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Hop Breeding Research

Detached Leaf Assay to Test for Downy Mildew Tolerance in Hops

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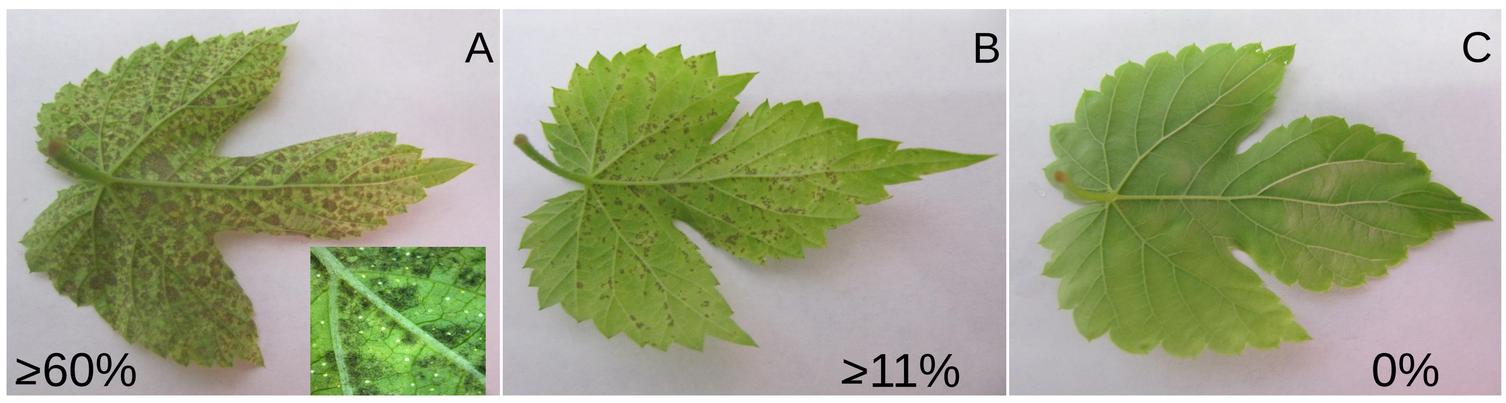
Downy mildew (DM) caused by the fungus P*seudoperonospora humuli* is a destructive disease in hop production. Breeding of hops with enhanced tolerance towards this fungus is a crucial contribution to solve this problem. Assessment of tolerance is quite complex due to the occurrence of systemic infections starting from “inside” as well as leaf or cone infections as non-systematic form of DM disease symptoms (2, 3). A seedling test system with artificial inoculation in the growth hall had been in use for decades. In recent years a detached leaf assay in the laboratory based on standardized inoculation and incubation conditions was elaborated to assess the tolerance of advanced hop selections towards this fungus.

Methods

Leaves of vigorously growing test plants were taken from the third node (using at least three replicates) and the abaxial side of each leaf was inoculated with a suspension of *P. humuli* at 2 - 5 x 10^4 sporangia / ml. Leaves were visually evaluated and finally assessed 14 days after inoculation (dpi). Reaction towards DM were sporulation, chlorosis and necrosis on a scale of 0 to 5: 0 = no symptoms, 1 = 1-10% of leaf area infected; 2 = 11-30%; 3 = 31-60%; 4 = 61-80%; 5 = 81-100%. Finally the index of disease severity according to Townsend-Heuberger (4) was calculated based on 6-19 observations obtained from 2016 to 2018 and statistically evaluated.



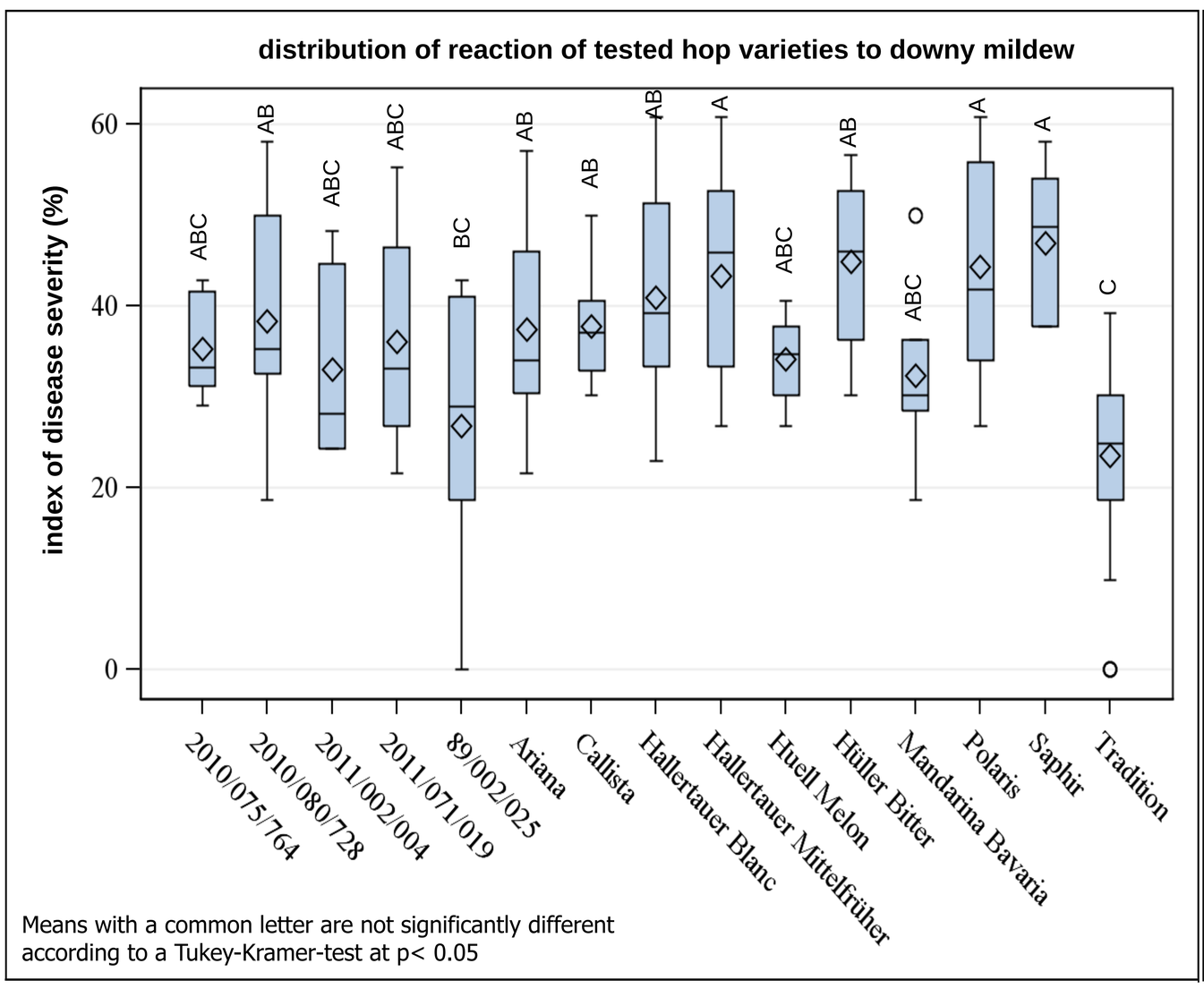
Rating of DM susceptibility or tolerance utilizing standard area photos (% of leaf area infected = sporulation; close-up in A) of DM infected leaves six days after inoculation

Parameters

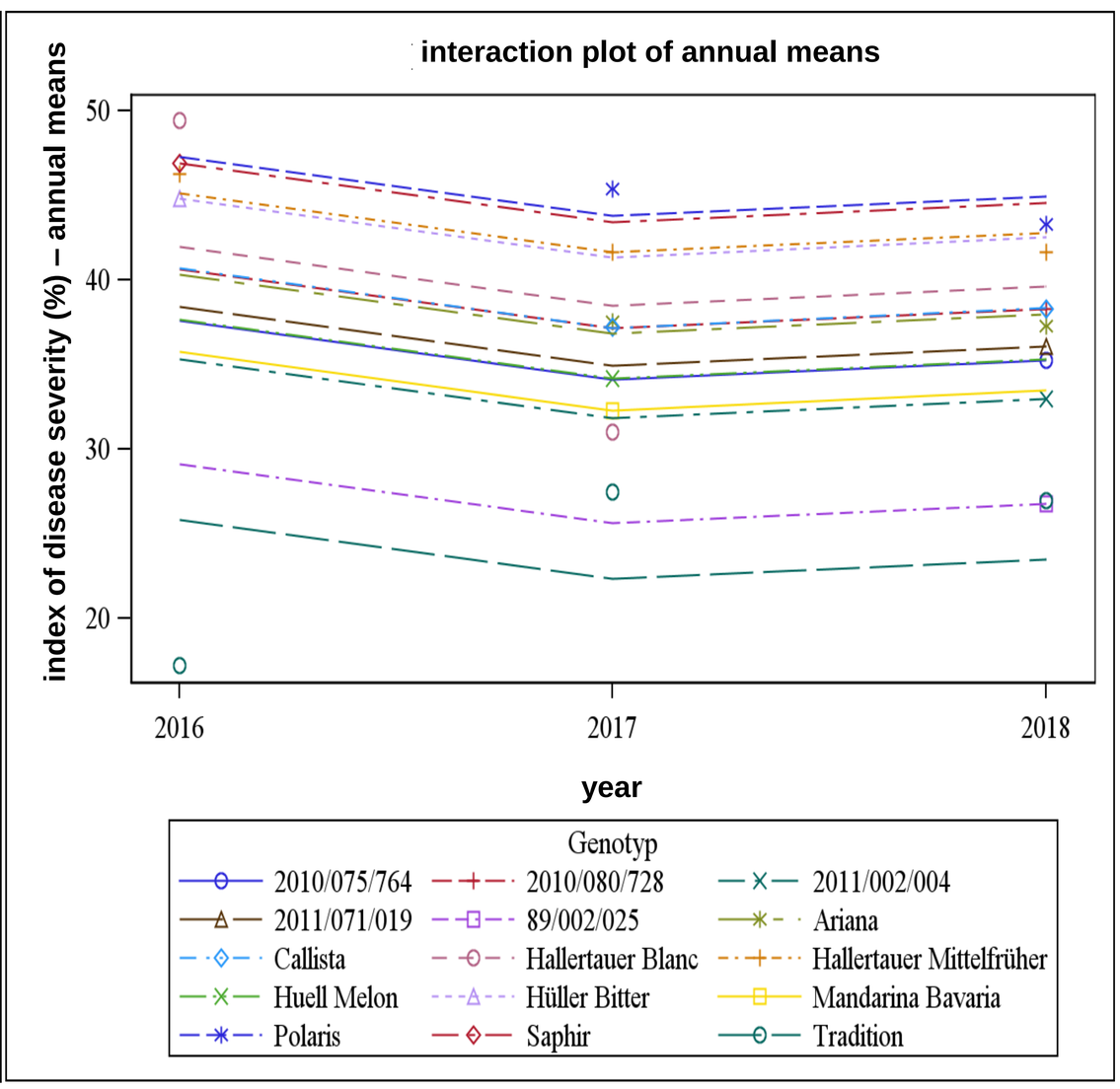
- **leaf age**: at least 3 leaves from the third node
- whole leaves instead of leaf discs
- **Inoculation material:** fresh spikes, dislodging of sporangia from leaves with 4 ^{0}C cold water
- **inoculation** by spraying the sporangial suspension (2 - 5 x 10^4 sporangia/ml) onto each leaf with an atomizer
- **incubation** 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 assessment of abaxial surface 5-14 dpi: chlorosis, necrosis and focus on sporulation

Results

A detached leaf assay to evaluate DM tolerance has been optimized (1) based on knowledge from UK (2, 3), USA (5, 6) and Germany (2). A temperature cycle of 22 ^{0}C during light phase and 13 ^{0}C during the 12-hour-darkness induced vigorous sporulation of the fungus on leaves of DM susceptible plants within the first days after inoculation. Later the necrotizing of host cells followed. A clear differentiation of both disease reactions (sporulation of the fungus and/or necrosis of host cells) could be achieved. Sporulation ratings were most suitable to assess non-systematic tolerance towards DM (3). Preliminary results on the tolerance or susceptibility of Hüll cultivars and experimental lines to DM over two to three years of testing showed highly tolerant individuals with Hallertauer Tradition as the most tolerant one, highly susceptible with Polaris and Saphir as examples and the majority of cultivars and experimental lines with medium level of tolerance and susceptibility, respectively.



Means with a common letter are not significantly different according to a Tukey-Kramer-test at p< 0.05



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