EMPHASIS

Effective Management of Pests and Harmful Alien Species - Integrated Solutions

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WP 2 Practical solutions for surveillance and monitoring

Neil Boonham

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 634179.

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Duration:
48 months
(2015-2019)

EU funding:
6.526.038 €

22 partners
out of which 11
companies

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More than
1.000
person
months

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EUROPE **CANADA**

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PREDICT

pest management challenges and opportunities will be evaluated according to stakeholder-focused criteria and through pathway analysis.

PREVENT

practical solutions for surveillance in different pathways to enhance preparedness will be provided to end-users, and monitoring tools following outbreaks and eradication will be developed.

PROTECT

practical solutions for managing native and alien pests in agriculture, horticulture and forestry will be developed, their technical and economic feasibility will be demonstrated and their market uptake will be enhanced.

PROMOTE

a **mutual learning process with end-users** will be developed, and the solutions identified by the project will be promoted through training and dissemination.

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Agri-ecosystem/Target plants	Target pests
(Field crops) - Cereals	*Puccinia* spp. on wheat
	Aphids
	Ambrosia artemisiifolia on summer cereals
(Field crops) – OSR and Wheat	*Mycosphaerella graminicola Leptosphaeria maculans*
	Seedlings insects
(Protected crops) Veg and high-value crops	*Bemisia tabaci* and associated viruses
	Downy mildew Soil-borne diseases
(Orchards) - Pome fruit	*Cydia pomonella*
(Forestry and amenity plants) - Fraxinus	*Chalara fraxinea*
(Forestry and amenity plants) - Conifers	*Heterobasidion irregulare* *Heterobasidion* spp.
(Open land) - Amenity plants, Non-agricultural areas	*Ambrosia artemisiifolia* *Ailanthus altissima* *Heracleum* spp.

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Overview of WP2

- Rapid assays based on LAMP
- LAMP hardware development
- Surveillance of fungal diseases
- Surveillance of insect pests

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- Xylella fastidiosa in Italy
  - First recorded in 2013
  - Land-scape scale devastation

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Taxonomy and existing diagnostics

- Sub-species

3 formally described;

X. f subsp. *pauca*

X. f subsp. *fastidiosa*

X. f subsp. *multiplex*

3 proposed;

X. f subsp. *sandyi*

X. f subsp. *morus*

(*X. f* subsp. *tashke*)

X. taiwanensis (pear leaf scorch)

- qPCR and LAMP

Development of LAMP and Real-Time PCR Methods for the Rapid Detection of *Xylella fastidiosa* for Quarantine and Field Applications

S. J. Harper, L. I. Ward, and G. R. G. Clover

Plant Health and Environment Laboratory, Investigation and Diagnostic Centre, MAF Biosecurity New Zealand, P.O. Box 2095, Auckland 1140, New Zealand.
Accepted for publication 18 August 2010.

- 7 to 10 gene MLST

Xylella fastidiosa MLST Databases

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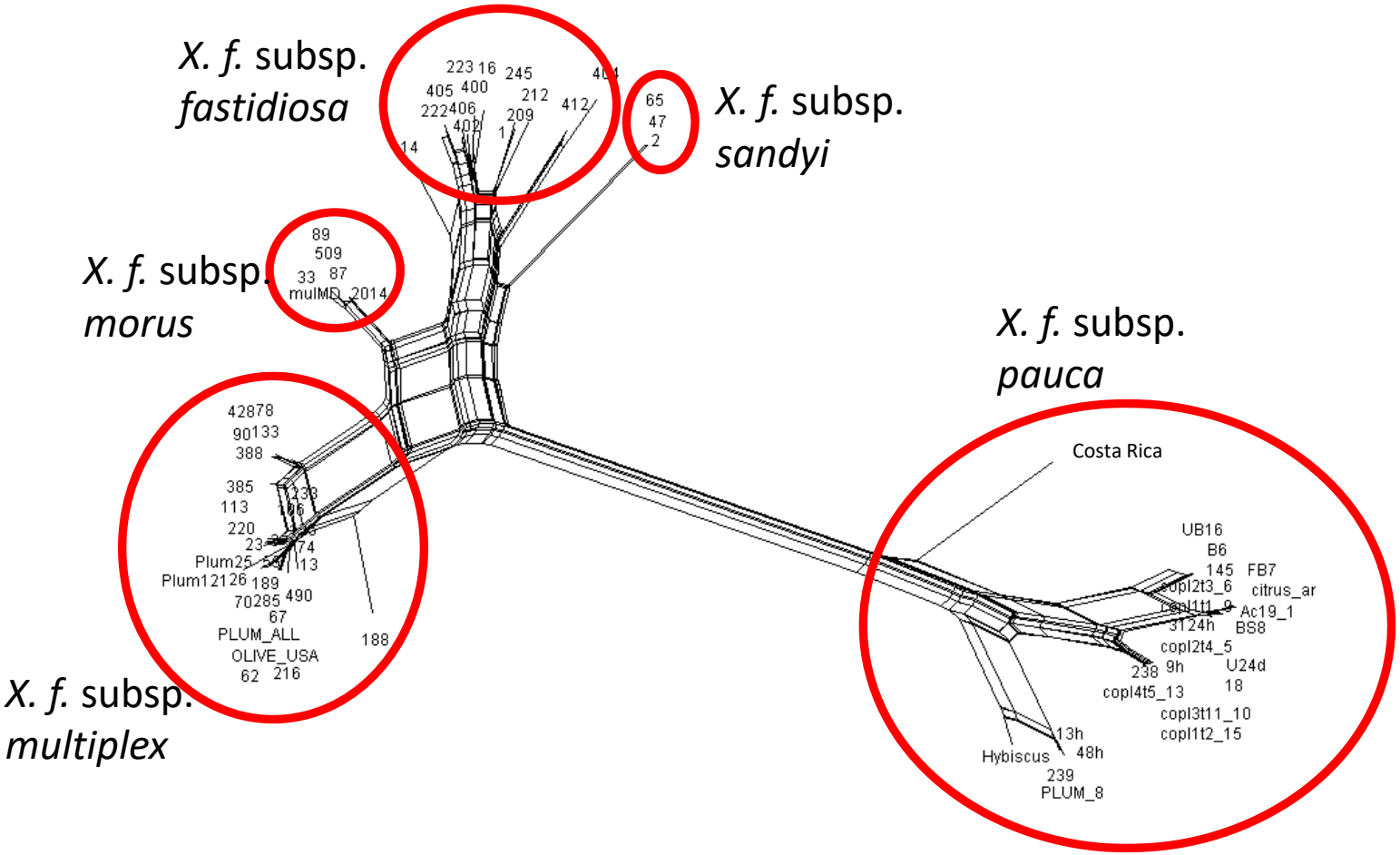
Conventional phylogenetics



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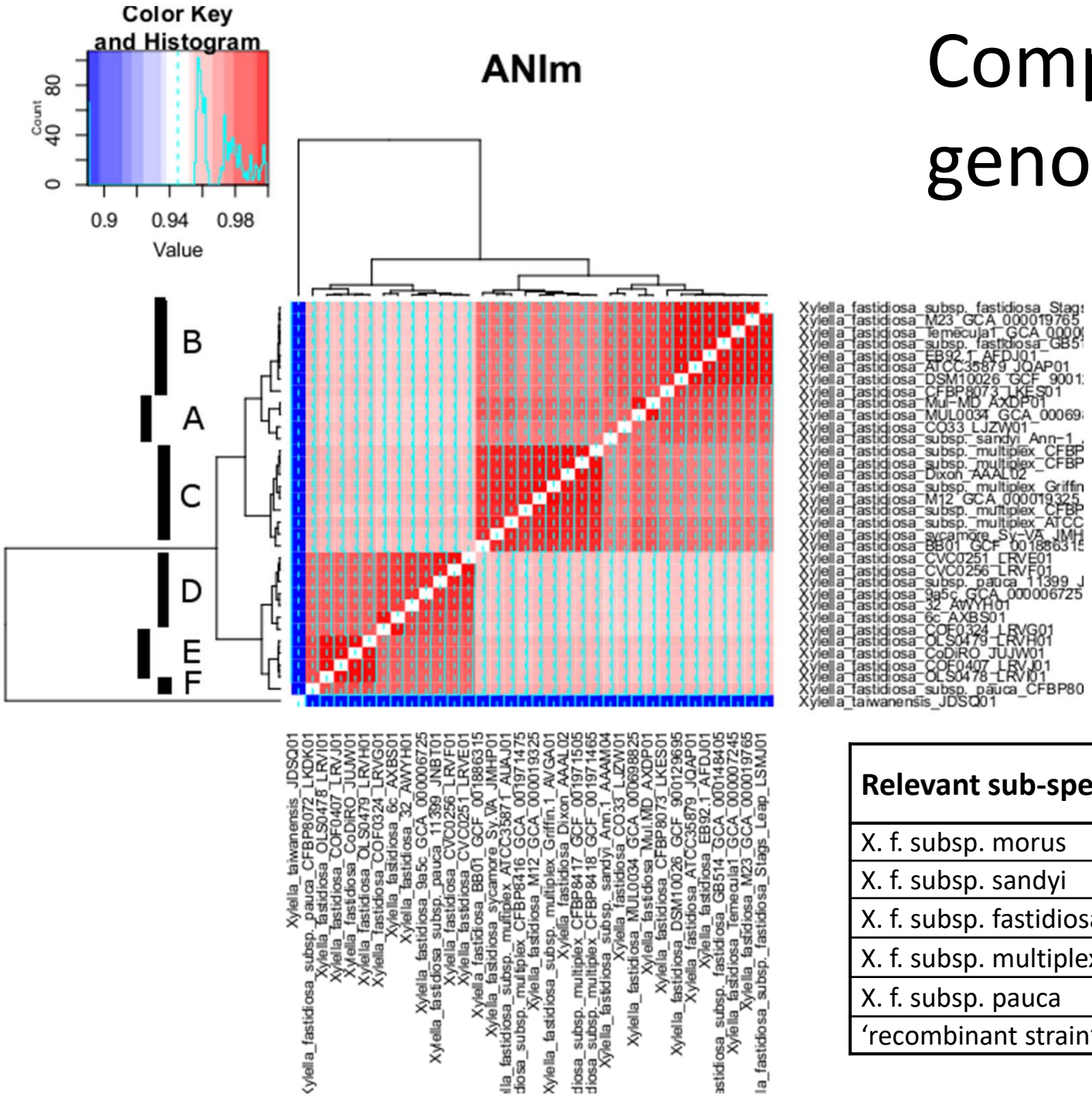
- Marker identification via comparative genomics
- Using publically available genomes (n=33)
- ANI analysis to identify sequences
- Aim = Sub-species level identification
- Develop qPCR and LAMP tests

Comparative genomics



Relevant sub-species	Corresponding sequence cluster
X. f. subsp. morus	A
X. f. subsp. sandyi	
X. f. subsp. fastidiosa	B
X. f. subsp. multiplex	C
X. f. subsp. pauca	D and E
‘recombinant strain’ ???	F

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Real-time PCR assays

Species / *Xylella* sequence cluster		A-1-Ra	A-1-Rb	B-1-Ra	B-1-Rb	B-2	B-3-Fa	B-3-Fb	C-1	C-2	C-3	DEF-1
X. f. subsp. *morus*	A	15.7	15.4	-	-	-	-	-	-	-	-	-
X. f. subsp. *fastidiosa*	B	-	-	17.5	17.9	16.3	16.7	18.0	-	-	-	-
X. f. subsp. *fastidiosa*	B	-	-	15.8	15.8	12.9	16.1	16.2	-	-	-	-
X. f. subsp. *fastidiosa*	B	-	-	17.9	16.8	16.4	16.0	17.1	-	-	-	-
X. f. subsp. *multiplex*	C	-	-	36.2	34.7	26.0	-	-	27.0	25.1	25.8	-
X. f. subsp. *multiplex*	C	-	-	36.7	-	33.3	-	-	18.2	17.3	18.1	-
X. f. subsp. *pauca*	DEF	-	-	-	-	-	35.1	-	-	31.6	-	16.9
A. tumefaciens		-	-	-	-	-	-	-	37.4	-	-	-
P. syringae pv. *persicae*		-	-	-	-	-	-	-	-	-	-	-
X. fuscans subsp. *aurantifolii*		-	-	-	-	-	-	-	-	-	-	-
X. campestris pv. *campestris*		-	-	-	-	-	-	-	-	-	-	-
NTC (water)		-	-	-	-	-	-	-	-	-	-	-

- Sub-species specific assay designed for ‘groups’ A, B, C and DEF

Shading
red = negative reaction
green = specific positive reaction
yellow = cross-reaction (non-target)

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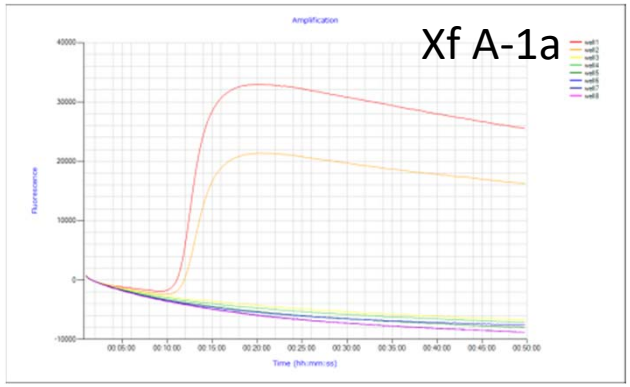
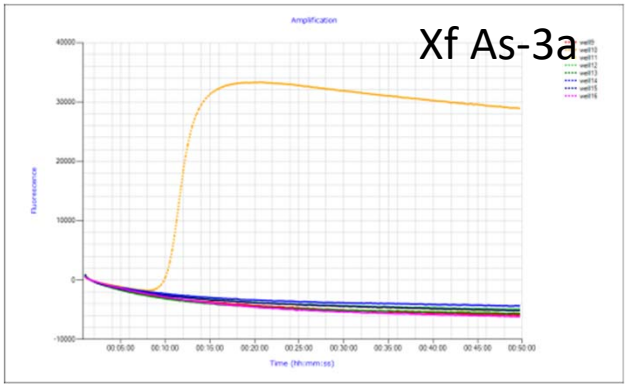
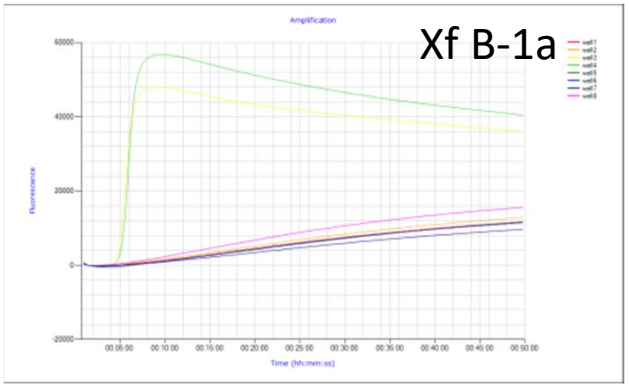
LAMP assays

Xylella sequence cluster		Xf-A 1a	Xf-A 1b	Xf-Am 2	Xf-As 3a	Xf-As 3b	Xf-B 1a	Xf-B 1b	Xf-B 2a	Xf-B 2b	Xf-C 1	Xf-C 2a	Xf-C 2b
X. f. subsp. *morus*	A / Am	12:30	15:30	07:45	-	-	-	-	-	-	-	30:00	-
X. f. subsp. sandyi	A / As	13:00	16:00	(41:15)	11:30	11:30	-	-	~ 34	~ 34	-	28:15	-
X. f. subsp. *fastidiosa*	B	-	-	-	-	-	06:00	05:45	~ 18	~ 18	-	25:00	-
X. f. subsp. *fastidiosa*	B	-	-	-	-	-	06:15	06:00	~ 20	~ 20	-	26:00	-
X. f. subsp. *multiplex*	C	-	-	-	-	-	-	-	-	-	-	11:30	40:00
X. f. subsp. *multiplex*	C	-	-	-	-	-	-	-	-	-	~ 49	08:15	26:45
X. f. subsp. *pauca*	DEF	-	-	-	-	-	-	-	-	-	-	18:00	-
NTC (water)		-	-	-	-	-	-	-	-	-	-	-	-







Shading
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yellow = cross-reaction (non-target)

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Deliver an effective platform for laboratory use and on-site deployment

- **Genie HT high-throughput platform**
- Hardware development
  - Prototype assembled and tested
  - Final modifications implemented
  - Drawings and parts list issued for quotation
- Software development
  - Individual modules operating
  - Central command unit software to be developed
- Available Q3 2017

- 12 sample blocks – 96 tubes
- 10.1” touchscreen interface
- Wired USB communications
- Bluetooth and **WiFi**
- Two-colour fluorescence
- Mains power only
- Forced-air cooling
- **Integrated barcode reader**
- **Supports instruction videos**

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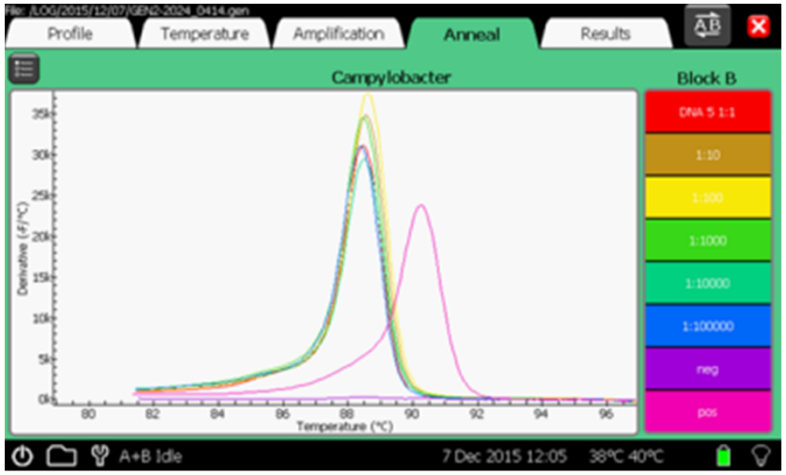
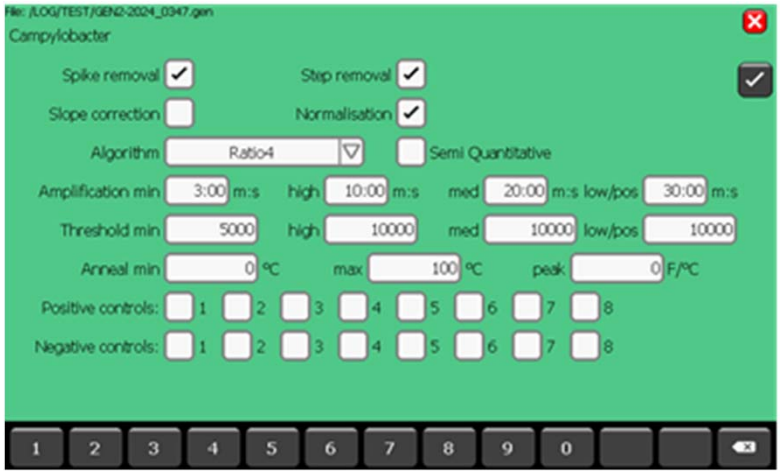
Promote ease of use

- Automated data entry and result interpretation
  - 1-D / 2-D barcode reader included within **Genie HT**
  - **D2.1** ***Automated result calling algorithms developed for LAMP assays implemented on the Genie platform***
    - Deliverable report issued February 2017 on-schedule





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Practical kit design

- Implement a tube sealing method for pre-dispensed or dried reagents
  - New plate for sealing 8-strips simultaneously manufactured
  - Fitted to pneumatic press December 2016

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Data management and connectivity

- WiFi connection (to **Genie III**)
  - Requirement to collect run files from **Genie III** over WiFi link
  - Software developed for **Genie III** to serve as an Access Point
  - Android app developed to enable connection and file transfer
  - Additional functionality planned

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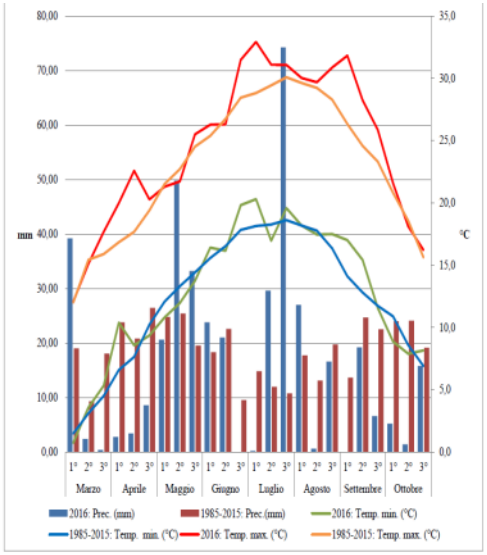
Validation of protocols combining spore trapping and the use of taxon-specific primers



Meteorological conditions

Spore trap

Disease index

	Pyricularia oryzae symptoms on leaves						
	19.7.16	26.7.16	2.8.16	10.8.16	17.8.16	23.8.16	30.8.16
CONTROL	3.00	3.75	4.00	3.25	6.50	8.25	9.00

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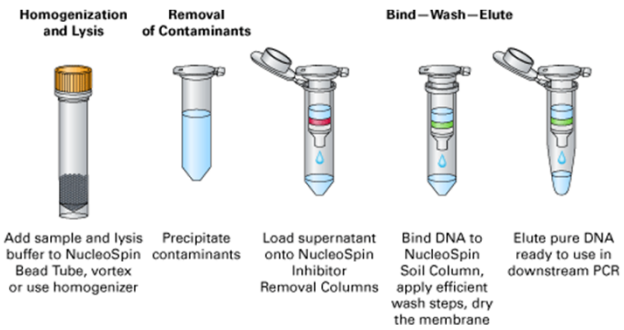
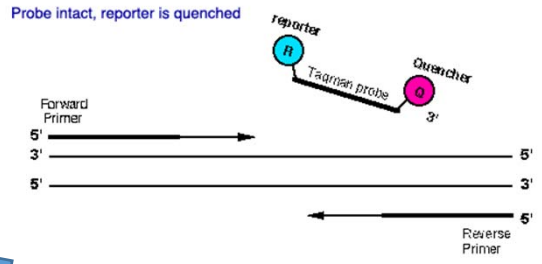
Samples collected in two different rice paddies in ENTERISI (Ente Nazionale Risi, Castello d'Agogna (PV), Italy)

Weekly samples

Cut tapes



DNA extraction of daily samples



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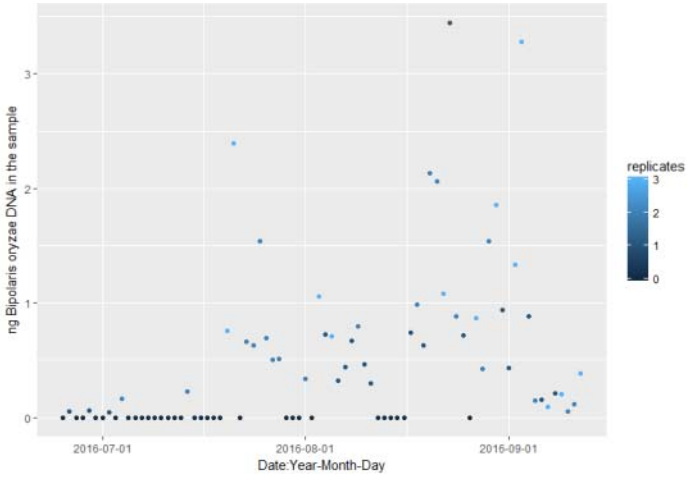
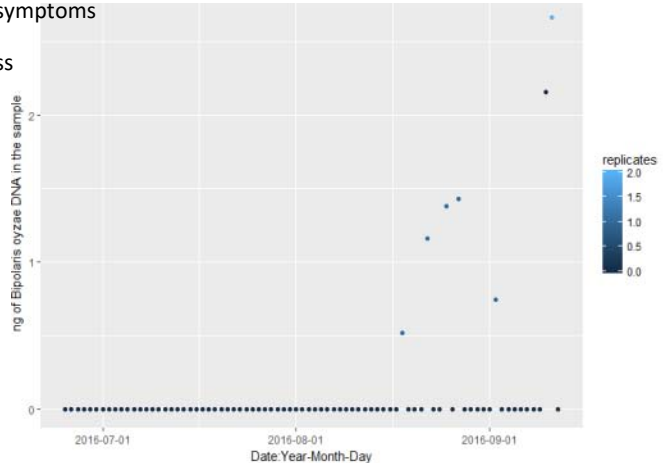
Quantification of *Magnaporthe oryzae* in the field showing blast

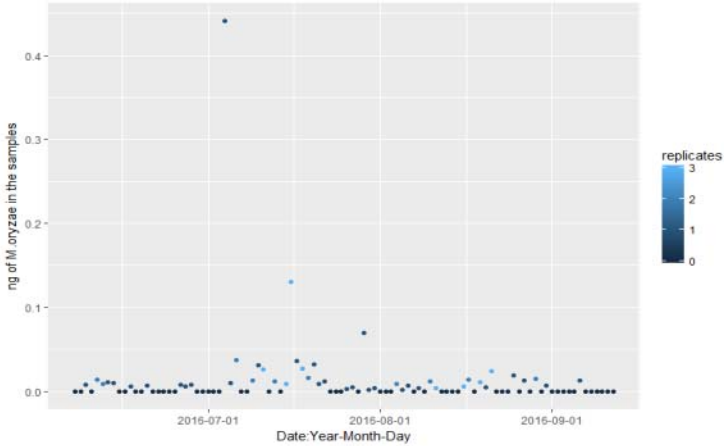


Quantification of *Bipolaris .oryzae* in the field showing blast rice symptoms

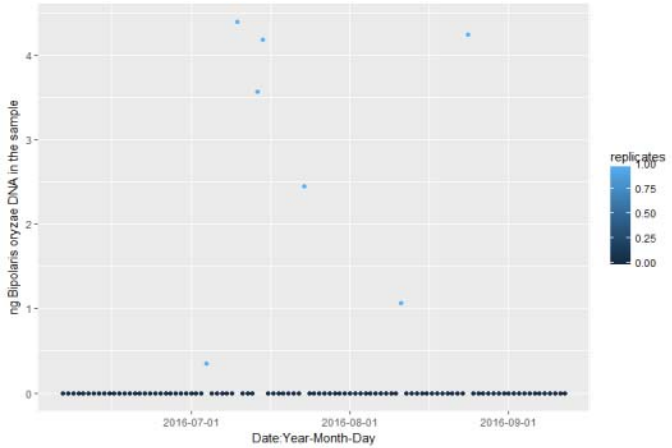
ss



Quantification of *Magnaporthe oryzae* in the field showing bakanae symptoms



Quantification of *Bipolaris .oryzae* in the field showing bakanae symptoms



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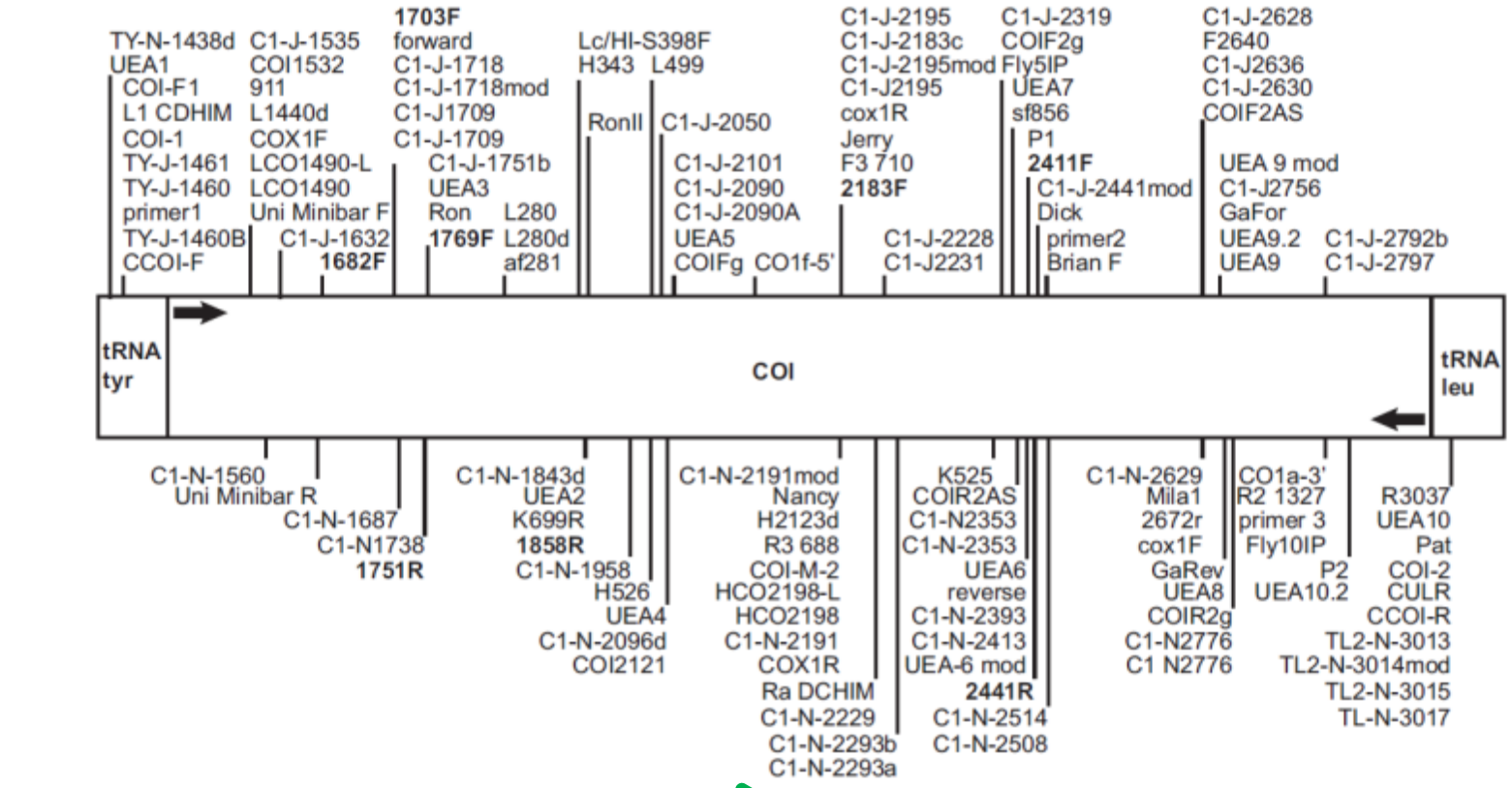

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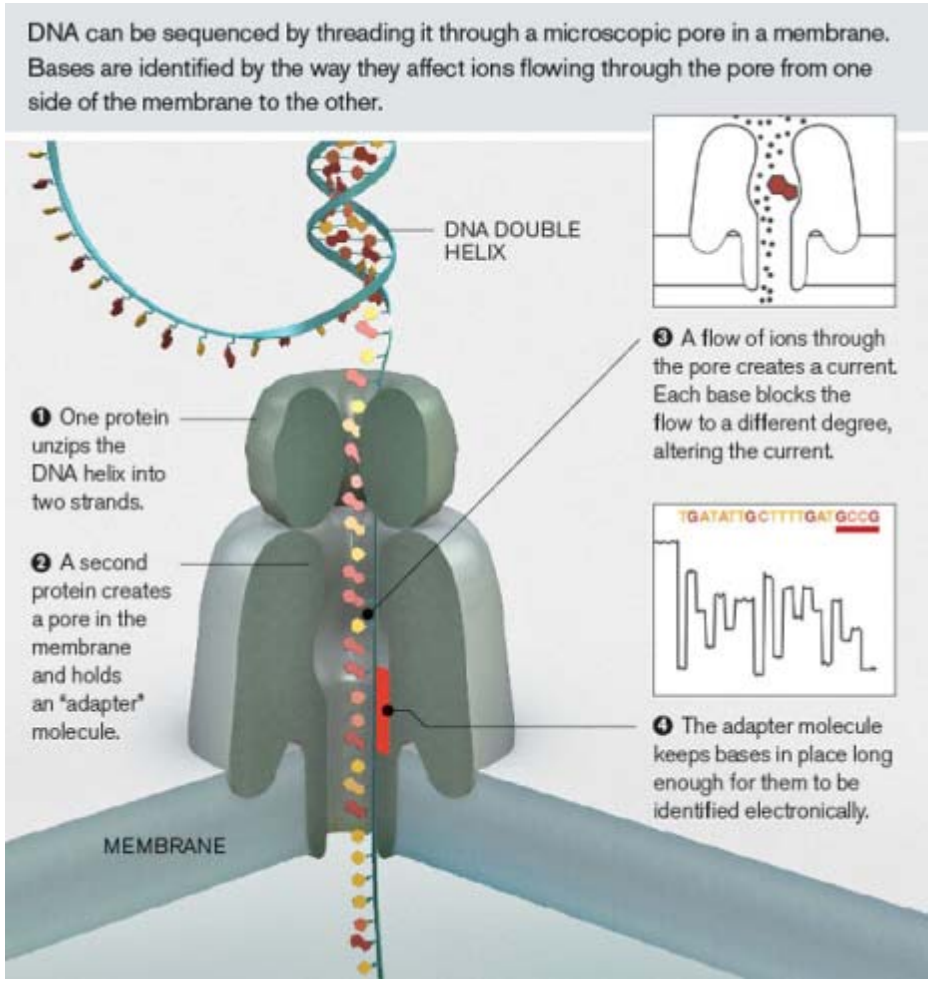
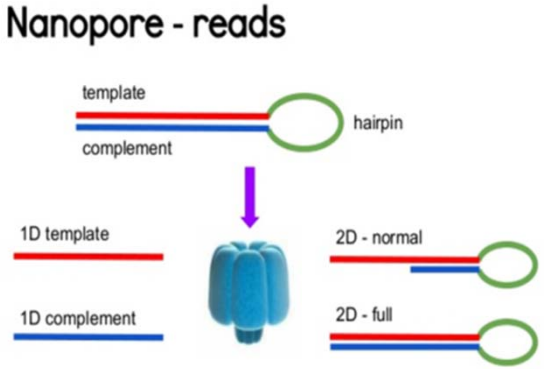
Oxford nanopore MinION





Latest pores offer 95% accuracy with better chemistry / basecalling algorithms

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- *Lyriomyza hubrensis*, *Bemisia tabaci* and *Frankinella occidentalis* as pure extracts
- Mixtures of extracted DNA
- Mixtures of insects extracted
- Leaf from *Lyriomyza hubrensis* culture with no evidence of insects
- Control leaf

Sequenced on R9 flow cell

12 barcoded samples run for 48hrs produced 35081 reads of which 17802 gave good quality 2D sequence.





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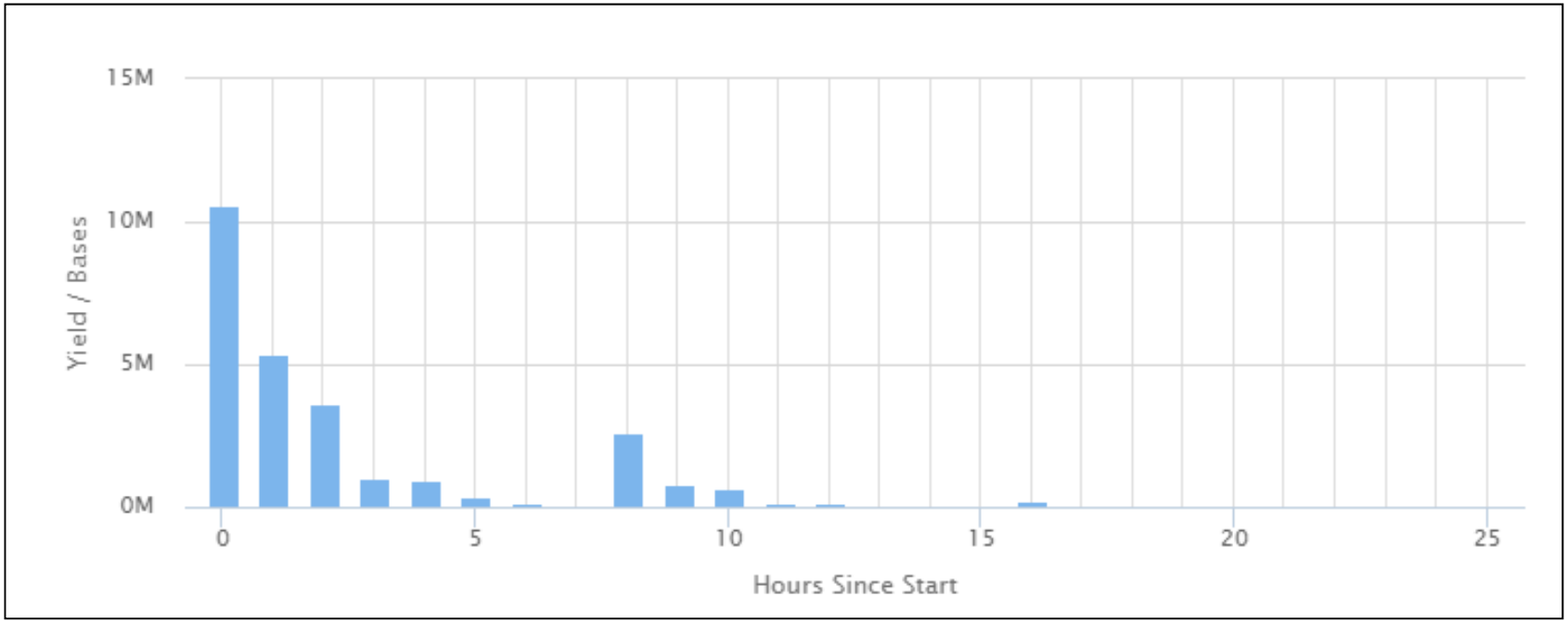

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Sample description	*Frankinella occidentalis*	*Lyriomyza hubrensis*	*Bemisia tabaci*	plant
Equal amounts of *Frankinella occidentalis* and *Lyriomyza hubrensis* DNA	35.0%	48.5%	0.0%	0.0%
Lyriomyza hubrensis mined leaf	0.1%	59.8%	0.1%	33.1%
Control leaf	0.0%	0.4%	0.0%	49.5%
Mix of *Frankinella occidentalis* and *Lyriomyza hubrensis* insects	30.0%	42.3%	0.0%	0.3%
Mix of *Frankinella occidentalis*, *Bemisia tabaci* and *Lyriomyza hubrensis* insects	3.4%	45.0%	4.8%	0.3%

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Acknowledgments

- Jen Hodgetts, Ian Adams & Rachel Glover (Fera)
- Sarah Franco Ortega & Davide Spadero (Agrinnova)
- Tom Wood & Rosa Caiazzo (NIAB-EMR)
- Michael Andreou (Optisense)

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FUTURE PROOFING Plant Health

A Defra Network partnership delivering interdisciplinary plant health research to improve biosecurity and build capability