Early detection tool for *Agrilus anxius* (Bronze Birch Borer): laboratory and field validation of a LAMP molecular assay

Sezer Olivia Kaya
SLU, Southern Swedish Forest Research Centre

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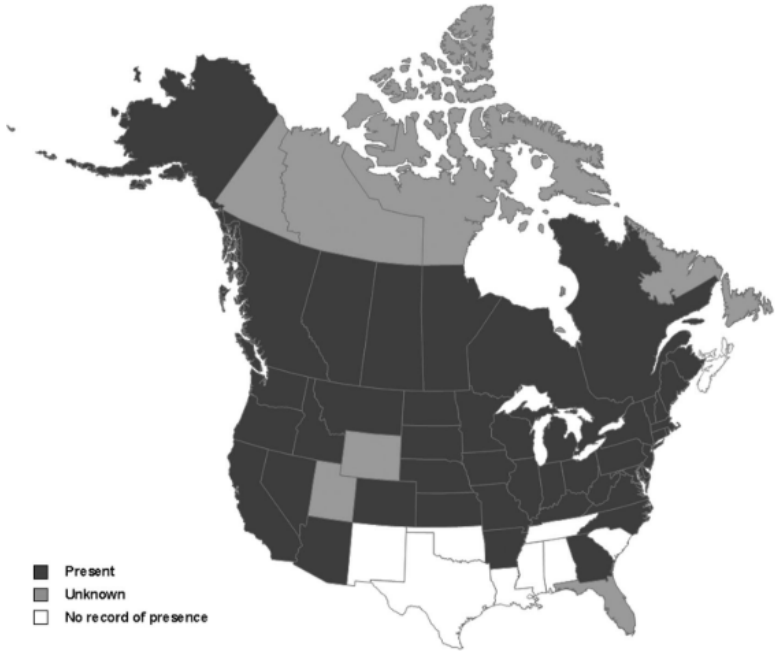
Bronze Birch Borer (BBB)
Agrilus anxius Gory, 1841

Major factor causing birch decline in North America



Muilenburg and Herms, Environmental Entomology, 2012

Declining *B. papyrifera* in Edmonton

Agrilus spp.

Kelnarova et al, Bulletin of Entomological Research. 2019.

Corfoto / Istock, Emerald Ash Borer Traces on a Dead Tree Trunk

BBB damage

Swellings on bark

Serpentine galleries

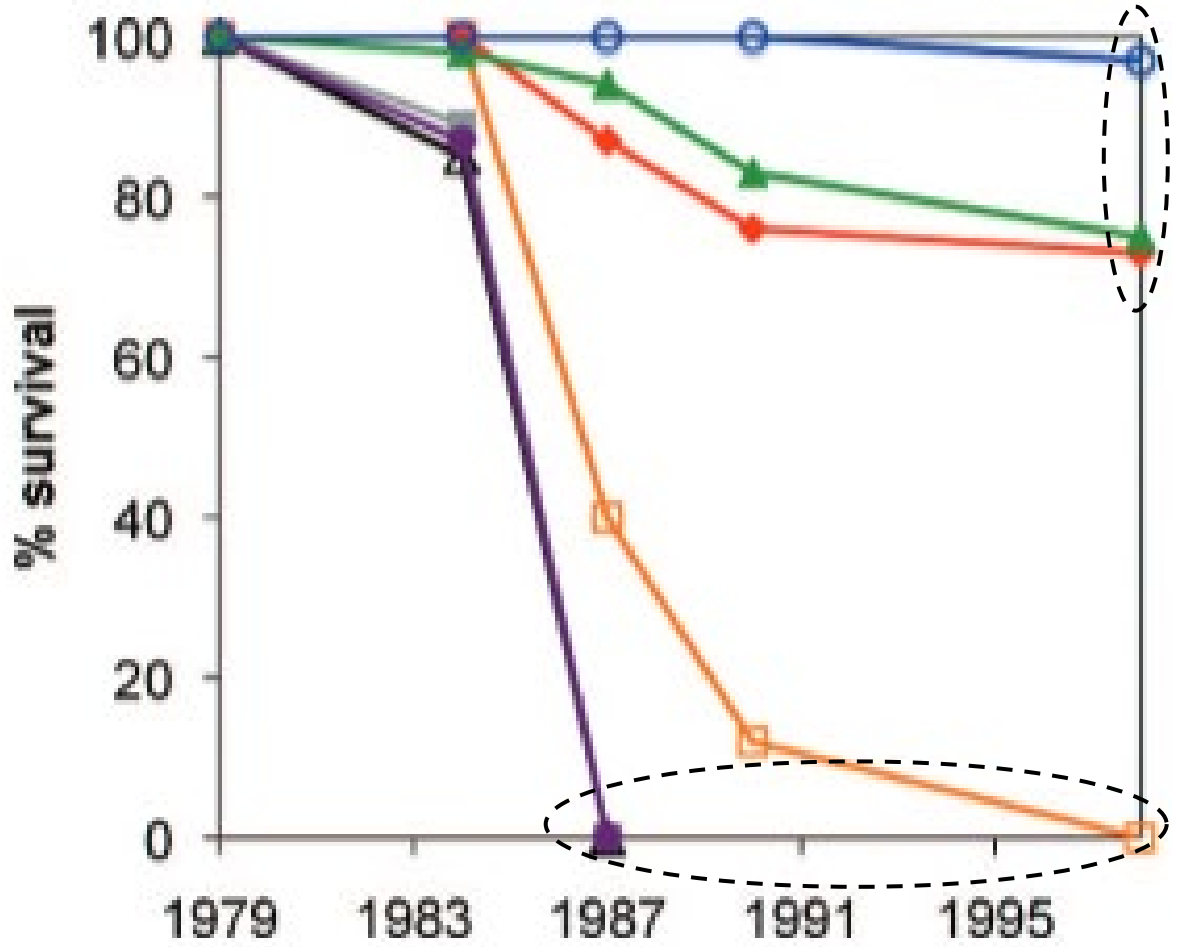
D-shaped exit holes

Can cause extensive dieback and mortality

Naturally regenerated paper birch

Planted silver birch

Bronze birch borer kills healthy Eurasian birch trees



North American birch species

European & Asian birch species

(Nielsen et al, 2011)

BBB lifecycle, cryptic

May-June

July

July- Sept

Oct - April

Photos: Adult: Steven Katovich, Bugwood.org
Pupa: David G. Nielsen, The Ohio State University, Bugwood. org

Can we develop a tool for rapid detection of BBB?

Early detection with rapid results

- qPCR > 3 hours
- Loop-mediated isothermal amplification (LAMP)
  - Single, constant temperature of 65℃
  - Rapid results in ~30 minutes
- Genie II or III
  - Portable units that can amplify DNA in the field
  - Tube strips for eight or 16 samples

A molecular tool for detecting BBB in the field

Developing the assay
- Sensitive
- Specific

Field validation
- eDNA
- Larvae

LAMP and qPCR assay design

25 insect species collected from Europe and N. America

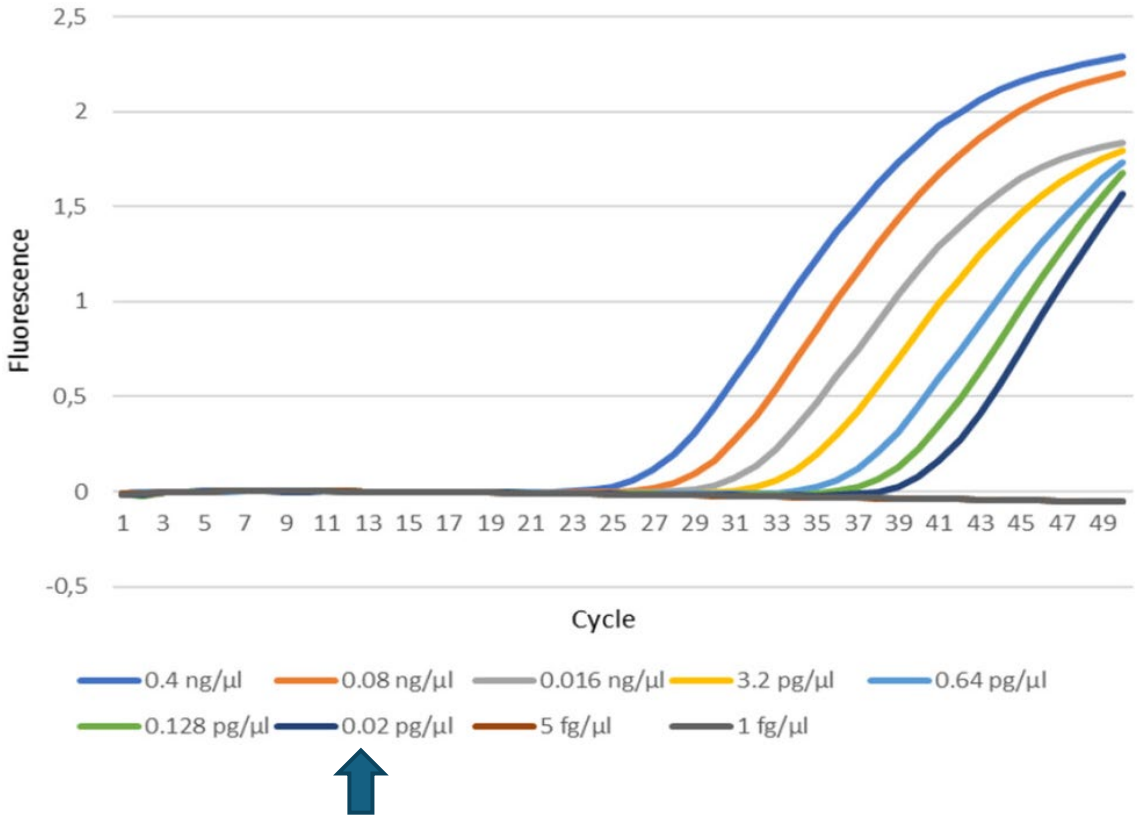
BBB-specific LAMP and qPCR assay

- Tested with 25 species:
  - 13 European Agrilus
  - Four other Buprestids
  - Two Ambrosia beetles
  - Five Cerambycids

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

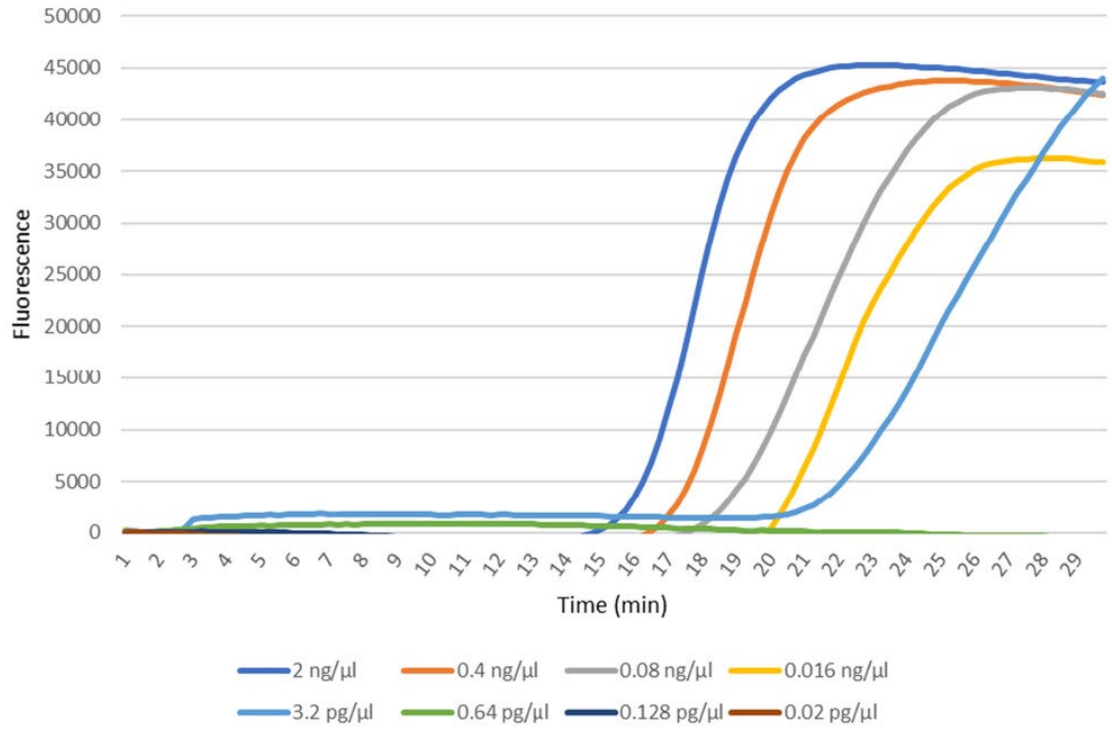
Detectibility even in low DNA concentration

qPCR



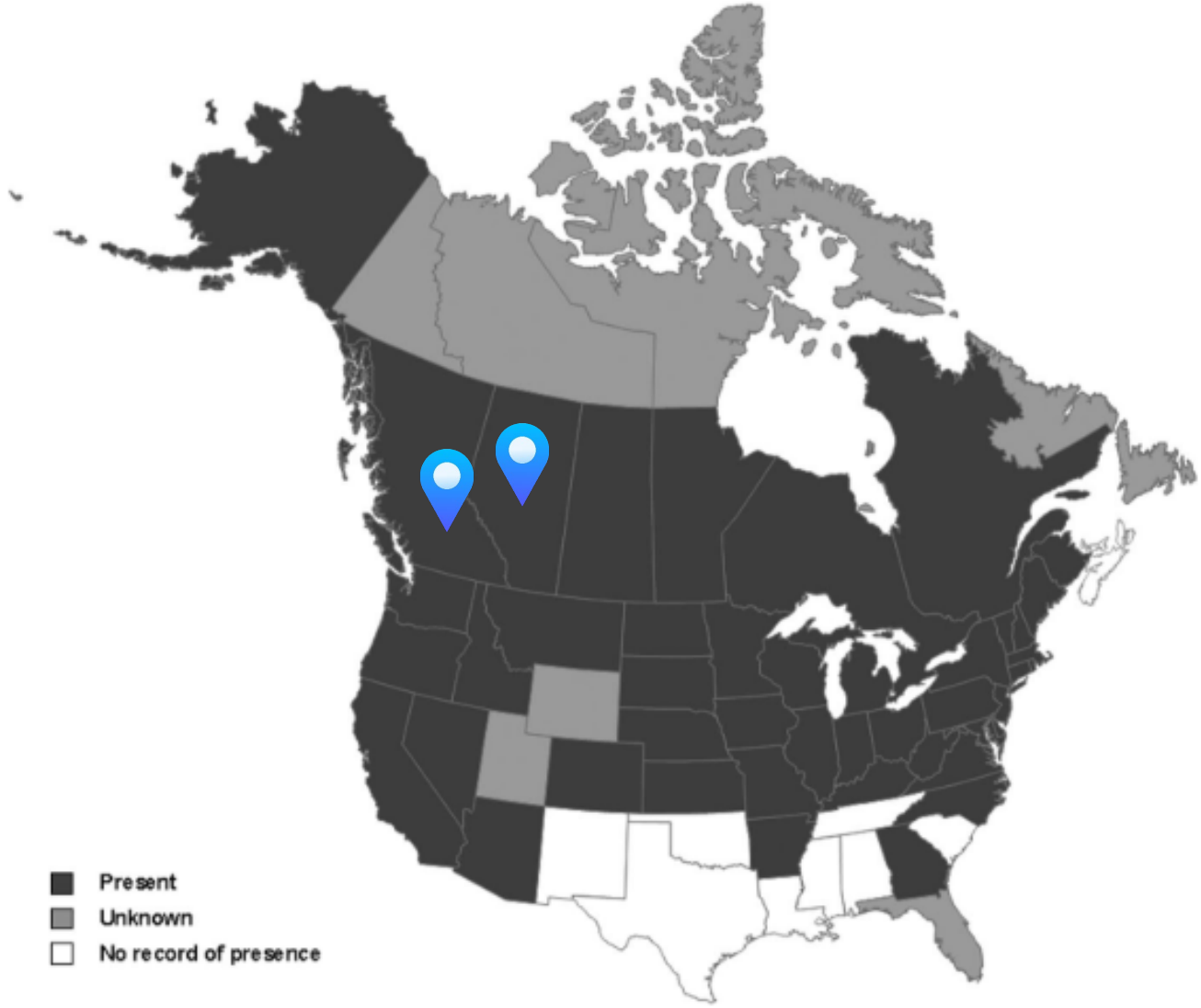
0.02 pg/µl

LAMP



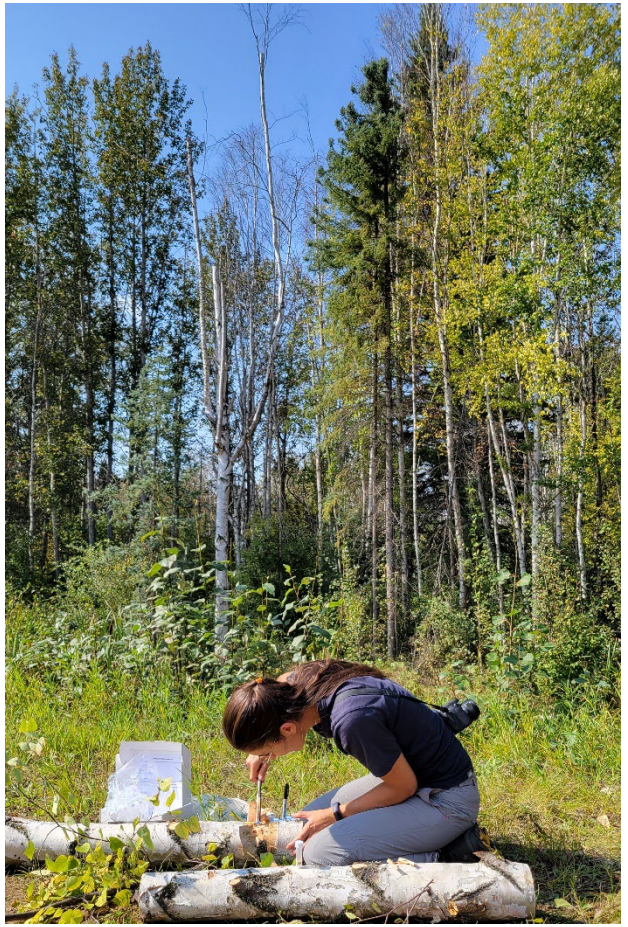
3.2 pg/µl

Field Validation of LAMP Assay in Alberta and BC

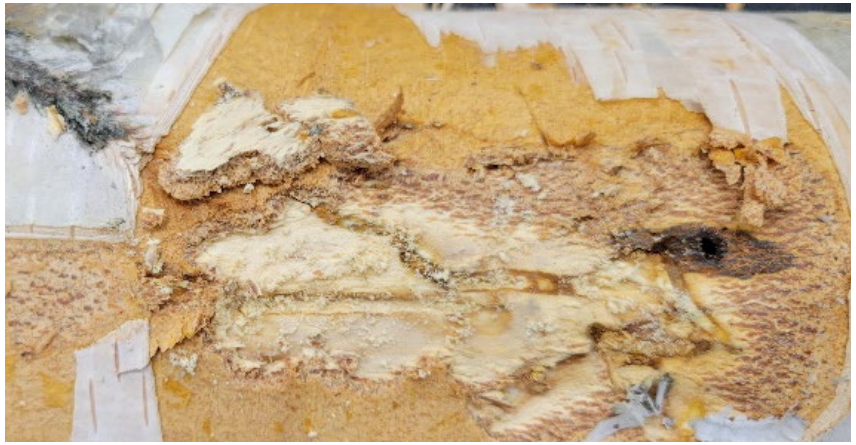


Muilenburg and Herms, Environmental Entomology, 2012

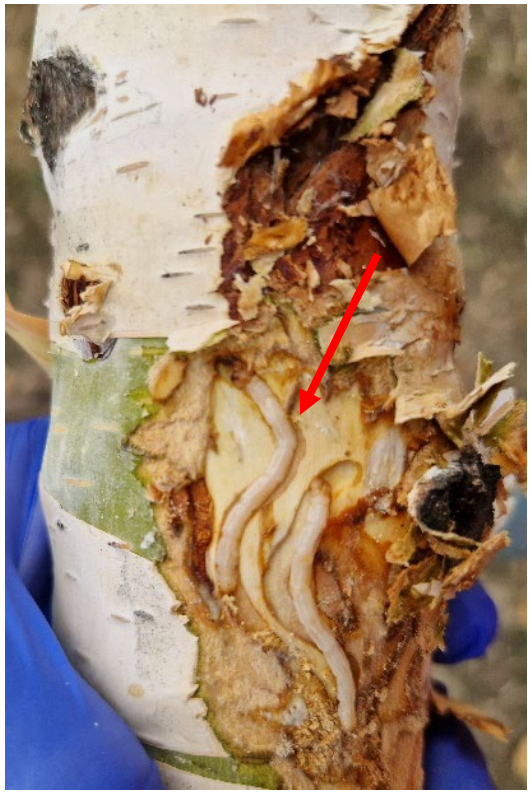
Larvae and frass (eDNA) tested in BBB infested sites



Frass Collection

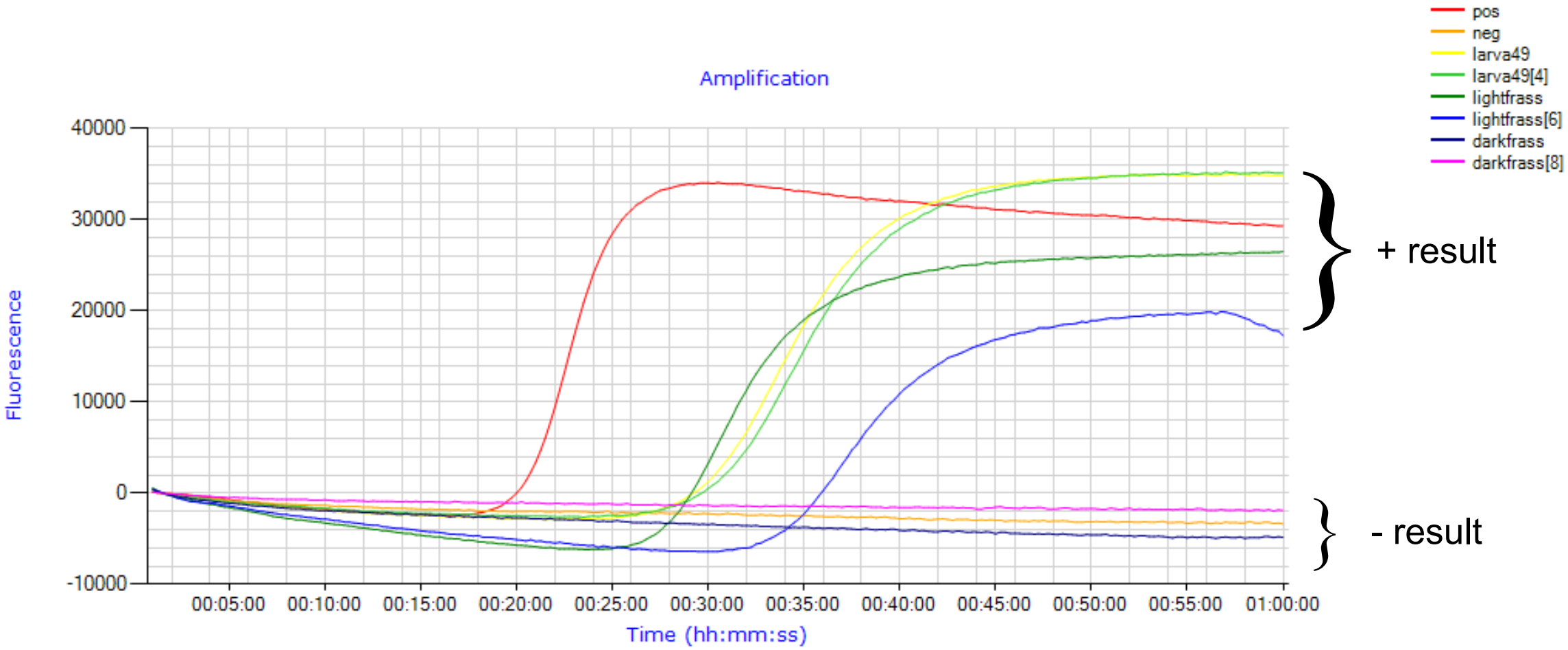


Larvae Collection



Genie in the field

BBB detection using larvae and frass



Avoiding doomsday

- Specific and sensitive assay
- Field validated using eDNA
- Fast and portable tool

USDA Forest Service - Northeastern Area Archive, Bugwood.org

Thank you!

Sezer Olivia Kaya
sezer.olivia.kaya@slu.se

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METHOD | Open Access

Development of novel LAMP and qPCR assays for rapid and specific identification of Bronze birch borer (*Agrilus anxius*)

Donnie L. Peterson, Francesco Pecori, Nicola Luchi, Duccio Migliorini, Alberto Santini, Kathleen E. Kyle, Claire Rutledge, Aurélien Sallé, Sezer Olivia Kaya, Tod Ramsfield, Michelle Cleary

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Alberto Santini

Tod Ramsfield

Kathleen E. Kyle

Claire Rutledge

Aurélien Sallé

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