Differences in Epicuticular Wax Layer in Tulip Can Influence Resistance to *Botrytis tulipae*

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Abstract

***Botrytis tulipae* is one of the major diseases of tulip. The pathogen is a necrotroph and infection normally results in host cell death, resulting in serious damage to plant tissues and culminating in rotten plants. Several defense strategies are required to counter attack this aggressive invader. Two different methods were used to proof if tulip wax layer had an influence on *Botrytis* resistance. Wet inoculation method (standard method) with removal of wax layer and dry inoculation method with no removal of wax layer were applied in cultivars Christmas Marvel, Leen van der Mark and Ile de France. The wet inoculation showed that Christmas Marvel was susceptible, Leen van der Mark was partial resistant and Ile de France was resistant. However, when dry method was applied in the same cultivars the level of resistance changed, leading to an increase of the level of resistance in Christmas Marvel, a decrease in Ile de France and equal levels in Leen van der Mark compared to the wet method. These results suggest that wet and dry inoculation altered the level of resistance, meaning that differences in the epicuticular wax layer provides an extra defense strategy against the pathogen in Christmas Marvel, enhanced infection in Ile de France and it had no effect in Leen van der Mark. Moreover, differences in the amount of wax layer in several species of the genus *Tulipa* are present. In general *T. fosteriana* is more susceptible to *B. tulipae* than *T. gesneriana*. The results showed that *T. fosteriana* had less wax layer than *T. gesneriana.* However, there was no complete correlation between amount of wax layer and resistance in all tested cultivars.**

INTRODUCTION

Tulips have been cultivated for more than 400 years in The Netherlands. The earliest mention of them was heard from a Persian poem in 1258. Tulips (Genus *Tulipa* L.) belong to the *Liliaceae* family and they are the most important ornamental bulb crop in the world (Van Tuyl and Van Creij, 2004). Cultivated tulips can be divided into bulb production (forcing and garden), cut flower and pot plant industry. In 2002/2003 (season), The Netherlands acreage was about 10.800 hectares (BKD-statistics, 2002). The most important commercial hybrid groups of tulips are: *T. gesneriana* and Darwin Hybrids (interspecific cross between *T. gesneriana* and *T. fosteriana*). In general, *T. fosteriana* is more resistant to virus diseases and more susceptible to *Botrytis* sp. than *T. gesneriana.*

Botrytis tulipae is a specialized *Botrytis* species that presents a specific parasitism and interaction with tulip. *B. tulipae* was first identified in 1830 by Madame Libert, and in 1902 this pathogen was spread out from Holland to America (Hopkins, 1921). Since 1911, considerable losses by *Botrytis tulipae* have been reported (Hopkins, 1921). The rapid rate of spore germination, infection, mycelia growth and sporulation makes chemical control sometimes not satisfactory (Coley-Smith et al., 1980). Breeding for resistance against this pathogen is one of the sustainable alternatives to overcome the problem.

The interaction between the pathogen and the host starts in the phyllosphere, where the conidia germinate on the plant cuticle in a layer of water containing nutrients (plant exudates) (Elad, 1997). The plant cuticle consists of an epicuticular wax layer and is the first barrier that the invader encounters. This layer is composed of a complex mixture of long chain fatty acids (Kirkwood, 1987). This leaf film provides a physiochemical obstacle to increase resistance to drought and disease (Shepherd et al., 1995). The resistance mechanism is not completely understood. The epicuticular waxes of several plants contain fungistatic compounds and acts as a blockage to leaching of nutrients from the host (Inyang et al., 1999; Alcerito et al., 2002). Epicuticular waxes have demonstrated to influence germination and virulence of several plant pathogenic fungi (Conn and Tewari, 1989; Blakeman and Atkinson, 1976; Kumar and Sridhar, 1987; Inyang et al., 1999). In tulip, a thick wax layer has been distinguished among different species (Straathof et al., 2002). Now the question is: Does the difference in epicuticular wax layer in tulip influence *Botrytis tulipae* resistance?

This paper describes the research that had been done at Plant Research International (PRI, The Netherlands) as part of a Master in Science thesis at Wageningen University.

MATERIALS AND METHODS

Plant Material and Experimental Set Up

The material used for the experiment was obtained from commercial sources and available from the breeding program at PRI. This plant material was divided into two experiments:

A) Wax layer test with control plants,
B) Wax layer test in different *Tulipa* species.

In experiment A, the control cultivars Ile de France, Leen van den Mark and Christmas Marvel were used, with a total number of 20 plants per genotype. The experiment was done twice with 10 plants per cultivar each time. In experiment B, several cultivars belonging to the *T. gesneriana* (21 genotypes), *T. fosteriana* (18 genotypes), *T. greigii* (12 genotypes), *T. vvedensky* (7 genotypes), *T. alberti* (3 genotypes), *T. eichleri* (2 genotypes), *T. viridiflora* (2 genotypes) and *T. kaufmanniana* (3 genotypes) were used. A total of 5 plants per genotype was used.

Wax Layer Tests

In experiment A the removal of the epicuticular wax layer was the main variable. Wet inoculation method with removal of wax layer was used as described by Straathof et al. (2002). Tulip leaves were rubbed gently and sprayed with a prepared *Botrytis* inoculum. Afterwards, the inoculated plants were maintained at 90% relative humidity and from 15°C to 20°C temperature. Plants were kept from 5 to 7 days in those conditions. The scoring was performed by counting the uninfected plants in the sample. The percentage of resistance plants in the population was calculated. Dry inoculation method with no removal of wax layer was used. Dry spores from Petri dishes (propagation media) were push over the plants. Afterwards, the inoculated plants were located at the same conditions as explained previously.

In experiment B several genotypes were scored for their leaf hydrophobicity. This parameter could give us an indication of the relative amount of epicuticular wax layer on the leaves. This was done by spraying tap water over the plants and scoring water retention on the leaf surface using a subjective scale. The scale consisted of 1, 5, 10, 25 and 50, with the lower the parameter equaling the higher content of epicuticular wax layer or hydrophobicity.

RESULTS AND DISCUSSION

Experiment A showed that the level of resistance of Christmas Marvel (CM), Leen van den Mark (LVM) and Ile de France (IDF) was from low, medium and high, respectively, with the standard wet inoculation method. However, when the dry inoculation method was used, the level of resistance changed. There was an increase in the level of resistance in Christmas Marvel, a decrease in Ile de France and no change in resistance for Leen van der Mark when compared with the wet inoculation method (Fig. 1).

In these experiments the results showed significant differences (α = 5%) in the level of resistance within the cultivars tested. These outcomes suggested that removal of the wax layer changed the level of resistance, meaning that differences in the epicuticular wax layer provides an extra defense strategy against the pathogen in Christmas Marvel, enhance infection in Ile de France and it had no effect in Leen van der Mark. The extra resistance in Christmas Marvel could be explained by the unfavorable conditions for the pathogen to germinate. The most possible explanation could be the absence of free water on the host surface, lack of exogenous nutrients, or presence of inhibiting compounds (Elad, 1997). However, more detailed research is needed to confirm these hypotheses. The other interactions between the two other cultivars (Ile de France and Leen van der Mark) and *Botrytis tulipae* suggested that the epicuticular wax layer does not improve resistance. This could be explained by assuming that the conditions for the pathogen were favorable or/and some compounds of the epicuticular wax layer enhanced the infection, especially in Ile de France. In the future, characterization of these waxes could tell us if the epicuticular wax layer in tulips triggers appresorium formation and later produces a successful infection (e.g. in Ile de France) or to see if any fungistatic compounds are presented (e.g. in Christmas Marvel). This characterization could also help eliminate the possible involvement that epiphytic bacteria (pathogenic bacteria) are involved in the interaction. Epiphytic bacteria are known to interact with *Botrytis* and other fungi as a source of food (Blakeman and Brodie, 1976).

In experiment B a significant difference in the epicuticular wax layer between species in the genus *Tulipa* (Fig. 2) was found. This dissimilarity was mainly due to the high value of leaf coverage presented in the *T. fosteriana* species. In general, as a rule of thumb, *T. fosteriana* is more susceptible to *Botrytis* than *T. gesneriana*. Meaning that there is strong indication that the lack of epicuticular wax layer in *T. fosteriana* could be one of the main reasons for its susceptibility against *Botrytis tulipae*.

However, there was no complete correlation between amount of wax layer and resistance in all cultivars tested from all the species when the wax layer was removed. In the future, more cultivars need to be tested in order to determine if the epicuticular wax layer influence the level of resistance in other species.

It is important to mention that the amount and chemical composition (related to the morphology) of epicuticular wax is known to be affected by growing conditions such as temperature, relative humidity, irradiance and by wind (Percy and Baker, 1987). This means that future studies need to take growing conditions in account in order to elucidate under which circumstances the amount of wax layer is formed or/and which chemicals are produced in order to inhibit *Botrytis* infection in tulips.

More research of the interaction between *Botrytis tulipae* and the epicuticular wax layer in tulips has to be done in order to learn more about this possible mechanism of resistance. Nevertheless, breeding for resistance using this interesting trait could help in the battle against this important pathogen.

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Figures

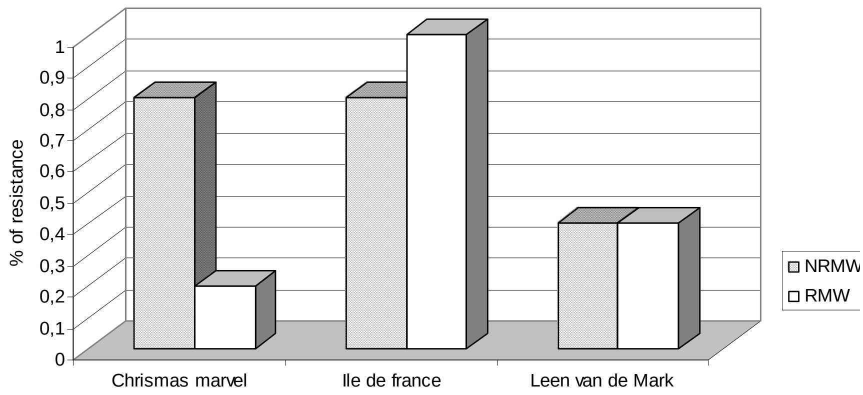

Fig. 1. Percentage of resistance with the removal of the wax layer method (RMW) and not removal (NRMW) in three tulip cultivars.

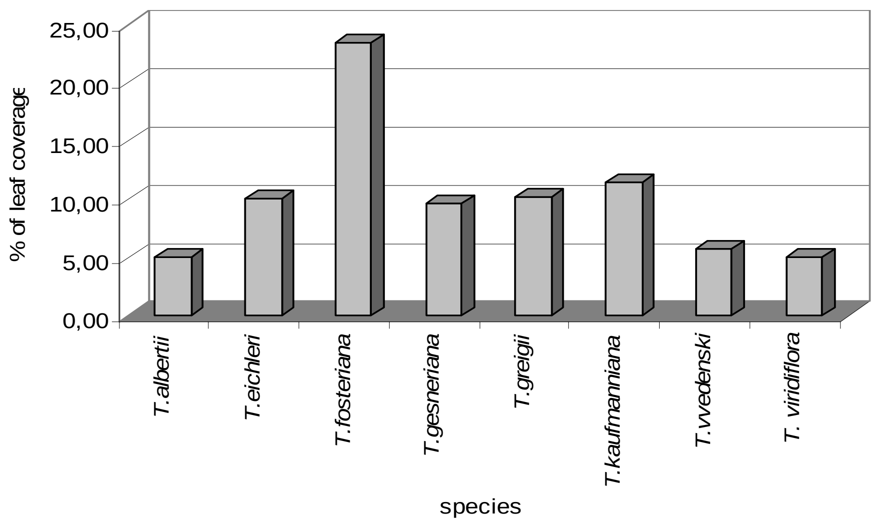

Fig. 2. General results on leaves hydrophilicity test in different *Tulipa* species.