Efficiency of the EPPO protocol for preventing the introduction and dissemination of fire blight in two Spanish areas
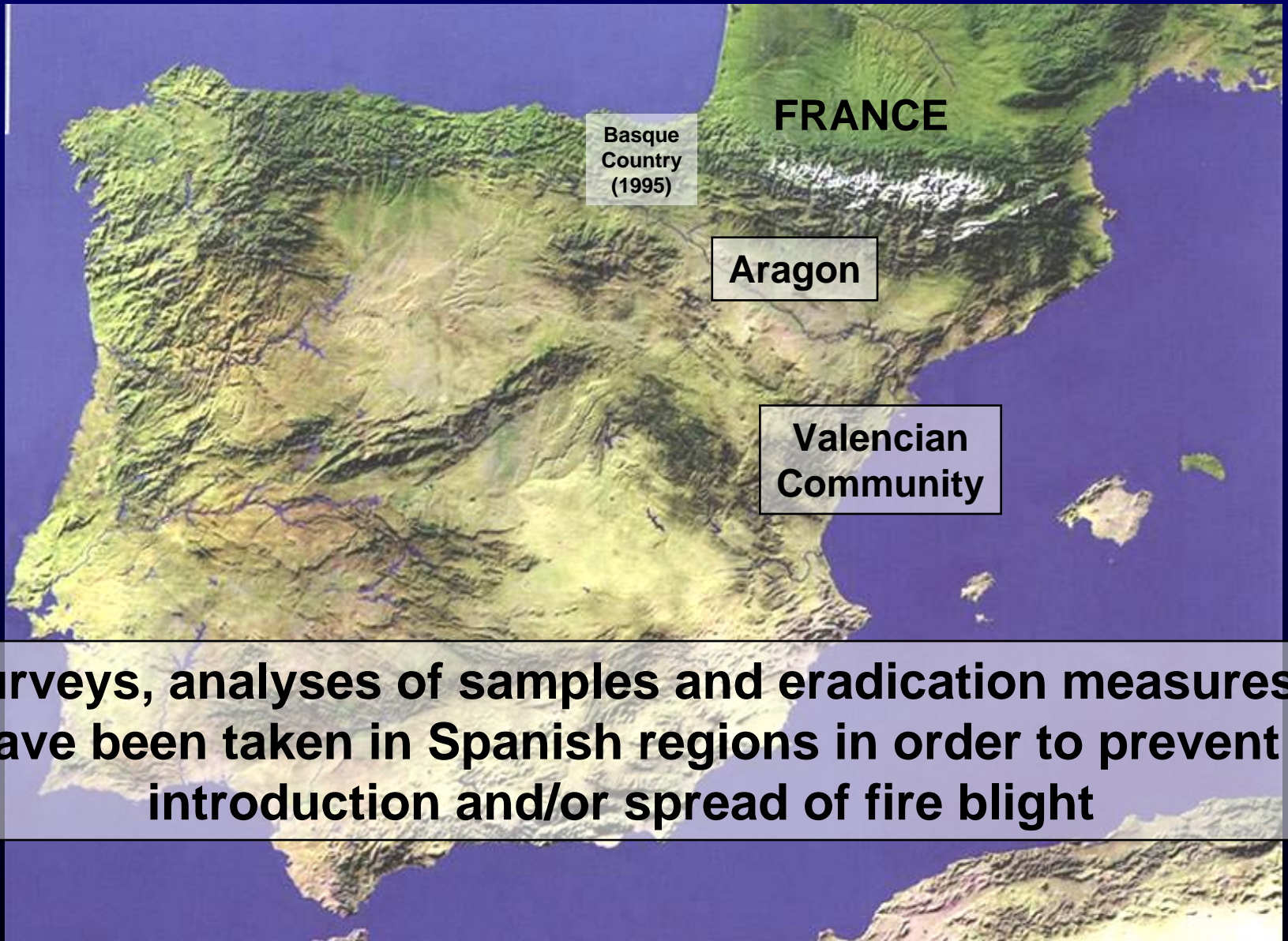
Palacio-Bielsa A., Cambra M.A., Roselló M., Gorris M.T., Peñalver J., Montesinos E., López M.M.

FRANCE

Basque
Country
(1995)

Aragon

Valencian
Community

Surveys, analyses of samples and eradication measures have been taken in Spanish regions in order to prevent introduction and/or spread of fire blight

Diagnosis of *E. amylovora*

http://www.csl.gov.uk/science/organ/ph/diagpro/

Quick
■Search:

You are here: Science / Areas of Study / Plant Health / DIAGPRO (diagnostic protocols)

DIAGPRO (DIAGNOSTIC PROTOCOLS)

SMT PROJECT SMT-4-CT98-2252
DIAGNOSTIC PROTOCOLS FOR ORGANISMS HARMFUL TO PLANTS

DIAGNOSIS OF *Erwinia amylovora*

PROTOCOL FOR THE DIAGNOSIS OF QUARANTINE ORGANISM

Erwinia amylovora

Identity

Name: *Erwinia amylovora* (Burrill) Winslow et al.

Synonyms: *Micrococcus amylovorus* Burrill.
Bacillus amylovorus (Burrill) Trevisan.
Bacterium amylovorus (Burrill) Chester.
Erwinia amylovora f.sp. rubi Starr, Cardona and Falson.

Common name: Fire blight.

Taxonomic position:
Proteobacteria, γ Subdivision, orden *Enterobacteriales*,
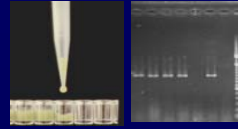family *Enterobacteriaceae*, genus *Erwinia*.

Quarantine status: EPPO A2 list, EU Annex II/A2.
Bayer computer code: ERWIAM

Bulletin OEPP
EPPO Bulletin

SCHEME FOR SYMPTOMATIC FIRE BLIGHT SAMPLES

Plants with symptoms

RAPID SCREENING TESTS
IF, Enrichment DASI-ELISA, nested PCR

ISOLATION, ENRICHMENT- ISOLATION

Test all negative

Positive

Colonies with typical morphology

Yes
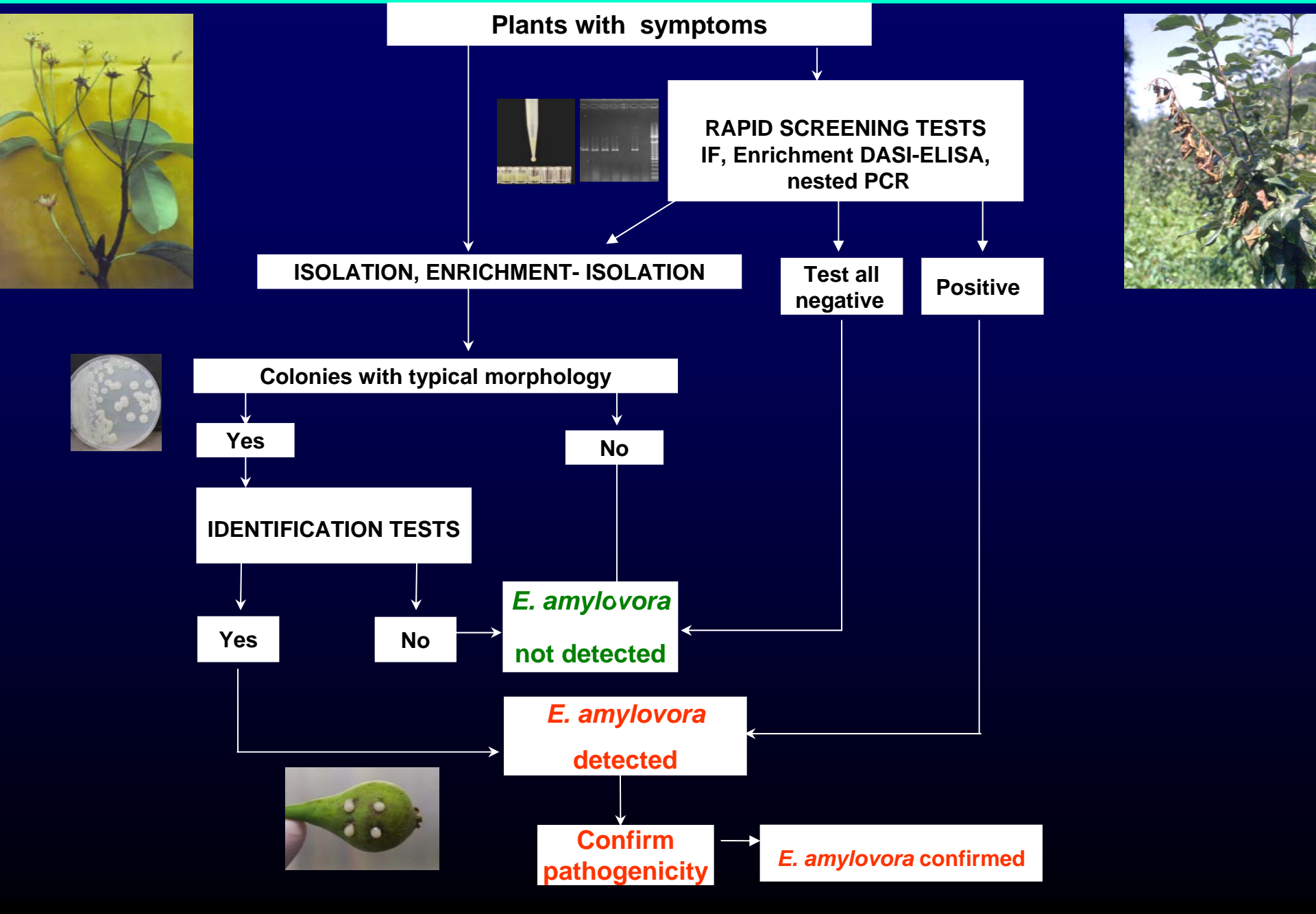
No

IDENTIFICATION TESTS

Yes

No

E. amylovora not detected

E. amylovora detected

Confirm pathogenicity
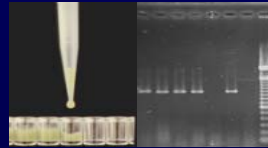
E. amylovora confirmed

SCHEME FOR ASYMPTOMATIC FIRE BLIGHT SAMPLES

Asymptomatic sample

↓

Pathogen extraction, optative concentration and enrichment

↓

SCREENING TESTS
IF, enrichment-isolation, enrichment DASI-ELISA, enrichment-PCR

↓

At least one test positive

Tests all negative

↓

Isolation and/or enrichment isolation

↓

Colonies with typical morphology → **No** → *E. amylovora* **not detected**

Yes

↓

IDENTIFICATION TESTS → **No**

Yes

↓

E. amylovora **detected** → **Confirm pathogenicity** → *E. amylovora* **confirmed**

FRANCE

Valencian
Community

VALENCIAN COMMUNITY: PREVENTIVE MEASURES

Surveys and analyses of asymptomatic and suspicious samples in nurseries, orchards and forests every year

ANALYZED SAMPLES (1996-2008)

HOST	Nurseries	Orchards/Forests	TOTAL
Pear	1.213	364	1.577
Apple	1.116	101	1.217
Loquat	695	158	853
Medlar	15	-	15
Quince	290	7	297
Crataegus spp.	193	63	256
Pyracantha spp.	2.298	1	2.299
Cotoneaster spp.	2.090	-	2.090
Sorbus spp.	116	-	116
Photinia spp.	25	-	25
Wild pear	-	1	1
TOTAL	8.051	695	8.746

E. amylovora HAS NOT BEEN DETECTED

ISOLATES CHARACTERIZATION AND IDENTIFICATION

✓ Bacterial isolates with colony morphology resembling *Pseudomonas* were obtained from suspicious samples:
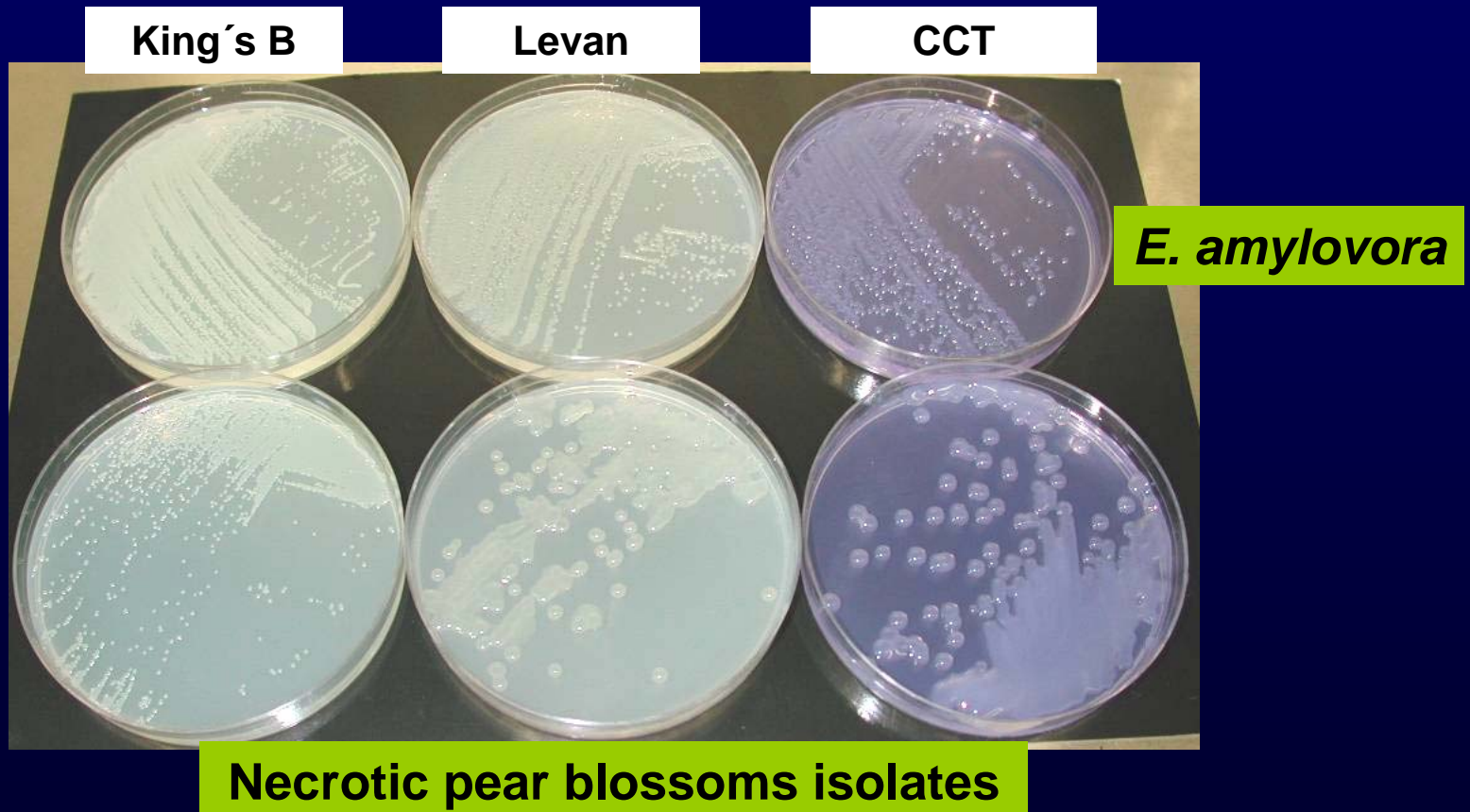
CROP	YEAR	IDENTIFICATION
Pear	2001, 2003, 2005, 2006	*Pseudomonas syringae* pv. *syringae*
Apple	2006	*Pseudomonas syringae* pv. *syringae*
Apple	2007	*Pseudomonas* sp.

✓ **Bacterial isolates with colony morphology resembling *E. amylovora* were obtained from necrotic pear blossoms**

NECROTIC SYMPTOMS SIMILAR TO *E. amylovora* ONLY PEAR BLOSSOMS ARE AFFECTED
(No progress to other parts of the tree)

Colony morphology (48h after 25 ºC incubation)

King´s B   Levan   CCT

E. amylovora

Necrotic pear blossoms isolates

Polyphasic approach characterization:

- **Phenotypic** (biochemical and physiological, fatty acid profiles, serological tests)
- **Genetic** (PCR, rep-PCR, plasmids profile, 16S rRNA, G+C content and DNA-DNA hybridization)

Identification of an *Erwinia* sp., different from *E. amylovora*, and responsible for necrosis on pear blossoms (proposed as *Erwinia piriflorinigrans*) (Roselló *et al.,* 2008)

Interest of the use of an integrated approach, with specific tools for accurate detection of *E. amylovora*, to avoid false positive detections

FRANCE

ARAGON

ARAGON:
ERADICATION MEASURES OF FIRE BLIGHT AND PREVENTION OF THE SPREAD

Jalon river fertile plain
All detections inside a 5 km radius area

* 2000
● 2001
● 2002
2003

FIRE BLIGHT ON FRUIT TREES IN ARAGON

Year	No. affected orchards (measures adopted)	Area (ha)*	No. infected trees
2000	12 (E)	19.6 (E)	249
2001	2 (E) 2 (SE)	7 (E) 12.4 (SE)	227 39
2002	1 (E) 2 (SE)	3.2 (E) 7.9 (SE)	15 25
2003	3 (E)	9.2 (SE)	4
TOTAL	15 (E) 7 (SE) (22)	29.8 29.5 (59.3)	491 68 (559)

NO NEW DETECTIONS SINCE 2004 UP TO DATE

(E): total eradication; (SE): selective eradication; *Aprox. 1.000 trees/ha

ANALYSES PERFORMED IN A PEAR ORCHARD AFTER SELECTIVE ERADICATION (3 laboratories)

	Date	Material[b]	No. samples	No. positive samples		
				E-ELISA	Isolation	PCR
24 plots[a]	March 2002	Blossoms	720 (240 x 3)	1	0	0
	June 2002	Shoots	720 (240 x 3)	2	0	0
	June 2002	Air	24	0	0	0
		Soil	24	0	0	0
13 plots[a]	May 2003	Shoots	390 (130 x 3)	0	0	0
	June 2003	Shoots	390 (130 x 3)	0	0	0
TOTAL			**2.268**			

[a] Ten trees per plot
[b] Three samples per tree (4 corymbs, shoots / sample)

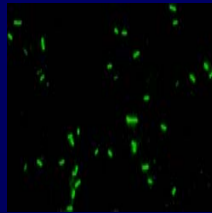**VERY LOW INOCULUM AVAILABLE (Maryblyt predicted high risk)**

OTHER ANALYSES PERFORMED IN THE ORCHARD

Material	Date	No. samples	No. positive samples [*]		
			E-ELISA	Isolation	PCR
Diverse plant material (Shoots, blossoms)	2002	1.164	9	1	1
	2003	1.650	0		
Honeybees (Full bloom)	2002	200	0	0	0
	2003	360	0	0	
Beehive pollen	2002	30	0	0	0
	2003	40	0	0	0
Flies	2003	90	5	0	0
TOTAL		3.606			

*One positive tree (only in 2002, no detection in further analyses)

VERY, VERY INOCULUM AVAILABLE IN THE PLOT, NO SYMPTOMS

CONCLUSIONS

- The EPPO protocol for diagnosis of *E. amylovora* has proved to give accurate data for fireblight prevention and eradication programs

- The efficient detection of *E. amylovora* in asymptomatic plant material requires an integrated approach

- The combination of intensive surveys and fast elimination of infected plant material has been successful in long-term eradication of fire blight in Aragon region (Spain)

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Thanks for your attention