Results of a worldwide trapping program using generic lures to detect cerambycid invaders at arrival on other continents

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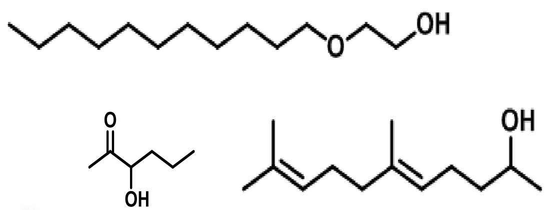




4th IFOPE Conference on pest invasions in Eurasian Forests, 18/01/2023

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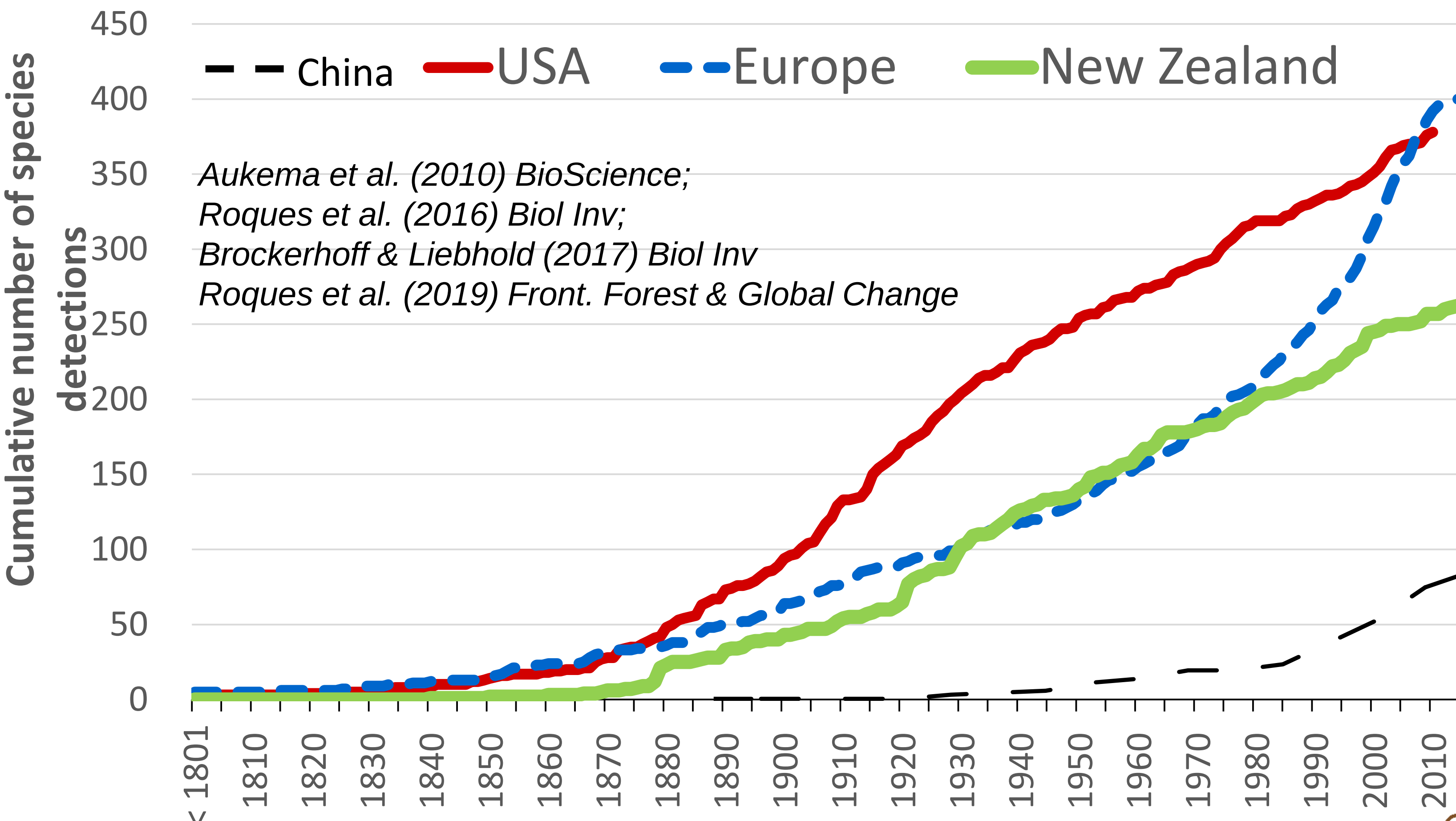
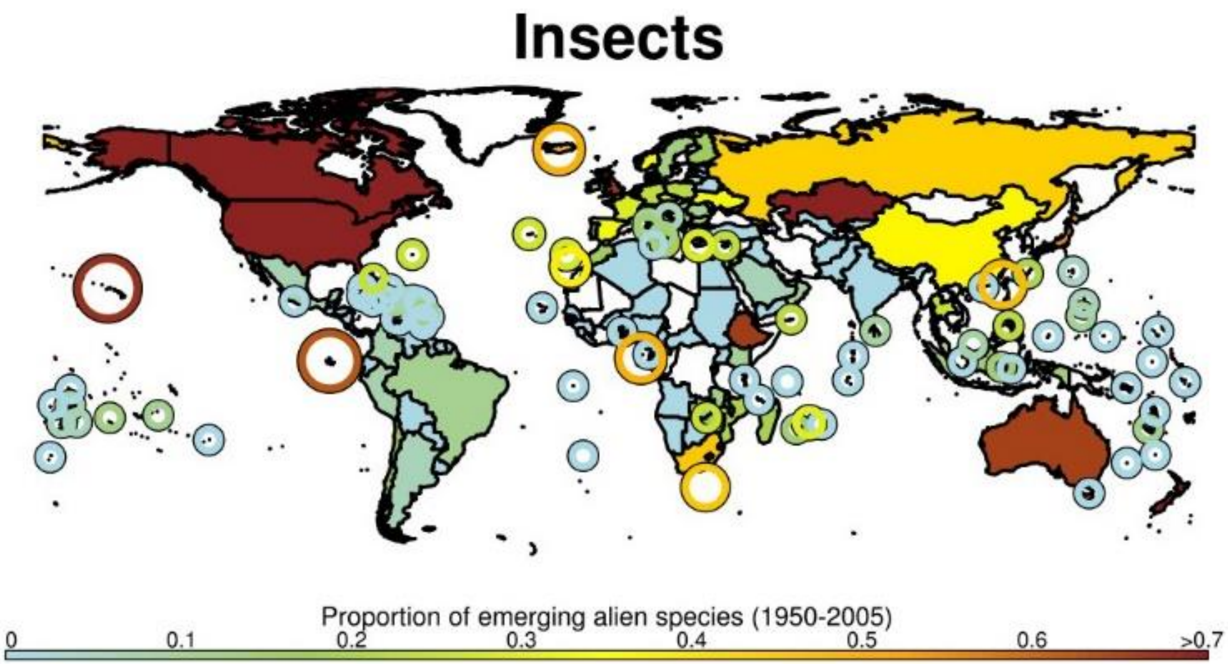
Establishments of non-native species associated to woody plants

An exponential or a linear increase in all regions despite strong differences in border controls



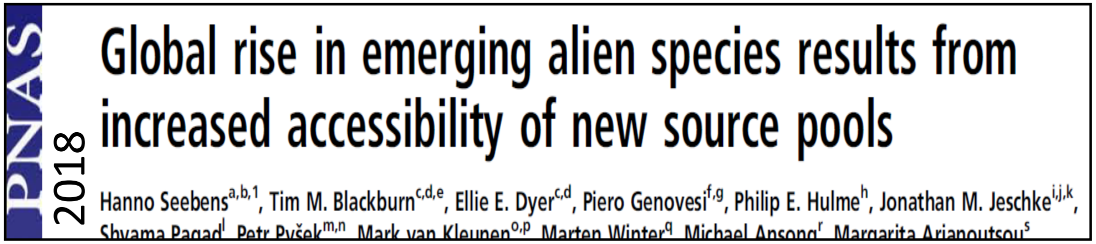
A new key pattern: a large proportion of recent invaders are « emerging », « unknown » species

« Emerging » invaders or « first-time » invaders = *species never found before outside their native range*



Arrival of new pools of invaders likely resulting from increasing access to these pools through

- **new trade routes**
- **new pathways including ornamental and horticultural trade**
- **changing climatic conditions**


PNAS 2018

Global rise in emerging alien species results from increased accessibility of new source pools

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Some recent woody plant insect invaders in Europe
Indicators of the diversity of the source pools

Contarinia pseudotsugae
Pityophthorus juglandis
Thaumastocoris peregrinus

Xylosandrus compactus
Platynota stultana
Batrachedra enormis
Lopholeucaspis japonica

Aromia bungii
Xylotrechus chinensis
Popilia japonica

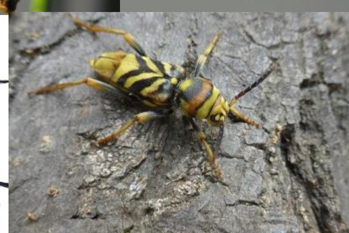
Octodonta nipae
Trachymela sloanei
Nematus lipovskyi
Neophyllaphis podocarpi

Among them, the « emergent » species


Contarinia pseudotsugae
Pityophthorus juglandis



Platynota stultana
Batrachedra enormis
Lopholeucaspis japonica

Aromia bungii
Xylotrechus chinensis




Octodonta nipae
Trachymela sloanei
Nematus lipovskyi
Neophyllaphis podocarpi

The big challenge:
Being capable of detecting the unknown « emerging » species upon arrival at ports of entry

- The NPPO quarantine lists or the lists of invaders in other continents are not very useful- most of these emergent species are not showing large damage in native areas (natural ennemies, coevolution with hosts, …)

- Detection cannot rely on species- specific lures since the species are unknown

- ***A possible strategy: design lures with potential generic attractiveness for xylophagous groups to carry out automatic trappings at ports-of-entry***

- A group of xylophagous species well adapted for developing such an approach: long-horned beetles- Cerambycidae

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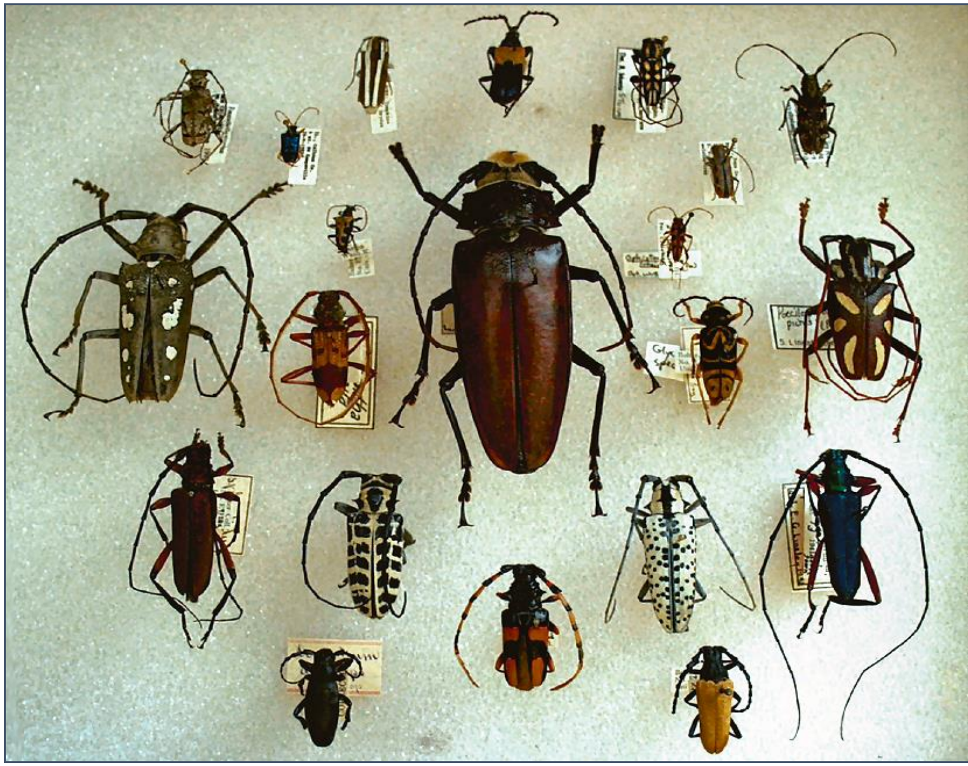
Why long-horned beetles ?

- >38,000 species described (http://titan.gbif.fr)
- Among the most damaging insect pests worldwide:
  - Forestry and plantations
  - Orchards
  - Ornamentals
  - Lumber and wooden structures



- Many species highly invasive

Damage of recent long-horned beetle invaders in Europe

Xylotrechus chinensis
China to Europe
Damage to mulberry trees

Saperda candida
North America to Europe
Damage to apple trees

Aromia bungii
China to Europe
and Japan
Damage to
Prunus trees

Since the late 1980s, large progresses in chemical ecology of cerambycids

> 400 pheromones (or pheromone-like) identified

Subfamily	Type	Producing sex	# Examples	Amount produced
Cerambycinae	Sex-Aggregation	Male*	>>200	Large >100µg per ♂
Lamiinae	Sex-Aggregation	Male	Many	Large >100µg per ♂
Spondylidinae	Sex-Aggregation	Male	~20	Large >100µg per ♂
Prioninae	Sex	Female	~30	Very small
Lepturinae	Sex	Female	~5	Very small

∗ A South African Cerambycinae has a female produced sex pheromone.

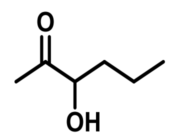
Millar & Hanks, 2017

A strong hope for getting a generic lure:

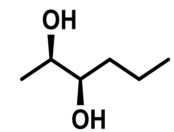
The pheromone structures seem highly conserved at world level among phylogenetically- related species

i.e., pheromones of European spp. could attract Asian or North American congeners and vice-versa

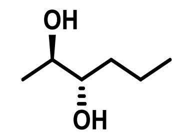
- ❑ **Hydroxyketones and diols** (short-chain 6–10 carbon)
  Pheromones/ likely pheromones
  for many species in Cerambycinae
  subfamily from all continents
- ❑ **2- Methylbutanol**
  Callidini tribe of Cerambycinae in North America
  and Europe
- ❑ **Fuscumol, fuscumol acetate, geranyl acetone**
  Many spp. in subfamilies Lamiinae and
  Spondylidinae from all continents
- ❑ **Monochamol**
  Many *Monochamus* spp. from North America,
  Asia, Europe and other spp. in several related
  genera
- ❑ **Prionic acid**
  Many species in the Prioninae tribe Prionini from
  North America, Asia and Europe



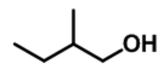
3R*-3-hydroxy-2-hexanone



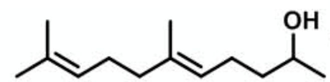
2R*,3R*-hexanediol



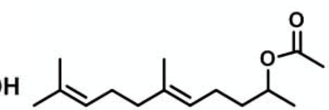
2R*,3S*-hexanediol



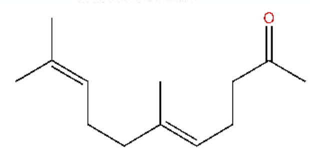
2-Methylbutanol



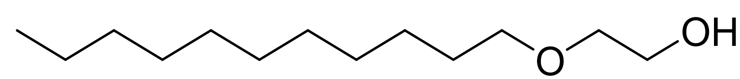
Fuscumol



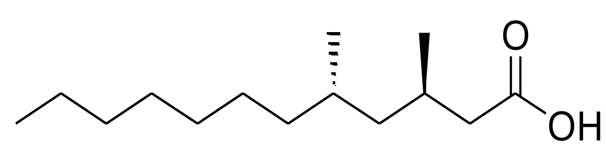
Fuscumol acetate



Geranylacetone



[2-(undecycloxy)-ethanol]- Monochamol

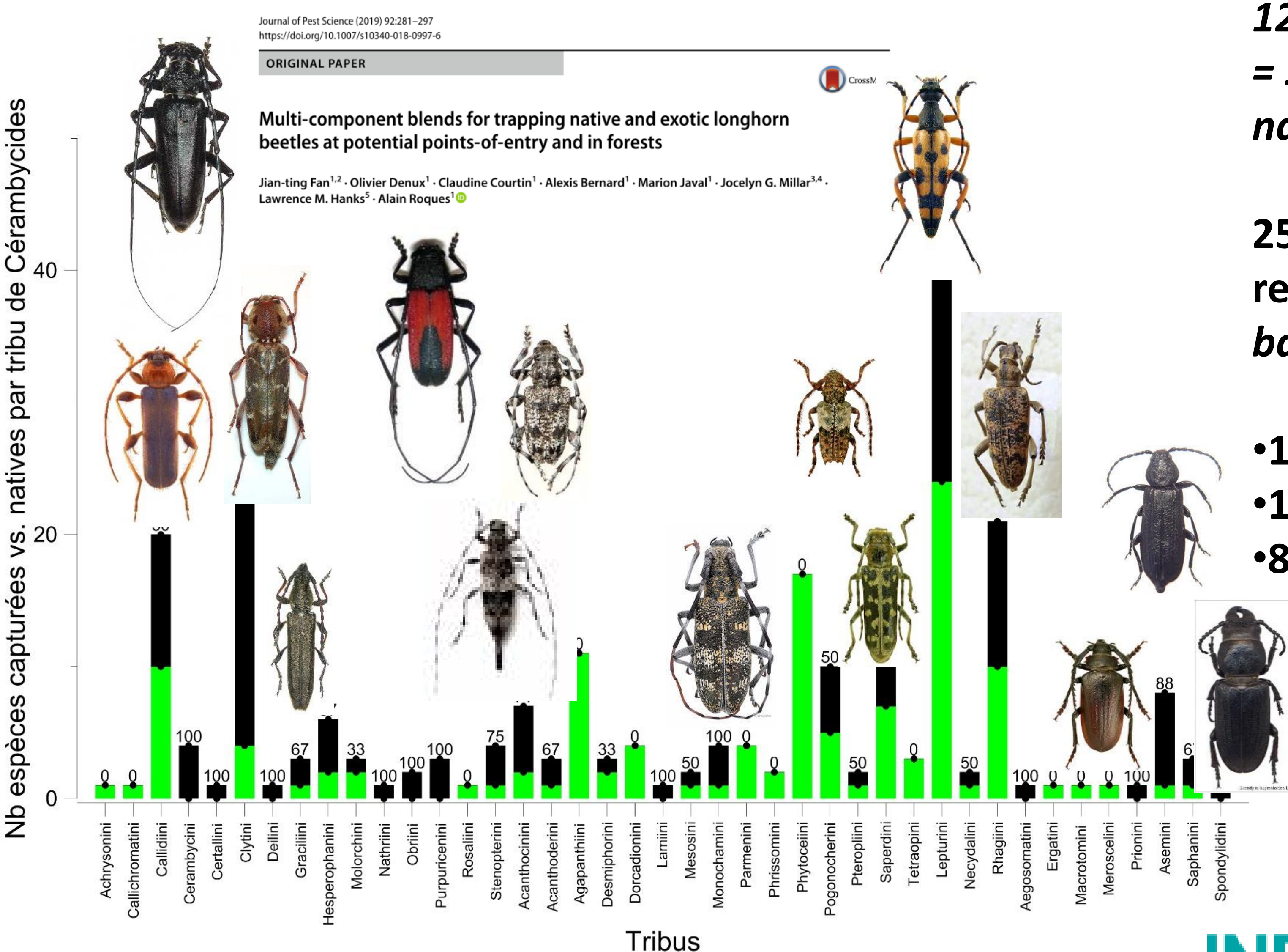


([3*R*,5*S*]-3,5-dimethyldodecanoic acid- Prionic acid

Combining 8 of these pheromones into one blend expected to result in simultaneous trapping of species from different tribes and subfamilies …*if no antagonisms*

Compound	Amount/lure (mg/ml)	Target Sex	Target tribe	Subfamily	Refs
Fuscumol + Fuscumol Acetate	50+ 50	M/F	Asemini	Aseminae	Millar et al. 2018
			Acanthocinini	Lamiinae	Millar et al. 2018
			Acanthoderini	Lamiinae	Hanks and Millar 2013
			Obriini	Cerambycinae	Millar et al. 2018
Geranyl acetone	25	M/F	Asemini	Aseminae	Halloran et al. 2018
			Acanthocinini	Lamiinae	Meier et al. 2016, 2019
Monochamol	50	M/F	Lamiini	Lamiinae	Wickham et al. 2014
			Monochamiini	Lamiinae	Hanks et al. 2018
3-hydroxyhexan-2-one (C6-ketol)	50	M/F	Callidiini	Cerambycinae	Millar et al. 2018
			Clytini	Cerambycinae	Hanks and Millar 2013; Wickham et al. 2014;Bobadoye et al. 2019
			Hesperophanini		unpub. data JGM
			Hylotrupini	Cerambycinae	Reddy et al. 2005
			Molorchini	Cerambycinae	none
Prionic acid	0.5	M	Prionini	Prioninae	Barbour et al. 2011
2-methylbutan-1-ol	50	M/F	Callidiini	Cerambycinae	Hanks et al. 2018
2R*,3S*-hexanediol	50	M/F	Clytini	Cerambycinae	Hanks and Millar 2013, Wickham et al. 2014

Preliminary tests in France during 2014- 2018 confirmed the high generic attractiveness of the 8- component blend



126 spp. trapped = 51.9% of the native fauna!

25 / 42 tribes well represented *(black bars)*

- **10 tribes at 100%**
- **15 tribes > 50%**
- **8 exotic species**

Marginal antagonist effects

A standardized worldwide trapping program using the 8-pheromone blend developed during 2018-2021

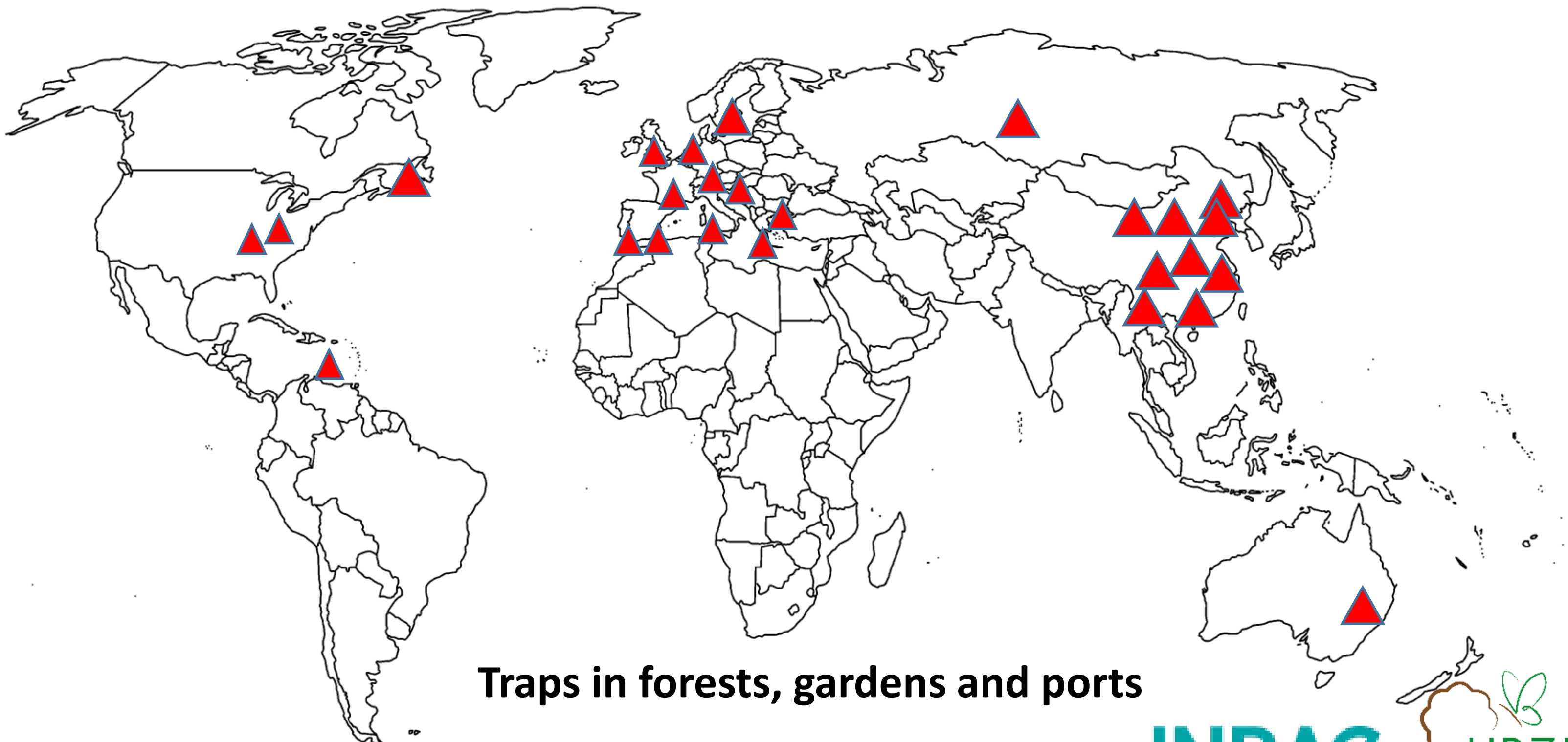
The hypotheses:

1- ***if a species is regularly trapped in significant numbers by the blend on a continent, that increases the probability that it can be detected upon arrival in other countries/continents***

2- ***if the blend shows an effective, generic attractiveness to multiple species, because of the high degree of conservation of pheromone structures within related taxa, it is likely that previously unknown and unanticipated species also could be trapped***

A trapping network of 1308 traps deployed at 302 sites in the world

- 244 sites in Europe- 13 countries,
- 38 in Asia- 35 in 10 provinces of China and 3 in Siberia,
- 11 in North America- 10 in the USA and 1 in Canada,
- 5 in the Carribean (Martinique)
- 4 in Australia



Traps in forests, gardens and ports

A standardized design:
Mostly 12- funnel black traps coated with fluon

8-pheromone blend
(1 ml on dental pad)
Changed every 3 weeks

802 Black traps

Ethanol

α-pinene

Plant volatiles for synergy and simultaneous trapping of bark beetles

A few variations

284 Green	37 Green/ Black	63 Purple	44 Yellow

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In press

NeoBiota @@: #–## (2022)
doi: 10.3897/neobiota.@@.91096
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Worldwide tests of generic attractants, a promising tool for early detection of non-native cerambycid species

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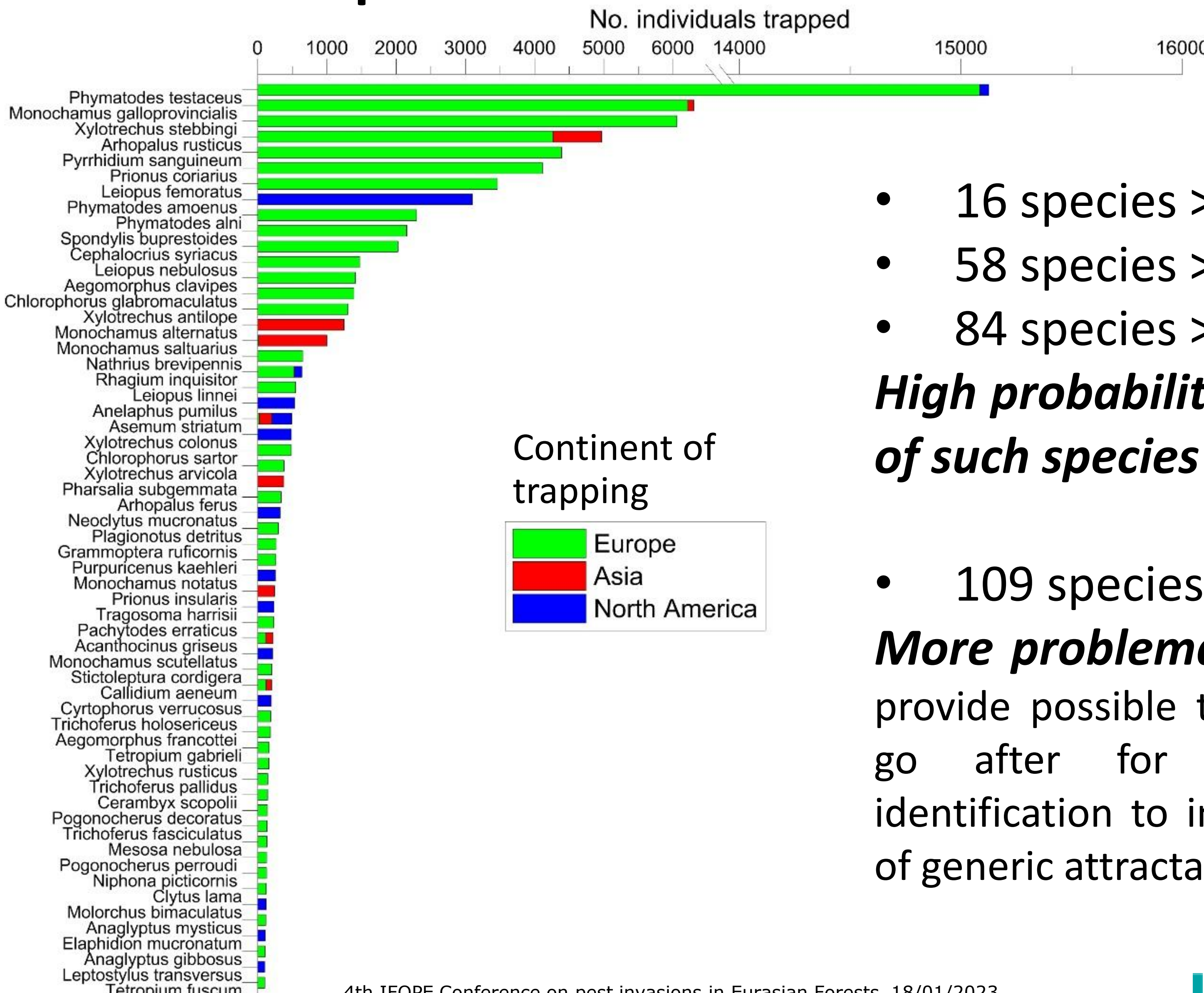
76891 cerambycid beetles trapped in total
376 species of 8 subfamilies and 60 tribes



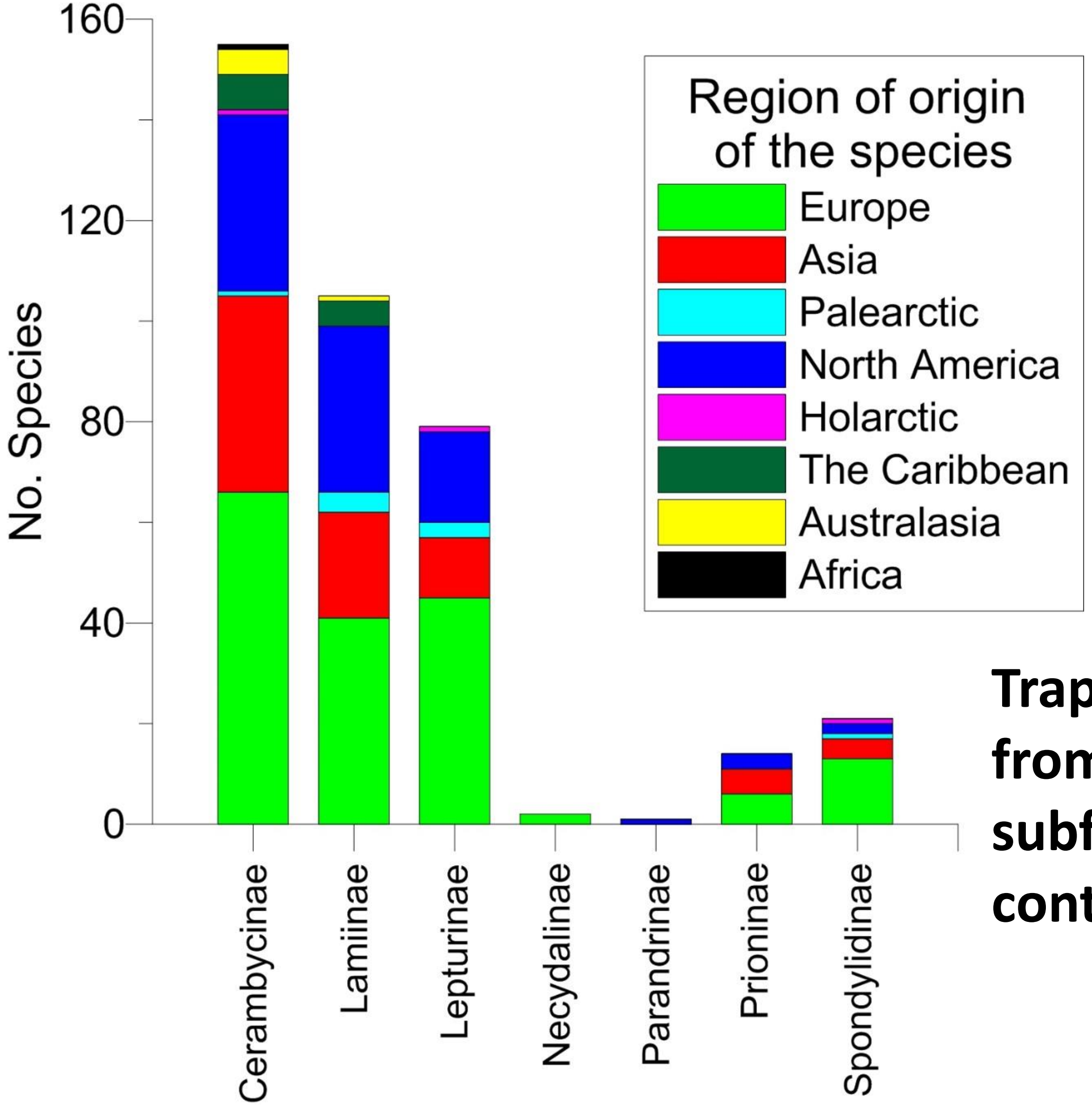
- 16 species > 1000 indiv.
- 58 species > 100 indiv.
- 84 species > 50 indiv.

High probability of detection of such species upon arrival

- 109 species: 1 or 2 indiv.

More problematic to say but provide possible target species to go after for a pheromone identification to increase the pool of generic attractants

Genericity at the subfamily level



Trapping of numerous species from the most important subfamilies in the different continents

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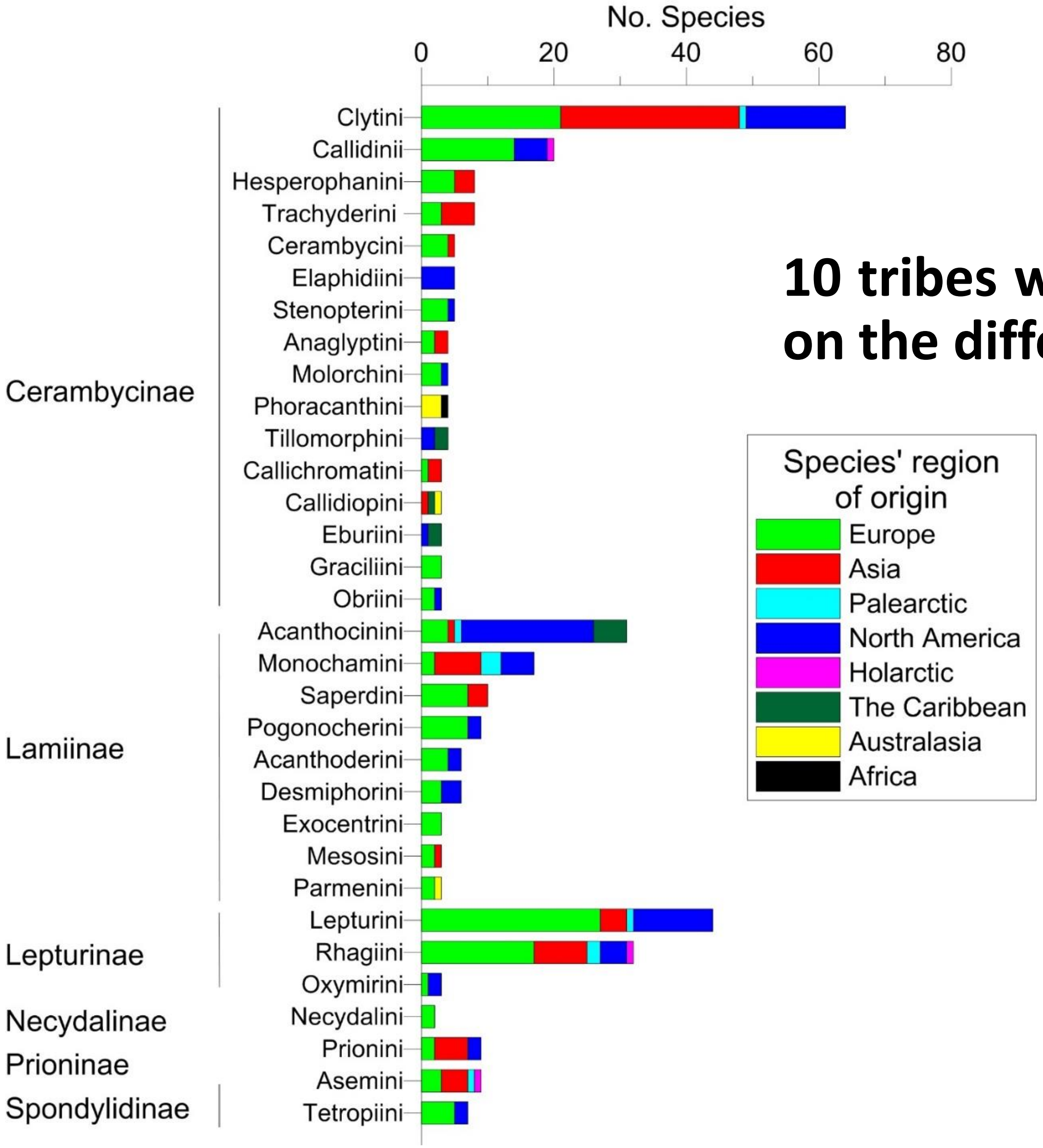
Genericity at the tribe level



10 tribes with > 9 species captured on the different continents

Genericity at the genus level

Trappings: EU-Europe, CH- China, NA-North America, RU- Russia

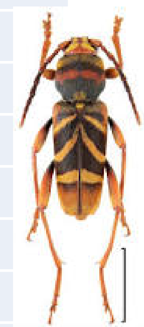
In red: trapped in invaded continent

12 spp *Monochamus*

Monochamus alternatus CH
Monochamus bimaculatus NA
Monochamus carolinensis NA
Monochamus galloprovincialis EU
Monochamus maculosus NA
Monochamus notatus NA
Monochamus saltuarius CH, RU, EU
Monochamus sartor EU
Monochamus scutellatus NA
Monochamus sutor EU
Monochamus sutor longulus CH
Monochamus urussovi RU, EU

19 spp *Xylotrechus*

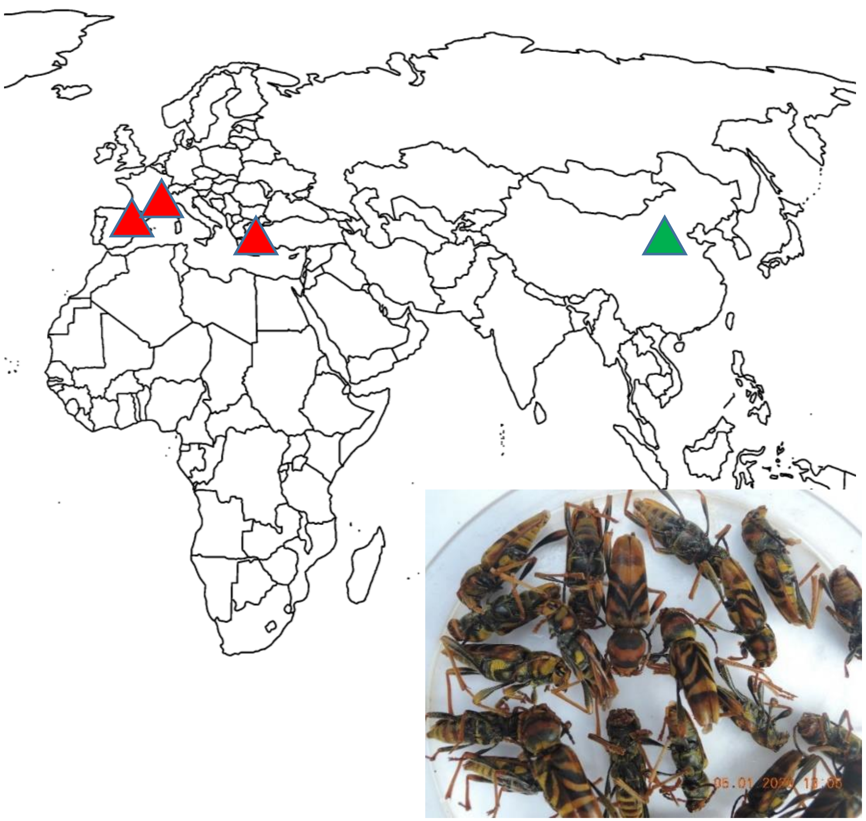
Xylotrechus antilope EU
Xylotrechus antilope sekerai EU
Xylotrechus arvicola EU
Xylotrechus atronotatus CH
Xylotrechus buqueti CH
Xylotrechus chinensis CH ***EU***
Xylotrechus clarinus CH
Xylotrechus colonus NA
Xylotrechus gratus CH
Xylotrechus integer NA
Xylotrechus latefasciatus CH
Xylotrechus magnicollis CH
Xylotrechus pantherinus EU
Xylotrechus pekinensis CH
Xylotrechus rufilius CH
Xylotrechus rusticus EU CH
Xylotrechus sagittatus NA
Xylotrechus stebbingi ***EU***
Xylotrechus undulatus NA



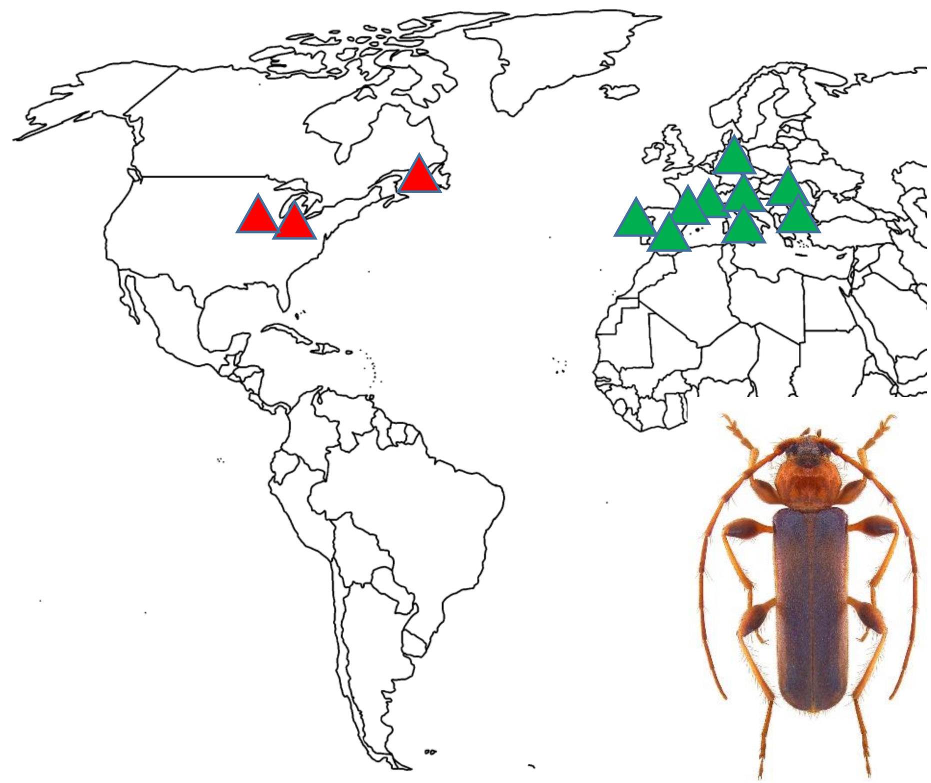
11 spp *Phymatodes*

Phymatodes aereus NA
Phymatodes alni EU
Phymatodes amoenus NA
Phymatodes dimidiatus NA
Phymatodes fasciatus EU
Phymatodes glabratus EU
Phymatodes lividus EU
Phymatodes pusillus EU
Phymatodes rufipes EU
Phymatodes testaceus EU ***NA***
Phymatodes varius NA

Exemples of species trapped in both native range ▲ and in invaded continent ▲



Xylotrechus chinensis, a major pest of *Morus*, trapped in both native China and in ports of France, Spain and Greece



Phymatodes testaceus, trapped in both native Europe (several thousands) and in Canada, Ohio and Michigan

Random trapping rather than blend attraction ?

- The question is obvious for all species <50 individuals trapped
- The subfamily Lepturinae especially concerned:
  - None of the very few female sex pheromones identified so far was present in the blend
  - 79 species trapped (49 Europe, 12 Asia, 18 North America) but only 3 of the 44 Lepturini with >50 indiv., most with 1-2
  - Probably related to trap color rather than lure, especially for flower-visiting species(Cavaletto et al. 2020)
  - However, 639 specimens of another Lepturinae but of tribe Rhagiini, *Rhagium inquisitor* in Europe and Asia
- What role for Ethanol and α-pinene ? Synergist or antagonist for the trapping of some species ? Not fully analyzed yet

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Pheromone identification by proxy

16 species >1000 individuals: only 8 have pheromone or pheromone-like already identified !

Very likely that major components of the pheromones of the 8 others were present in the blend, eg

- Cerambycinae Clytini *Xylotrechus stebbingi*- 6054 ind.
- Lamiinae Acanthocinini *Leiopus femoratus* -3461 ind.
- Spondylidinae Asemini *Cephalocrius syriacus*- 2024 ind.

May also concern a number of the remaining 46 species > 100 ind.

New insights about possible pheromones in not yet considered genera; e.g. the Asian Clytini *Raphuma*

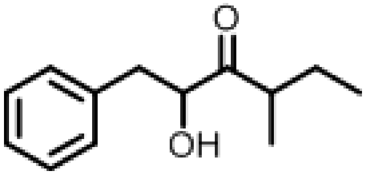
Rhaphuma anongi

Rhaphuma laosica

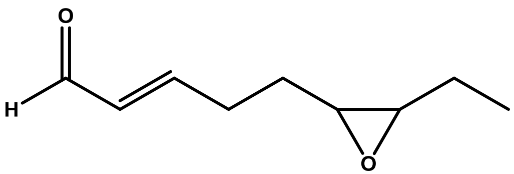
Is it possible to enlarge the blend with new constituents ?
A 10-pheromone blend tested in 2020 and 2021 (France and China)

Two additional compounds:

- Trichoferone, a modified hydroxyketone from the invasive Asian Cerambycinae *Trichoferus campestris* (Tribe Hesperophanini)



- (*E*)-2-*cis*-6,7-epoxynonenal, the pheromone of the invasive Asian Cerambycinae *Aromia bungii* (Tribe Callichromatini)



Results:

- ***No significant change in the cerambycid species richness compared to those of traps baited with the 8-pheromone blend in the same places***
- Large trappings of *Trichoferus campestris* in the native China and first detection in eastern France
- 3 other native species of *Trichoferus* trapped in Europe
- Large trappings of *Aromia bungii* in its native China

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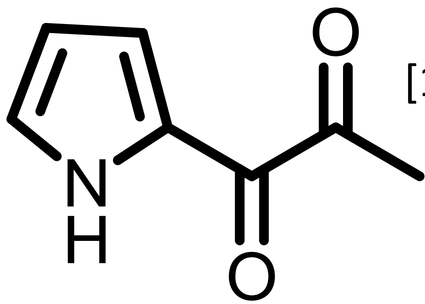
Other possible constituents to be added ?

Semanopyrrole appears to be another highly conserved pheromone in subfamily Cerambycinae



[1-(1*H*-pyrrol-2-yl)-1,2-propanedione]

North America:
Callidiini: *Callidium antennatum, C. pseudotsuga, Semanotus amethystinus, S. ligneus, S. litigiosus*
Asia*:*
Callidini: *Callidiellum villosulum, C. rufipenne, Semanotus spp.;* Phoracanthini *Allotreus asiaticus* (Wickham et al., 2016)
South America:
Elaphidini: *Ambonus distinctus* and *Ambonus electus* (Silva et al, 2017)

BUT…

- ❖ Numerous other species from each subfamily were not attracted to the blend
- ❖ Some other large tribes were not trapped at all (especially those not related to trees- Phytoceini, Agapanthini, … and apterous species)
- ❖ Completely different species-specific pheromones have been identified in some species, eg. *Rosalia alpina*
- ❖ No pheromones known yet from several smaller subfamilies or tribes

Conclusions

- A database of ca. 400 cerambycid species susceptible to be trapped by the multilure blend
- Convenient genericity for early detection of non-natives belonging to a number of tribes such as Clytini, Callidinii, Monochamini, Acanthocini, Acanthoderini, Prionini, …
- Trap location could not be standardized between forests and ports, canopy/understory, edge/forest interior, and some species likely missed
- Probably a number of species are attracted to the trap color/shape and/or plant volatives but… catches are catches!
- Increasing the number of blend constituents is a way to be capable of detecting other tribes, given no antagonistic effects
- However, it appears likely unrealistic to expect catches of species with fairly unique pheromones (e.g. *Rosalia alpina*) unless their pheromones are part of generic blends.
- ***Keep in mind that the probability that insects arrive at the adult stage or near adult stage in ports-of-entry is low, and so affects the probablity of being trapped !***

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Thank you your attention !