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Smart-traps combined with molecular on-site detection to monitor *Monochamus* spp. and associated pine wood nematode

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INTRODUCTION

The pine sawyer beetles Monochamus spp. (Coleoptera Cerambycidae) are the main vectors of the Pine Wood Nematode (PWN), Bursaphelenchus xylophilus, the agent of pine wilt disease in various parts of the world (Mamiya 1983). In Europe, M. galloprovincialis (Olivier) gained importance as a vector after the finding of the PWN in Portugal in 1999 (Sousa et al. 2001). An effective monitoring method based on early detection of both vector insects and associated nematode is needed in order to adopt appropriate phytosanitary measures (Rassati et al. 2012 and 2013).

MATERIALS AND METHODS

The present study shows a new technology for the remote detection of beetle catch combined with on-site molecular detection of both vector and nematode identity. A multi-funnel trap, baited with either specific or generic blend, and equipped with a specifically modified security camera (BioCam, Mi5 Security, Auckland, New Zealand), composed by a wide-angle lens, 1 or 3 MegaPixel sensor, rechargeable battery pack and internal modem for General Packet Radio Service (GPRS) connection was used. The interval between images taken by the camera can be programmed and saved in a Secure Digital (SD) memory card. The images can be stored in the same SD card and simultaneously sent to a safe repository accessible through the web, from which they are downloadable. On the same repository it is possible to check the level of battery charge of each camera and the GPRS coverage as well.

When a target beetle is detected, an on-site visit is planned, during which a fragment of the thorax is analyzed using a Loop Mediated Isothermal Amplification (LAMP) portable

device (Genie II, Optigene, UK) to identify the trapped species of *Monochamus* spp. and to detect the PWN possibly vectored by the beetles. Currently, primers were deloped for the endemic *M. galloprovincialis* and *M. sutor*, and for the exotic *M. alternatus* and *M. carolinensis*. For the beetles identified as *Monochamus*, presence of PWN is also tested with the same device using slighty modified LAMP primers from Kikuchi *et al.* (2009), specific for the nematode ITS1 region. A positive control for the nematode is included in the test. The technique allows amplifying target DNA in a few minutes visualizing the results immediately.

RESULTS

Images obtained by cameras are definitely adequate to visually recognize large longhorn beetles such as *Monochamus* spp.. All the main morphological traits of the species are detectable (Fig. 1). The system works also under sub-optimal light conditions. LAMP primers designed to amplify the ITS2 region of *M. galloprovincialis*, *M. sutor*, *M. alternatus* and *M. carolinensis* show to be specific, giving a positive result only for these species after 10-15 minutes after the test start (Fig. 2). On the other hand, no positive insects for PWN have been detected until now.

CONCLUSIONS

Both technologies are designed for quick and cheap on-site analyses, and can be used by non-expert staff with a short training. In case of positive samples, they must be taken to the laboratory and analyzed more accurately with standard protocols for official confirmation.

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Figure 1 Picture taken by 3MP trap camera. One individual of *Monochamus* spp. is clearly recognizable on the left, together with several individuals of the longhorn beetle *Acanthocinus griseus*, one of the western seed bug *Leptoglossus occidentalis* (above) and several small bark beetles (right).

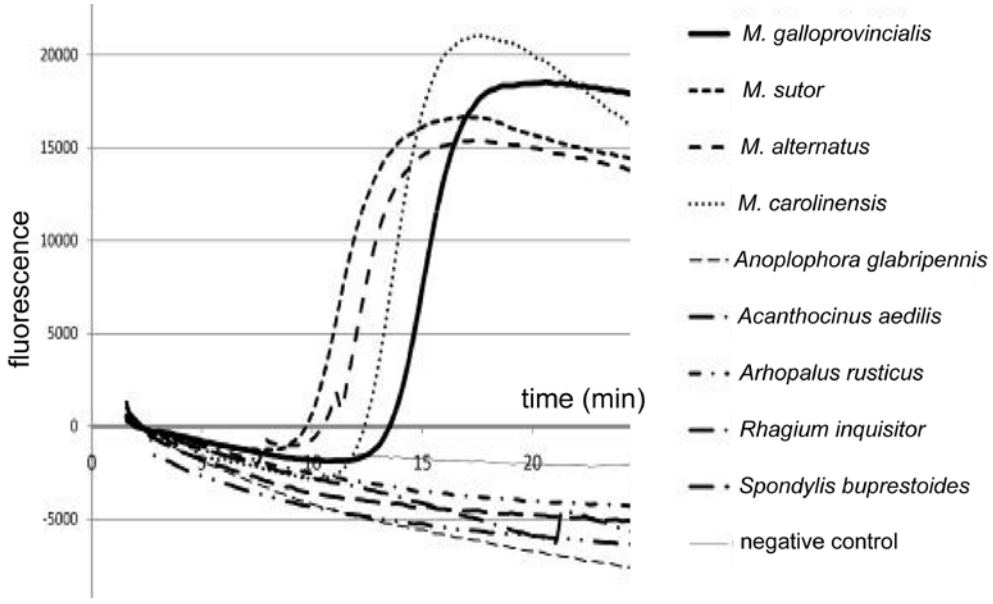

Figure 2. Amplification profile for the LAMP assay carried on *Monochamus* spp. and other cerambycid beetles. Positive curves are obtained in 10-15 minutes only for *M. galloprovincialis*, *M. sutor*, *M. alternatus* and *M. carolinensis*.