SEPTORIA IN FLAX

FIRST MILESTONES

towards varietal resistance

Major advances have just been made in understanding pasmo disease in flax. Varietal characterisation tools are now available. They open the door to genetic control, which is likely to become one of the major solutions for integrated control of this disease.

Field trials (artificial inoculation) revealed varietal differences in the level of susceptibility to septoria.

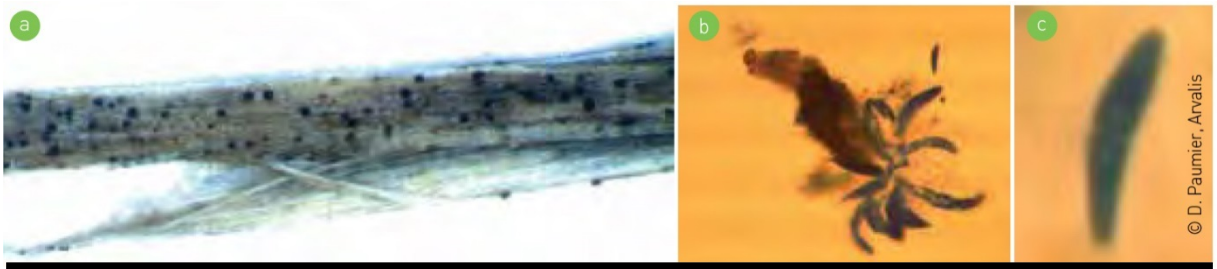
More than 170 strains of *Septoria linicola* (see insert) were isolated from flax plants (linen and linseed) in different production areas in France, as part of the SeptoLIN project(1). This fungi culture collection preserved by Arvalis is an important source of material for the whole sector (microbiological studies, artificial inoculation, etc.).

The genomes of two strains of *S. linicola* were also sequenced, creating the first genomic resources for this pathogenic species. Both strains were selected on the basis of their original host (linen or linseed) and their geographical origin (Paris basin or southwestern France). A phylogenetic analysis revealed the genetic similarity of *S. linicola* and the *Zymoseptoria tritici* (wheat septoria), *Cercospora zeae* (maize cercosporiosis) and *Septoria musiva* (poplar septoria) species. These new genomes, which will soon be published, may form the basis of studies in this species' molecular function and evolution.

A first in France: identification and characterisation of sexual reproduction

The asexual form of the fungus (*S. linicola*) is suspected of being its main propagating form from one epidemic season to the next. The epidemic period during the growing season sees the formation of pycnidia on the lesions, that produce pycnidiospores scattered through splashing or by animals and insects. Because of their large size, it is assumed that pycnidiospores are not dispersed by the wind. However, *S. linicola* can survive in the form of *pseudothecia*, which are fructifications that produce ascospores (spores derived from sexual reproduction) carried by the wind over long distances. *Pseudothecia* are found on seeds and crop residue, where they can survive for up to four years. The sexual form (*Mycosphaerella linicola*) has not been identified in all parts of the world and is poorly documented in Europe.

Arvalis's plant pathology laboratory revealed, for the first time in France, the presence of this sexual form. Fibre flax straw collected in the field was studied using an adaptation of a technique developed by INRA (Frédéric Suffert, Bioger Unit) for septoria leaf blotch of wheat. This technique helped to observe *pseudothecia*, to isolate ascospores (Figure 1), to grow them, and to use the isolated strains in order to verify the pathogenicity of the isolates on flax (verification of the Koch postulate). Typical symptoms as well as the presence of pycnidia and cirruses undoubtedly confirm that the pseudothecia observed belong to the *S. linicola* species and that sexual reproduction of the fungus exists in France.



SEPTORIA LINICOLA: a sexual form undoubtedly observed
(a) M. linicola pseudothecia, binocular magnifier x10. (b) Ascus and ascospores, microscope x100. (c) An ascospore, microscope

Figure 1: Photos of the fungus's sexual form.

Straw, a dissemination medium

Terres Inovia then studied the dynamics of the appearance and maturation of pseudothecia on the straw during the three-year project. The fructifications analysis confirmed the presence of pseudothecia on most of the straw. Some pycnidia could be observed but in low quantities.

The analysis shows the presence of mature pseudothecia on flax straw as early as from harvest time (July). There was an abundance of mature pseudothecia (capable of producing wind-dispersed ascospores) from October to November. In the spring, there were very few of these mature pseudothecia, if any. The comparison of the pseudothetic maturation kinetics for each of the three seasons highlights similarities between the different years. This epidemiological data leads to the strong hypothesis that sexual reproduction plays a major role in the epidemic development of flax septoria and that attention should be paid to straw management.

VARIETAL SENSITIVITY:
(a) Pycnidia at D+19 after inoculation on cotyledons. (b) Symptoms at D+6 after inoculation on leaves. (c) Symptoms at D+19 on stems.

Figure 2: Observations made during the test carried out to characterise varietal sensitivity to septoria in flax.

Proven difference in varietal susceptibility

In order to characterise varietal resistance to septoria, a test in controlled conditions was developed as part of the SeptoLIN project. The purpose of this test was to express symptoms on the different organs of the plant (cotyledons, leaves, stems and capsules; Figure 2). Symptoms can appear as early as six days after inoculation on cotyledons and leaves. The test also revealed symptoms on the stems, but with low reproducibility. Similarly, symptoms on capsules were noted but the effectiveness of the method, which is not easily replicated, very much depends on varietal earliness. In addition, the duration of the test (over two months) makes it difficult to use it routinely for varietal screening. Accordingly, pathological tests are validated for cotyledons and leaves but still need to be improved for stems and capsules. Further experimental work has shown that the seeds of contaminated capsules can be affected and therefore that the disease could be transmitted through the seeds.

The varietal sensitivity of 22 genotypes (15 linen varieties and 7 linseed varieties) was assessed for six strains of *S. linicola*. The main results show significant and correlated varietal differences for cotyledons and leaves. Another major result is the difference in varietal susceptibility between linen and linseed cultivars. In experiments, linen varieties are significantly more susceptible than linseed varieties.

These trials also revealed the absence of total resistance to the six strains tested. The difference observed between varieties is therefore probably related to quantitative resistance (the combined action of several genes confers resistance). As far as the pathogen is concerned, this work reveals differences in the level of virulence between the strains and depending on the original host (fibre flax or linseed).

Better characterisation of varieties

These results are very encouraging for the industry because they show that genetic progress is possible. In addition, the disease susceptibility divide that tends to exist between flax and linseed is providing clues to help seek quantitative resistance in easily accessible but nonetheless quality material. The project included numerous trials with artificial inoculation carried out in the field. They highlighted the value of artificially inoculating varieties and identified the best methods for gathering representative data on disease pressure. In the short term, these protocols will help to characterise varieties more precisely with regard to septoria. Although the trials did not accurately measure the disease's detrimental impact on linseed and fibre flax, they confirmed, in field conditions, that there are differences in susceptibility between varieties. Selection work should therefore improve flax resistance to septoria.

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A COMMON PATHOGEN

Septoria linicola (Speg.), a causal agent of septoria in flax historically known as pasmo, affects flax production in many parts of the world, including France. This ascomycotina fungus thrives in damp temperate conditions and attacks all the parts of the plant above ground. It can cause significant loss of leaves and capsules, and breaking of stems that severely penalise oil and fibre yields. The capsules can be sterile or produce contaminated seeds.