General information

Description	**Tools for fast disease diagnostic - *Spore Traps Combined with qPCR***
Geographical area	Europe
Group of tree species	*Pinus* sp.
Date	September 2018
Authors (affiliation)	Helena Bragança INIAV, Eugénia Andrade INIAV, Eugénio Diogo INIAV
Contact e-mail	helena.braganca@iniav.pt
Tool type	Survey results/protocol
Tool format	Text
Language	English
Risk management plans to which the tools can be added	https://plurifor.efi.int/wp-content/uploads/WP2/plans/Fusarium-risk-plan_ES.pdf
Risk management plans link	https://plurifor.efi.int/wp-content/uploads/WP2/plans/Fusarium-risk-plan_ES.pdf
This tool is…	☒ a new tool

Topic

Risk	Pine pitch canker
Risk component	☒ hazard
Risk area	Risk management
Risk phase	Surveillance/monitoring/early warning
Risk phase (alternative terms)	Prevention
Level	Choisissez un élément.
Sendai priorities	☒ Priority 1: Understanding disaster risk ☒ Priority 2: Strengthening disaster risk governance to manage disaster risk ☐ Priority 3: Investing in disaster risk reduction for resilience ☐ Priority 4: Enhancing disaster preparedness for effective response and to “Build Back Better” in recovery, rehabilitation and reconstruction
Contribution to Sendai targets	☒ Reduce global disaster mortality ☐ Reduce the number of affected people ☐ Reduce the direct disaster economic loss ☐ Reduce disaster damage to critical infrastructure ☐ Increase the number of national and local disaster risk reduction strategies ☐ Enhance international cooperation to developing countries ☒ Increase availability of and access to multi-hazard early warning systems and disaster risk information and assessment

Description and analysis

Summary
Tools for fast disease diagnostic using Spore Traps Combined With Real-Time PCR (qPCR) aiming to allow authorities to detect new outbreaks of the disease and to prevent spread to new forest areas

Place in national/regional policy
National Control Plan

Goals and achievements
The aim of this tool is to provide fast disease diagnostic- early warning

Stakeholders involved
A workshop was held in Aveiro to present the new diagnosis tool to researchers, forests owners and representatives from Spain and Portugal. Furthermore, ICNF, the National Forest Authority is involved as well as nursery owners and managers.

Implementation stage
In process

State of technical knowledge
In process

Regulatory and/or socio-economic contexts
The tool can help authorities to detect new outbreaks and prevent spread to new forest areas.

Impacts of the tool

If we prove that the tool is efficient , it can help authorities to detect new outbreaks and to prevent the fungus dispersion, controling/irradication of new outbreakss aiming to avoid the expansion of the disease

Implementation requirements and durability

Description of the implementation steps
The analytical detection methodology was implemented and optimized before sampling in field. The most crucial step was DNA extraction from the spore traps sticks. In order to validate DNA extraction methods, spore traps sticks were spiked with known copy numbers of spores. As soon as a DNA extraction method was selected among the tested ones, spore traps were installed in areas where the fungus had been previously detected. The stiks were collected into sterile Eppendorf tubes, brought to the laboratory and DNA exctracted. qPCR were performed according to currently available methods. Further implementation stages would include the use of this methodology to generate information to make decisions about plant trade and forest operations.

Governance
An Operational procedure is currently in place in order to coordinate the efforts and actions of all actors involved, namely, the National Phytossanitary Authority, the NRL and all the producers and nurseries

Regulatory framework
Portaria n.º 294/2013, Diário da República, 1.ª série — N.º 187 — 27 de setembro de 2013
National Plan for Eradication & Survey of F. circinatum

Human resources requirements
Implementation of the tool requires phytossanitary technicians from the regional forest services with availability and means to travel to the field (installation of the spores traps and collection of the sticks) and analysts experience in Molecular Biology protocols

Financial requirements As the detection of *F. circinatum* constitutes a National Control Plan, partially financed by the Europena Commission, some financial requirements, specially for the installation and collection of the spores traps are expected to implement this tool.
Technical requirements For the time being, there are no additional needs.
Priorities identified for successful implementation of the tool (political, technical, human, financial…) The priorities for the implementation of the tool are (i) further optimization; (ii) articulation between forest authorities and analytical laboratories;(iii) more human resources; (iv) means to travel to different forests whenever necessary
Challenges or risk factors (legal, financial, safety…) expected during the implementation and solutions proposed Other diseases may be detected. Spores traps are unspecific traps, therefore allowing for the capture of spores of other fungi. As a challenge, this tool may help in the early detection of the other emergence diseases
Additional and non-formal experiences to help the implementation of good practice

SWOT analysis

Strengths	**Weaknesses**
The information can provide early warning by rapid disease diagnosis around areas/nurseries with previous reports and or areas with high probalilitiy of occurrence. qPCR (with fluorescence probe) increases the accuracy in comparison with conventional PCR	The available qPCR protocols to detected *F. circinatum* are not 100% sensitive. The available qPCR protocols to detected *F. circinatum* can failed in low % of spores.
Opportunities	**Threats**
New information may be included in the Good Practice manual to prevent the dispersion of the disease.	Globalization and vegetal trade may increase the spread of the pathogen. The price of the methodology (spore traps and/or lab costs) can difficult the massive utilization by the stakeholders .

Lessons learnt

Evaluation process, if exists (internal or external) N.A.
Assessment of results (quantitative and qualitative) and comparison with main goals **???** The data collected so far show that spore traps are useful and effective in some periods of the year. This is correlated with the concentration of spores in the air and, therefore, with the life cycle of the fungi. Quantitative detections of spores from the sticks installed in the field were performed by means of real-time qPCR based on calibration curves. The former were established with the DNA extracted from sticks spiked with known counts of spores. As the main goal of this tool is to provide a fast disease diagnostic- early warning, it is a huge advantage if air inoculum can be detected before the appearce of devastating symptoms.

Negative aspects identified The used qPCR protocol to detected *F. circinatum* was not 100% effective. An alternative method, with better performance values, is under implementation.
Unexpected consequences (short- / mid- / long-term) and corrective measures implemented N.A.