Acknowledgements

Inoculations on cut shoots have visualised the differences between the most susceptible and the most resistant cultivars. This test can be performed on small greenhouse areas or in a climate chamber and the results can be obtained within 4-6 weeks; very useful when there is a need for infected material for other experiments, e.g. qPCR, microscopy etc. However, the infection was not considered possible influence of the rootstock and environment. Inoculations on trees allow observations of the disease and defence reactions and repeated measurements of the lesions developed very quickly, which requires numerous data recordings, preferably every fifth day. Different cvs showed different kinds of symptoms which can complicate phenotyping. The method is therefore important to detect quantitative differences in resistance among cultivars.

European canker, caused by the fungus *Nectria galligena*, is a severe problem in apple production both in Sweden and in many other North-European countries. Even when applying fungicides and good horticultural practices, canker damage occurs almost yearly in nurseries and orchards. Some years, devastating outbreaks can destroy a large number of trees. To date, complete resistance to *N. galligena* in the presumably infected tissue, but we were not able to detect the fungus in presumably healthy tissue. However, further improvement of the method is necessary before any conclusions about usefulness of this method as an alternative/complement to phenotyping can be drawn. This documented variation in apple cultivar-specific response to the infection calls for further studies on the genetic control of this trait.

Introduction

European canker, caused by the fungus *Nectria galligena*, is a severe problem in apple production both in Sweden and in many other North-European countries. Even when applying fungicides and good horticultural practices, canker damage occurs almost yearly in nurseries and orchards. Some years, devastating outbreaks can destroy a large number of trees. To date, complete resistance to *N. galligena* is not known to occur in apple. Therefore it is important to detect quantitative differences in resistance among cultivars.

Screening for resistance: inoculations on cut shoots and two-year-old trees

To gain information about cultivar-specific response to *N. galligena* infection, length of cankers induced by inoculation was measured at regular intervals throughout a period of one–three months.

55 cultivars were originally screened for resistance to *N. galligena* by inoculating one-year-old shoots from mature trees in the greenhouse in two plastic tents on two different occasions with a standardised volume of macroconidia suspension (1000 conidia/wound) using different inoculation methods: inoculation in bud wounds (M1) as well as inoculations in bark wounds (M2). One shoot was ‘inoculated’ with distilled water and one was left uninoculated to monitor possible internal infection. Clear and consistent results were obtained for 39 cvs with the M1 method (Table 1).

Furthermore, two-year-old trees of 5 cvs: Cox La Vera (sport of Cox Orange), Elise, Globstar, Rubinola and Rubinstar were inoculated in the field in a shelter as above. Both methods indicated the same pattern with Cox La Vera being the most susceptible and Rubinstar being the most resistant, and with the M1 method being superior due to stable disease progression. Furthermore, with the M1 method, we observed correspondence between the cultivar response to *N. galligena* for cut shoots and trees, respectively (Fig. 1a, b).

Alternative/complementing approach: qPCR

Relative amounts of *N. galligena* were determined using qPCR (quantitative PCR) analysis with primers specific to a variable region of the *Nectria* genome in presumably diseased and presumably healthy tissue. The amplification was performed in duplicates and repeated twice in time. The amplified *Nectria* DNA was normalized to that of the apple ubiquitin gene in order to compensate for differences in DNA loading during amplification, to an internal control sample (calibrator) normalized to a control tissue. The amplification was measured at regular intervals throughout a period of one–three months.

Conclusions and future prospects

Inoculations on cut shoots have visualised the differences between the most susceptible and the most resistant cultivars. This test can be performed on small greenhouse areas or in a climate chamber and the results can be obtained within 4-6 weeks; very useful when there is a need for infected material for other experiments. However, the infection development very quickly, which requires numerous data recordings, preferably every fifth day. Different cvs showed different kinds of symptoms which can complicate phenotyping. The method doesn’t consider possible influence of the rootstock and environment. Inoculations on trees allow observations of the disease and defence reactions and repeated measurements of the lesions under longer time. It is possible to inoculate trees at different seasons (different state of the trees, different inocula), which results in a better modelling of the situation in orchards. However, the time (about a year) and considerable investments needed for producing trees limits the amount of cultivars to be tested. qPCR procedure developed at our laboratory allows for estimation of the biomass of *N. galligena* in the presumably infected tissue, but we were not able to detect the fungus in presumably healthy tissue. However, further improvement of the method is necessary for any conclusions about usefulness of this method as an alternative/complement to phenotyping can be drawn. This documented variation in apple cultivar-specific response to the *N. galligena* infection calls for further studies on the genetic control of this trait.

References


Acknowledgements

Thanks to ass. prof. Jan-Eric Englund for his help with statistical analyses. This project is supported by a grant from SLF.