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Hop Breeding Research

Detached Leaf Assay to Test for Downy Mildew Tolerance in Hops

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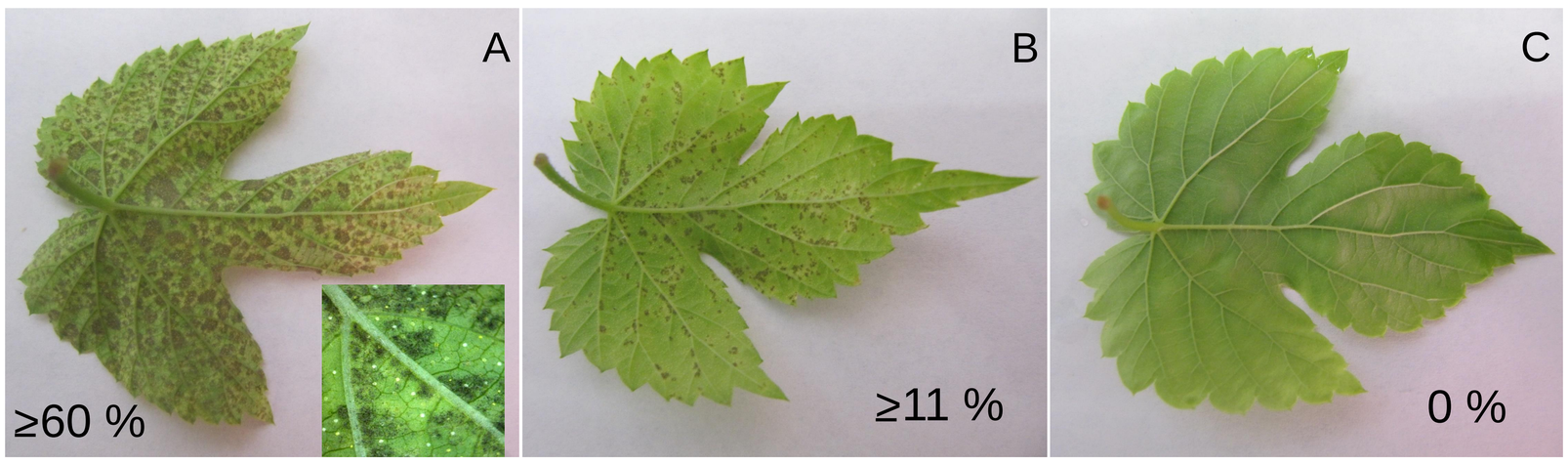
Downy mildew caused by the fungus *Pseudoperonospora humuli* has been a serious threat in hop production in the Hallertau region in recent years. A crucial contribution to solve this problem with downy mildew (DM) is the breeding of hops with enhanced tolerance towards this disease. Assessment of tolerance is quite complex due to the occurrence of systemic infections starting from “inside” as well as leaf or cone infections as non-systematic form of DM disease symptoms (1, 2). Since decades, each year thousands of seedlings have been tested for tolerance towards DM in the growth hall. This seedling screening system was optimized by using plastic sheets to ensure high humidity at the crucial stages of infection favoring sporulation of DM on susceptible hops. Thus, a more reliable estimation of the seedlings` reaction towards DM in the greenhouse could be achieved. Here, a detached leaf assay in the laboratory was elaborated to provide additional information based on standardized inoculation and incubation conditions to predict and specify the tolerance of advanced selections towards this fungus.

Methods

Hop plants to be tested were grown in the greenhouse. Two pairs of leaves randomly taken from the third node of vigorously growing test plants (at least two replicates) were harvested and very rapidly the abaxial side of each leaf was inoculated with a suspension of *P. humuli* (2 – 5 x 10^4 sporangia / ml) using a hand-held atomizer until fully wet. Inoculum derived from fresh spikes of field-grown downy mildew susceptible experimental lines from breeding plots in Freising or Huell. Each leaf was incubated in small plastic containers bottom-side up under fully water-saturated conditions for 20 hrs. Thereafter, residual fungal suspension was dried from the leaf using a paper tissue and leaves were fixed with their petiole in the agar plate abaxial side down. Five to seven days after inoculation (dpi) leaves were visually evaluated based on the occurrence of chlorosis, necrosis, sporulation and finally assessed 14 dpi. Ratings for sporulation, chlorosis and necrosis were on a scale of 0 to 5: 0 (highly tolerant) = no sporulation, 1 (tolerant) = 1-10 %; 2 (medium) = 11-30 %; 3 (susceptible) = 31-60 %; 4 (highly susceptible) = 61-80 %; 5 (extremely susceptible) = 81-100 % of leaf area infected. Results of each genotype are based on at least 4-5 independent inoculations per year in 2015 and 2016.



Rating of DM susceptibility or tolerance utilizing standard area photos (% of leaf area infected = sporulation; close-up in photo A) of DM infected leaves six days after inoculation

Parameters

- **leaf age**: at least 4 leaves from the third node of various plants
- whole leaves instead of leaf discs
- **inoculation material:** fresh spikes, dislodging of sporangia from leaves with 4 ^{0}C cold water
- **inoculation density:** 2 - 5 x 10^4 sporangia/ml
- **inoculation** by spraying the sporangial suspension onto each leaf with an atomizer
- **incubation** of leaves on 0.7 % water agar in water saturated plastic containers at 22 ^{0}C with 12 h-photoperiod and 13 ^{0}C with 12 h in the dark
- rating of tolerant /susceptible reaction based on visual examination of abaxial surface 5-14 dpi: ratings of chlorosis, sporulation and necrosis

Results

Parameters of a detached leaf assay for testing DM tolerance have been optimized (3) based on information from UK (1, 2), USA (4, 5), CZ (6) and Germany (1, 7, 8). The age of leaves was identified as being crucial. Vitality of downy mildew spores during the inoculation procedure was improved (8) resulting in a better reproducibility of disease symptoms on leaves. Moreover, the temperature regime was optimized (9,10). While constant temperature conditions of 20 - 22 ^{0}C throughout the dark and light phase promoted necrotic reactions as sign of dying host cells which stopped sporulation very rapidly. Performing a temperature cycle of 13 ^{0}C during the 12-hour-darkness and 22 ^{0}C during light phase vigorous sporulation of the fungus occurred on leaves of DM susceptible plants within the first days after inoculation followed by the necrotizing of host cells in a later phase of infection (large necrotic spots). A clear differentiation of both disease reactions (sporulation of the fungus and/or necrosis of host cells) could be achieved. Sporulation ratings (intensity, area) on leaves were most suitable to assess non-systematic tolerance toward DM (in agreement with references 2 and 11).
Further investigations are necessary to estimate the validity of this leaf test to estimate DM tolerance in the field.

Table 1: Comparison of non-systemic DM tolerance of various hop cultivars based on field records or detached leaf assay

Assessment based on	Hallertauer Mittelfrüher	Saphir	Hallertauer Tradition	Hüller Bitterer	Mandarina Bavaria	Huell Melon	Hallertau Blanc	Herkules	Polaris
Field records	- - -	- -	+ + +	+ +	+ / -	+	+	-	-
Leaf assay	- -	- -	+ + +	+	-	+/-	-	- -	- -

Rating: - - - extremely susceptible; - - highly susceptible; - susceptible; +/- medium; + tolerant; ++ and +++ highly tolerant

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