



# ANOPLORISK-II

Further Development of Risk Management for the EC listed  
*Anoplophora* species, *A. chinensis* and *A. glabripennis*  
01 Jan 2014 to 31 December 2015



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**WP2: Detection methods for woodboring stages (BFW, FERA)**

**WP3: Detection of adult beetles with pheromone baited traps (FERA, BFW)**

**WP4: Molecular tools for diagnosis (FERA, BFW, JKI)**

**WP 5: Development of contingency plans (JKI, BFW, FERA)**



## WP2: Detection methods for woodboring stages

Evaluation and further development of

Acoustic detection

Dog detection



## Acoustic detection

Various sensors designed  
and tested

Sensitivity tests

The systems have been  
found to be successful  
and have additionally  
been employed in the  
laboratory at Fera for long  
term recording

Further recordings/  
evaluation in progress at  
Fera



# Evaluation of ALB Detection Dog Method

Quantification of detection of different ALB scent material under abstract, standardized conditions and in realistic environments

Two meetings with 10 and 14 dog teams, respectively (all trained at BFW; various levels of experience)



## Quantification of

**Sensitivity** = correct positives / all positive samples

**Specificity** = correct negatives / all negative samples

## Experimental setup:

8 samples: 2 positive and 6 negative (randomized)

3 repeats per test

Random order of the dog teams

Blind search (dog and handler)



# Standardized conditions

## Hollow building blocks



...with ALB frass, wood shavings



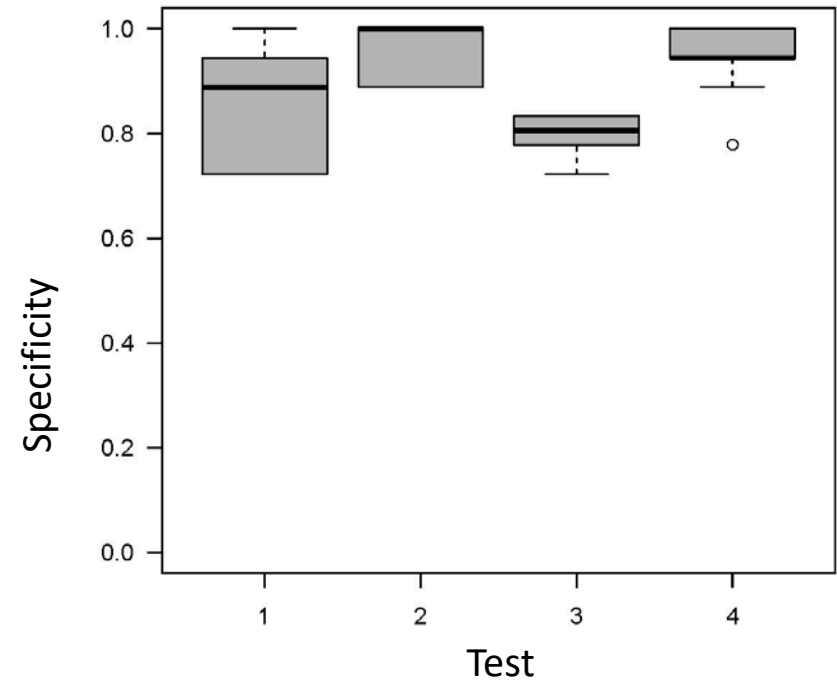
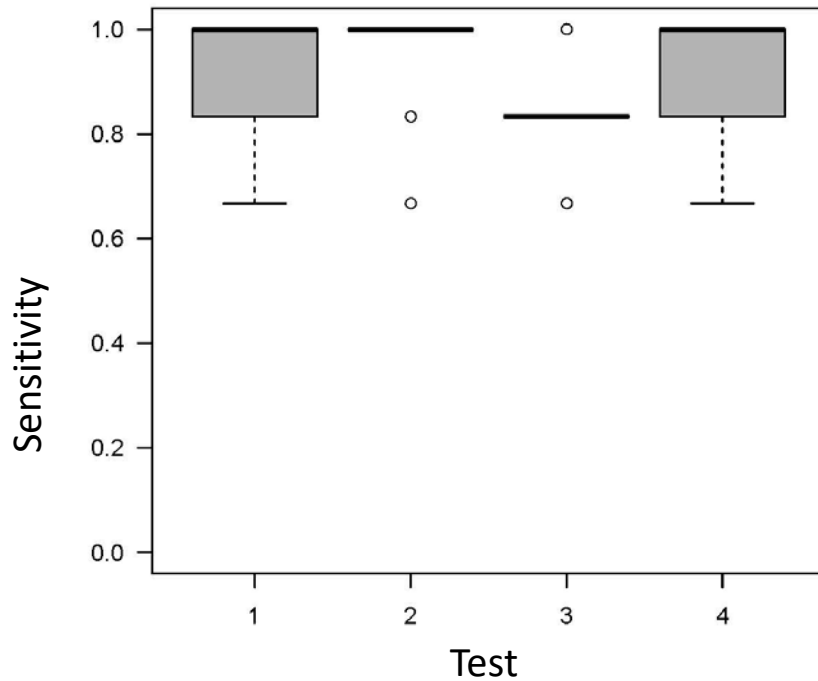
...with living ALB larva

...with 2x living ALB larva plus infested wood piece  
(negative samples contained uninfested wood)



## Abstract standardized conditions (Feb. 2015)

Test	Sensitivity	Specificity	total samples
1 saw dust	0,917	0,856	240
2 wood under logs	0,950	0,961	240
3 larvae	0,850	0,794	240
4 larvae + wood	0,926	0,944	216





# More realistic environments

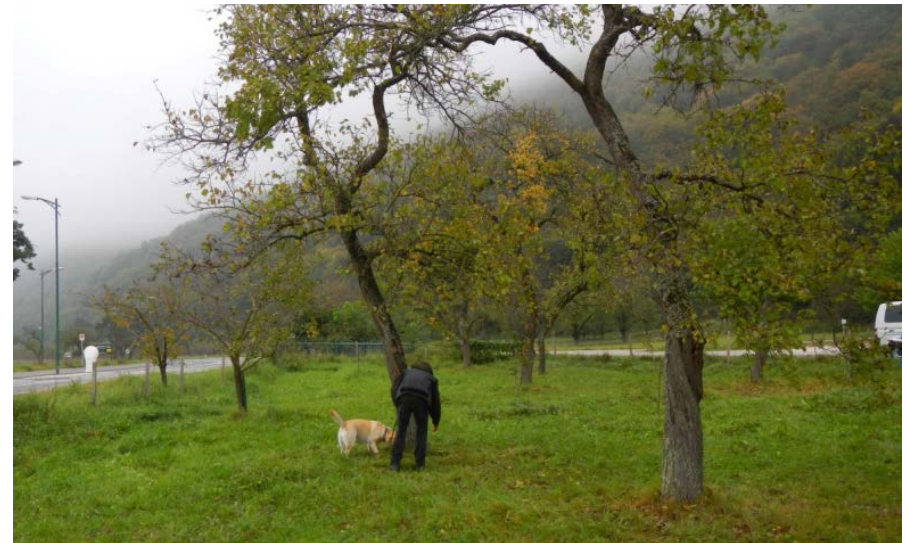
ALB frass, wood shavings, saw dust hidden



...in the grass at base of poplar tree

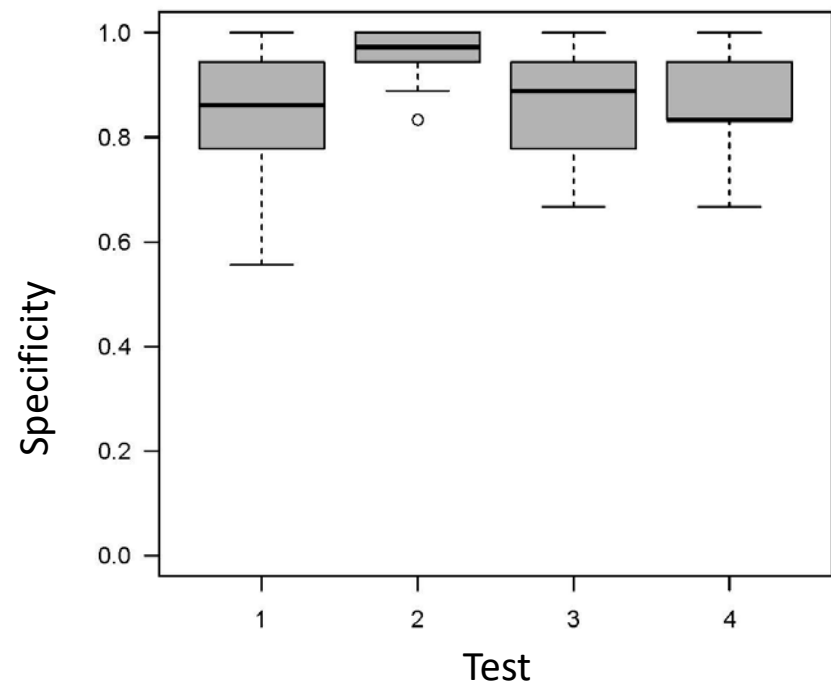
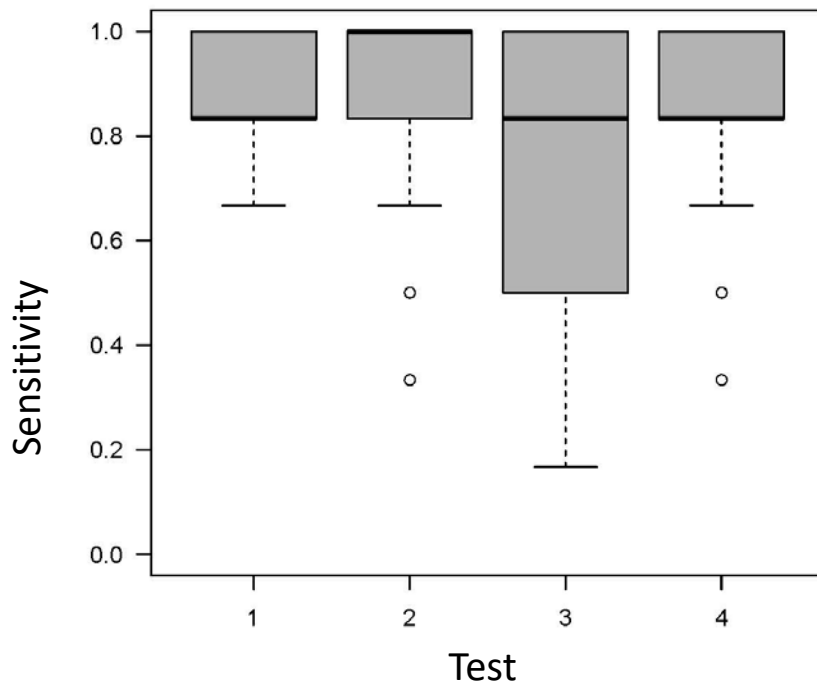
...in a tube at the height of 1.8 m on poplar tree

...in holes and crevices at ca. 1.8 m on old orchard trees



## ALB frass /saw dust in realistic environments (Oct. 2014)

Test	Sensitivity	Specificity	total samples
1 larva in blocks	0,857	0,857	336
2 at base of poplar	0,881	0,956	336
3 in tube at 1.8 m on poplar	0,750	0,865	336
4 orchard tree (1.8 m), hidden	0,833	0,853	336





## WP4: Molecular tools for diagnosis

**Loop-mediated isothermal amplification for the detection of *Anoplophora glabripennis* and *A. chinensis***

Technology transfer from FERA to JKI  
Validation of method at JKI



# LAMP assays validation

## 1. Organisms

*Anoplophora glabripennis* ✓

*Anoplophora chinensis* ✓

*Aromia moschata* ✓

with extended run time of 50 min

*Saperda carcharias* ✗

*Cossus cossus* ✗

## 2. Matrices

• Adult beetles dry material ✗

• Adult beetles fresh material ?

• Larval fresh material ✓

• Frass ?

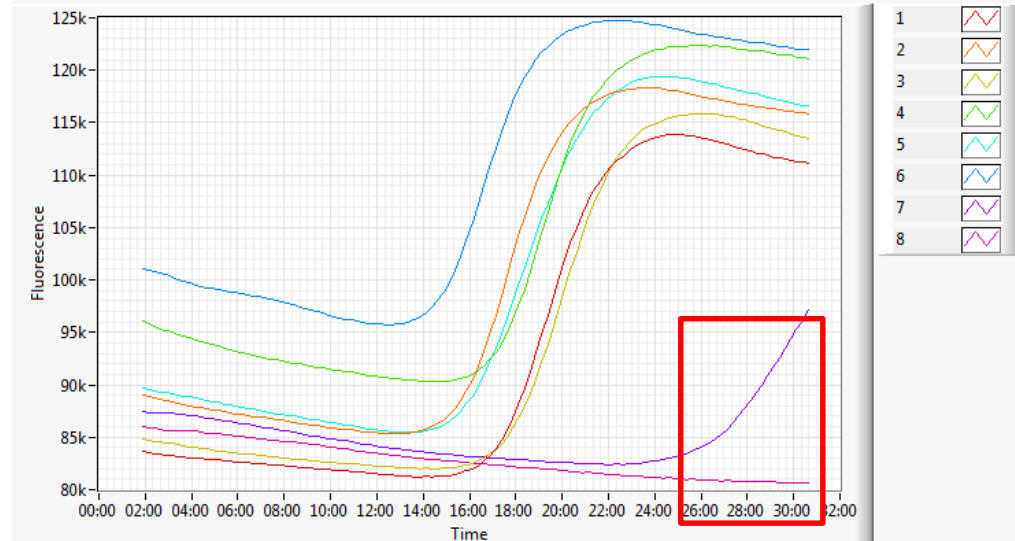
# LAMP assays validation

## 3. Analytical Specificity

*Anoplophora glabripennis* ( ✓ ) **false positive CLB at 26+ min**

*Anoplophora chinensis* ✓

*Aromia moschata* ✓

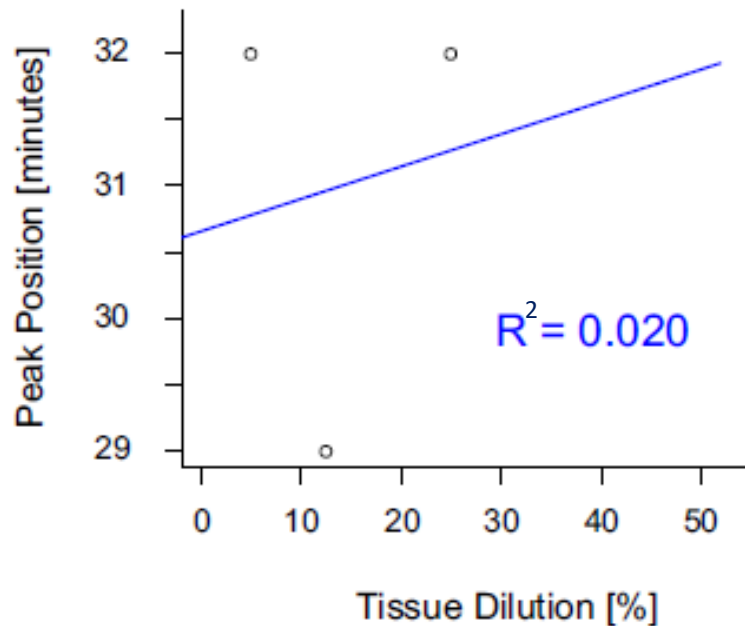


Solution: LAMP assay run of ALB and CLB in parallel

**Definitive answer for methods specificity is limited due to the lack of fresh reference material!!!**

## 4. Analytical Sensitivity

### ALB LAMP assay tested on serial dilution of beetles dry material

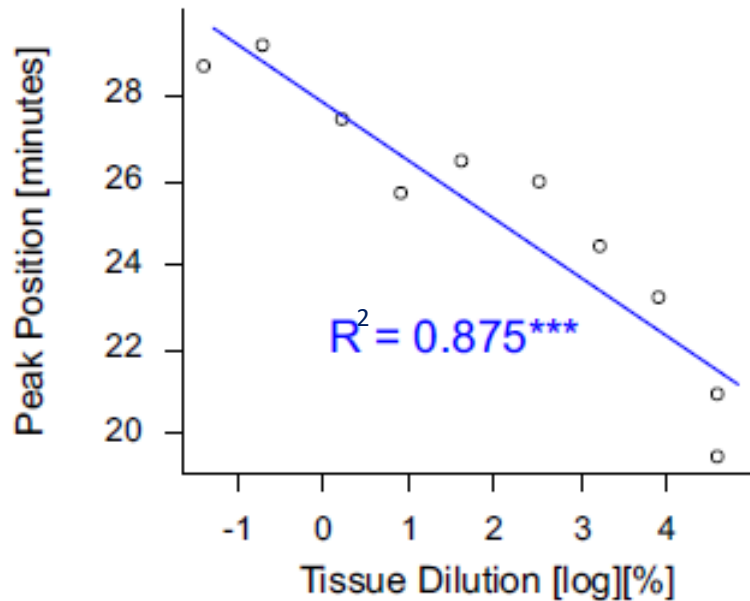


No linear signal detection

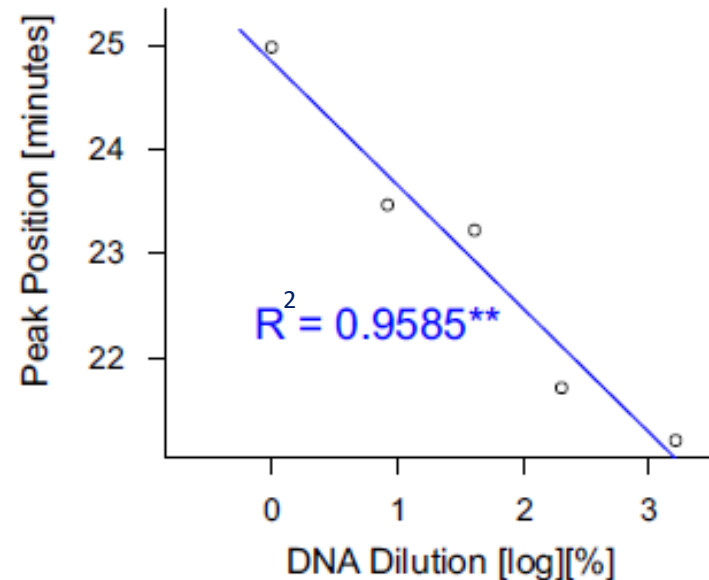
**Low reliability** due to degraded DNA and probable inhibition effects

## 4. Analytical Sensitivity

ALB LAMP assay tested on serial dilution of larval fresh material



ALB LAMP assay tested on serial dilution of DNA



Significant reverse proportional decrease of detection time by increasing amounts of homogenized material indicates reliable detection of different tissue amounts!

Serial DNA dilution with not significant higher linearity than the tissue dilutions indicate a high accuracy of both DNA extraction and LAMP detection

## Conclusions

- Successful LAMP assays generated for the detection of ALB, CLB and *Aromia moschata*
- Fresh material enables most reliable results
- Specificity analysis showed false positive CLB detection in the ALB assay and needs therefore to be extended on fresh material of *Anoplophora* spp. and indigenous species
- LAMP sensitivity allows the detection of less than 1 mg fresh material
- Standard method to compare with LAMP diagnostic potential should be the **RFLP assay** as established at the **BFW Vienna**





## WP3: Detection of adult beetles with pheromone-kairomone baited traps

Available lures for ALB based upon

### (1) Male produced pheromone components:

4-(n-heptyloxy)butan-1-ol

4-(n-heptyloxy)butanal

### (2) Host tree volatiles:

Linalool, (Z)-3-hexen-1-ol, trans-caryophyllene, (linalool oxide, trans-pinocarveol)



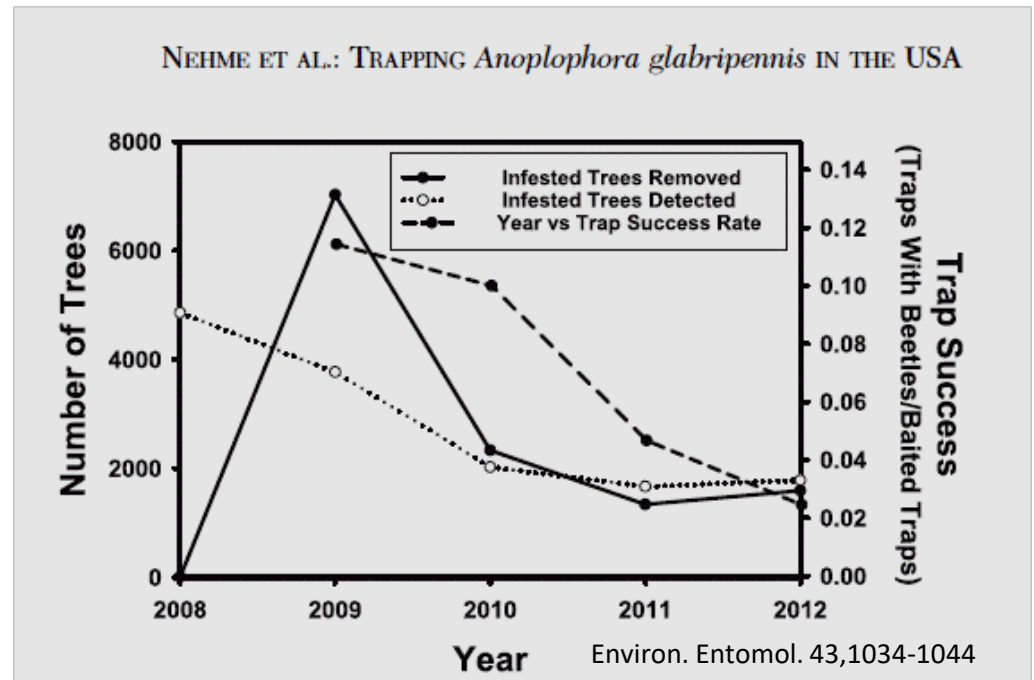
# Experiences in the USA (Worcester, MA)

Nehme et al. 2014: Environ. Entomol. 43,1034-1044

45 ALB captured in 40 traps from a total of 876 baited traps (= 4.6%) from 2009 through 2012

No ALB in 137 unbaited traps

Reduction of number of caught beetles with reduction of infested trees



# ALB pheromone traps in UK and Austria 2014-15

## ALB infestation site Paddock Wood, Kent, UK

- 18 ALB traps

## ALB infestation site Gallspach, Austria

- 20 (27 in 2015) ALB traps

## Stone importer I, Austria

- 5 ALB traps, 3 *Monochamus* traps

## Stone importer II, Austria

- 10 ALB traps, 2 *Monochamus* traps

- ALB traps and lures: ChemTica Internacional S.A. (Costa Rica); additional traps and lures from Witasek (Austria) in 2015
- *Monochamus* traps from Econex (Spain) and lures (Gallopprotect-2D; alpha-pinene added in 2015) from SEDQ (Spain)
- Traps deployed from June to October

## Trap catches in ALB outbreak areas

		Traps	ALB
Paddock Wood, UK	2014	18	0
	2015	18	0
Gallspach, Austria	2014	20	0
	2015*	27	1



### Other cerambycids caught in traps in Gallspach, Austria

2014	no other cerambycids
2015*	1 <i>Phymatodes testaceus</i>
	1 <i>Leptura rubra</i>
	1 Lepturinae

### *Osmoderma eremita* (priority species in EU habitat directive)

2014	1 specimen
2015*	2 specimens



\*2015: preliminary data

# Traps in infestation area Gallspach



## Trap catches at stone importers

		ALB	<i>M. gallo.</i>
Stone Importer I	2014	<b>0</b>	<b>1</b>
	2015*	<b>0</b>	<b>2</b>
	Traps	5	3
Stone Importer II	2014	<b>0</b>	<b>0</b>
	2015*	<b>0</b>	<b>1</b>
	Traps	10	2



\*2015: preliminary data

# Trap catches at stone importers

other cerambycids, 2014 and 2015\*

	ALB		G2D	
	SI	S II	SI	S II
<i>Acanthocinus griseus</i>	-	-	-	1
<i>Anisarthron barbipes</i>	1	-	-	-
<i>Aromia moscata</i>	1	-	3	-
<i>Arhopalus rusticus</i>	-	2	-	-
<i>Chlorophorus figuratus</i>	-	-	-	1
<i>Chlorophorus sp.</i>	-	-	-	1
<i>Hylotrupes bajulus</i>	-	7	-	-
<i>Leptura rubra</i>	-	-	-	1
Lepturinae	1	-	2	1
<i>Obrium brunneum</i>	-	-	1	-
<i>Phymatodes testaceus</i>	-	3	-	1
<i>Rhopalopus clavipes</i>	-	2	-	1
<i>Spondylus buprestoides</i>	1	1	15	3
<b><i>Trichoferus campestris</i></b>	-	-	<b>1</b>	-
<i>Xylotrechus sp.</i>	-	1	-	-



\*2015: preliminary data

## WP5: Development of contingency plans



Notfallplan und Leitlinie  
zur Bekämpfung des  
Asiatischen Laubholzbockkäfers  
*Anoplophora glabripennis*  
in Deutschland  
(Stand Juli 2015)







# Thank you for your attention!



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