Brillouin elastography in horticultural sciences: impact of *D. rosea* on rose leave biomechanics

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Abstract: Using Brillouin light scattering spectroscopy as a non-destructive, label-free method to determine the effects on leave biomechanics after inoculation of *Diplocarpon rosea* in two different resistive rose genotypes.

Keywords: biomechanics, Brillouin spectroscopy, plant science, rose blackspot

1. Introduction

The black spot disease due to the fungus *Diplocarpon rosea* is one of the most important diseases of roses, since most modern cultivates are affected with commercial deficits [1,2]. The growth of the fungus impacts the cellular structure as seen in [1].

Brillouin light scattering spectroscopy (BLS) is an arising optical method to determine biomechanical properties [2]. In BLS, the incident light scatters on spontaneous excited density fluctuations which introduce a small frequency shift. This frequency shift and the linewidth can be linked to the elasticity and viscosity of the sample. Compared to different methods of measuring biomechanics it is label-free, non-contact, non-destructive and reaches subcellular resolution.

Here, two types of roses, Pariser Charme which is susceptible to *D. rosea* and the more resistant genotype 91/100-5 carrying the *Rdr 1* gene [3,4], where inoculated to investigate the impact on Brillouin frequency shift.

2. Material and Methods

To determine the Brillouin frequency shift due to the black spot disease a Brillouin spectrometer based on the Tandem-Fabry-Pérot design was used (JRS Scientific Instruments) with a narrowband 532.1 nm wavelength continues-wave laser source. The spectrometer was coupled to a confocal microscope in order to reduce the axial resolution.

The twigs of two different types of roses (genotype 91/100-5 and Pariser Charme) were stored in an enclosed box with a wet paper towel to ensure hydration and were stored in a well-lit room at 20° C. Only the leaves on one side of the twig were inoculated with *D. rosea* by applying droplets of a mixture of 10^5 spora/ml in distilled water (Fig. 1). Measurements were taken two, four, nine and 14 days after inoculation. For each measurements the same regions were scanned for 500 s with laser power of 65 µW. This ensures no significant heating of the cells. As reference the not inoculated leaves on the same twig were taken to reduce influence of hydration.

3. Result

In Pariser Charme leaves the Brillouin frequency shift lowers in the inoculated areas compared to the not treated leaves (Fig 1a). In comparison to the resistant genotype 91/100-5, Pariser Charme shows a decrease in frequency shift rather than an increase in the inoculated areas. After day 9 severe drying was noticed in the boxes and the paper towel was rewetted. This leads to the increase of frequency shift after day 9 as shown in Fig. 2

4. Conclusion

While BLS is already used in biomedical applications, not much effort is taken to use BLS in plant sciences. Here we demonstrated the use of Brillouin scattering as a tool for understanding the fungus growth in rose leaves. We could show that different genotypes of roses react different upon inoculation. Future experiments will further investigate the relationship between the frequency shift and the biological context.

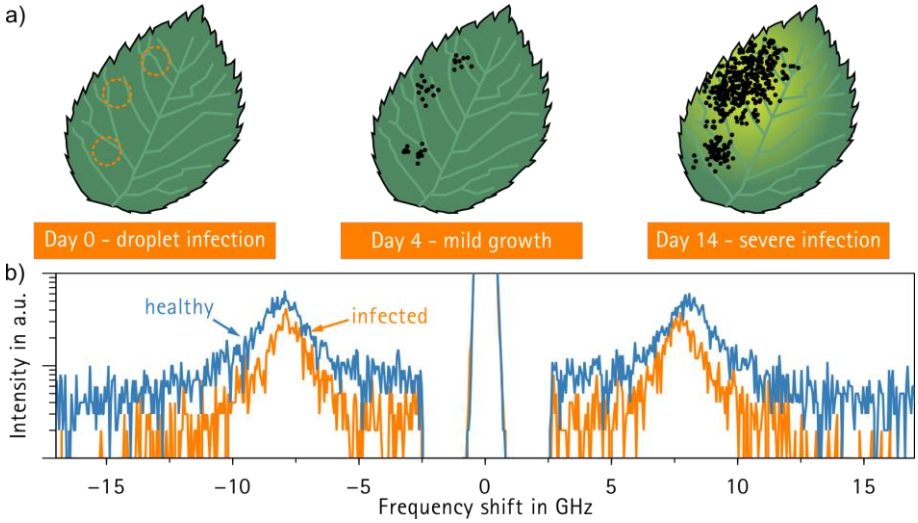

Figure 1 a) typical disease states of *D. rosea*. b) Brillouin shift (anti-Stokes and Stokes shift) of Pariser Charme on day nine after inoculation. The Infected areas show a reduced frequency shift compared to healthy regions.

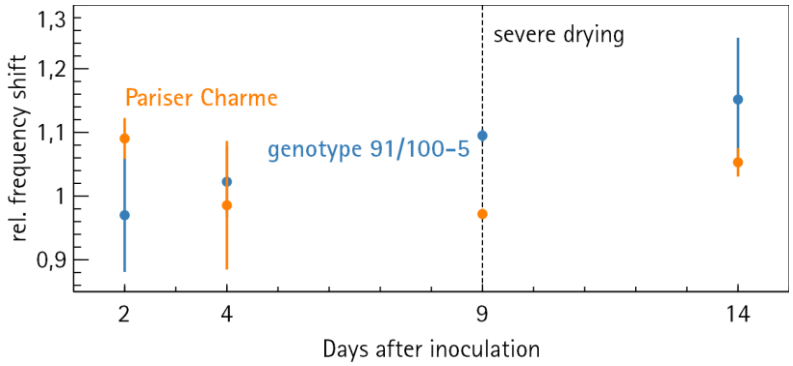

Figure 2 relative frequency shift of Pariser Charme and genotype 91/100-5 leaves over the period of 14 days. After day 9 severe drying was notices, perceptible due to drastic increase of frequency shift.

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