Study of umbel browning and stem necrosis on carrot in France: isolation and characterization of the fungal pathogen: *Phomopsis dauci/Diaporthe angelicae*

Franck Bastide (1,2), Isabelle Sérandat (2), Pascal Poupard(1), Philippe Simoneau(1), Valérie Grimault (2), Julie Gombert(3), Elise Morel(4), Juliette Kolopp(5), Pierre-Louis Guillermin(6), Emmanuelle Laurent(3)

Background

Since 2007, symptoms of umbel browning and stem necrosis have been regularly observed in carrot seed production areas in France. The first symptom is a triangular necrotic lesion on one part of the umbel, which spreads over the entire umbel and then often progresses to the stem. Black spots are present on plant debris (Fig.1.). Diseased umbels dry prematurely, and this has a negative effect on seed development. In the 2007 and 2008 harvests this resulted in a reduction in yield of approximately 8%. The symptoms of the disease are the same as those caused by *Phomopsis dauci,* described by Von Arx in the Netherlands in 1951, and so may indicate the re-emergence of the fungus. This pathogen is being studied as part of the DIAPOCAR project.

Fig.1. Umbels lesions and black spots on carrot debris by *P.dauci*

Characterization

A reference collection of 82 isolates (69 from carrot and 13 from parsley), which mainly come from the centre of France, has been established. Strains are characterized using phenotypic and molecular criteria.

Phenotypic criteria:

Fig.2. Colony of *Diaporthe angelicae*

Fig.3. Pycnidia of *Diaporthe angelicae*

On Malt-agar mycelium is white and downy (Fig.2.). On media, pycnidia (asexual stage) appears after 19 days (Fig.3.) and produces two types of conidia, α (6.3x2.2µm, unicellular, ovoid) and β (23.9x0.9µm, unicellular, filliform). β conidia appear after 21 days and α conidia after 26 days. However, after 35 days, only α conidia are present.

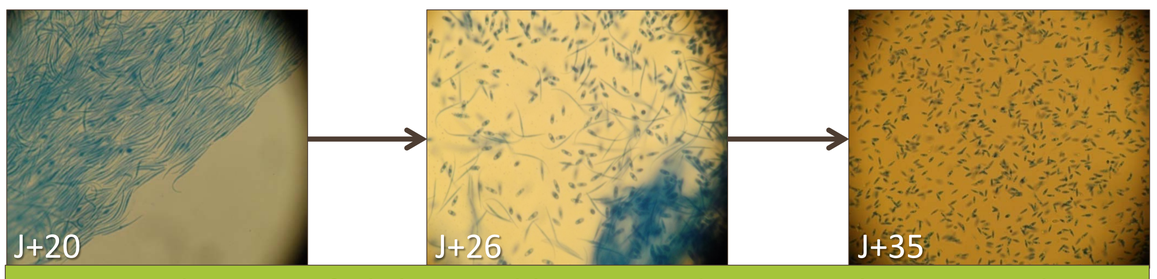


Fig.4. Production of alpha- and beta conidia on malt-agar medium

Molecular criteria:

ITS region analysis has proved that *Phomopsis dauci* and *Diaporthe angelicae* are the same fungus. *Diaporthe angelicae* is the sexual stage of *Phomopsis dauci*.

The whole collection is being characterized through the study of ITS regions to determine the species (Fig.5.) and IGS regions to study the variability of strains.

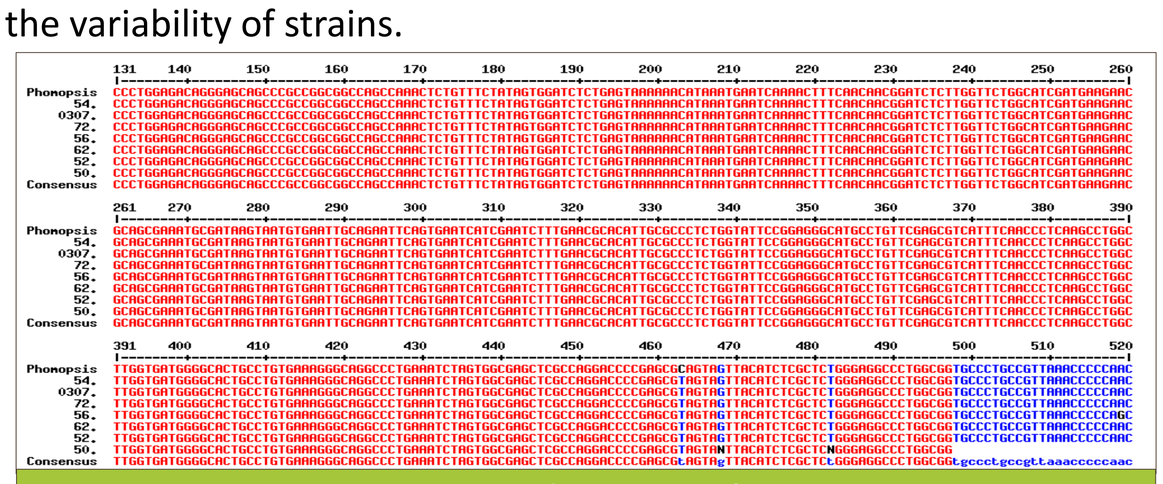


Fig.5. Sequence alignment of ITS region

Development of sexual stage

Using black spots from plant debris in artificial conditions, perithecia appear after 11 days and produce asci with 6 to 8 ascospores (10.2x2.1µm, bicellular, ovoid). After 24 days, cirrhi are observed containing free ascospores (Fig.6.).

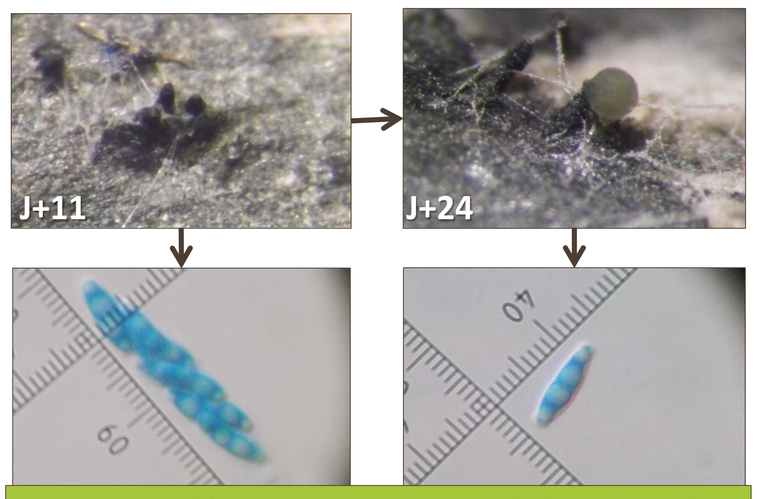
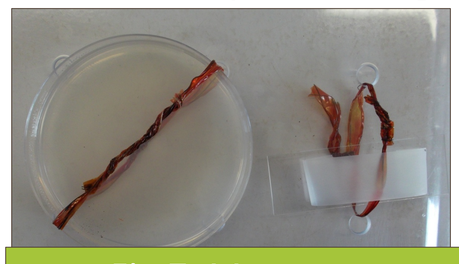


Fig.6. Different structures of the sexual stage : perithecia, asci and ascospores

Substrate : moist sand

Conditions :
-T°C : 20°C
-Humidity : 80%
-Photoperiod : 12h light/12h darkness

Petri dishes and pieces of cellotape placed at different heights above debris, to collect ascospores in order to measure dispersion. (Fig. 7.). The maximum dispersion height was 60 cm.



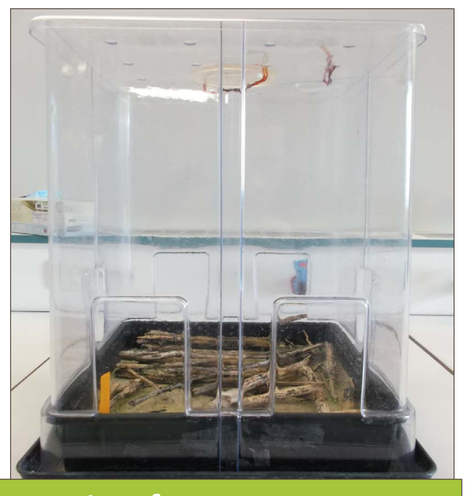


Fig.7. Measurement of ascospore dispersion from mature perithecia

Artificial contamination

To contaminate plants, either conidia suspensions (artificial conditions) or plant debris showing black spots (natural conditions) were used. This allows verification of Koch postulates.

Artificial conditions:

Umbells were collected in field, preserved in growth chambers and contaminated by spraying of conidia suspension (α/β at 10^7 conidia.ml^{-1}). The first symptoms appeared after 10 days and plants were completely dry after 20 days (Fig.8.).

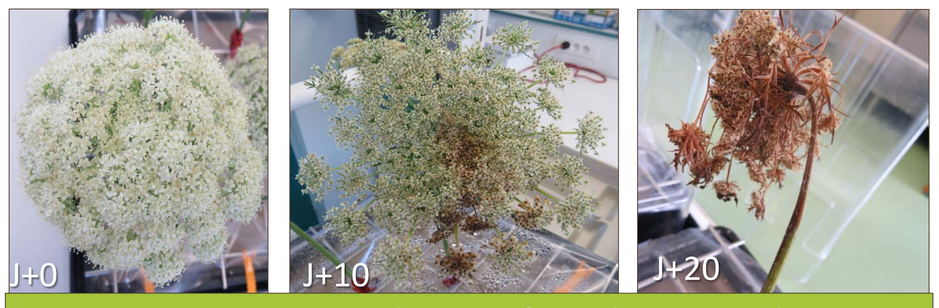


Fig.8. Symptom evolution after plant inoculation in artificial conditions

Substrate :
moist sand + nutrient solution

Conditions :
-T° : 20°C
-Humidity : 80%
-Photoperiod : 12h light/ 12h darkness

Natural conditions:

Fig.9. Symptoms observed in field after contamination with plant debris

In controlled/natural conditions, *Diaporthe angelicae* was re-isolated using inoculated plants.

Conclusion

Sexual and asexual stages obtained in artificial conditions allowing the in vitro production of inoculum for future tests. Protocols of artificial contamination using conidia or debris are operational : Koch postulates were verified, confirming that symptoms are caused by *Diaporthe angelicae/ Phomopsis dauci.*

Create protocol for artificial contamination with ascospores, expand reference collection, study variability of strains (IGS regions).

1-UMR 1345 IRHS-INRA-Université d'Angers Agrocampus Ouest, 49045 Angers, France / 2-GEVES, 25 rue Georges Morel, 49071 Beaucouzé, France / 3-FNAMS, Impasse du Verger, 49800 Brain-sur-l'Authion, France
4-FNAMS, 45 voie Romaine, 41240 Ouzouer-le-Marché, France / 5-VILMORIN, Route du Manoir, 49250 La Ménitré, France / 6-HMCLAUSE, 1 chemin Moulin des Ronzières, 49800 La Bohalle, France