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Detection Of Latent Infections Of Apples Caused By *Neofabraea* Spp. and *Monilinia* Spp. Fungi Using LAMP Method

INTRODUCTION

Bitter rot of apples (Fig. 1), caused by fungi of the genus *Neofabraea* (*N. vagabunda*, *N. kienholzii*, *N. perennans* and *N. malicorticis*), is considered the most important storage disease of apples, due to significant yield losses. The presence of brown rot of pome trees (Fig. 2), caused by fungi of the genus *Monilinia* (*M. fructicola, M. fructigena* and *M. polystroma*) is much less frequent on stored fruit. Only fungi present in the lenticels of the fruit at the time of harvest are responsible for the development of bitter rot on stored fruit, while the fungi causing brown rot infect through the lenticels, as well as directly through the cuticle or mechanical damage. **Therefore, in the context of assessing the risk of occurrence and severity of these diseases, it is important to assess the health status of fruits prior placing them in cold storage, which is enabled by quick, sensitive and accurate diagnostic tests.**

MATERIALS & METHODS

The aim of the study was to develop a method requested from phytosanitary service (PIORiN) enabling the detection of fungi causing bitter rot of apples and brown rot of apples in infected fruit, but without symptoms of the disease. The realisation included the following stages:

1. Inoculation of apples cv. Topaz, growing in the experimental orchard in Dąbrowice, with spores of fungi of the genera *Neofabraea* and *Monilinia*, in both cases: species found on apples. After a month, the apples were harvested and placed in a cold store. 2. Development of a protocol for DNA isolation from the whole apple peel. 3. Design of LAMP primers for *Monilinia* and *Neofabraea* fungi. 4. Optimization of the LAMP reaction conditions with these primers and development of a method for pathogen DNA detection in fruits without disease symptoms. 5. Validation of the method using apples with symptoms of the disease and application of the method to the detection of pathogens in asymptomatic apples.

SUMMARY

The developed methods, based on the LAMP technique, enabled sensitive and specific detection of pathogens responsible for the development of bitter rot in apples and brown rot on apples at an early stage of disease development. The entire procedure is carried out: from the stage of apple peel preparation to obtaining the result of the LAMP reaction, it takes two days, including the LAMP reaction itself, and allows to obtain a positive result within 35 minutes from reaction start point. The whole procedure makes it possible to assess the health of apples before the development of disease symptoms.

The presented research was carried out in the frame of task 5.1 (Developing strategies to control pests in the country and supporting activities to acquire new markets for domestic products derived from plants), financed as a targeted subsidy from the Ministry of Agriculture and Rural Development.

RESULTS

Fig. 1. Symptoms of the bitter rot of apples on the stored fruits.

Fig. 2. Symptoms of the bitter rot of apples on the stored fruits.

1. As a result of the conducted research, two sets of primers for the detection of fungi of the genus *Neofabraea* were finally selected: Aspa_Nvaga for the detection of N. vagabunda and GTP_NKienPer for *N. perennans*, *N. kienholzii* and *N. malicorticis*, while in the case of fungi of the genus *Monilinia*, the set of primers HSP_Moni enabled detection three species of fungi in one reaction.
2. These primer sets enabled the detection of the target DNA in the LAMP reaction with a sensitivity of about 5 pg/µl for Neofabraea DNA (Fig. 3, 4, 5 and 6), and 2 pg/µl for *Monilinia* DNA (Fig. 7 and 8).
3. For samples of artificially infected apples, stored for 30, 60 or 120 days after harvest, only 1 apple sample from the second time point (i.e. 60 days after harvest) and 1 sample from the third time point (120 days after harvest) gave a positive result LAMP reaction for the fungus *N. vagabunda*, and only for 2 samples of apples (120 th day after harvest) a positive LAMP reaction for the fungi *N. kienholzii* and *N. perennans* was obtained. For samples of artificially infected apples, stored for 30, 60 or 120 days after harvest, only 2 samples of apples (120 th day after harvest) showed a positive LAMP reaction for *M. polystroma* and *M. fructicola.*

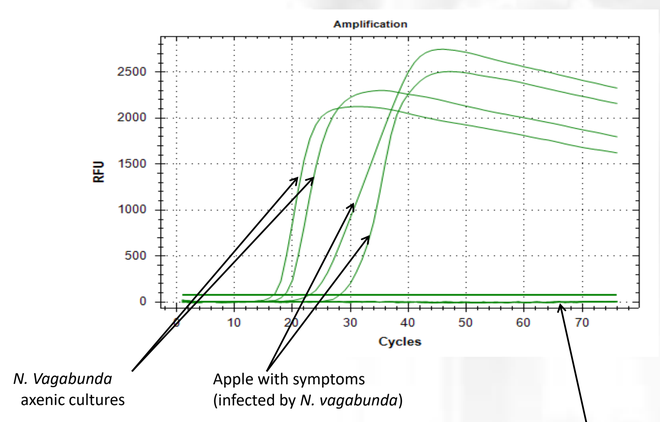

FIG. 3. Amplification curves obtained in LAMP reaction with ASPA_Nvaga primer set and DNA of *N. vagabunda.*

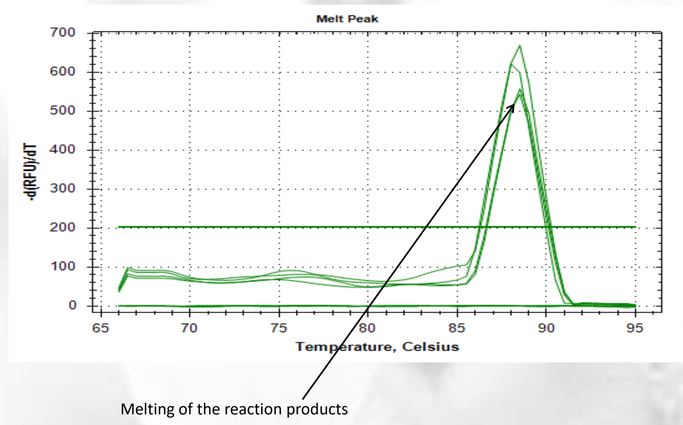

FIG. 4. Melting curves obtained after LAMP reaction with ASPA_Nvaga primer set and DNA of *N. vagabunda.*

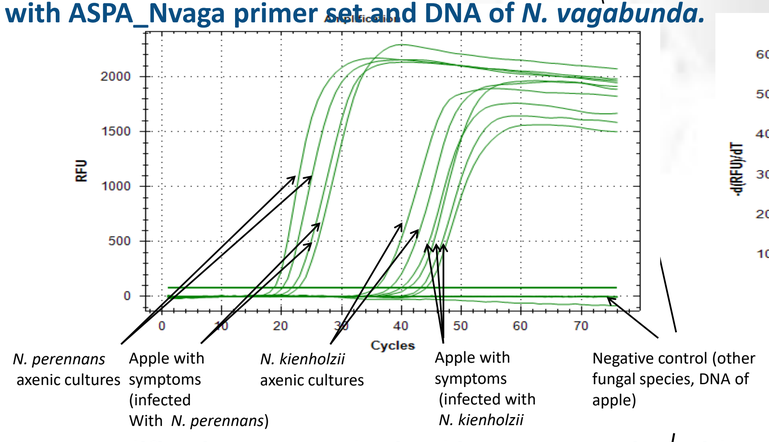

FIG. 5. Amplification curves obtained in LAMP reaction with GTP_NKienPer primer set and DNA of *N. perennans* and *N. kienholzii.*

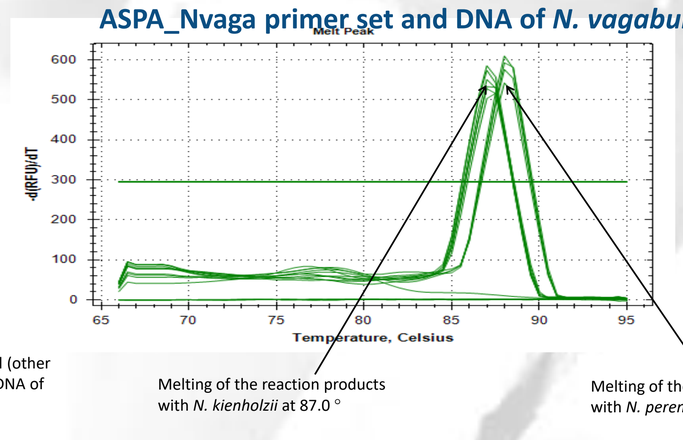

FIG. 6. Melting curves obtained after LAMP reaction with GTP_NKienPer primer set and DNA of *N. perennans* and *N. kienholzii.*

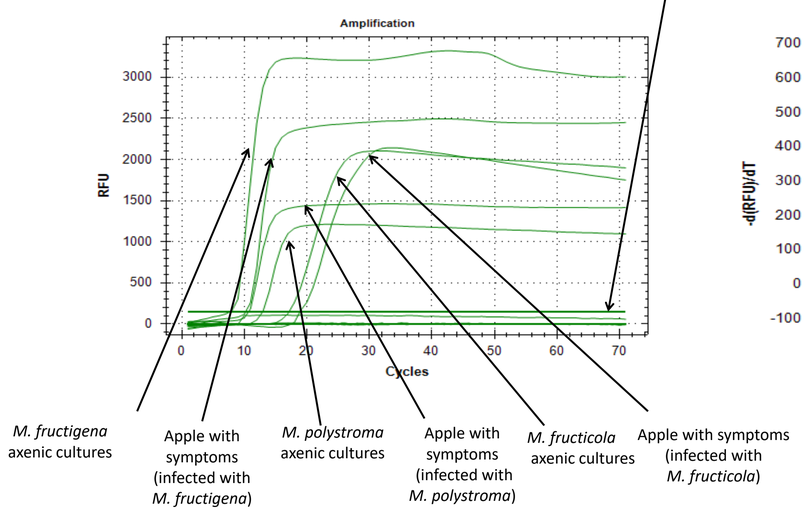

FIG. 7. Amplification curves obtained in LAMP reaction with HSP_Moni primer set and DNA of *Monilinia* spp.

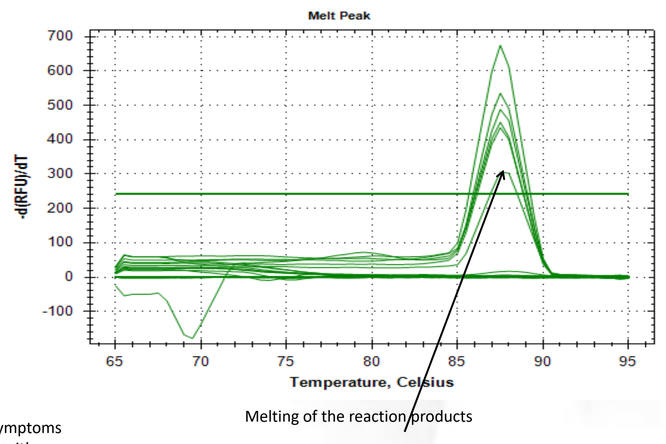

FIG. 8. Melting curves obtained after LAMP reaction with HSP_Moni primer set and DNA of *Monilinia* spp.