Specificity and sensitivity of LAMP assays for early detection of two Agrilus pests: Emerald ash borer (A. plannipennis) and bronze birch borer (A. anxius)

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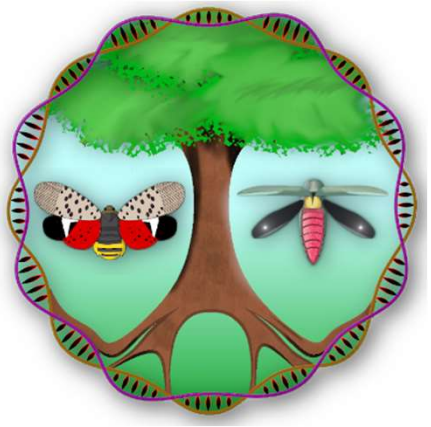
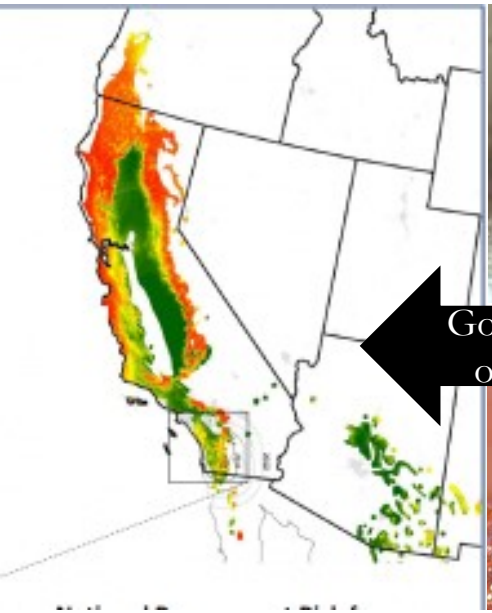
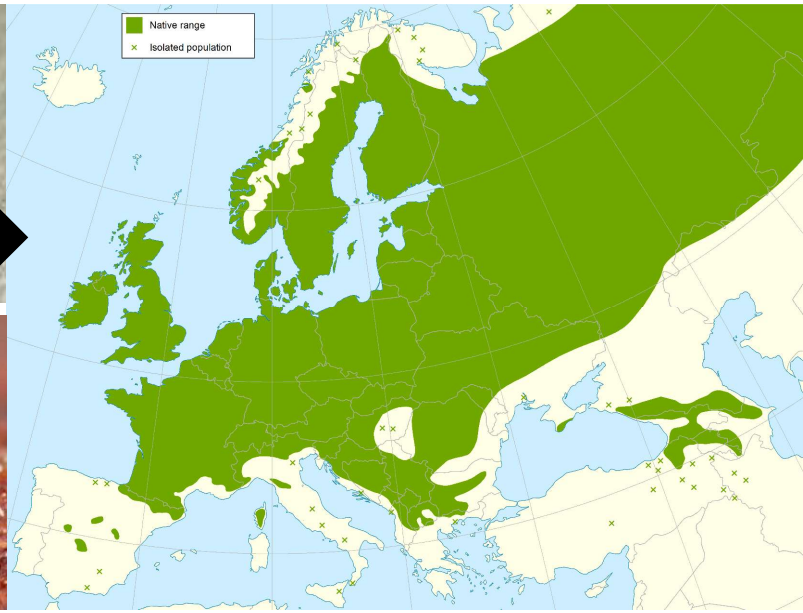


Buprestidae

- Buprestidae: tree specialists that select stressed hosts
- Bronze birch borer (BBB, *Agrilus anxius*) kills healthy Eurasian birch trees (*Betula* spp.) (Nielsen et al. 2011, Muilenburg and Herms 2012).
- Goldspotted oak borer (*A. coxalis*) threatens novel California oak





Two lined chestnut borer (TCB) *Agrilus bilineatus*

Native to North America - Oak *(Quercus spp.*) & chestnut (*Castanea dentata*)

Reported in Turkey in 2018 (Hızal and Arslangündoğdu 2018*)
*The first record of two-lined chestnut borer Agrilus bilineatus (Weber, 1801)(Coleoptera: Buprestidae) from Europe. Entomological News. 2018 Apr;127(4):333-5.

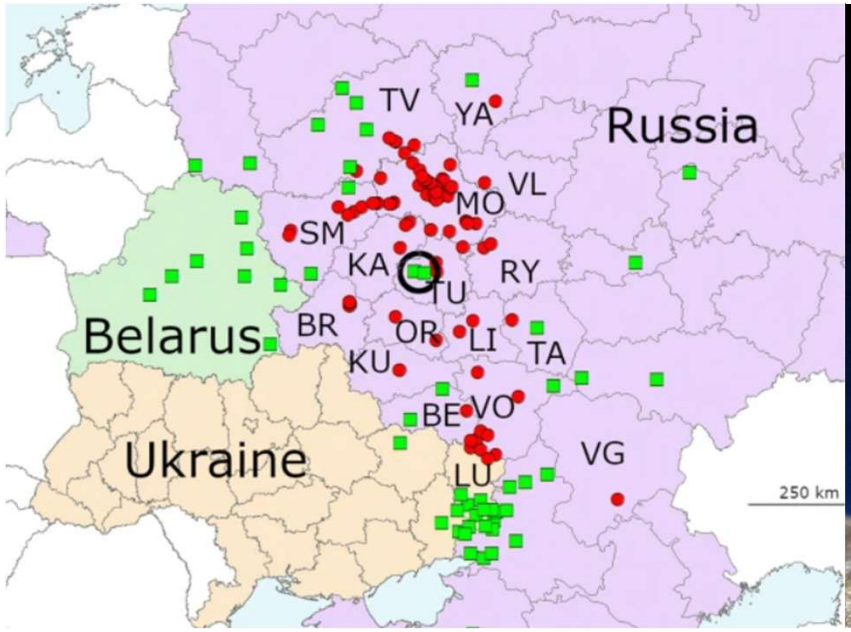
Prefers weakened/stressed trees

One-two year life cycle

Adults (spring–summer): emerge, feed on oak foliage, mate, and females lay eggs in bark crevices of stressed trees

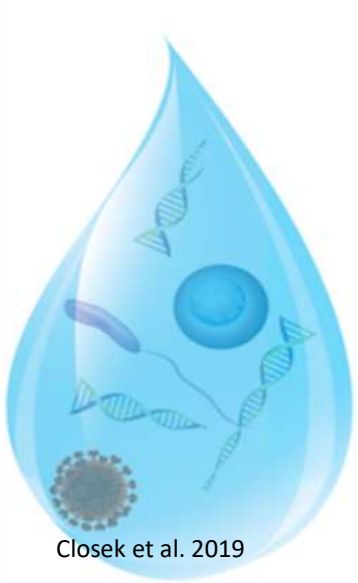
Larvae (summer–winter): tunnel under bark (phloem/xylem), form J-shaped pupal cells to overwinter → pupate in spring → new adults emerge

Emerald ash borer, *Agrilus planipennis* (EAB)



- Found 2000s
- Kills healthy ash trees (*Fraxinus* spp.) native to North America and Europe
  - >99% are killed
- Damage >$10 billions with >100 million dead ash, in the US

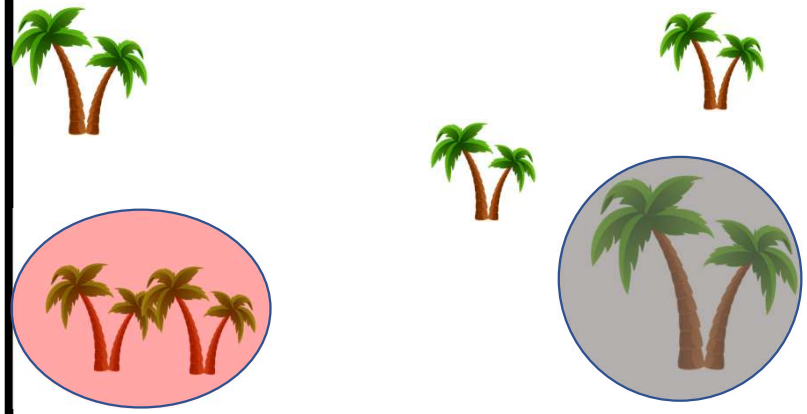
eDNA



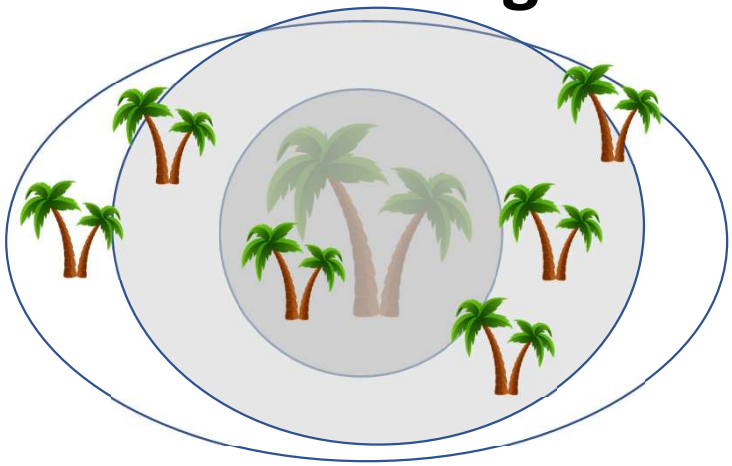
Closek et al. 2019

BioSecurity

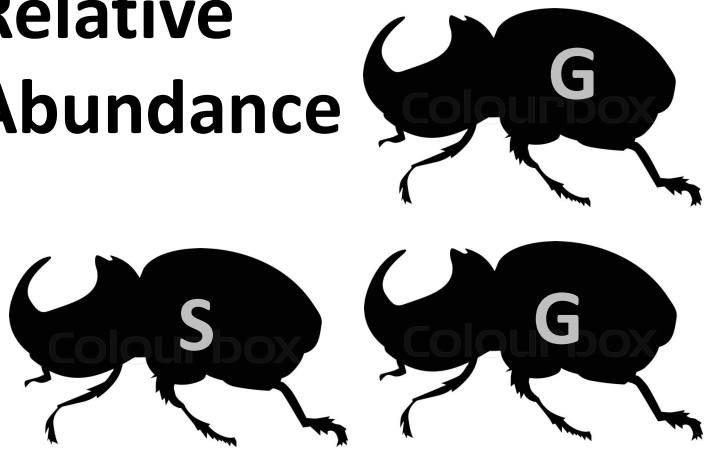
Satellite Populations



Delimiting



Relative Abundance



Early detection with rapid results

- qPCR can take 3+ hours for results
- Loop-mediated isothermal amplification (LAMP)
  - Single, constant temperature of 65°C
  - Less expensive than PCR devices
  - Rapid results <30 minutes

- Genie II or III
  - Portable units that can amplify DNA in the field
  - Tube strips for eight samples or 16 samples

Molecular Entomology

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Research

OXFORD

Development of a Loop-Mediated Isothermal Amplification Assay as an Early-Warning Tool for Detecting Emerald Ash Borer (Coleoptera: Buprestidae) Incursions

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- Specificity
  - In European forests
- Sensitivity
  - Lowest level DNA for detection
- TCB
  - Design assay for detection

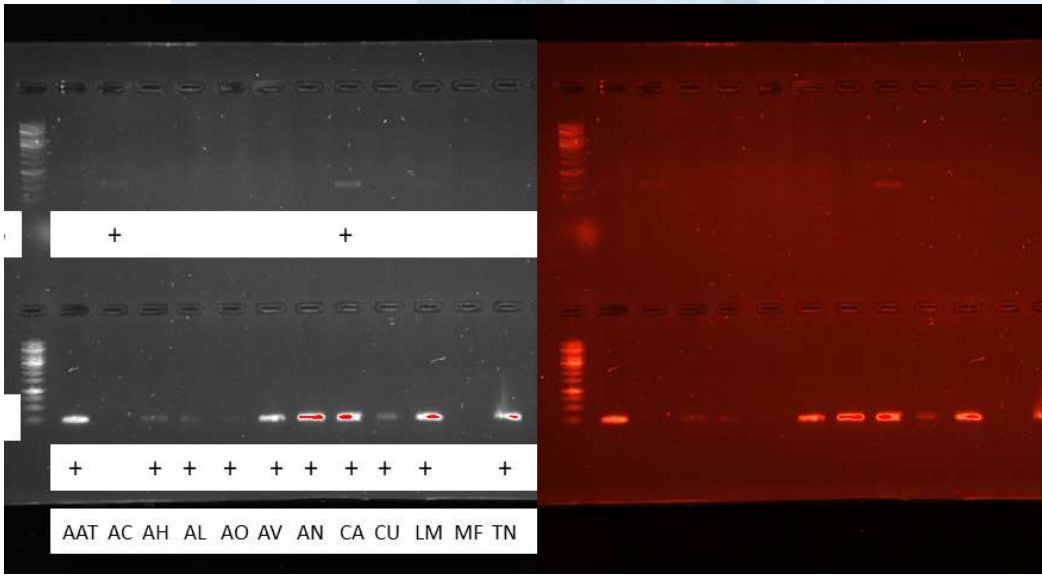
Laboratory research

- EAB and BBB Qiagen extractions
  - Rutgers University
  - Shipped to Italy
- Other species collected in funnel traps
  - Italy (2021)
  - France (2021)

Extractions

- Crushed samples
- Extracted DNA
  - Qiagen blood and tissue kit
    - 100-200 uL/specimen

- Confirmation of DNA with general insect primers
  - LCO1490-HCO2199



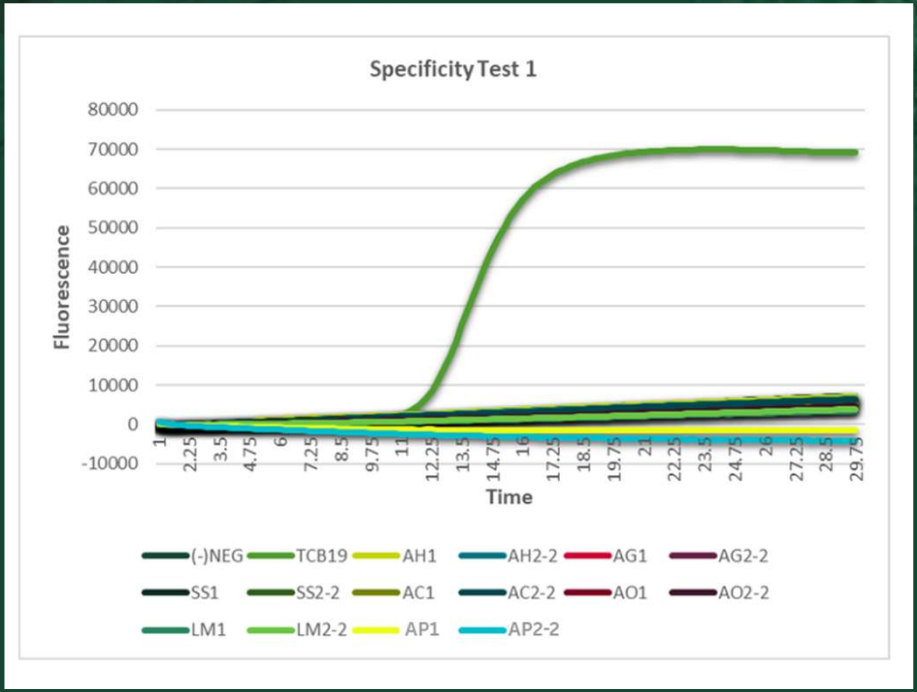
Results

- LAMP Designer (OptiGene Ltd, Horsham, UK) for BBB

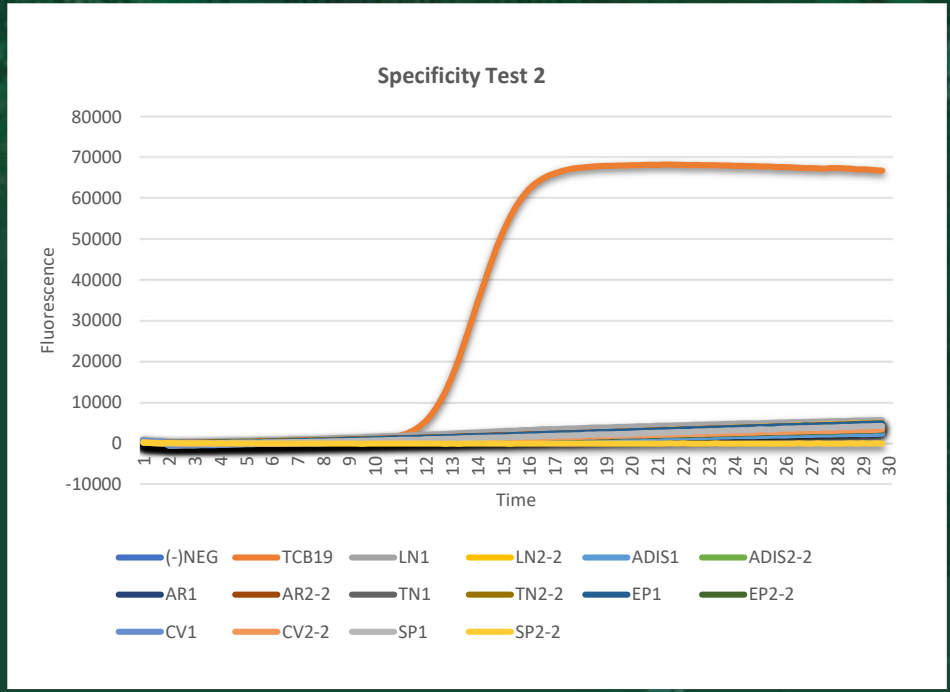
- Both assays are species specific
  - 25 species tested
  - 13 European *Agrilus*
  - Four other Buprestids
  - Two Ambrosia beetles
  - Five Cerambycids

Insect Species
Agrilus angustulus
Agrilus anxius (BBB)
Agrilus ater
Agrilus convexicollis
Agrilus curtulus
Agrilus graminis
Agrilus hastulifer
Agrilus laticornis
Agrilus obscuricollis
Agrilus olivicolor
Agrilus planipennis (EAB)
Agrilus roscidus
Agrilus sulcicollis
Agrilus viridis
Anthaxia nitidula
Chrysobothris affinis
Coraebus undatus
Lamprodila mirifica
Meliboeus fulgidicollis
Curculionidae: Scolytinae
Anisandrus dispar
Xyleborinus saxesenii
Cerambycidae
Aegomorphus clavipes
Exocentrus punctipennis
Leiopus nebulosus
Saperda punctata
Trichoferus pallidus

Specificity Tests

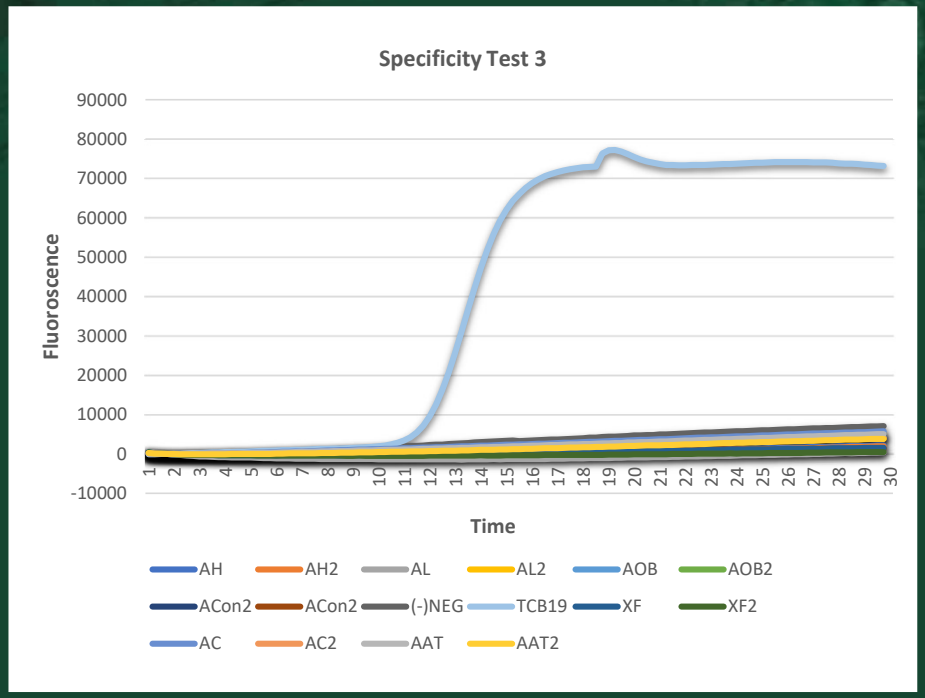


Amplification curves from the first LAMP specificity assay run. DNA from Agrilus bilineatus (TCB19) and non-target species - Agrilus hastulifer (AH1, AH2-2), Agrilus graminis (AG1, AG2-2), Saperda scalaris(SS1-SS2), Agrilus olivicolor (AO1, AO2-2), Agrilus curtulus (AC1, AC2-2), Lamprodila mirifica(LM1-LM2), Agrilus planipennis(AP1-AP2)

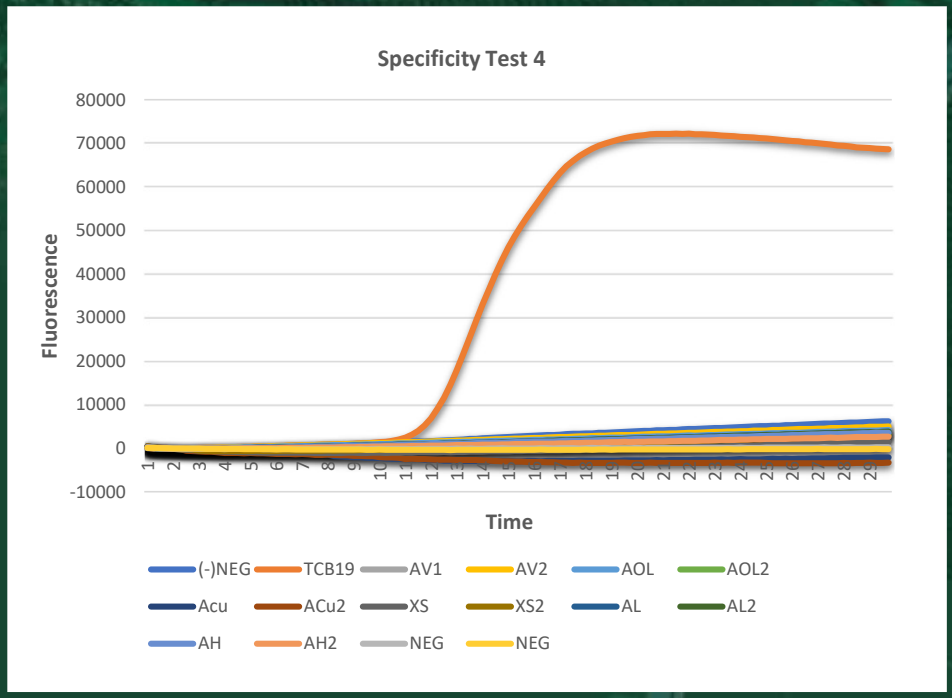


Amplification curves from the second LAMP specificity run. Agrilus bilineatus (TCB19) and non-target species—including Leiopus nebulosus(LN1-LN2), Anisandrus dispar (ADIS-ADIS2), Agrilus roscidus(AR1-AR2), Trichoferus pallidus(TN1-TN2), Exocentrus punctipennis(EP1-EP2), Coraebus undatus(CV1-CV2),and Saperda punctata(SP1-SP2) tested.

Specificity Tests



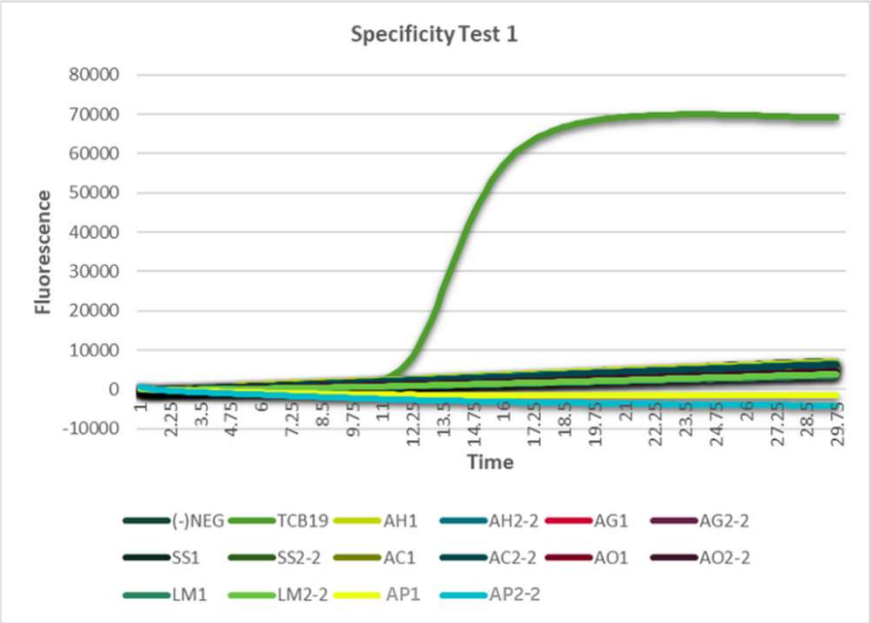
Amplification curves from the third LAMP specificity run. Only the A. bilineatus positive control (“TCB19”) produces a steep increase in fluorescence (starting ~12 min), whereas all other samples—including Agrilus hastulifer (AH-AH2), Agrilus laticornis (AL-AL2), Agrilus obscuricollis(AOB-AOB2), Agrilus convexicollis (ACON-ACON2), Agrilus ater(AAT-AAT2), and the negative control—remain flat near baseline, confirming the high specificity of the assay.



Amplification curves from the second LAMP specificity run. Agrilus bilineatus (TCB19) and non-target species—including Leiopus nebulosus(LN1-LN2), Anisandrus dispar (ADIS-ADIS2), Agrilus roscidus(AR1-AR2), Trichoferus pallidus(TN1-TN2), Exocentrus punctipennis(EP1-EP2), Coraebus undatus(CV1-CV2),and Saperda punctata(SP1-SP2) tested.

Results

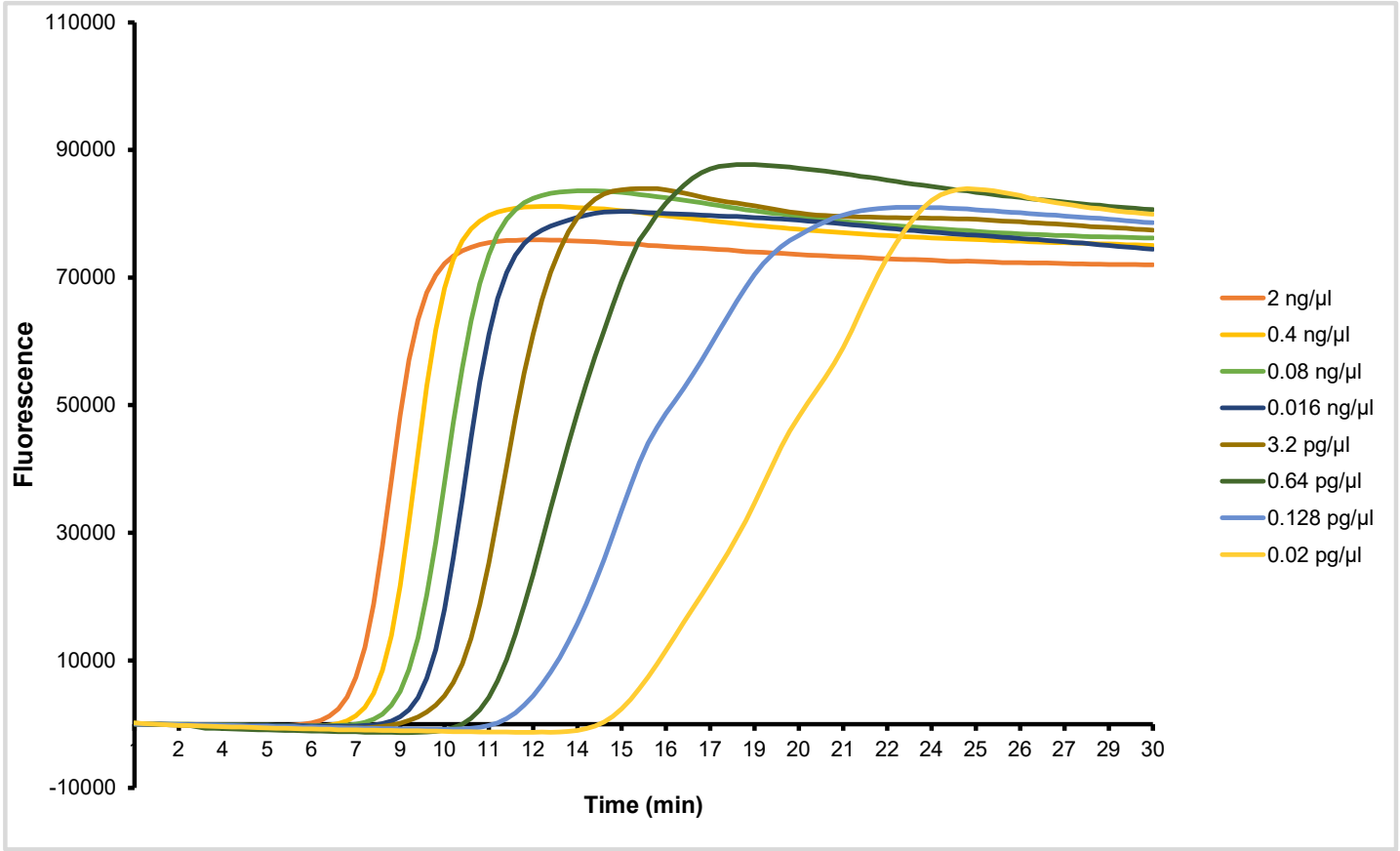
- Both assays are specific



Insect Species	EAB	TCB
Agrilus angustulus	-	-
Agrilus anxius (BBB)	-	-
Agrilus ater	-	-
Agrilus bilineatus	-	+
Agrilus convexicollis	-	-
Agrilus curtulus	-	-
Agrilus graminis	-	-
Agrilus hastulifer	-	-
Agrilus laticornis	-	-
Agrilus obscuricollis	-	-
Agrilus olivicolor	-	-
Agrilus planipennis (EAB)	+	-
Agrilus roscidus	-	-
Agrilus sulcicollis	-	-
Agrilus viridis	-	-
Anthaxia nitidula	-	-
Chrysobothris affinis	-	-
Coraebus undatus	-	-
Lamprodila mirifica	-	-
Meliboeus fulgidicollis	-	-
Curculionidae: Scolytinae		
Anisandrus dispar	-	-
Xyleborinus saxesenii	-	-
Cerambycidae		
Aegomorphus clavipes	-	-
Exocentrus punctipennis	-	-
Leiopus nebulosus	-	-
Saperda punctata	-	-
Trichoferus pallidus	-	-

Sensitivity

- Sensitivity:
  - 0.02 pg/uL for EAB assay
  - 0.064 pg/µL for TCB assay
  - 3.2 pg/uL for BBB assay



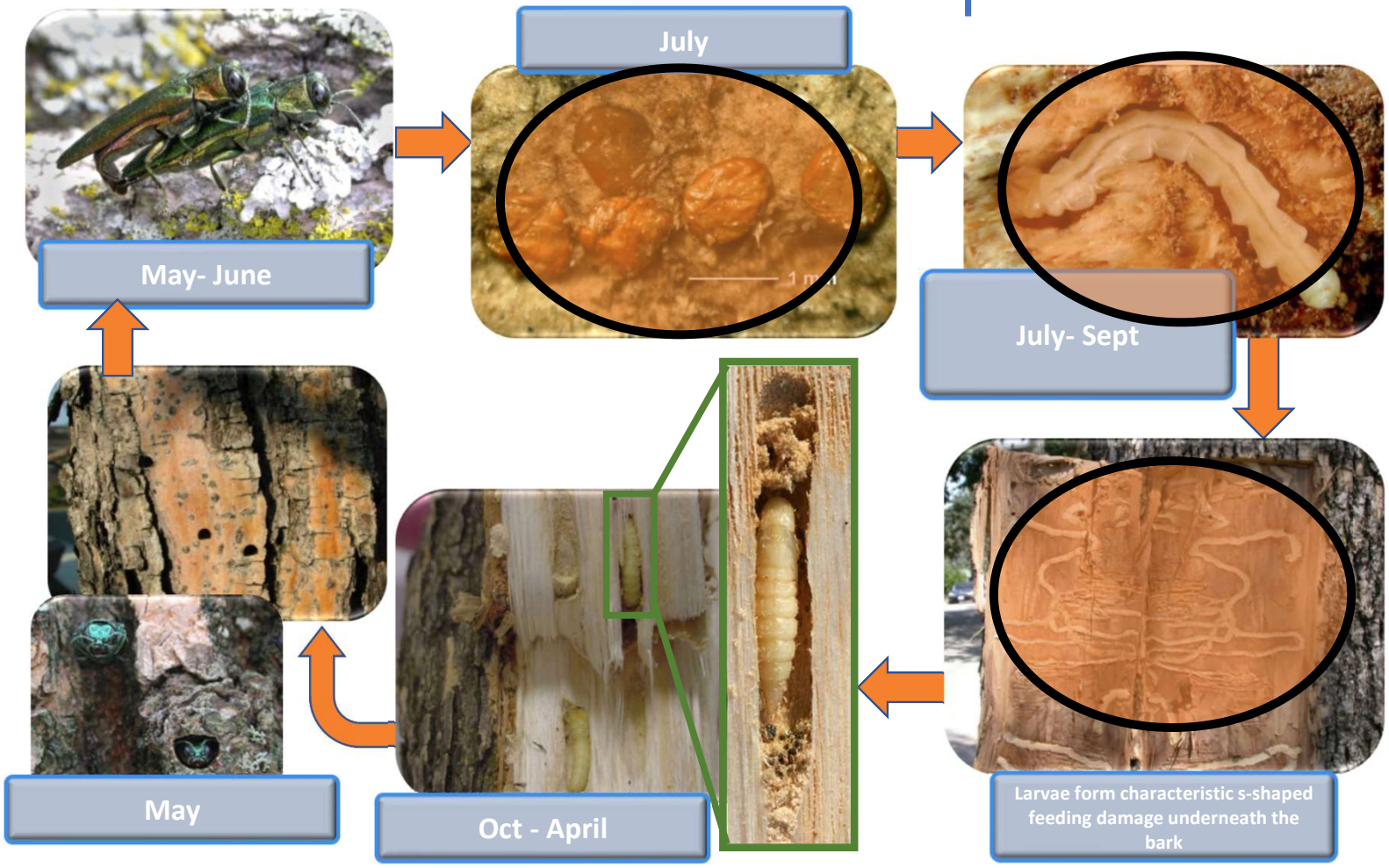
EAB TCB and BBB LAMP

- EAB LAMP
  - Very sensitive and specific
  - More robust than previously published.
- TCB LAMP
  - Very sensitive and specific
- The BBB LAMP
  - Less sensitive but specific

So what is the next step

- We have field validation for BBB and EAB (Peterson et al. 2023, Kyei-Poku et al. 2020)
  - But is needed for TCB studies
- How does plant chemistry influence on DNA detection?
  - Lower DNA concentration = higher inhibitory influence
  - Sampling with wood tissues lowers detection (Peterson et al. 2023)
- How best methods too sample for Buprestid/Woodboring?
  - Targeted approaches

EAB TCB and BBB life cycle, cryptic
Where and when to sample?



How to sample?

- Exit holes
- Larval galleries
- Plant tissues, limit detection (Peterson et al. 2023)
  - Foliage
  - Bark
- Insect frass
  - How much is needed for detetion?

Frass limit of detection, 20-80 mg

	20 mg		40 mg		80 mg	
Site	100 µl	200 µL	100 µl	200 µL	100 µl	200 µL
Smoky Lake, AB LAMP	✓ (2/2)	✓ (1/2)	✓ (2/2)	✓ (1/2)	✗ (0/2)	✓ (1/2)
qPCR	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)
Bailey Orchard, BC LAMP	✓ (2/2)	✓ (1/2)	✓ (2/2)	✓ (1/2)	✓ (1/2)	✓ (2/2)
qPCR	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)
Skimikin, BC LAMP	✓ (2/2)	✓ (2/2)	✓ (1/2)	✓ (2/2)	✓ (2/2)	✓ (2/2)
qPCR	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)	✓ (3/3)

Frass is detectable

	10 mg	5 mg	2 mg	0.5 mg
BBB Frass	✓ (6/6)	✓ (6/6)	✓ (6/6)	✓ (7/9)
Transfer amount for detection	50 mL	25 mL	10 mL	
	6/15 40%	8/25 32%	10/28 36%	

Discussion

- **High specificity**: TCB and EAB consistent with other forestry LAMP assays (Peterson et al., 2023; Rizzo et al., 2021)
  - Specific to European forest fauna
- **High sensitivity**: detection limit 0.064 pg/µL → comparable with EAB, BBB, *A. bungii*, and others.
- **qPCR vs LAMP**: qPCR offers higher sensitivity but is less field-suitable; LAMP is rapid, portable, and tolerant to inhibitors.
  - Future work can use qPCR to confirm LAMP field samples and questionable samples
- **Diagnostic tool** to support surveillance of TCB, EAB, and BBB in Europe.
- Detection from frass, larvae, adults (Peterson et al. 2023)
  - Phloem tissue possible with limits (Kyle et al. 2024)
  - Frass can be quite low amount (0.5 mg) for detection (unpublished data DLP)

Kyle, K.E., Allen, M.C., Siegert, N.W., Grabosky, J. and Lockwood, J.L., 2024. Design of an eDNA sampling method for detection of an endophagous forest pest. *NeoBiota*, *95*, pp.149-164.

Acknowledgments

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