Effects of crop management on the incidence and severity of fungal diseases in sunflower

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Chapter

EFFECTS OF CROP MANAGEMENT ON THE INCIDENCE AND SEVERITY OF FUNGAL DISEASES IN SUNFLOWER

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ABSTRACT

The main fungal diseases of sunflower are black stem disease (*Phoma macdonaldii*), downy mildew (*Plasmopara halstedii*), Phomopsis stem canker (*Phomopsis helianthi*) and white mold (*Sclerotinia sclerotiorum*). Thanks to genetic improvement, the main method of disease control is the use of varieties with good tolerance or resistance. Crop management includes a set of measures to reduce the risk of fungal attacks and to limit the impact of these attacks on the crop. Cultural control is based on prevention (e.g. by crop rotation), escape (e.g. by adjusting the sowing date) or the promotion of microclimatic conditions unfavorable to pathogens (through control of vegetative growth), all of which have been shown to be effective. A suitably timed combination, organized at the regional level, of cultural, genetic, chemical and biological control methods is the key to an effective, integrated and sustainable control of diseases in sunflower.

Keywords
Sunflower, fungal diseases, crop management, Phomopsis helianthi, Phoma macdonaldii

INTRODUCTION

Sunflowers were grown in France on 680 000 ha in 2012 and the grain yield averaged 2.3 $t.ha^{-1}$, while potential yield is about 4 $t.ha^{-1}$ in experimental plots where most limiting factors are controlled. Water stress and fungal diseases are the two most frequent and detrimental factors limiting crop yield (Jouffret et al., 2011). A dozen phytopathogenic fungi are currently reported as potential problems for this oilseed crop, but only four diseases cause significant yield losses nationally: downy mildew (*Plasmopara halstedii*), phoma (*Phoma macdonaldii*), phomopsis (*Phomopsis helianthi*) and sclerotinia (*Sclerotinia sclerotiorum*) (Cetiom, 2002). Their main biological and ecological characteristics are reported in Table 1.

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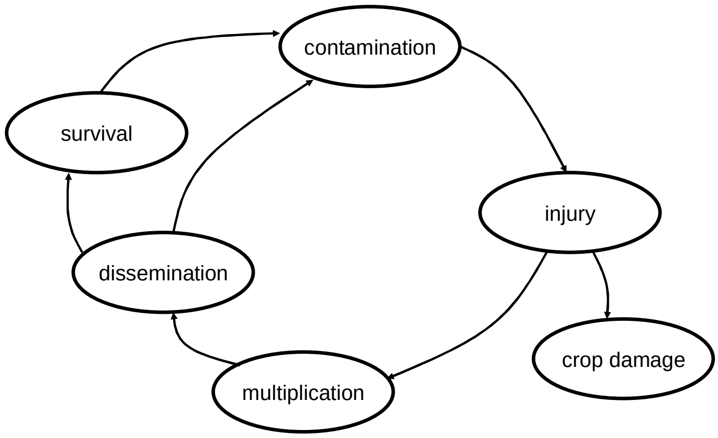
The incidence and severity of a disease result from complex interactions between a pathogen, a host plant and their common biophysical and biological environment defined by soil, weather and crop management.
The biological cycle of sunflower diseases conforms with the general scheme proposed by Lucas (2007) in Fig.1.

Figure 1: Simplified life cycle of a fungus on a plant, within a crop, or at landscape scale (Lucas, 2007).



Reducing the harmfulness of disease attacks in a field in a given year is the main objective of crop protection. But reducing injury (sporulating lesions), even in the absence of detrimental effects on annual crop yield, is a way to reduce the production of primary inoculum and thus future epidemics. For example, in Argentina, where verticillium is a serious problem on sunflower, the response of varieties is evaluated from the symptoms they express, but also on their ability to multiply the fungal inoculum (by assessing the amount of microsclerotia in the stem pith). Hence the planning of disease control methods must consider both temporal and spatial dimensions as soon as the fungus is able to spread widely.

To avoid (or at least limit) the injury (symptoms) and the damage (loss of yield and quality of the crop), farmers have three possible control strategies to trigger (Delos et al., 2004; Attoumani-Ronceux et al., 2010):
- prevent the disease risk (prophylactic methods)
- avoid the contamination when inoculum is still present
- mitigate crop injury and damage after infection

There are four ways to control fungal diseases: genetic (through the choice of resistant or tolerant varieties), chemical (fungicidal), biological (e.g. *Coniothyrium minitans* against sclerotinia) and agronomic (or cultural) (Aubertot et al. 2005).

During the last two decades there has been a lot of progress in controlling sunflower diseases by genetic methods (Vincourt et al 2011; Vear and Muller, 2011); nowadays it is surely the most efficient, practical and repeatable method to control most of the diseases. Nevertheless, the other methods should not be neglected, especially cultural control (Sackston, 1992). Most of these effects are presented in Table 2.

Table 1 : Characteristics of four main sunflower diseases in France (from CETIOM, 2002 ; Delos *et al.*, 2000 ; Delos *et al.*, 2004; Gulya *et al.*, 1997 ; Moinard *et al.*, 2009).

		Phoma	Downy mildew	Phomopsis	Sclerotinia
					
Type of fungus		necrotrophous widespread	biotrophous local attacks	necrotrophous widespread	necrotrophous widespread (except for stem base attacks)
Most detrimental symptoms		Stem base necrosis and premature ripening	Dwarf plants (for primary systemic attacks)	Girdling spots on stem	Stem base and head rot
Potential yield loss		30 to 50 %	50 % (if primary attacks)	0.1 to 0.3 $t.ha^{-1}$ for 10 % of plants with girdling lesions	> 50 %
Attacks responsible for the spread of inoculum		Stem	Sporulating dwarf plants	Stem (girdling and non-girdling spots)	Stem, collar, head
Inoculum survival	Form	Mycelium	Oospores, mycelium	Mycelium	Sclerotia
	Support	Crop residues, seeds	Crop residues, seeds, soil	Crop residues	Crop residues, soil
	Life span	> 3 years	10 years	1 year	7 to 10 years
Key period for disease development		Attack at stem base early enough to provoke a stem base sheath at anthesis	Synchronism between plant emergence and presence of free water in the soil	Time to reach the stem from an attack at leaf margin	Anthesis coincides with spore emission
Favorable conditions for the establishment of the fungus		Strong inoculum pressure, environmental conditions moderately limiting	50 mm of precipitation required within 10 days around sowing time, soil temperature > 10°C	Relative humidity > 90% during 36h, optimal temperature range 20-24°C	Free water during 42h at floret surface
Time for symptoms to appear after contamination		8 to 15 days	10 days to 7 weeks (primary infection)	20-25 days (leaf spots) then 20 more days before stem lesions	3-8 weeks on heads
Distribution (France) Rare Present Frequent Very frequent No data					

Table 2 : Efficacy of control methods (from Aubertot *et al*., 2005).
(+) efficacy level of the method, (-) no method available

	Downy mildew	Phoma	Phomopsis	Sclerotinia
Genetic control	+++	+	+++	++
Chemical control	+++	+	+++	-
Biological control	-	-	-	+
Cultural control	+	+	+	++

In order to reduce the application of pesticides for environmental and public health concerns, and to preserve the sustainability of genetic resistance, agronomic control should also be part of crop protection programs. For this, the interactions between the pathogen, its host plant, the biophysical and biological environment and crop management must be dissected and modeled in a comprehensive system approach (Desanlis et al., 2013).

When genetic control is based on the use of specific resistance (e.g. mildew), agronomic control is necessary for maintaining the effectiveness of this resistance over time.

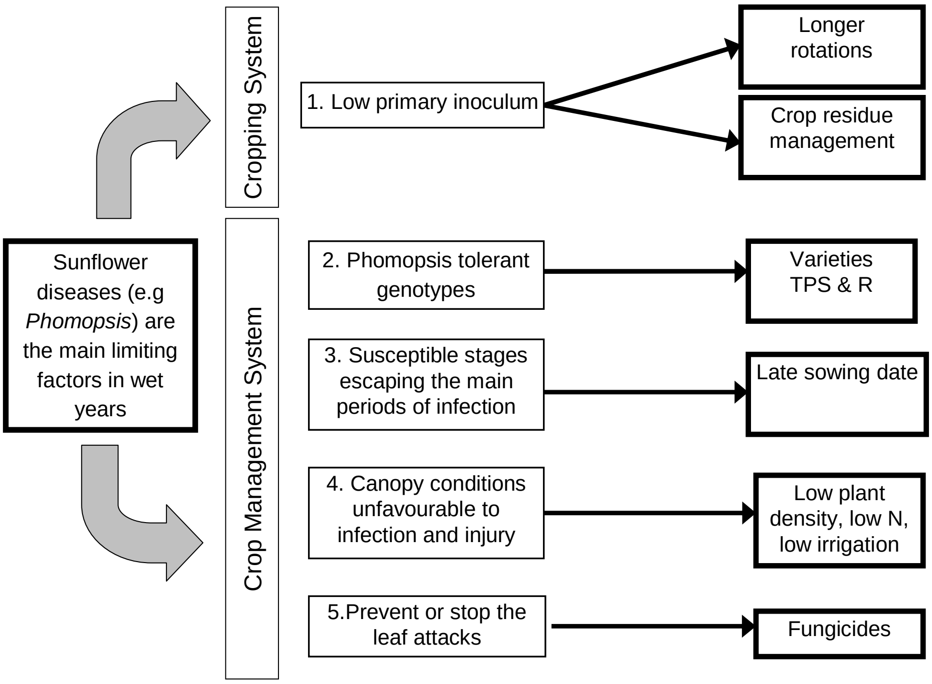
The reduction of the initial inoculum is the first step in the disease control strategy. The main method is lengthening the crop rotation, i.e. delaying the return of the same crop on the same field but also the 'previous effect' of each crop. The rotation could be diversified by alternating host and non-host species, together with soil tillage methods, in order to break the reproductive cycle of soil-borne diseases. At regional level, an optimal landscape mosaic should be arranged to prevent unwanted spore dissemination from sources of primary inoculum to host plants. The second lever is the management of infected crop residues which are the main source of primary inoculum: crushing and burying the crop residues reduces the inoculum's life span and its ability to continue its cycle. The sanitary quality of seeds is also an important criterion.

Avoidance strategies consist of reducing the risk of synchronism between the period of maximal crop receptivity and the phase of emission of contaminating spores. The main lever is the choice of the sowing date, associated with the choice of suitable varieties (when earliness is possible).

Mitigating the impact of diseases in crops consists of reducing the magnitude of disease injury and damage when contamination occurs. Usually the management objective is to manipulate the canopy vigor through various cultural practices (sowing date, seeding rate, nitrogen fertilization, irrigation). Variety choice is also involved, through its level of genetic resistance to the initial infection but also other characters such as earliness, plant architecture and tolerance (i.e. ability to limit the damage caused by the pathogen injury).

Studies on the impact of crop management on the expression of sunflower diseases began in the mid-80s, but are still limited as compared to other crops (for instance wheat). The purpose of this chapter is to review the results of these studies with a special focus on two major fungal diseases (phomopsis and phoma) which have been intensively studied in the last decade by agronomists and pathologists because they are significantly influenced by cultural practices.

Figure 2: A schematic diagram representing the strategies for controlling *Phomopsis helianthi* and the technical solutions than can be applied: 1. prophylactic methods ; 2. tolerance (genetic control) ; 3 escape ; 4. avoidance; 5 attenuation (chemical control). Cultural control is 1 (cropping system level, before sunflower crop or on adjacent fields), 3 and 4 (crop management system, intra-field).



REDUCE PRIMARY INOCULUM

The inoculum of major fungal diseases persists in various forms, either as spores produced by asexual or sexual reproduction (oospores, ascospores) or by mycelium and sclerotia, on different organs of the plant (vegetative parts, seeds) or diffuse in the soil. Its survival time is variable (1-10 years) depending on environmental conditions and agricultural practices. Knowing this duration is critical to evaluate the effectiveness of the methods applied for inoculum reduction.

1.1. ***Longer rotations: a more effective practice when the inoculum is not widespread at regional level***

In general, short rotations or landscapes with a high concentration of sunflower fields both increase the risk of disease. They contribute to the frequent soil enrichment in contaminated residues (mildew, phomopsis, phoma) or various forms of storage (sclerotia of *Sclerotinia*, oospores of mildew, microsclerotia of *Macrophomina* or *Verticillium*), which constitute different sources of inoculum for the following sunflower crop. Spreading the sunflower crop on the largest possible number of plots to delay the return of sunflower on the same plot usually helps reduce risk. Whatever the cropping system, crop rotation is always an effective practice to prevent the risk of disease. Two successive sunflower crops are banned in France to prevent the spread of mildew (Delos et al., 2004). Masirevic and Gulya (1992) recommended a minimum period of three years between two successive sunflower crops.

In the case of mildew, although weather conditions around sowing are critical for infection, the risk is particularly great in areas where rotations are very short, because the level of infestation of a field depends on the presence of plants with mildew symptoms in previous years. A survey of 225 fields monitored between 2007 and 2008 in southwestern France revealed a significant effect of crop rotation duration on the percentage of infected plants and on the infectious potential of the field, these two effects being closely associated with soil type: the risk was higher in calcareous clay soils and for short rotations (one sunflower every two years) compared to rotations where sunflower returned not less than one year in three (Moinard et al., 2009).

In the case of sclerotinia, the risk of attack increases with the amount of sclerotia in the soil. The return frequency of susceptible crops (e.g. oil or protein crops) in the rotation potentially increases the risk of contamination of the field. Lengthening the crop rotation is a good way of reducing the bank of sclerotia in the soil, part of which disappears naturally each year due to insufficient melanisation or because of mycoparasites (Huang and Scott Erickson, 2008). This measure will be all the more effective if it is applied as soon as the first attacks occur (Heffer-Link and Johnson, 2007), and if other susceptible crops are considered (such as oilseed rape, pea, soybean) (Delos et al. 2004). Gulya et al. (1997) consider that a minimum of 5 years without a susceptible crop in the rotation is necessary to reduce the infectivity potential of a plot.

In the case of phomopsis and phoma, the short life span of the inoculum on crop residues and the long distance dissemination of ascospores both reduce the effect of the return period of sunflower cropping (Jouffret, 2005). When the next crop (often a cereal) is established after simplified tillage, the sunflower-infected debris remains on the surface in wheat plots, thus constituting a source of inoculum for the surrounding sunflower plots. In the case of phoma, this was verified in recent years: in the Poitou-Charentes region (center-west of France): despite sunflower returning less often than in the southwest for many years (10 to 15% of plots with less than one sunflower crop every 3 years against 40 to 50% in the southwest, Wagner and Lieven, 2010), significant attacks of phoma and premature ripening syndrome were still observed in 2010.

1.2. Management of primary inoculum: at field level, but not only ....

Assessing the amount of primary inoculum is critical to predict the risk of disease; this amount is dependent both on the severity of attack in previous years and how infected crop residues are managed after harvest.
Crop residues infected by *Phomopsis* and *Phoma* are a source of inoculum for attacks in sunflower fields in subsequent years. These two fungi continue their cycle by producing fruiting bodies (perithecia and/or pycnidia) on residues, which constitute new sources of contaminating spores. Thus, the fate of these residues after harvest determines the level of inoculum that can infect sunflowers grown in the following year. Crushing sunflower stalks also accelerates the decomposition of stalks and limits the trophic support for different fungi which are sources of inoculum (Delos et al., 2004). In addition, burying residues disrupts the cycle of these fungi (Jinga et al., 1992), the formation of fruiting bodies being determined by light.

Given the ability of the inoculum of *Phoma* and *Phomopsis* to be released into the air, measures promoting the rapid degradation of infected residues will be more effective if they are applied in all the fields in a region (Delos et al., 1994). In the case of phomopsis, the dissemination of contaminating spores generally follows the direction of prevailing winds (Masirevic and Gulya 1992); for phoma, ascospores may be dispersed over long distances by rain and wind (Maric et al., 1998) and were identified in former Yugoslavia as the source of attacks on sunflower in places where it had never previously been grown.

Regarding sclerotinia, soil tillage influences the survival of sclerotia and their ability to germinate in the mycelial form (responsible for the stem base attacks) or to form apothecia, sources for airborne contamination by ascospores. However, some results on soybean in the United States appear to be contradictory: a deep tillage can bury sclerotia, avoiding the formation of apothecia, but may encourage attacks at the stem base. Maintaining sclerotia less than 5 cm deep with a shallow tillage would promote their germination but also would expose them to degradation by mycoparasites present in this layer (Duncan et al., 2005; Heffer, 2007).

According to Quiroz et al. (2009), the combination of no-tillage and highly resistant cultivars promises to be an interesting tool to manage *V. dahliae* and Verticillium wilt in sunflower. Doran and Linn (1994) found that conservation tillage (no tillage) increased the level of microbial populations from 0.5 to 2.7 times in the top 15 cm of soil. Increases in soil microbial activity would provide a highly competitive environment, leading to competition effects between soil microbes and encouraging disease suppression (Chen et al., 1988).

1.3. Biological control against sclerotia of Sclerotinia

Among mycoparasites, *Coniothyrium minitans* is a fungus that can destroy hyphae and sclerotia of *Sclerotinia sclerotiorum*. Mycelium penetrates into sclerotia through their pigmented bark or by altering the surface, and fully colonizes them (Huang and Kokko, 1987). Under optimal conditions, sclerotia can be destroyed in 15 days. Today, an organic product based on spores of this fungus is available on the market: Contans ® WG. Studies on the effectiveness of this control method against sclerotinia in oilseed rape suggest that parasitized sclerotia cease to produce apothecia, resulting in a reduction in crop attacks of 20 to 30% (Penaud and Michi, 2009). Moreover, this biological control is more effective when it is applied to each crop in the rotation (Penaud et al., 2003), because not all sclerotia are fully destroyed after a single application.

1.4. ***The sanitary quality of seeds***

The sanitary quality of seeds is an important method of control for all fungi that can be transmitted through this way. Mildew is the disease for which the transmission of the disease by seeds played a crucial role in the spread of the 703 and 710 races in France. Sunflower seeds can harbour the mildew in the form of oospores and mycelium. In case of a severe attack, the proportion of infected seeds can reach 100% (CETIOM, 2000). The use of certified seeds is imperative to avoid the intake of inoculum from outside.

Today, phoma is a fungus whose ability to be transmitted by seed (especially in unclean seed lots) has been clearly shown, which explains its sudden onset in countries where sunflower was not grown till now (Luo et al., 2011). This would not be the case for phomopsis.

REDUCE THE RISK OF DISEASE BY ESCAPING INFECTION

The success of an infectious event and its expression (injury) depends on the crop growth stage when it occurs.
The choice of sowing date should be based on the notion of an escape strategy, which consists of optimizing the delay between the phase of crop receptivity and the usual periods of contaminating events, as derived from historical records, i.e. the date of ripening of perithecia for *Phomopsis* and *Phoma*, and rainy periods favoring the emission of *Sclerotinia* spores or the germination of mildew oospores in soil.

2.1. Adjusting sowing date according to weather conditions: a key factor in mildew control.

Primary mildew attacks are the most serious: they are responsible for dwarf plants and head sterility. Rainfall plays a major role in the success of infections: these occur at the time of crop germination, as contaminating oospores (arising from the dormant form of the fungus) need free water in the soil to come into contact with sunflower rootlets. The cumulative rainfall between 10 and 20 days around sowing time is thus a risk factor, especially if it exceeds 50 mm in 10 days around sowing. Soil temperature is also involved, the success of infections being greatly reduced for temperatures less than 10°C (Delos et al., 2000).

2.2. Phomopsis: higher risk for early sowing

The period of crop receptivity to *Phomopsis* ranges between flower bud stages E1 (star bud stage) and E5 (pre-anthesis). According to several studies, early sowings often result in severe attacks while delaying planting can shorten the overlap between the period of crop receptivity and the time when the risk of attack is at its maximum (spring rainy events).

In 1992, Jinga *et al.* were the first to demonstrate the effect of sowing date on phomopsis incidence: a sowing made 20 days later than usual (late April instead of early April), reduced the fraction of injured plants by nearly 30%.

From two experiments done in Toulouse in 1996 and 2000 it was evident that delayed sowing significantly reduced the fraction of plants infected: delaying sowing by 12 days (2000: from April 22 to May 3) or 35 days (1996: 10 April to 15 May) together with low input management, resulted in 3% attacks in 1996 (against 73% in high input, early sown management) and 42% in 2000 (against 97%) (Debaeke et al., 2003). However, a delay such as that tested in 1996 has a major drawback, as potential yield was affected too.

The escape effect can be attributed to the phenological stage of the crop during the period highly conducive to infection: although infections are possible as long as green leaves are present, the highest proportion of infected stems results from attacks that occur in the early stages of flower bud development. Late planting can usually limit the number of infections due to less favorable weather conditions (less frequent rainfall events and higher frequency of days with lethal temperatures for fungus) coinciding with a shorter duration of the crop receptivity phase and of the time of canopy closure (Debaeke et al., 2003).

These experimental approaches have been confirmed by the use of the Asphodel model, an epidemiological model which was applied to a range of annual weather patterns and sowing dates to simulate the risk of infection (Debaeke et al., 2001).

2.3. Phoma: the random effect of sowing date

Sackston (1950) first described a wilt and stalk rot of unknown etiology as ‘‘premature ripening’’, and earlier evidence showed that stem base girdling canker caused by *P. macdonaldii* was its primary cause. Crops show loss of vigor from mid- to late summer; leaves become wilted and necrotic, the stalk turns dark brown to black, and this is followed by senescence and plant death a few weeks before physiological maturity (Donald et al., 1987).

Regarding phoma, the periods of crop receptivity and emissions of contaminating ascospores are both very broad : attacks can take place throughout the growing season. Consequences of delaying planting appear less clear than with phomopsis: according to several authors (Fayazalla and Maric, 1981; Delos et al, 1998), the risk of attack on stems and its severity would be lower with late plantings. Indeed, late plantings tend to reduce the infection rate because of lower rainfall before flowering (escape strategy). On deep soils, avoiding too early planting (late March) can reduce *Phoma* attacks which are generally frequent on these high potential situations (Taverne, 2005). However, deferring planting to late spring is not always effective, especially if June and July are wet: in three experiments carried out in different regions of France (1999 and 2000), it was observed that the attacks increased by an average of 23% on stems and 12% at stem base when delaying sowing by three to four weeks (Debaeke and Peres, 2003).

Besides, although early plantings (early April) are often more exposed to attacks on stems (including phoma), they can escape terminal water stress more easily, with limited risk of premature ripening.

The optimum planting date is thus a balance between sacrificing potential yield (which needs a long season), escaping water stress (through early sowing) and mitigating/escaping disease attacks by delaying sowing and thus avoiding risky periods for disease infection.

Other examples of sowing date effects on disease incidence were given for *Alternaria helianthi* in Brazil where sunflower is planted as a second crop after soybean (Leite et al., 2008).

MITIGATE DISEASE IMPACT

Several elements of crop management can reduce disease development and the final proportion of diseased plants. This is the case for phomopsis and phoma at stem level, phoma and sclerotinia at stem base level, and sclerotinia on heads.
The severity of symptoms can also be reduced: in the case of phomopsis, harmful symptoms are girdling lesions; for phoma, crop management can have a major influence on premature ripening due to attacks at stem base level (Seassau et al., 2010).

3.1 Practices to reduce the proportion of infected plants

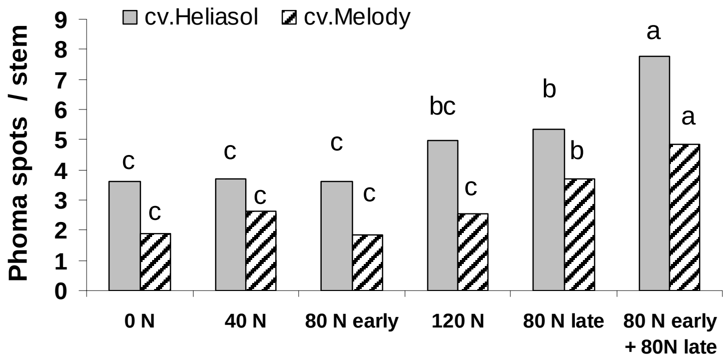
Phomopsis
In the case of phomopsis, plant density has a significant effect on the fraction of plants with lesions on leaves and stems.
From nine experiments done at INRA Toulouse between 1994 and 2001, Debaeke et al. (2003) observed that increasing plant density from 5 to 7.5 plants.m^{-2} resulted in an increase of 22% in infected stems.
Similarly, when early infections and rainfall are conducive to the onset of symptoms on stems, crop irrigation around anthesis favors and secures these attacks: from 1997 to 2000, on 41 experimental situations, irrigation has contributed to a 22% increase in the percentage of attack on stems (Debaeke et al., 2003).
Regarding the effect of nitrogen fertilization under conditions of low inoculum pressure, the proportion of infected stems increased by 36% when increasing N fertilization from 60 to 120 kg N.ha^{-1} (Debaeke et al., 2003).
When weather conditions are such that the inoculum is not limiting, relative humidity conditions within the canopy are favorable to the success of infections even if the field is sparsely covered. Under these extreme conditions, the dissemination of contaminating spores would be favored within a more open canopy, causing more spots on leaves; the reduced plant growth (smaller leaf size and petiole length due to nitrogen deficiency in these conditions) would facilitate the leaf-to-stem passage of the fungus (Desanlis et al., 2013). Disease severity was thus accentuated by the smaller stem diameter due to high plant density (Debaeke et al., 2003; Delos et al., 2004).

Hence, on the same soil, with the same weather and for the same inoculum pressure, changing plant density, nitrogen fertilization and irrigation can vary the percentage of infected stems for a susceptible genotype from 0 to 100% (Debaeke et al., 2003).

Phoma
The impact of crop management on *Phoma* attacks at stem and collar level have been reported in several studies (Formento and Velasquez, 2000; Debaeke and Peres, 2003; Seassau et al., 2010).

The influence of N fertilization on phoma incidence was illustrated by the 2005 experiment in Toulouse (Debaeke and Taverne, 2006). The number of *Phoma* spots per stem was evaluated for 2 cultivars and 6 nitrogen treatments differing in N amount and splitting. Irrigation was fully supplied. The number of *Phoma* spots per stem increased dramatically with excessive N fertilization (120-160 kg N.ha^{-1}), cv.Melody being always less infected than cv.Heliasol (Fig.3).

Figure 3 - Effect of N management on phoma incidence for 2 cultivars (Toulouse, France - 2005) – LSD at 5 %



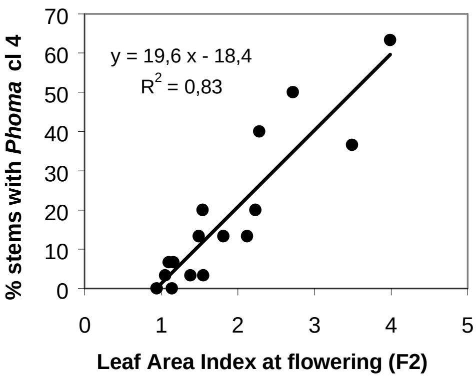
Fifteen field experiments between 1996 and 2000, in four French regions, with different combinations of sowing date, sowing density, nitrogen fertilization (0 to 120 kg $N.ha^{-1}$) and irrigation (rainfed or different dates and amounts of irrigation) were subjected to natural *Phoma* attacks (Debaeke and Peres, 2003).
The fraction of plants with stem spots was higher under wetter conditions in June (heavy rainfall or irrigation): 94% cf. 77%. This percentage and the number of spots per stem were both closely related to the N available to the crop: on average, 5.9 stem spots with 120 kg $N.ha^{-1}$ and 4.2 with 60 kg $N.ha^{-1}$. The higher the number of spots per plant, the higher the inoculum potential for future sunflower crops in the area (Debaeke and Peres, 2003).

At a given plant density, the proportion of infected plants increased steadily with N fertilization rate. An interaction between the level of N fertilization and plant density was also shown: when the crop was N-limited, the number of *Phoma* spots on the stem increased with plant density. Conversely, in non-limiting N conditions, the highest number of spots was observed in low-density conditions (Debaeke and Peres, 2003). This effect can be attributed to the fact that the troughs at the insertion points of petioles on the stem, which act as receptacles for free water, are deeper in low density and high N conditions and that the frequent bursting of petiole tissue at groove level may favor the penetration of the fungus within the plant (Delos et al., 2000).

Regarding stem base attacks of *Phoma*, a positive correlation was found between the fraction of plants with a girdling lesion at stem base and leaf area index at flowering (Fig. 4).

Figure 4: Relationship between the proportion of plants with harmful lesions of *Phoma macdonaldii*: (a) on stems (class 4) ; (b) on stem base (class 2), and leaf area index at flowering. Field trial in Saint-Florent (central France), year 2000 (Debaeke and Peres, 2003).

a)



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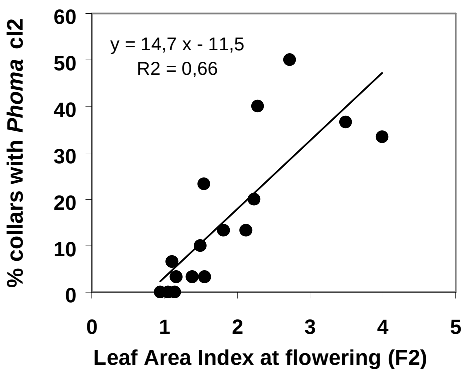


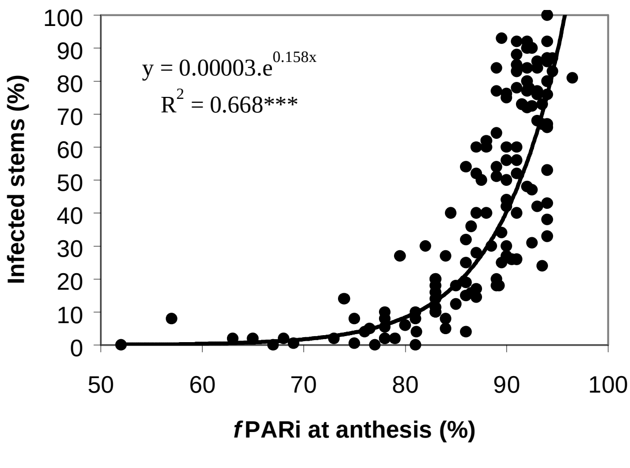
Thus, below a leaf area index of 2.5 at flowering, the risk of potentially harmful attacks of *Phoma* at stem base remains low (below 30%). This synthetic index (LAI) being the result of complex interactions between each of the factors of crop management (sowing date, plant density, water and nitrogen availability before flowering), probably reveals the underlying existence of an impact of any or all of these factors on the extent of collar attacks, even if each individual relationship was not demonstrated in this series of experiments.

Phomopsis and Phoma: the extent of canopy development for predicting the attacks on stem

The extent of canopy development is commonly expressed by leaf area index, which is a synthetic indicator of crop growth and development. The fraction of photosynthetically active radiation intercepted by the canopy ($f\mathrm{PAR}_i$) is closely related to LAI and thus to the intensity of management before flowering (nitrogen fertilization, plant density, irrigation) and partly to the variety.

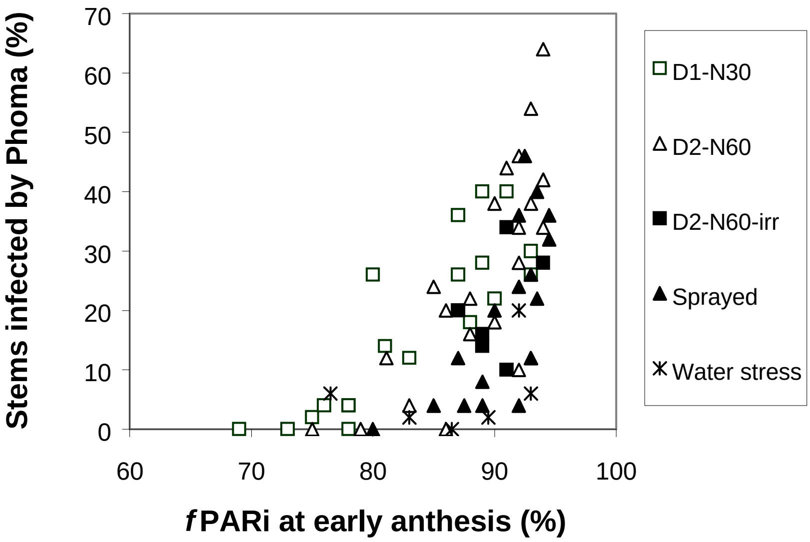
Concerning phomopsis, in experiments where the weather conditions and crop management (early sowing, pre-flowering irrigation) favored natural attacks, the percentage of infected stems increased exponentially from 0 to 100% with the fraction of radiation intercepted by the canopy (55 to 95%), whatever the susceptibility level of the varieties (Susceptible, Moderately Susceptible, Tolerant). Beyond $f\mathrm{PAR}_i$ of 85% at flowering (i.e. LAI > 2.5), the proportion of stems with infected lesions increased rapidly; below this threshold, the attack rate remained below 20% (Fig. 5). Therefore, obtaining a high leaf area index by increasing plant density and nitrogen fertilization increased the fraction of infected stems, regardless of the intrinsic susceptibility of the varieties (Debaeke and Estragnat, 2003).

Figure 5 – Relationship between the proportion of stems infected by *Phomopsis* and the fraction of PAR intercepted by the sunflower crop at anthesis for weather conditions promoting leaf and stem infection (n = 129) – Genotypes were susceptible, moderately susceptible or tolerant (Debaeke et al., 2003).



This relationship is also valuable in the case of *Phoma* attacks on stems (Debaeke and Peres, 2003): within a range of variation of $f\mathrm{PAR}_i$ from 65% to 95% (corresponding to a LAI range of 1.5 to 5 at flowering), the proportion of infected stems increased with the value of $f\mathrm{PAR}_i$. The number of spots on stems increased significantly above a $f\mathrm{PAR}_i$ of 85% (up to 12 spots per plant) while it remained below 7 per stem below this $f\mathrm{PAR}_i$ threshold (Fig. 6).

Figure 6: Relationship between the proportion of stems with simple *Phoma* spots (class 2) and the fraction of PAR intercepted by sunflower (cv. Select) at early anthesis for different crop management systems (INRA, 1994). D1 = 5.2 plants.m^{-2}, D2 = 6.8 plants.m^{-2}, N30 and N60: N fertilization at sowing time (kg N.ha^{-1}), irr = one irrigation before anthesis, sprayed =one application of Punch CS 0.8 L.ha^{-1}, water stress : plots where senescence was observed before anthesis as a result of water stress (Debaeke and Peres, 2003).



For *Phomopsis*, this close relation can be explained by the effect of crop management on the dynamics of canopy closure: with a high population density (> 6.5 plants.m^{-2}), canopy closure is faster, creating suitable microclimatic conditions (notably relative humidity) for leaf infection (Debaeke and Moinard, 2010; Desanlis et al., 2013); these conditions give rise to early attacks, causing the most severe injury to yield and oil concentration. In addition, dense stands are characterized by smaller leaves and thinner stems, which are more quickly destroyed by *Phomopsis* (Desanlis et al., 2013). N concentration of plant tissues does not appear to affect the fungus's growth within the tissue, and thus the leaf-to-stem passage (Berault, 2000; Desanlis et al., 2013). Changes in the rate of spread of symptoms are related to the susceptibility of the variety and the microclimatic conditions (temperature and relative humidity), irrigation causing an acceleration of the fungus's progression, which on a susceptible variety may vary from 1.5 to 4 cm.day^{-1}, depending on weather conditions.

For phoma, unlike phomopsis, successful infections at the collar seem more dependent on the weather than on microclimatic conditions. Changes in microclimate within the canopy appear more critical for attacks on leaves than at the stem base, where soil moisture concentrated near the collar generally provides favorable conditions for the infection to succeed. This "lesser requirement" may explain the high incidence of collar attacks under natural conditions in areas where the inoculum is abundant (Bordat et al., 2011). Sometimes, however, low levels of attack are observed when weather conditions in June and July are particularly hot and dry. Regarding attacks on the stem, they are widespread in all areas of sunflower cultivation. Unlike *Phomopsi*s, where the fungus necessarily infects the leaves before infecting the stem, *Phoma* attacks on the stem can occur at its insertion point with the petiole. The trough that forms at this level (generally from the 10-12 leaf stage) is conducive to water storage. This creates a microenvironment extremely favorable for the germination of *Phoma* spores as soon as free water is maintained.
Intensifying crop management in sunflower (nitrogen fertilization, plant density, and irrigation) thus increases the fraction of plants infected by *Phomopsis* and *Phoma*.

Sclerotinia at stem base: an underground transmission of the disease

Germination of sclerotia and mycelial growth of *Sclerotinia* affect the root system of sunflower plants. The disease is more common in soils rich in organic matter (deep silt soils, marshland). Reducing plant density (plants spaced at least 40 cm apart) reduces plant-to-plant contamination. Similarly, the distribution of sclerotia down the soil profile affects the attack rate: it decreases from 50% to about 20% when sclerotia are buried at 20 cm, rather than 10cm, depth (Huang and Hoes, 1980).

Sclerotinia on heads: avoid synchronizing flowering and irrigation

The period of sunflower susceptibility to *Sclerotinia* attacks on the capituluum starts from the beginning of flowering and lasts until mid-flowering: ascospores from apothecia settle at floret level on one side of the head. Conditions of high humidity are necessary for spore germination and early colonization of the inflorescence: the presence of continuous free water for at least 39 hours determines the success of the infection phase (Pauvert and Lamarque, 1981). Attacks take place during rainy periods in July. In case of dry weather, the main element of crop management that affects the percentage of infected heads is irrigation at flowering : in low inoculum_pressure conditions, for the same total amount of irrigation (100 mm), infection rates can increase from 1% with two applications to 27% with 5 applications around flowering (Peres and Allard, 2000). Stand structure, through inter-row spacing, also appears to have a significant effect on the percentage of heads with *Sclerotinia*: for the same population density (60,000 plants.ha^{-1}), a row spacing of 50 cm increases the attack level on heads (17% of injured plants) compared to an inter-row of 80 cm (10% of injured plants) (Peres et al., 1992; Peres and Allard, 2000).

3.2. Practices to reduce the severity of symptoms

Phomopsis – The harm caused by Phomopsis to yield and oil content is related to the fate of spots on stems, the most detrimental symptom being at the girdling lesion, where the pith is completely destroyed, leading more or less rapidly to a wilted plant.

Delaying sowing till May helps to reduce the extent of attacks on stems and also has a significant effect on the fraction of plants with at least one girdling spot: depending on the delay (between 12 and 35 days), late planting associated with low-input management results in reduced vegetative growth that can limit the infection rate to between 2% and 31%, as against 73% to 85% with high-input management (Debaeke et al., 2003). In addition, the hot dry periods most common after late infections are unfavorable to fungal growth on plants.

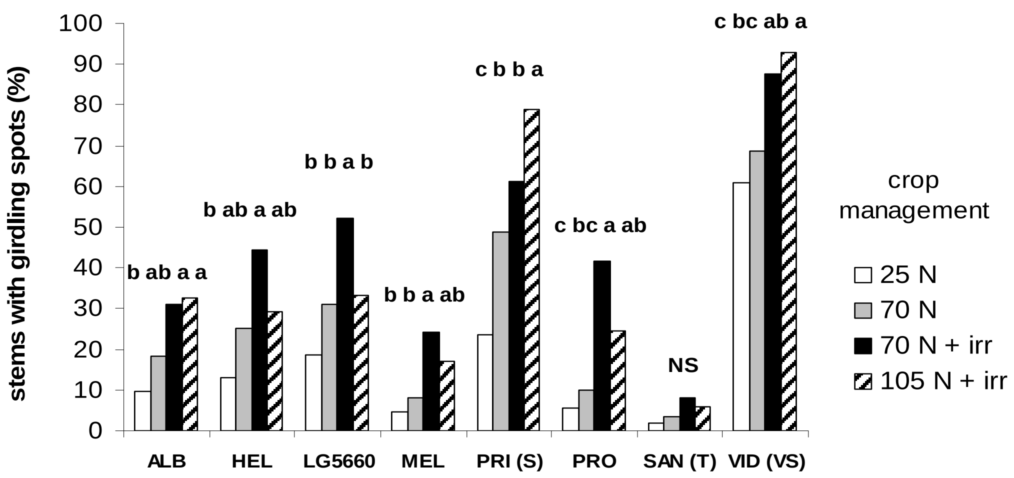
Debaeke et al. (2003) also showed in nine experiments that increasing plant density from 5 to 7.5 plants.m^{-2} increased the proportion of plants developing girdling spots by up to 82%. The effect of plant density on disease severity is the same regardless of the varietal susceptibility (Debaeke and Moinard, 2010).

Irrigation around flowering is the third crop management factor which may affect the severity of attacks: with reinforced semi-natural infection, the fraction of plants with at least one girdling spot increased by 48 % (from 27 to 40%) with two water applications of 35 mm around flowering (Debaeke and Moinard, 2010). Irrigation at flowering increases leaf area duration and thus offers more chances for the fungus to progress on the leaves and develop girdling lesions on stems.

From 1994 to 2005, several field experiments were done in south-west France by INRA and CETIOM to quantify the influence of crop management systems on disease occurrence and yield loss in sunflower (Debaeke et al., 2003).

To illustrate the main results on phomopsis, the 2002 experiment is presented here: 8 cultivars (from tolerant to very susceptible) were submitted to 4 management systems differing in plant density (5 vs 7 plants.m^{-2}), N fertilization (25, 70, 25+80 kg N.ha^{-1}), and irrigation (0 vs 40 mm at anthesis) (Debaeke and Taverne, 2006). The proportion of stems with necrotic lesions was observed on early August. The treatments had a big effect on the proportion of stems severely infected by *Phomopsis* (5 - 95 %) confirming previous results (Debaeke et al., 2003). Disease incidence increased with plant density, N rate and irrigation, irrespective of genotypic tolerance (Fig.7). Splitting nitrogen at sowing and star bud stage appeared to be a good strategy to limit phomopsis occurrence, but an interaction with genotype susceptibility was observed: increasing total N rate was detrimental to susceptible cultivars in spite of splitting. In 10 plots out of 32, yield loss was below the injury threshold (15 % of stems with girdling lesions) and chemical treatment could have been replaced by cultivar tolerance and canopy management in these situations.

Figure 7 - Effect of crop management (N fertilization and irrigation) on phomopsis incidence for 8 cultivars differing in their susceptibility to this disease: (T) tolerant, (S) susceptible, (VS) very susceptible, (-) moderately S or moderately T (Toulouse, 2002) – Comparison of means by LSD at P < 0.05 (Debaeke and Taverne, 2006)



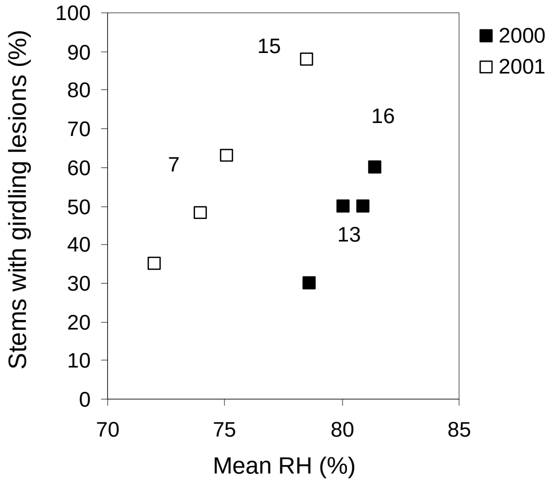
A relationship between fPAR$_i$ at flowering and the fraction of plants with at least one girdling lesion due to *Phomopsis* (PHO) was suggested for susceptible to moderately susceptible varieties (Debaeke and Estragnat, 2009):

$$\text{PHO (\%)} = 1.10\, f\text{PAR}_i\text{ (\%)} - 38.3 \quad (r^2 = 0.661,\ P < 0.0001)$$

On two experiments done in 2000 and 2001 in Auzeville (31), the fraction of plants bearing at least one girdling lesion of *Phomopsis* was closely related to relative humidity (RH) during the infection period (Fig. 8). RH was monitored by placing thermo-hygrometers at 40 cm above the soil. Intensifying crop management through plant density and nitrogen fertilization resulted in higher values of LAI, fPAR$_i$ and RH, with a greater risk of disease development in sunflower (Debaeke and Moinard, 2010).

The observation of the onset of symptoms on a susceptible variety studied in 2000 in Toulouse showed that the earliest attacks on leaves, affecting the bottom layers (leaf nodes 1 to 9), have a lower chance of reaching the stem than those infecting the plant later at upper nodes (leaf nodes 10 to 16). There are two reasons for this: physiological leaf senescence, which begins at the bottom of the plant due to leaf age and shading, and the attacks of *Phoma* on the stem, which begin at the bottom of the plant and prevent the passage of *Phomopsi*s from leaf to stem (Debaeke and Moinard, 2010).

Figure 8 - Relationship between the percentage of plants with girdling spots on the stem and the average relative humidity (RH) in the canopy during the infection period. The figures indicate the number of days suitable for infection as simulated by the Asphodel model in 2000 and 2001 (Debaeke and Moinard, 2010).



The effect of high-input management on *Phomopsis* is therefore obvious on both its incidence and severity. Although under favorable weather conditions associated with abundant inoculum the effects of crop management, however, remain limited (Delos et al., 1994), tests of crop management systems have shown that a given level of *Phomopsis* attack could be achieved either by conventional management based on fungicide or by an integrated management without fungicide, provided crop management is adapted to disease risk; i.e. by reducing plant density and N fertilization and delaying sowing date in a coordinated way (Debaeke and Estragnat, 2003).

Table 3 gives an overview of the effects of irrigation, N fertilization, plant density and variety on the three disease components of phomopsis : number of leaf symptoms, leaf-to-stem passage (%) and fraction of girdling symptoms (%) which all contribute to the final number of girdling symptoms of *Phomopsis* per plant (Desanlis et al., 2013).

Table 3 - Effects of crop management on the three components of phomopsis injury (+: factor promoting the disease; -: factor impeding the disease).

	Leaf symptoms (number)	Leaf-to-Stem passage (% leaf symptoms)	Girdling symptoms (% stem symptoms)
High plant density	early symptoms → microclimate + late symptoms → green leaf area -	small leaves + early senescence -	thin stems +
High Nitrogen	Microclimate + Green leaf area +	big leaves - more phoma - high N tissue content +	thick stems -
Irrigation	Microclimate + green leaf area +	delayed senescence +	
Susceptible genotype	low tissue resistance (leaf) +	low tissue resistance (stem) +	low tissue resistance (stem) +

Phoma and premature ripening

Regarding premature ripening caused by phoma at the stem base, which is the most harmful form of the disease, the idea emerged in 1984 that a combination of biotic stress (related to the pathogen:

nature, quantity, aggressiveness) and abiotic stress (related to water status and crop nitrogen status) could be the cause of this syndrome (Gulya et al., 1984).
Once *Phoma* has infected the plant collar, the onset and progression of premature ripening are strongly influenced by crop water and nitrogen status, resulting from the combined actions of soil, weather and management (N fertilizer, irrigation, plant density).

Four experiments were carried out at INRA Toulouse between 2006 and 2009 to study the effects of crop management on this syndrome (Seassau et al., 2010, 2012). The tests were conducted on two varieties with different susceptibility to phoma (cv. Melody vs cv. Heliasol), during very contrasting growing seasons, combining:
- two water regimes (rainfed vs irrigated after flowering)
- three levels of nitrogen fertilization (0, 50/75 and 150 kg $N.ha^{-1}$)
- three densities, 4, 6.5 and 9 $plants.m^{-2}$.

The effects of these factors were characterized after natural or artificial infection of plants at stem base by mycelium of a single conidium culture of *Phoma macdonaldii* selected for its aggressiveness.

The analysis of the percentage of prematurely ripened plants (i.e. ripened about 2-3 weeks before plants in disease-free conditions) observed from 2006 to 2009 was used to estimate the proportion of variability explained by each crop management factor (water regime, nitrogen availability, variety, population density) and by the method of contamination in order to rank their own contribution to the pathological syndrome (Table 4).

Table 4 – Contribution of five agronomic factors to the total variance of prematurely ripened plants (PR, %) – about 1500 plots observed ; - : the factor was not studied (Seassau, 2010)

Factor	2006	2007	2008	2009
Variety	26,5%	23,4%	-	-
N availability	13,7%	60,2%	84,1%	72,5%
Post-flowering water regime	31,4%	0	0	7,9%
Plant density	-	-	3,9%	7,8%
Contamination method	13,3%	3,9%	-	-

Of the factors studied, N availability contributed the most to the explanation of the PR fraction. Its contribution ranged from 14% (2006: a dry year) to 84% (2008: a wet year) to the variance of PR.
Varietal susceptibility appears as the second most explanatory factor. Water regime may have a strong effect on the disease, but this factor is highly variable depending on precipitation in spring and summer. In 2006 and 2009, when post-flowering stress was pronounced, water regime had a bigger effect on the disease.
In 2007 and 2008, the effect of water regime was concealed by the importance of the nitrogen effect, which alone accounted for between 60 and 84% of PR expression.
Population density contributed little to the variability of PR expression (4-8 %).
Crop management appears to be sufficient by itself to modify disease expression and to increase the proportion of PR plants: a variety susceptible to PR, under conditions of natural contamination, highly fertilized and not irrigated can sustain 100% of PR plants when N deficiency and water satisfaction may result in totally healthy plants. This should be considered in trials used for testing the susceptibility of inbreds and commercial hybrids.

From these experimental results, a simple linear model was built to relate PR plants (from artificial and natural contamination) to crop indicators of N and water status. NNI, the Nitrogen Nutrition Index was selected to represent the crop N status at flowering and the ratio of actual evapotranspiration (ETa) to maximum evapotranspiration (ETo) was used to estimate the plant water status during the post-contamination period (Seassau et al., 2010):

$$PR\ (\%) = 27.8 + 118.3\ NNI - 102.1\ (ETa/ETo)\ (n = 35\ \text{plots},\ r^2 = 0.787)$$

This relationship emphasizes how the adjustment of nitrogen fertilization and satisfaction of water requirements both affect the control of premature ripening.

Plant density is a management factor that would constitute a further control lever of PR. Increasing plant density (D) increased the proportion of PR plants in 83 % (2008) and 100% (2009) of the paired plots (low vs high density) (Seassau et al., 2012).
For this reason, in spite of the slight quantitative effect of plant density, Seassau et al. (2012) proposed a second regression model with 4 variables describing leaf area (LAI), stem diameter growth (SBD, mm), N shoot content (Nm, %) and water satisfaction rate (ETa/ETo, %) explaining 73 % of the variability of PR:

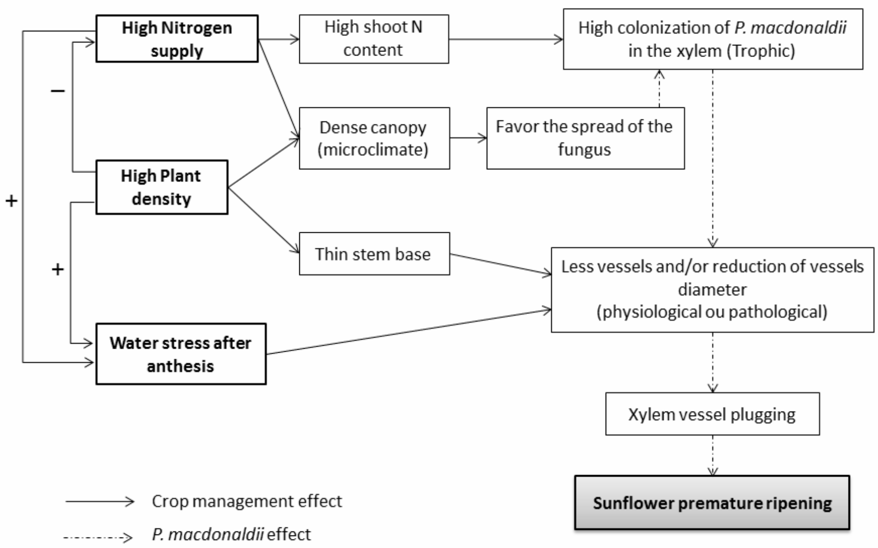
$$PR\ (\%) = 105.79 + 45.3\ Nm - 103.0\ (ETa/ETo) - 5.0\ (SBD) + 12.4\ LAI$$
$$(n = 36\ \text{plots},\ r^2 = 0.731)$$

PR increased with LAI and Nm (indicators of plant growth and N status) and decreased with SBD and ETa/ETo, indicators of plant density and water status

In these experiments, the average stem diameter at collar level for plants grown at different densities (4-9 plants.m^{-2}) can differ from 8 mm between extreme densities. Thus, a population density greater than 7 plants.m^{-2}, by reducing the stem diameter, accelerates the establishment of PR and the resulting damage. By contrast, highly fertilized plants are more susceptible to PR in spite of their thicker stems. It could be a dominant effect of nitrogen: plant susceptibility to disease is independent of collar diameter, but due to a trophic effect of nitrogen which stimulates the development of fungus inside the plant (Seassau, 2010).
Unlike *Phomopsis*, the severity of *Phoma* attacks at collar level appeared less related to the canopy microclimate (Seassau et al., 2012).
From the canopy characterization by agronomic indicators related to N shoot status, plant growth and architecture, stem base morphology, plant water satisfaction rate and microclimate, Seassau et al. (2012) proposed a conceptual framework of the effect of plant density on the expression of the disease (Fig. 9). The microclimate apparently has a moderate effect on disease epidemiology and PR, unlike the other variables that directly affect PR. High leaf area index (due to high N fertilization and high plant density) accelerates soil water exhaustion and a drop in transpiration after anthesis. Avoiding excessive N fertilization by using the soil N balance method could significantly reduce disease severity. Also, manipulating the stem diameter, mainly through planting density and N supply, could be exploited more, instead of resorting to fungicide use. This is probably a morphological trait that breeders could exploit in the future. Indeed, genetic tolerance to PR should be evaluated in order to achieve complete non-chemical control (Bordat et al., 2011). Promising cultivars, with thick stems, should therefore be tested at high density and N supply under water-limited conditions, a procedure which could be used in resistance tests during breeding programs for an effective control of sunflower PR.

Sclerotinia
Regarding white mold attacks on heads, the severity of symptoms depends on the level of genotype resistance and on weather conditions. The head symptoms appear within 3 to 8 weeks after infection at flowering. In areas at risk, the choice of sowing date and the use of early maturing varieties allow wet weather at maturity and hence poor drying conditions for the heads (after mid-September) to be avoided, and may limit the losses at harvest provided that sowing is not too late in spring (CETIOM, 2002). Plant nutrition has a substantial influence on the predisposition of plants to be attacked or affected by diseases. Miladinovic et al. (2008) found a high positive correlation between N content of infected sunflower plants and resistance, which indicates the important role of this nutrient in sunflower defense from *Sclerotinia* attack.

Figure 9 - Potential effect of crop management (water deficit, high nitrogen supply and plant density) on crop canopy development (architecture, morphology) inducing favorable conditions for the spread of *P. macdonaldii* in the plant (xylem) leading to premature ripening (from Seassau et al., 2012)



CONCLUSION

Cultural control must be seen as a combination of practices which can exert some control over the development and expression of fungal diseases (Table 5). Crop management, especially through techniques that control canopy establishment and its duration (plant density, row spacing, nitrogen fertilization, irrigation), can significantly modify the expression of sunflower diseases which develop during the vegetative period.

In the case of phomopsis, manipulating plant density, nitrogen fertilization and irrigation can vary the percentage of stems from 0 to 100% under the same conditions of soil, weather and inoculum pressure. However, in high-risk conditions and with abundant rains in late spring, agronomic measures are not sufficient alone to maintain a level of infection below the threshold of economic profitability of fungicide when susceptible varieties are grown (Debaeke et al., 2003).

In the case of phoma and premature ripening, the range of variation is similar to phomopsis and efficient levers are available (Seassau et al., 2010, 2012). These practices are most effective when they are combined with other practices to help reduce primary inoculum and disease impact on the crop, varietal choice being one of the main levers.
However, although significant genetic progress has been made by breeders to increase the level of resistance / tolerance of sunflower varieties to major fungi, varietal choice may not be the only method to control disease.
In the case of phomopsis, in very favorable conditions for disease, from the observation of 125 varieties over 10 years of experimentation in south-western France, it was concluded that even varieties with good levels of tolerance may result in 10 to more than 30% of plants with girdling spots which is more that the economic injury threshold (Debaeke and Estragnat, 2009).
In the case of sclerotinia diseases, for which breeding is particularly difficult given the highly quantitative genetic determinism of plant-pathogen interactions, all the varieties identified with a good

response could be 100% infected when faced with highly conducive weather conditions, or could bear sclerotia before harvest with harmful consequences for following crops and penalties for the harvest. Finally, with mildew, as the sustainability of specific resistance is uncertain, the choice of varieties with different types of resistance is a crucial decision to be made from year to year (Moinard et al., 2009).

In addition, cultural practices can contribute to the decision of whether to use chemical control as in the case of phomopsis: for instance, LAI or plant height values at star bud stage can be assessed to estimate the risk of disease and influence the spraying decision (Debaeke and Estragnat, 2009).

It is therefore important to enter a virtuous circle, where cultural control has its own place and will be more effective when the disease pressure is lower. The combination of all control methods with partial effects, by their additive or complementary effects, makes for the appropriate overall strategy (Lucas, 2007). In the case of sunflower, current surveys on farming practices in France provide opportunities for progress (judging nitrogen fertilization, suitable sowing time, longer rotations) (Wagner and Lieven, 2010; Jouffret et al., 2011). In addition, the implementation of appropriate farming practices can help to reduce the number of sprays (2.1 treatments; Butault et al, 2010.) which is one of the main agronomic advantages of this low input and drought-tolerant oilseed crop

The sound association, in time and at regional level, of cultural, genetic, chemical and biological control methods is the key to an effective, integrated and sustainable control of sunflower diseases.

Table 5 - Assessment of the effects of agronomic control and other control methods on sunflower diseases.
+ Favorable to the disease, 0: no effect or random effect, - unfavorable to the disease, Ø: not applicable.

Disease		Cultural control						Genetic resistance or tolerance	Fungicide	Biological control
		Rotation lengthening (field level)	Management of infected residues (crushing & burying)	Early sowing	High plant density	High N fertilization	Irrigation			
Mildew		-	0	-	0	0	0	-	-	Ø
Phomopsis	Leaf spots	0	-	+	+	+	+	-	-	Ø
	Non girdling spots (stem)	0	-	+	+	0	+	-	0	Ø
	Girdling spots (stem)	0	-	+	+	+	+	-	0	Ø
Phoma	Stem spots	0	-	0	+	+	+	-	-	Ø
	Stem base spots	0	-	0	+	+	0	-	-	Ø
	Premature ripening	0	-	-	+	+	-	-	0/-	Ø
Sclerotina	Stem base	-	-	0	+	+	0	-	Ø	-
	Capituluum	-	-	-	-	+	+	-	Ø	-

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