MILAROM project: Study of downy mildew caused by *Peronospora belbahrii* on basil in France

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BACKGROUND

Since 2001, basil crops are attacked by a fungal pathogen: *Peronospora belbahrii* which causes mildew. This pathogen causes disease symptoms on the leaves of fresh plants (Fig.1), which make basil unfit for consumption or processing (deep freezing, dehydration). It affects the main production regions in France and in Europe, causing significant economic losses (Belbahrii et al., 2005).

A research project, called MILAROM, was set up in France. Part of this project is studying identification of the fungus on fresh plants and on seeds.

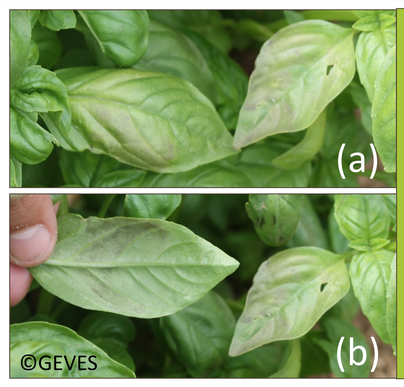


Fig. 1. Chlorosis on the adaxial surface (a) and sporulation on the abaxial surface (b) of the leaves in fields

CHARACTERIZATION OF THE PATHOGEN ISOLATES

P. belbahrii is an obligatory parasite belonging to the class Oomycetes. Symptoms are due to the presence of sporangiophores on the abaxial surface of leaves (Fig. 2).

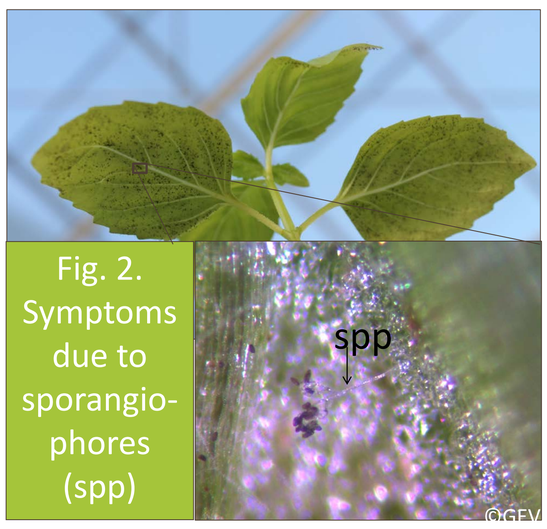


Fig. 2. Symptoms due to sporangio-phores (spp)

Their branches bears, at their tips, airborne spores in humid condition. These spores can infect the leaves and contaminate a new plant (Fig. 3).

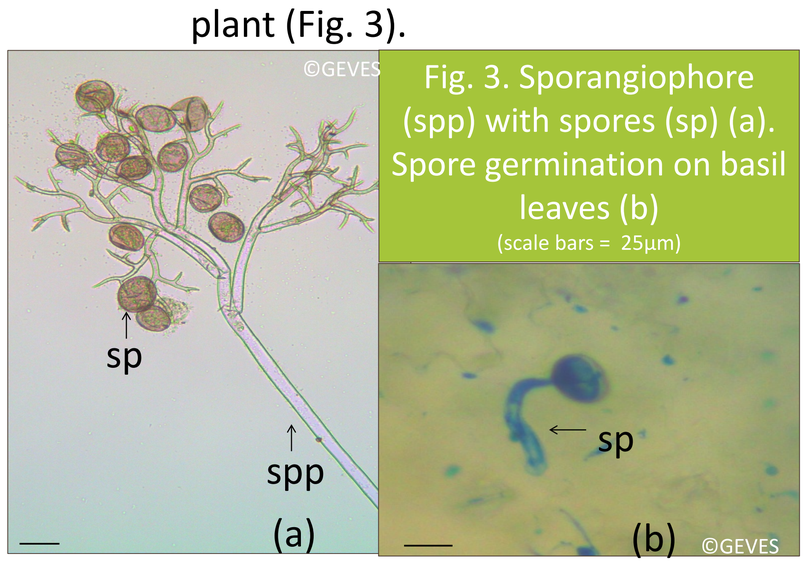


Fig. 3. Sporangiophore (spp) with spores (sp) (a). Spore germination on basil leaves (b) (scale bars = 25µm)

A protocol of leaves discoloration and mycelium staining confirmed the emergence of sporangiophores from stomata of basil leaves contaminated for 21 days (Fig.4.), creating a new source of contamination.

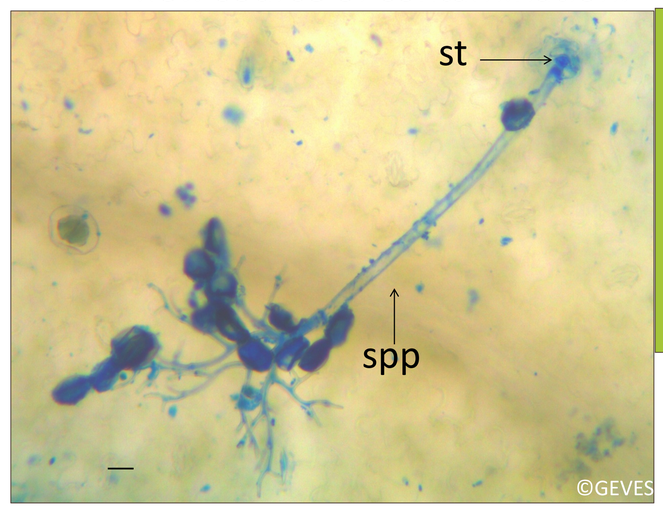


Fig. 4. Sporangiophore (spp) emerging from stomata (st) of basil leaves infected for 21 days (scale bar = 25µm)

PATHOGENICITY OF STRAINS AND SUSCEPTIBILITY OF VARIETIES

18 different varieties were separately inoculated with 8 strains of *P.belbahrii* (Fig.5).

For inoculation, a solution of 4.10^4 spores.ml^{-1} in distilled water+Tween 20 (1%) was sprayed on the adaxial surface of basil leaves at two leaf stages. Symptoms notation was realized at 21 days post-inoculation with a rating scale from 0 to 5 .

Each strain and variety were compared according to the observed symptoms (Fig.6).

Fig. 5. Comparison of susceptibility of 18 varieties mean of 8 strains

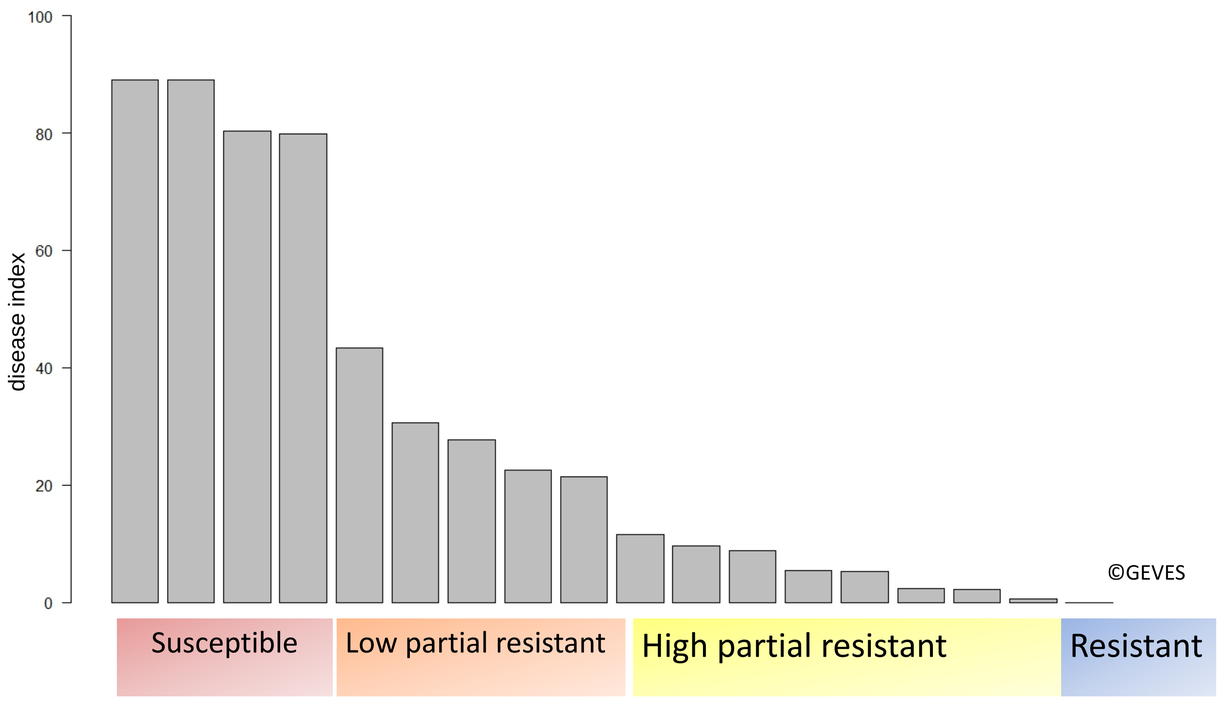
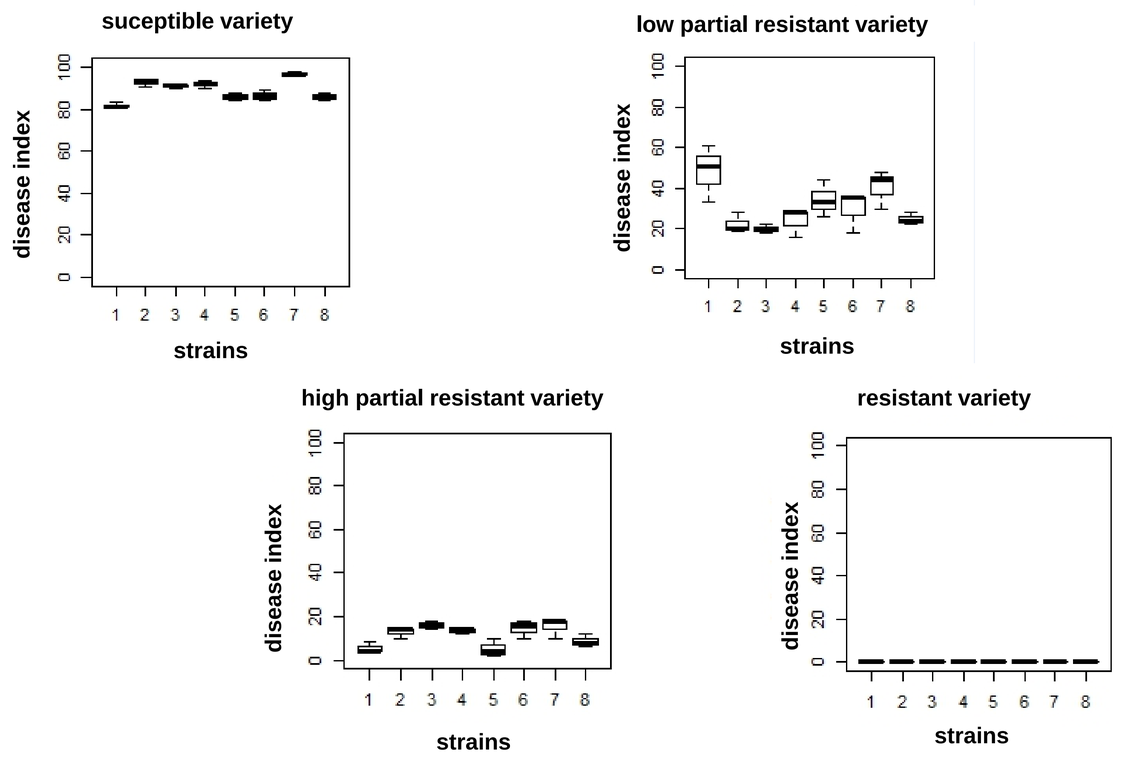


Fig.6. Example of pathogenicity for 8 different strains



→ 3 strains (1, 7 and 8) with different behaviour selected for breeding.

SEED DETECTION

A protocol of detection has been developed for the detection of *P. belbahrii* on seeds.

To detect if a seed lot is contaminated, 400 seeds/lot are sown and grown with high humidity (Fig. 7).

Weekly reads are realized to quantify the percent of contamination.
Symptoms appears after 20 to 30 days post-sowing (Fig. 8).

In 2015, 109 lots were analyzed and 7 were positive to *P. belbahrii*. The percentage of seed contamination of these lots is estimated to 1% -5%.

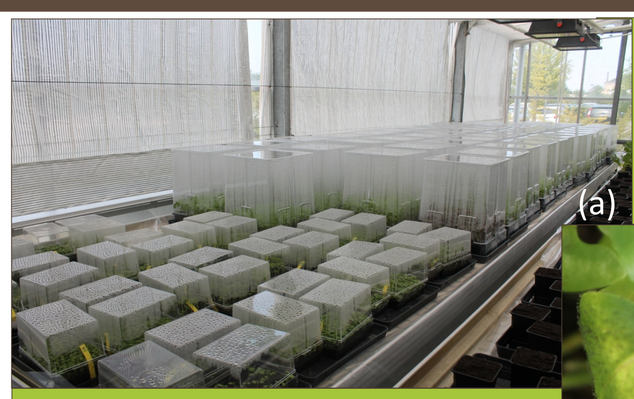


Fig. 7. Experimental device (a)

Fig. 8. Sporulation 28 days after seedling (b)

CONCLUSION

Protocol of artificial inoculation allowed to maintain and characterize the strains.
Seed detection allowed us to detect seed lots contaminated by *P. belbahrii*.

We now know the susceptibility of a range varieties for each strain of *P. belbahrii*.
We will select resistant plants obtained for crossings of different resistant varieties.

References:

- Belbahri, L. et al., 2005. *Phylogenetic analysis and Real Time PCR detection of a presumbably undescribed Peronospora species on sweet basil and sage*. Mycological Research, 109(11), p.1276-1287.

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