POnTE training course

The theoretical and practical training on the detection of *Xylella fastidiosa* and *Candidatus* Liberibacter solanacearum

University of Belgrade - Faculty of Agriculture (UB-FA), Department of Plant Pathology, Belgrade, Serbia
June 24-26, 2019

Trainers:

1. Giuliana Loconsole, UNIBA-DiSSPA, Italy, (giuliana.loconsole@ipsp.cnr.it)
2. Bruno Legendre, ANSES-LSV, France, (bruno.legendre@anses.fr)
3. Marianne Loiseau, ANSES-LSV, France, (marianne.loiseau@anses.fr)
4. Ester Marco-Noales, IVIA-PPBC, Spain, (emarco@ivia.es; marco_est@gva.es)
5. Teresa Gorris, IVIA-PPBC, Spain, (mtgorris@ivia.es)
6. Caroline Freye-Minks, LOEWE Biochemica GmbH, Germany, (caroline.freye@loewe-info.com)
7. Lilia Formica, AGRITEST, Italy, (l.formica@agritest.it)

TRAINING PROGRAM

Monday, June 24

Time	Session	Speaker/Trainer	Room
8:30-9:00	Arrival at the University of Belgrade - Faculty of Agriculture and registration	/	Institute library, room 15, 3rd floor
9:00-9:15	Welcoming note	A. Obradović, POnTE WP 10 Leader; UB-FA representative	
9:15-9:30	Training introduction, orientation with coffee and refreshments	M. Ivanović	
9:30-10:15	Lecture 1: Latest information and results related to the detection of	G. Loconsole	Teaching room A11, 3rd floor

	X. fastidiosa by molecular and serological methods referring to experience in Italy		
10:15-10:25	Discussion on Lecture 1		
10:25-11:10	Lecture 2: Latest improvements and results related to the detection of *X. fastidiosa* by molecular methods and identification by MLST on plants and insects	B. Legendre	Teaching room A11, 3rd floor
11:10-11:20	Discussion on Lecture 2		
11:20-12:20	Lab work for *X. fastidiosa*: • Preparation of plant material for isolation on medium (1h)	B. Legendre	Lab 6, 3rd floor
12:30-13:30	Lunch break		Institute library, room 15, 3rd floor
13:30-17:30	Lab work for *X. fastidiosa*: • Samples preparation (plants and insects) for biomolecular methods (total time 2h 30min) • Preparation of buffers for ELISA test • DNeasy mericon food kit (QIAGEN) extraction • CTAB DNA extraction • LAMP for detection of *X. fastidiosa*	G. Loconsole B. Legendre C. Freye-Minks E.M. Noales T. Gorris	Lab 6, 3rd floor
	Coffee and refreshments (some time between 2 and 6 pm)		Institute library, room 15, 3rd floor
17:30-17:45	Results analysis and discussion of Day 1		Institute library, room 15, 3rd floor

Tuesday, June 25

Time	**Session**	**Speaker**	**Room**
8:30-8:45	Yesterday's lectures discussion, questions, suggestions, planning	Moderator: A. Obradović	Teaching room A11, 3rd floor
8:45-9:45	Lab work: • ELISA test (approx. 1h)	C. Freye-Minks E.M. Noales T. Gorris	Lab 6, 3rd floor
9:45-13:15	Lab work:	B. Legendre	Lab 6, 3rd floor

	• Special session for QuickPick DNA extraction (total time 3h 30min)		
	Coffee and refreshments (some time between 11am and 1pm)		Institute library, room 15, 3rd floor
13:15-14:15	Lunch break		Institute library, room 15, 3rd floor
14:15-17:15	Lab work: • ELISA test (continuation) • Conventional PCR for *X. fastidiosa* (Minsavage et al., 1994) • Real-time PCR for *X. fastidiosa* (Harper et al., 2010, erratum 2013)	C. Freye-Minks E.M. Noales T. Gorris G. Loconsole B. Legendre	Lab 6, 3rd floor
	Coffee and refreshments (some time between 3 and 6 pm)		Institute library, room 15, 3rd floor
17:15-17:30	Results analysis and discussion of Day 2		Institute library, room 15, 3rd floor
Optional	Belgrade visit tour (2–3 hours) with tourist guide, social dinner		

Wednesday, June 26

Time	**Session**	**Speaker**	**Room**
8:30-8:45	Yesterday's lectures discussion, questions, suggestions, planning	Moderator: A. Obradović	Teaching room A11, 3rd floor
8:45-9:15	• ELISA test (continuation)	C. Freye-Minks E.M. Noales T. Gorris	Lab 6, 3rd floor
9:15-10:15	Lectures 3, 4 and 5: • Comparison, characterization and validation of different DNA extraction procedures for the detection of *Ca*Lsol on plant hosts and insect vectors; • General introduction to conventional PCR and real-time PCR and interpretation of (real-time) PCR results and	M. Loiseau	Teaching room A11, 3rd floor

	sequencing results for haplotyping; • Interlaboratory test for validation of diagnostic procedures for the detection of *Ca*Lsol – preliminary results;		
10:15-10:25	Discussion on Lecture 3, 4 and 5		
10:25-11:10	Lecture 6: The use of monoclonal antibodies in detection of *X. fastidiosa* (experience from IVIA lab)	E.M. Noales	Teaching room A11, 3rd floor
11:10-11:20	Discussion on Lecture 6		
11:20-13:30	Lab work: • Conventional PCR for *Ca*Lsol (Jagoueix et al 1996; Li et al, 2009) • Real-time PCR for *Ca*Lsol (Teresani et al., 2014) • ELISA test (continuation)	E.M. Noales T. Gorris M. Loiseau C. Freye-Minks	Lab 6, 3rd floor
13:30-14:30	Lunch break		Institute library, room 15, 3rd floor
14:30-16:30	Lab work • ELISA (continuation: plate reading) • Conventional PCR for *Ca*Lsol (continuation) • Direct tissue blot immunoassay (DTBIA) for detection of *X. fastidiosa*	E.M. Noales T. Gorris C. Freye-Minks L. Formica G. Loconsole	Lab 6, 3rd floor
16:30-17:00	Lab work: • Results analysis and discussion	All trainers	Lab 6, 3rd floor
17:00-17:30	Concluding section: • Conclusions from the course • Certificate award	All trainers	Institute library, room 15, 3rd floor
	Coffee and refreshments (some time between 3 and 5 pm)		Institute library, room 15, 3rd floor