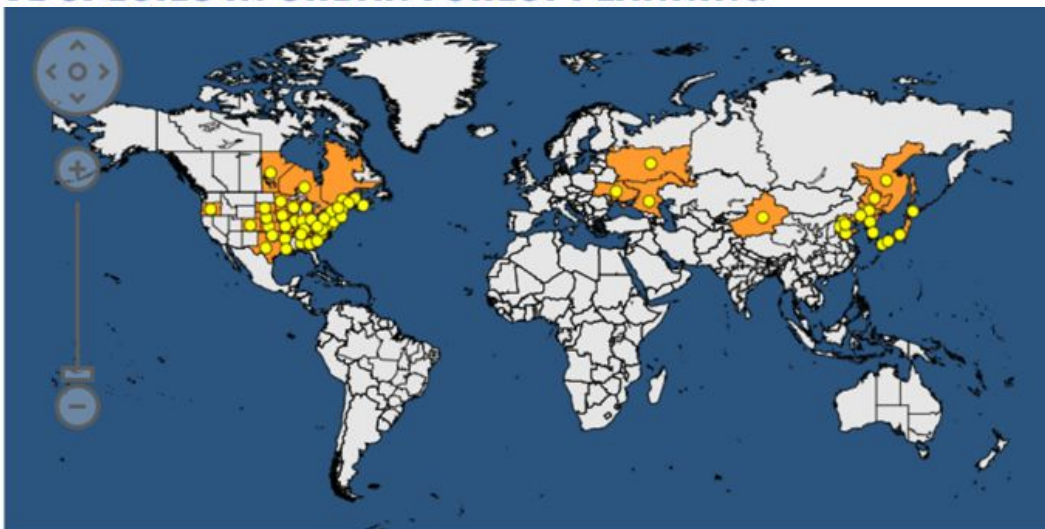
Ash dieback (ADB) caused by invasive fungus *Hymenoscyphus fraxineus*
ADB presents in most parts of the EU
F. excelsior is high susceptible



Emerald ash borer, invasive beetles, *Agrilus planipennis*, phloem-boring beetle
EAB presents in eastern Ukraine (2019)
F. pennsylvanica is high susceptible

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EAB

On its native range, EAB colonized *F. mandshui* and *F. chinensis* without significant damage. In secondary ranges, EAB displays a broader host range, infesting all Fraxinus species and even ash host, cultivated olive (*Olea europaea* L.). Over the past two decades, the range of EAB expanded, currently found in 36 US states, five Canadian provinces, and 20 regions of Russia. From 2019 – in Ukraine

EPPO (2024) *Agrilus planipennis*. EPPO datasheets on pests recommended for regulation. <https://gd.eppo.int> (accessed 2024-06-01)

ADB

On its native range, EAB colonized *F. mandshui* without significant damage. In secondary ranges, EAB displays a broader host range, infesting all Fraxinus species and even ash host, *Phillyrea angustifolia*. Over the past two decades, the range of EAB expanded, currently found in all European countries. From 2013 – in Ukraine

EPPO (2024). *Hymenoscyphus fraxineus*. EPPO database. <https://gd.eppo.int/taxon/CHAAFR/distribution> (accessed 2024-06-01)

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Ash dieback in Europe

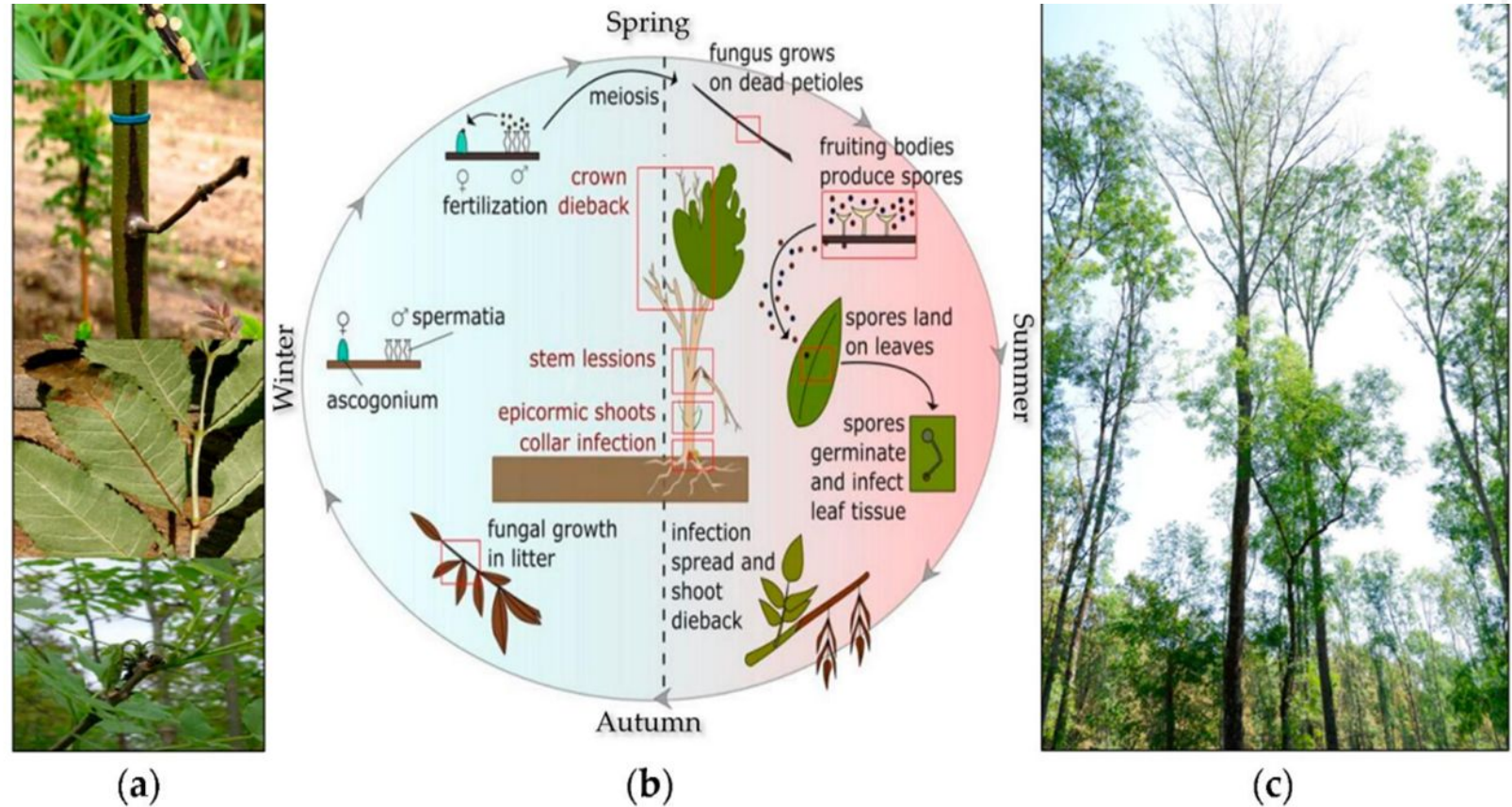


Figure 3. Ash dieback: symptoms and life cycle (Gašparović M, Pilaš I, Klobučar D, Gašparović I. (2023) Monitoring Ash Dieback in Europe—An Unrevealed Perspective for Remote Sensing? Remote Sensing): (a) *Hymenoscyphus fraxineus* disease symptoms: mushroom-like fruiting bodies (top); diamond-shaped lesions (middle upper); shoot wilting and leaf necrosis (middle lower and bottom) (source: Forest Research, www.forestresearch.gov.uk, accessed on 10 January 2023). (b) Lifecycle and symptoms (source: Teagasc—Agriculture and Food Development Agency, www.teagasc.ie, accessed on 10 January 2023) and (c) narrow-leaved ash (*Fraxinus angustifolia*) dieback; location Radinje forest, Croatia.



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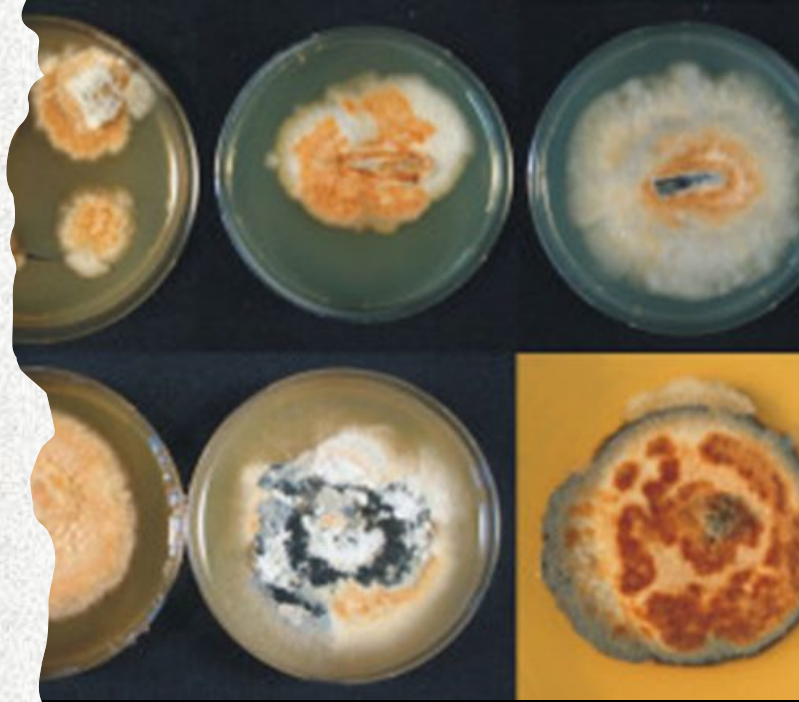
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ASH DIEBACK: SYMPTOMS, MONITORING AND MANAGEMENT





First detection 1992 (1996)
Chalara fraxinea (2006)
Hymenoscyphus albidus (2009)
Hymenoscyphus pseudoalbidus
(2010)
Hymenoscyphus fraxineus
(2014)

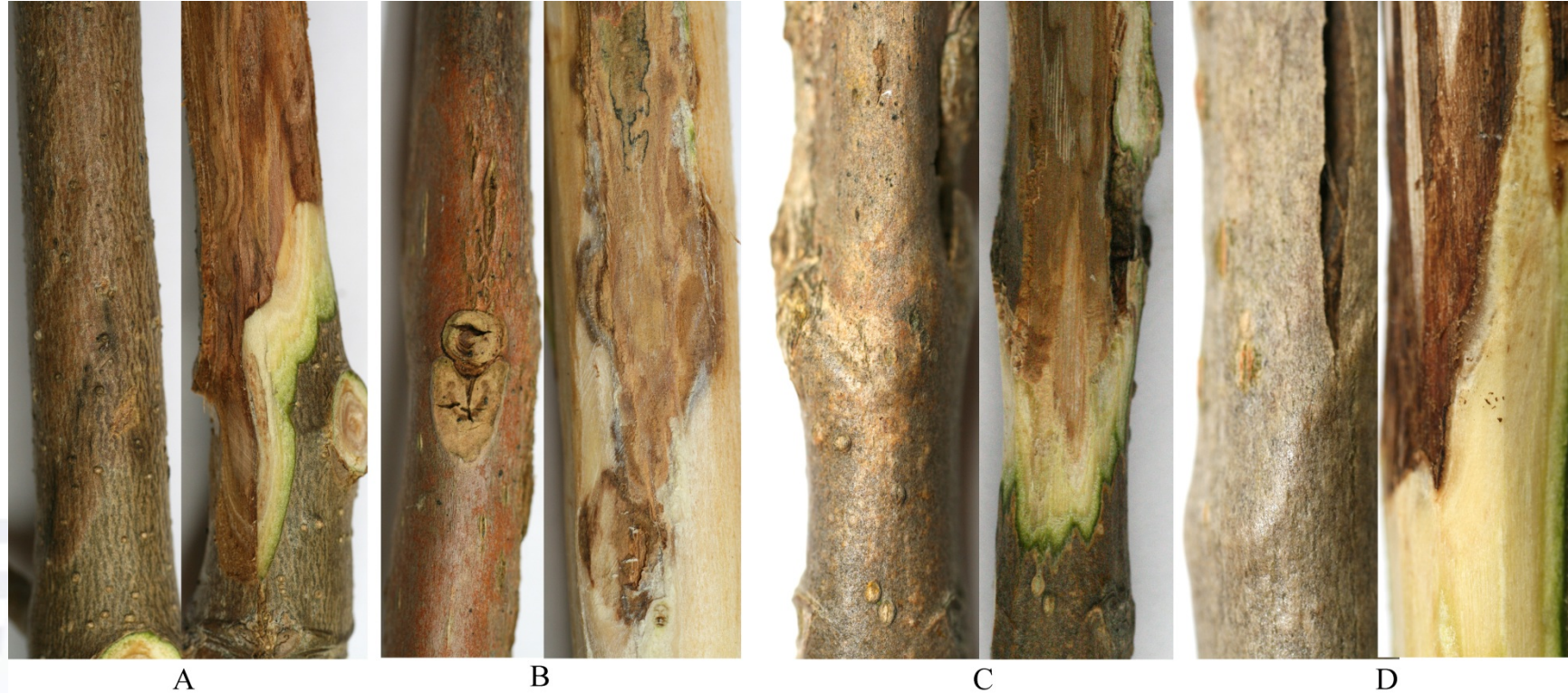




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Necrotic lesions of H. fraxineus on shoots



A - *Fraxinus nigra*, B - *F. pennsylvanica*, C - *F. americana*, D - *F. mandshurica*
(Drenkhan & Hanso 2010).





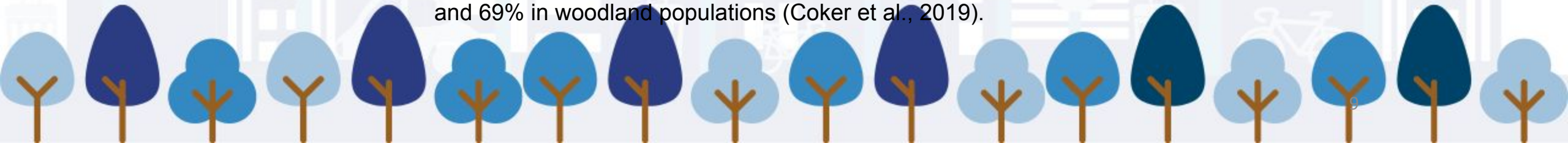
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SYMPTOMS



European ash trees infected with *H. fraxineus* have an estimated mortality rate of 85% in plantations and 69% in woodland populations (Coker et al., 2019).



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SYMPTOMS





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SYMPTOMS





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SYMPTOMS





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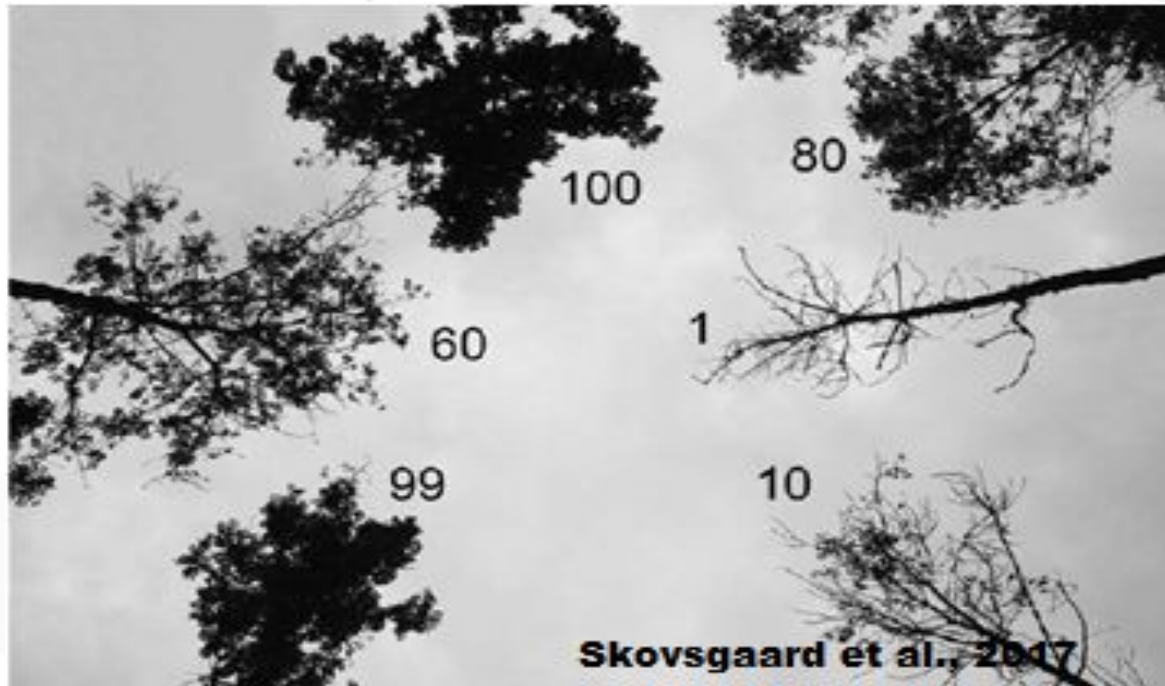
Infection still remains in litter and soil and can infect new shoots after cutting



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MONITORING



- Annual survey of ash stands
- Annual assessment of tree health condition throughout all monitoring plots
 - for forest health: healthy (1); weakened (2); severely weakened (3); drying-up (4); died (5-6);
 - for ADB: (0) no ash dieback-symptoms; (1) symptomatic shoots with necrosis in 10 % of crown; (2) symptomatic shoots with necrosis in 10–50 % of crown; (3) more than 50 % of all shoots are symptomatic and (4) tree mortality (Metzler et al., 2012);
 - Measuring the defoliation and part of stem circumference with collar rot (Skovsgaard et al., 2017)
 - Identification of *H. fraxineus* and other pathogens

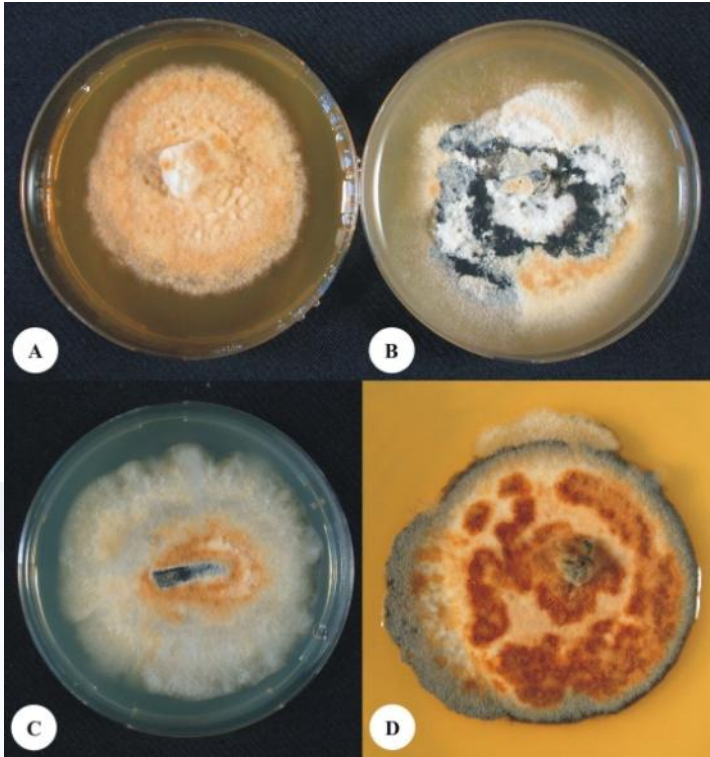




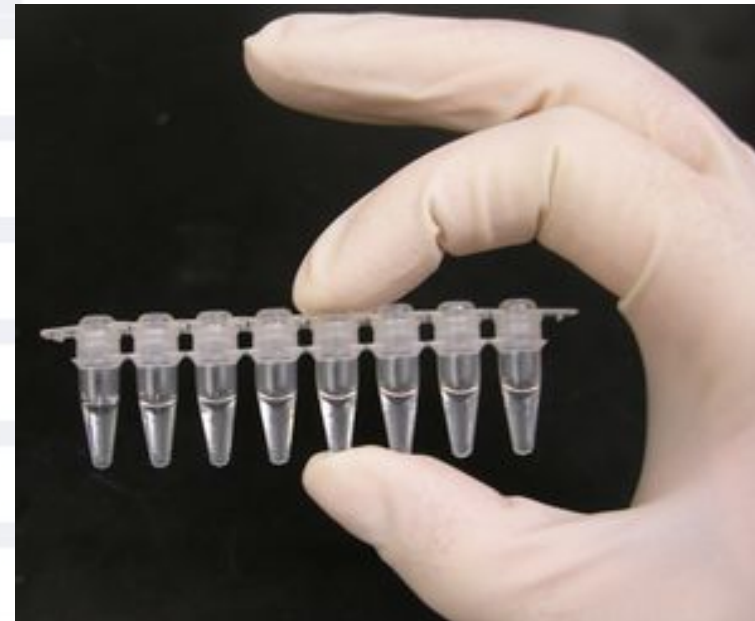
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IDENTIFICATION



Pure cultures, in lab, 2-3 weeks



PCR in lab, 1 day



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Emerald ash borer



LIFE CYCLE OF THE EMERALD ASH BORER

1 Female ash borers lay 40 to 70 eggs on the bark of an ash tree.



After hatching, the larvae bore into the tree layers just below the bark to feed. They remain there for 1 or 2 years, then pupate into adults.

2

Adults, which can fly, then seek out new trees, and the process begins again.

4



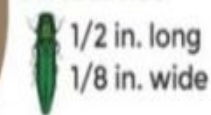
3

The adults then chew a telltale D-shaped exit hole in the bark.

Emerald Ash Borer
(enlarged view)

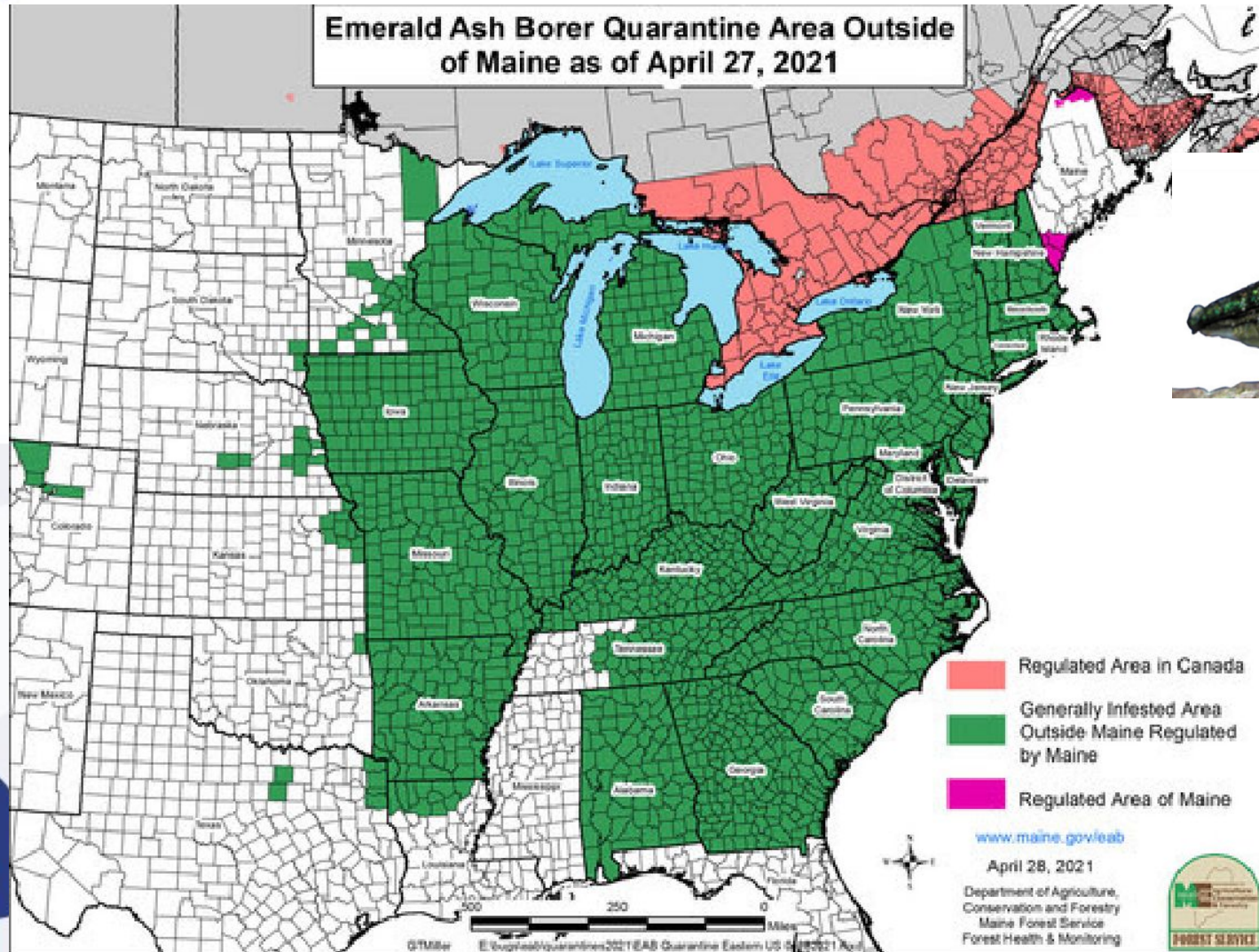


Actual size



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Emerald ash borer in NA

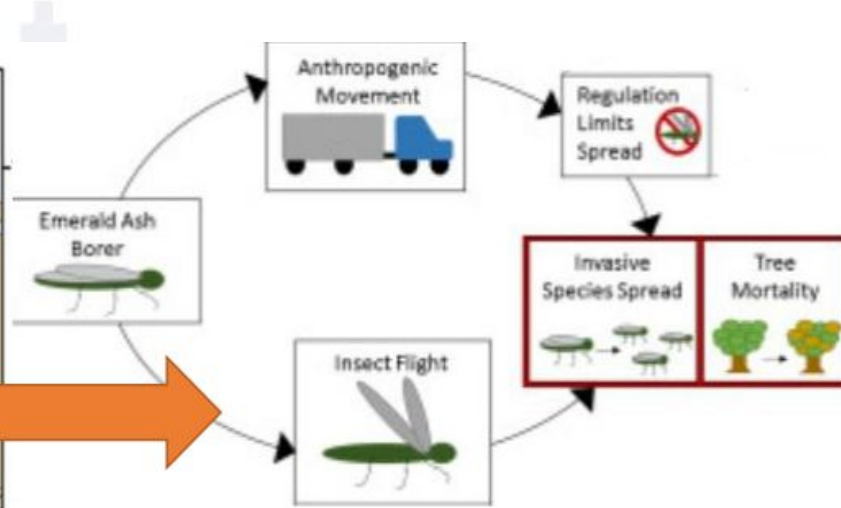
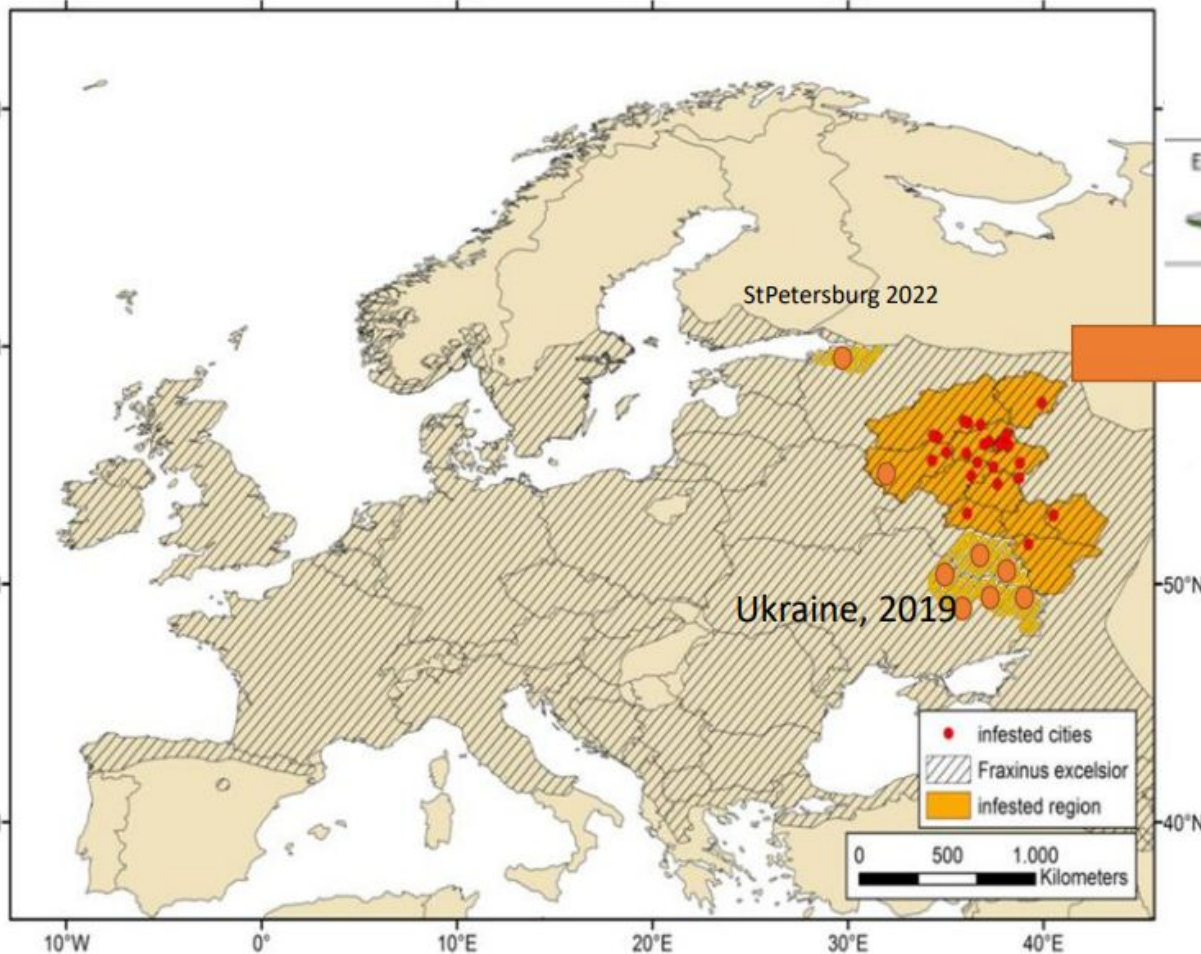


The costs of removal and replacement of ash trees...has been estimated \$ 12.5 billion from 2010 to 2020 (Hale 2021)

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Emerald ash borer in Europe



Spread of Emerald Ash Borer by flight and hitchhiking

(adapted figure: Hope et al. (2021).). Canadian efforts to slow the spread of Emerald Ash Borer (*Agrilus planipennis* Fairmaire) are economically efficient. *Ecological Economics*,





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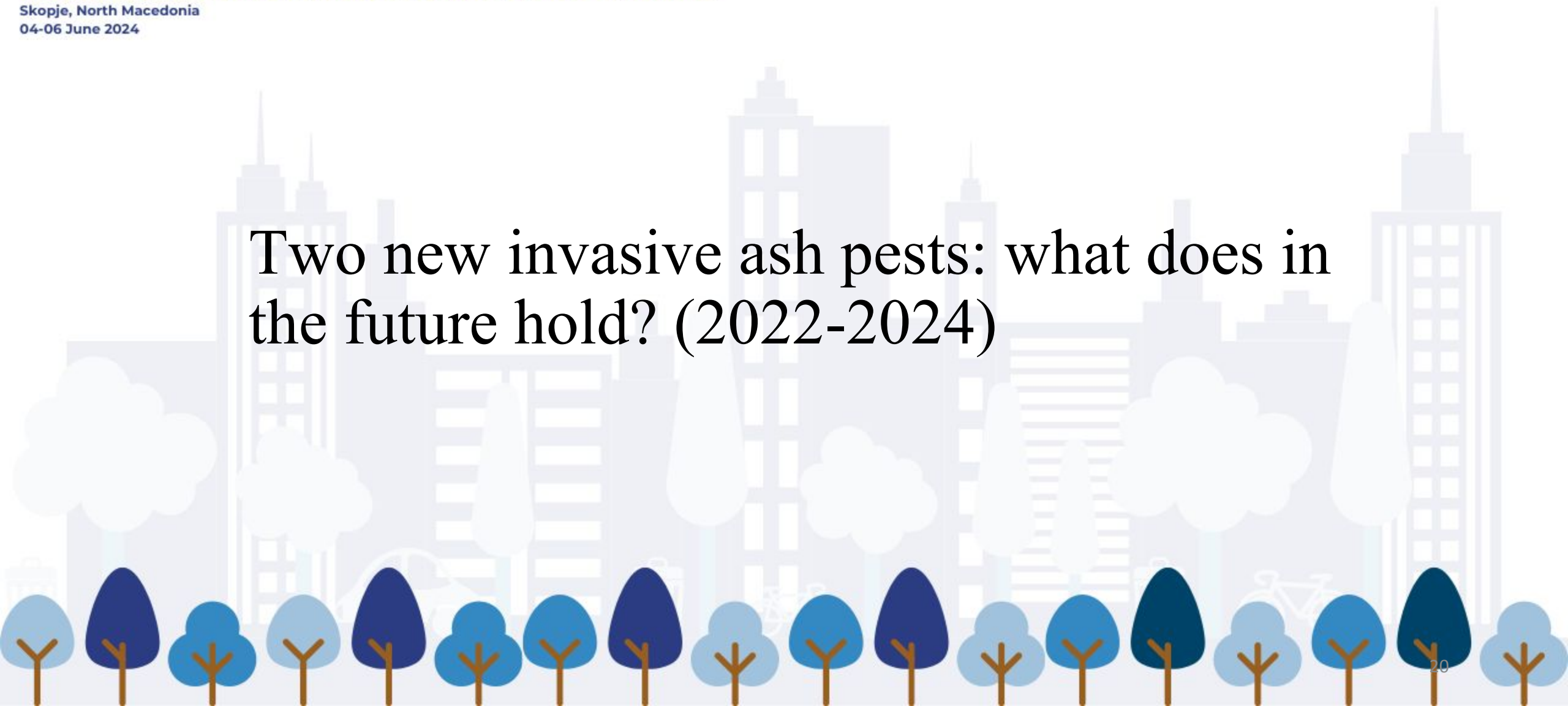


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Two new invasive ash pests: what does in
the future hold? (2022-2024)



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Management and control: to study the possible effects of co-infection of pest and pathogen

- mycobiome of ash trees showing different phenotypic response to ADB or/and EAB and their temporal variation in fungal/pest communities
- to identify the total fungal community and a complex of parasitoids associated with the invasive EAB and their galleries using molecular methods (next-generation sequencing)
- to provide the detailed information on potential agents of biological control and to model the effects of potential biological agents on EAB



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Management and control: to model the combined effect of ADB and EAB on ash

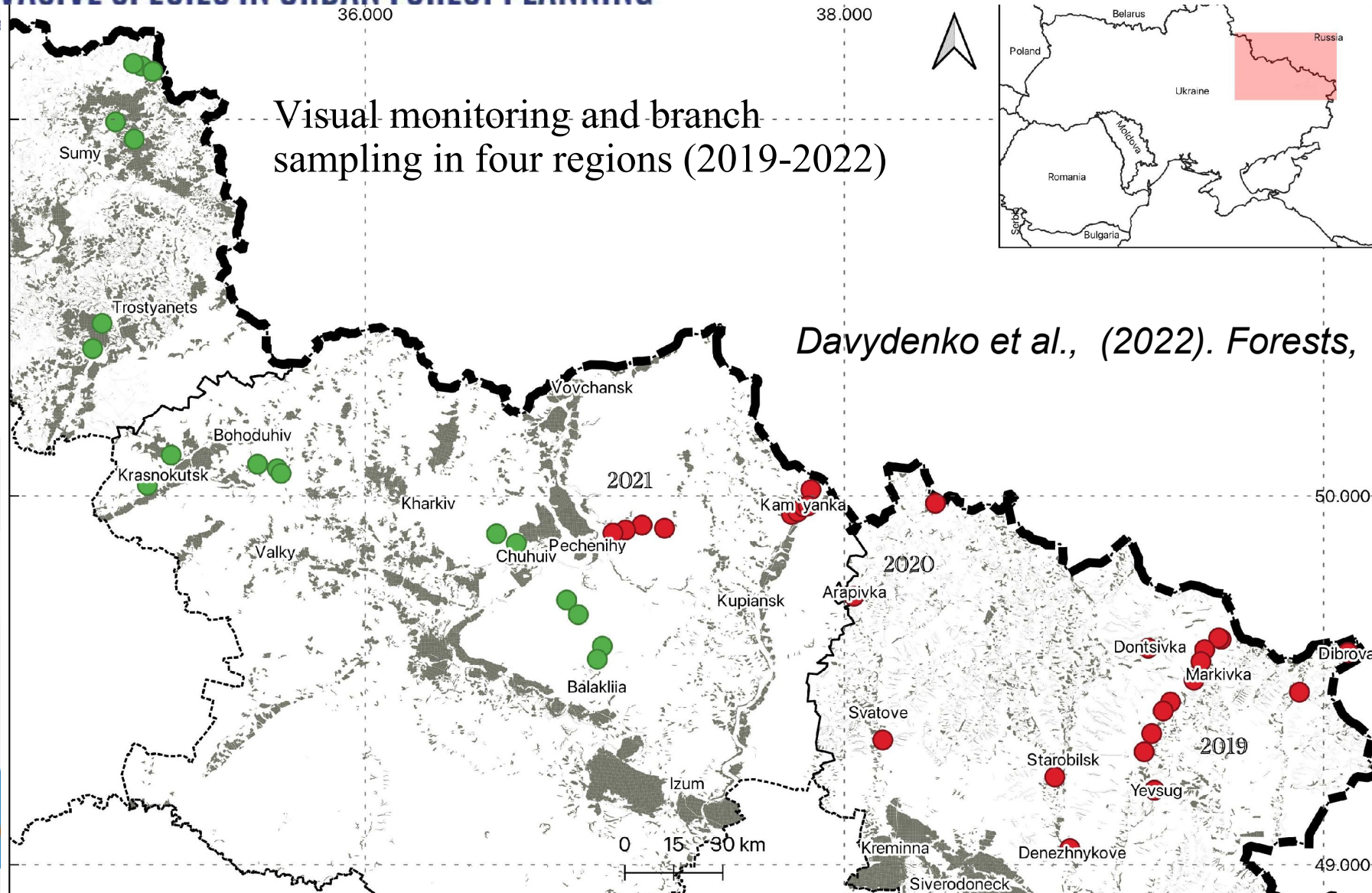
- to compare the traits of EAB seasonal development in different ranges and to reveal the climatic variables that may affect its viability;
- to predict the EAB expansion range in Ukraine and westward;
- to compare the most significant parameters outside the current EAB range with those in the native and invasive ranges.





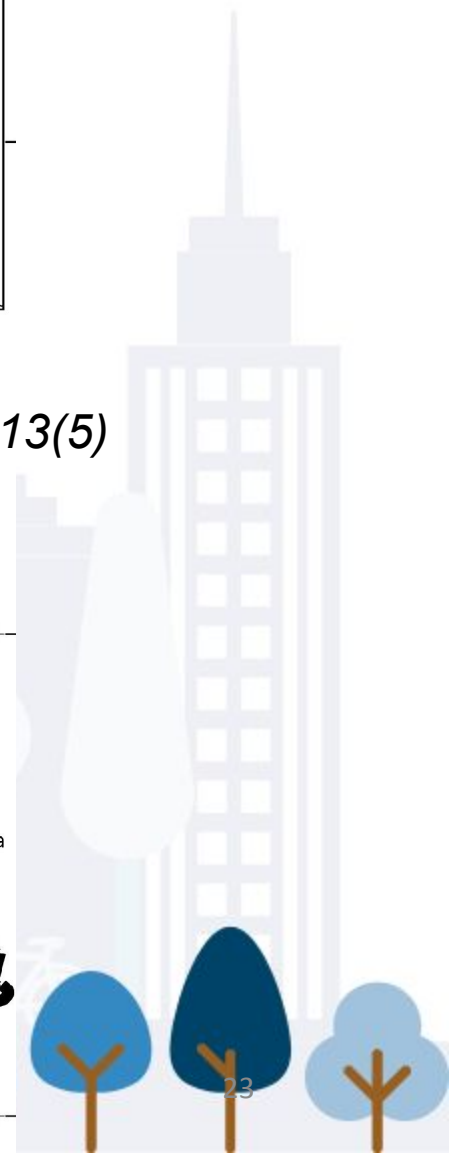
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Visual monitoring and branch sampling in four regions (2019-2022)

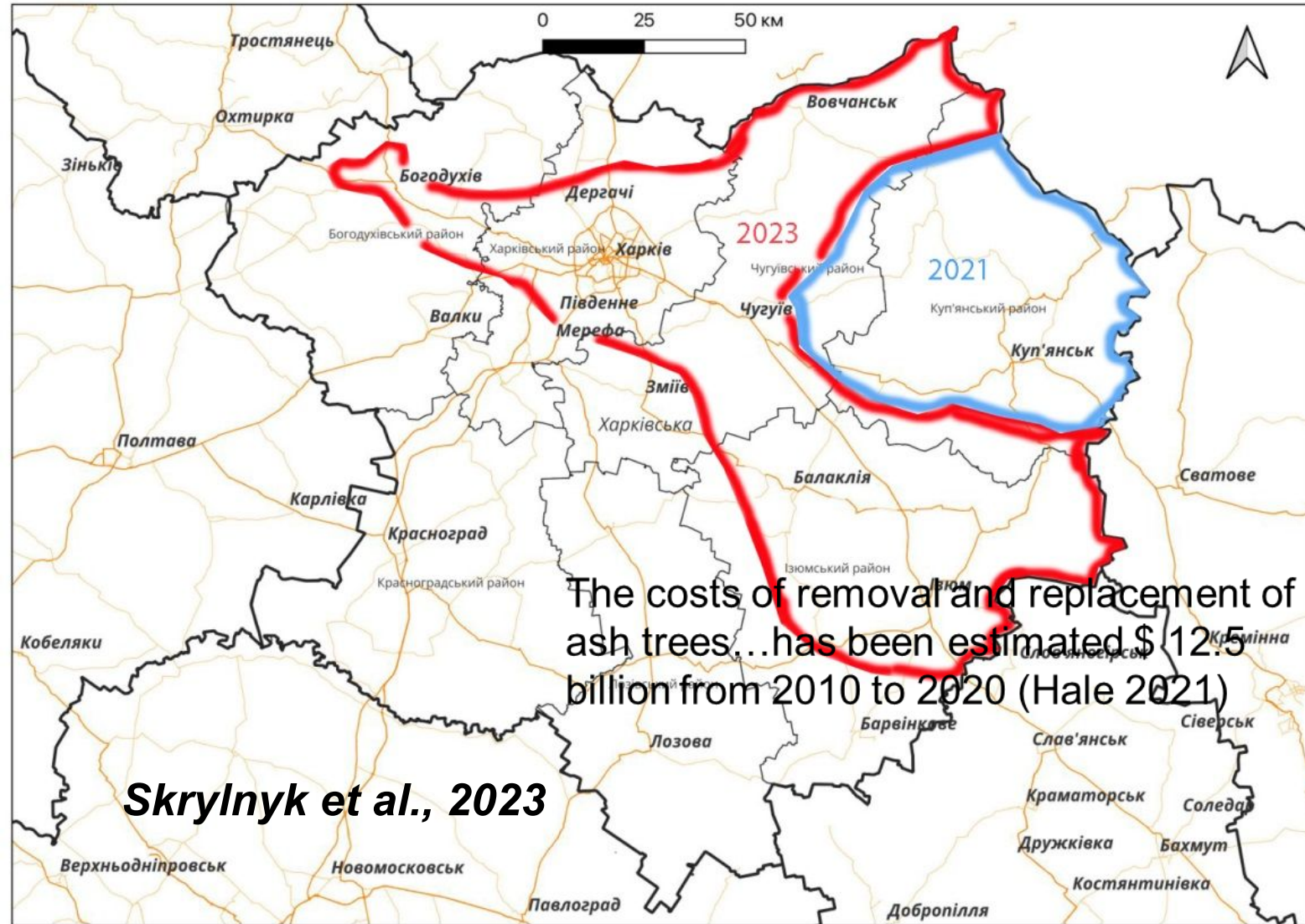
Davydenko et al., (2022). Forests, 13(5)



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Visual
monitoring
and branch
sampling in
2023



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Infestations of the ADB and EAB on *Fraxinus pennsylvanica* and *F. excelsior* trees in eastern Ukraine

Plot	<i>Fraxinus</i> spp.	Trees Monitored, no. (%)										
		All	ADB	EAB	Year 2020 Of those, ADB and EAB	Dead ^a	Visually Healthy	ADB	EAB	Year 2021 Of Those, ADB and EAB	Dead ^a	Visually Healthy
Luhansk region (LH)												
1LH	<i>F. pen.</i> ^b	38	1 (3)	19 (50)	0	12 (32)	18 (47)	2 (5)	37 (97)	1 (3)	29 (76)	0
2LH	<i>F. pen.</i>	25	0	16 (64)	0	7 (28)	9 (36)	1 (4)	22 (88)	0	14 (56)	2 (8)
3aLH	<i>F. pen.</i>	25	0	21 (84)	0	9 (36)	4 (16)	0	25 (100)	0	17 (68)	0
All LH <i>F. pen.</i>		88	1 (1)	56 (64)	0	28 (32)	31 (35)	3 (3.5)	84 (95)	1 (1)	60 (68)	2 (2)
3bLH, all LH <i>F. ex.</i> ^c		16	4 (25)	3 (19)	2 (13)	2 (13)	11 (69)	6 (38)	7 (44)	3 (19)	7 (44)	6 (38)
χ^2 test <i>F. pen.</i> vs. <i>F. ex.</i> ^d			***	**		n.s.	*	****	****	**	n.s.	****
Kharkiv region (KH, northwest from LH)												
4KH	<i>F. ex.</i>	60	-	-	-	-	-	15 (25)	17 (28)	9 (15)	9 (15)	37 (62)
5KH	<i>F. ex.</i>	55	-	-	-	-	-	18 (33)	12 (22)	6 (11)	9 (16)	31 (56)
6KH	<i>F. pen.</i>	52	-	-	-	-	-	7 (13)	31 (60)	4 (8)	23 (44)	18 (35)
7KH	<i>F. pen.</i>	45	-	-	-	-	-	3 (7)	24 (53)	2 (4)	19 (42)	20 (44)
All KH <i>F. pen.</i>		97	-	-	-	-	-	10 (10)	55 (57)	6 (6)	42 (43)	38 (39)
All KH <i>F. ex.</i>		115	-	-	-	-	-	33 (29)	29 (25)	15 (13)	18 (16)	68 (59)
χ^2 test <i>F. pen.</i> vs. <i>F. ex.</i>								**	****	n.s.	****	**
Sumy region (SU, northwest from KH)												
8SU	<i>F. ex.</i>	50	-	-	-	-	-	32 (64)	-	-	31 (62)	18 (36)
9SU	<i>F. ex.</i>	50	-	-	-	-	-	27 (54)	-	-	19 (38)	23 (46)
10SU, all SU <i>F. pen.</i>		25	-	-	-	-	-	8 (32)	-	-	5 (20)	17 (68)
All SU <i>F. ex.</i>		100	-	-	-	-	-	59 (59)	-	-	50 (50)	41 (41)
χ^2 test <i>F. pen.</i> vs. <i>F. ex.</i>								*			**	*
All plots (LH + KH + SU)												
All <i>F. pen.</i>		210	-	-	-	-	-	21 (10)	139 (66)	7 (3)	107 (51)	57 (27)
All <i>F. ex.</i>		231	-	-	-	-	-	98 (42)	36 (16)	18 (8)	75 (32)	115 (50)
χ^2 test <i>F. pen.</i> vs. <i>F. ex.</i>								****	****	*	****	****
Plots infested by the emerald ash borer (LH + KH)												
LH + KH <i>F. pen.</i>		185	-	-	-	-	-	13 (7)	139 (75)	7 (4)	102 (55)	40 (22)
LH + KH <i>F. ex.</i>		131	-	-	-	-	-	39 (30)	36 (27)	18 (14)	25 (19)	74 (56)
χ^2 test <i>F. pen.</i> vs. <i>F. ex.</i>								****	****	**	****	****

EAB distribution model

- The geographic distribution of EAB was predicted based on the ecological niche model devised in MaxEnt software, version 3.4.4

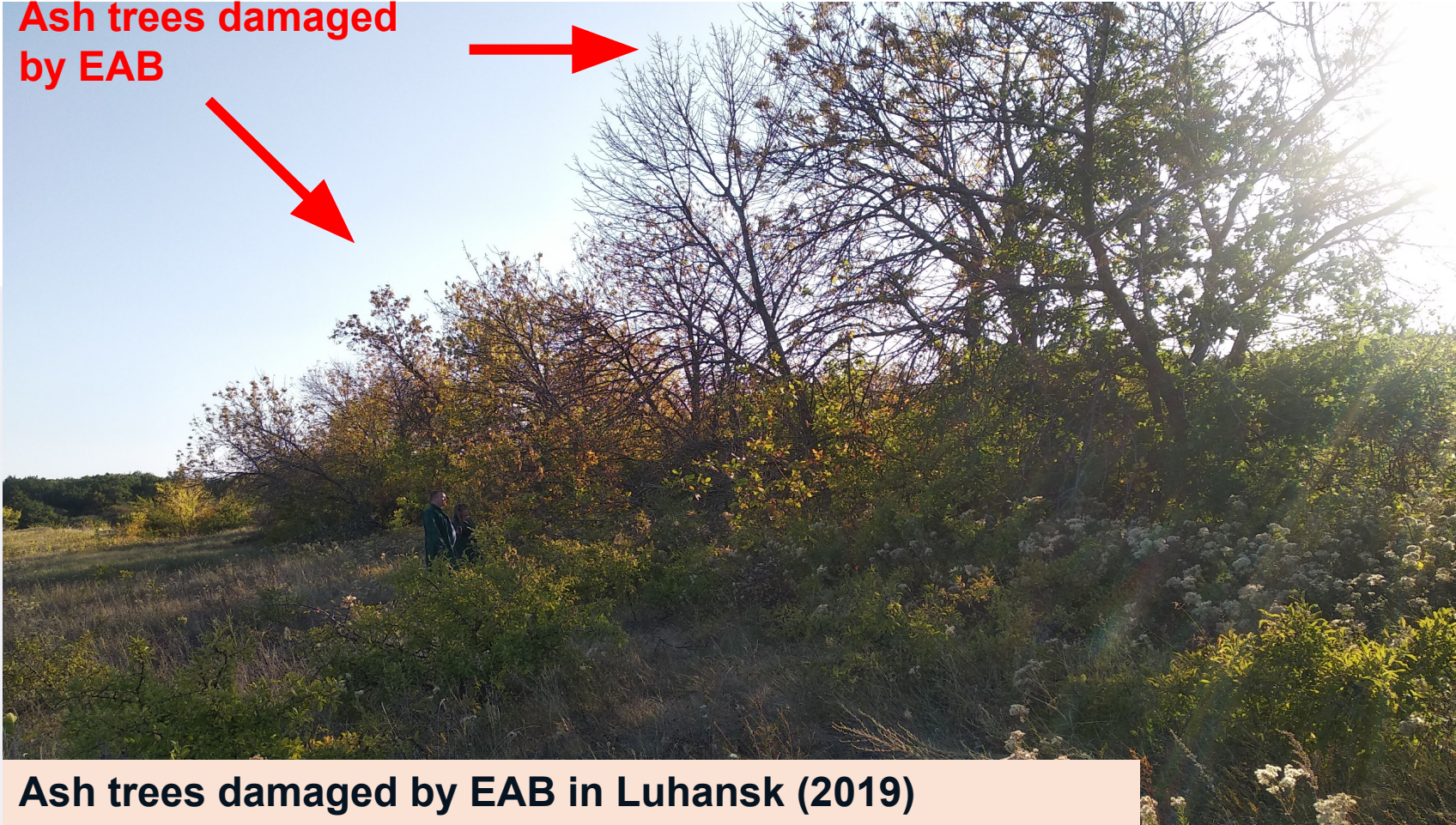
Variable	Variable name	Definition		
Bio_1, °C	Mean annual temperature	Annual mean temperature	Bio_11, °C	Mean temp. of the coldest quarter Average temperature for the three coldest months
Bio_2, °C	Mean diurnal range	The average difference between high and low daily temperature	Bio_12, mm	Annual precipitation Total annual precipitation
Bio_3, dimensionless	Isothermality	The ratio of the mean diurnal temperature range relative to the seasonal range	Bio_13, mm	Precipitation of the wettest month Total precipitation for the month with the most precipitation
Bio_4, °C	Temperature seasonality	Temperature variation over a year by monthly average temperature	Bio_14, mm	Precipitation of the driest month Total precipitation for the month with the least precipitation
Bio_5, °C	Max temp. of the warmest month	Monthly mean of daily high temperatures for the hottest month	Bio_15, fraction	Precipitation seasonality Precipitation variation over a year by monthly total precipitation
Bio_6, °C	Min temp. of the coldest month	Monthly mean of daily low temperatures for the coldest month	Bio_16, mm	Precipitation of the wettest quarter Total precipitation for the three months with the most precipitation
Bio_7, °C	Temperature annual range	Bio_07 = Bio_05 - Bio_06	Bio_17, mm	Precipitation of the driest quarter Total precipitation for the three months with the least precipitation
Bio_8, °C	Mean temp. of the wettest quarter	Average temperature for the three months with the most precipitation	Bio_18, mm	Precipitation of the warmest quarter Total precipitation for the three hottest months
Bio_9, °C	Mean temp. of the driest quarter	Average temperature for the three months with the least precipitation	Bio_19, mm	Precipitation of the coldest quarter Total precipitation for the three coldest months
Bio_10, °C	Mean temp. of the warmest quarter	Average temperature for the three hottest months	Elev, m a.s.l.	Elevation Elevation (altitude)



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Ash trees damaged
by EAB



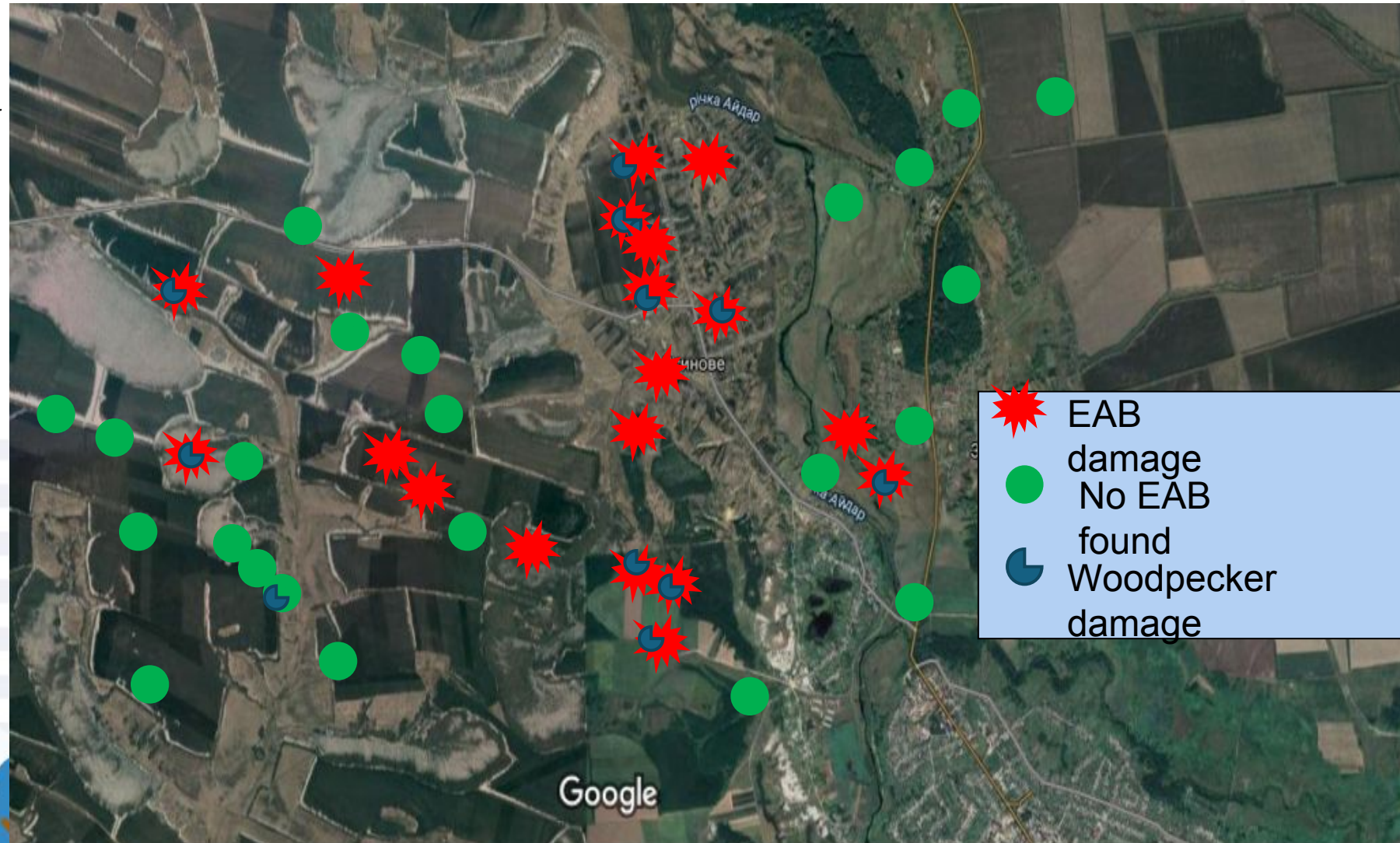
Ash trees damaged by EAB in Luhansk (2019)



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First field survey in Ukraine (2020)





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**Ash trees damaged by
EAB in Kharkiv
(Central park) in 2023
(photo by Y.Skrylnyk)**







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Management and control: to identify resistance of ash trees to EAB and ADB

- to develop markers for traits related to tolerance to ADB and EAB and to investigate whether genotypes selected for tolerance were genetically different from susceptible wild populations

Convergent molecular evolution among ash species resistant to the emerald ash borer

Laura J. Kelly^{1,2}✉, William J. Plumb^{1,2,3}, David W. Carey⁴, Mary E. Mason⁵✉, Endymion D. Cooper¹, William Crowther^{1,6}, Alan T. Whittemore⁵, Stephen J. Rossiter¹, Jennifer L. Koch⁴✉ and Richard J. A. Buggs^{1,2}✉

Recent studies show that molecular convergence plays an unexpectedly common role in the evolution of convergent phenotypes. We exploited this phenomenon to find candidate loci underlying resistance to the emerald ash borer (EAB, *Agrilus planipennis*), the United States' most costly invasive forest insect to date, within the pan-genome of ash trees (the genus *Fraxinus*). We show that EAB-resistant taxa occur within three independent phylogenetic lineages. In genomes from these resistant lineages, we detect 53 genes with evidence of convergent amino acid evolution. Gene-tree reconstruction indicates that, for 48 of these candidates, the convergent amino acids are more likely to have arisen via independent evolution than by another process such as hybridization or incomplete lineage sorting. Seven of the candidate genes have putative roles connected to the phenylpropanoid biosynthesis pathway and 17 relate to herbivore recognition, defence signalling or programmed cell death. Evidence for loss-of-function mutations among these candidates is more frequent in susceptible species than in resistant ones. Our results on evolutionary relationships, variability in resistance, and candidate genes for defence response within the ash genus could inform breeding for EAB resistance, facilitating ecological restoration in areas invaded by this beetle.

..to find candidate loci underlying resistance to the emerald ash borer... In genomes from these resistant lineages, we detect 53 genes with evidence of convergent amino acid evolution...., Seven of the candidate genes connected to the phenylpropanoid biosynthesis pathway and 17 relate to herbivore recognition, defence signalling or programmed cell death. (Kelly et al. 2020)

A high-quality reference genome for *Fraxinus pennsylvanica* for ash species restoration and research

Matt Huff¹ | Josiah Seaman^{2,3} | Di Wu⁴ | Tetyana Zhebentyayeva⁴✉ | Laura J. Kelly^{2,3} | Nurul Faridi^{5,6} | Charles D. Nelson^{5,7} | Endymion Cooper² | Teodora Best⁴ | Kim Steiner⁴ | Jennifer Koch⁸ | Jeanne Romero Severson⁹ | John E. Carlson⁴ | Richard Buggs^{2,3} | Margaret Staton¹✉

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³Royal Botanic Gardens, Richmond, Surrey, UK

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⁵USDA Forest Service, Southern Research Station, Saucier, Mississippi, USA

⁶Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas, USA

⁷Forest Health Research and Education Center, University of Kentucky, Lexington, Kentucky, USA

⁸United States Department of Agriculture, Forest Service, Northern Research Station, Delaware, Ohio, USA

⁹Department of Biological Sciences, Notre Dame University, Notre Dame, Indiana, USA

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Funding Information

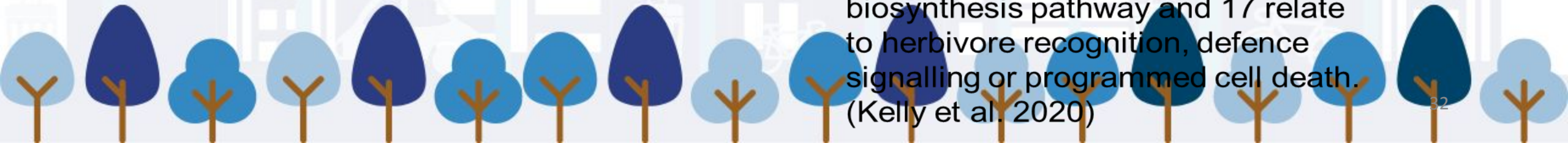
Erica Waltraud Albrecht Endowment Fund; National Institute of Food and Agriculture, Grant/Award Number: PEN04532; Living with Environmental Change (LWEC) Tree Health and Plant

Abstract

Green ash (*Fraxinus pennsylvanica*) is the most widely distributed ash tree in North America. Once common, it has experienced high mortality from the non-native invasive emerald ash borer (EAB; *Agrilus planipennis*). A small percentage of native green ash trees that remain healthy in long-infested areas, termed "lingering ash," display partial resistance to the insect, indicating that breeding and propagating populations with higher resistance to EAB may be possible. To assist in ash breeding, ecology and evolution studies, we report the first chromosome-level assembly

the identification of candidate genes for important traits including potential EAB-resistance genes, and an investigation of comparative genome organization among Asterids based on this reference genome platform (Huff et al. 2021).

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Identification resistance and less susceptible ash trees

1. Visual monitoring

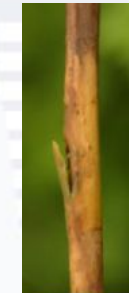
Less susceptible



Most susceptible

2. Inoculation

3. Data analysis



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Take-home messages

- International strategy with regular updates!
- Still gather science information (EAB +ADB)
- More research is required to identify ash genotypes possessing resistance to both ADB and EAB

what frequency of ADB resistance trees remains tolerant to EAB and

whether combined EAB and ADB attacks are expected to be more lethal?

individuals .



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Take-home messages



ADB + EAB =



What future awaits our ash trees?



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ACKNOWLEDGEMENT

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FORMAS 

